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Auxin–Cytokinin Interaction Regulates Meristem Development

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ABSTRACT Plant hormones regulate many aspects of plant growth and development. Both auxin and cytokinin have been known for a long time to act either synergistically or antagonistically to control several significant developmental processes, such as the formation and maintenance of meristem. Over the past few years, exciting progress has been made to reveal the molecular mechanisms underlying the auxin–cytokinin action and interaction. In this review, we shall briefly discuss the major progress made in auxin and cytokinin biosynthesis, auxin transport, and auxin and cytokinin signaling. The frameworks for the complicated interaction of these two hormones in the control of shoot apical meristem and root apical meristem formation as well as their roles in *in vitro* organ regeneration are the major focus of this review.

Key words: Auxin; cytokinin; interaction; shoot meristem; root meristem; development.

INTRODUCTION

Auxin and cytokinin play fairly important roles in many aspects of plant growth and development. The interaction between auxin and cytokinin is particularly important to control a few developmental processes, such as the formation and maintenance of meristems that are essential to establish the whole plant body. For example, the shoot meristems give rise to the above-ground parts of a plant, whereas the root meristems produce the below-ground parts. Many recent studies have provided important information for the understanding of the molecular mechanisms of auxin–cytokinin interaction in the regulation of meristem development.

Maintenance of the cellular optimum auxin concentration can be controlled at multiple levels, such as biosynthesis, transport, perception, and signaling. These multiple regulation pathways contribute to the differential auxin distribution within tissues at different developmental stages. Thus far, one tryptophan (trp)-independent pathway and four trp-dependent pathways for the biosynthesis of auxin/IAA have been proposed in *Arabidopsis* (Zhao, 2010). These four trp-dependent pathways include the indole-3-acetamide (IAM) pathway, the indole-3-acetaldoxime (IAQx) pathway, the tryptamine (TAM) pathway, and the indole-3-pyruvic acid (IPA) pathway. Two of these pathways—the TAM pathway, considered to be rate-limited through the YUCCA family, and the IPA pathway—have also been highlighted (Vanneste and Friml, 2009). Auxin polar transport is required to direct auxin flows and to form auxin gradients in plants, which are critical for developmental pattern formation. In *Arabidopsis*, three protein families are required to mediate auxin transport between cells: auxin efflux PINFORMD (PIN) proteins, MULTIDRUG RESISTANCE (MDR)-p-glycoprotein (PGP) proteins, and auxin influx AUXIN RESISTANT 1 (AUX1)/LIKE AUX1 (LAX) proteins (Benjamins and Scheres, 2008; Gao et al., 2008; Zazímalová et al., 2010).

Besides the biosynthesis and transport of auxin, auxin signaling through receptors and downstream signaling components has also been suggested to be the regulating mechanism for many developmental processes. One of the important auxin receptors in *Arabidopsis* has been identified as the TRANSPORT INHIBITOR REPONSE 1 (TIR1) protein. TIR1 protein is an F-box protein, a component of an SCF^{TIR1} ubiquitination E3 complex. This E3 complex is involved in proteasome-mediated protein degradation (Ruegger et al., 1998; Gray et al., 2001; Quint and Gray, 2006). Analysis of quadruple *tir1*-related mutants highlights the role of TIR1 and at least three other TIR1-related AUXIN BINDING F-BOX PROTEINs (AFB1-3) in the auxin signaling for plant development (Dharmasiri et al., 2005). Thus far, two classes of transcriptional

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regulators represent the core of auxin signaling: the AUXIN/INDOLE-3-ACETIC ACID (Aux/IAA) proteins and AUXIN RESPONSE FACTOR (ARF) proteins (Liscum and Reed, 2002; Quint and Gray, 2006). Aux/IAA proteins are known as a family of transcriptional repressors to negatively regulate auxin signaling (Ulmasov et al., 1997b). Recent studies have provided more extensive evidence that Aux/IAA proteins are the targets of SCF^{TIR1} complex (Paciorek and Friml, 2006; Benjamins and Scheres, 2008; Vanneste and Friml, 2009). Aux/IAAs interact with ARF proteins, a class of transcription factors that mediate auxin-dependent transcriptional regulation (Paciorek and Friml, 2006; Ulmasov et al., 1997b). The ARFs could function as either activators or repressors in the regulation of auxin-induced gene expression (Ulmasov et al., 1997a, 1999).

Like auxin, cytokinin is also a key regulator for various aspects of plant growth and development. Cytokinin homeostasis is spatially and temporally regulated by a fine balance between synthesis and catabolism. The first enzyme identified in the *Arabidopsis* cytokinin biosynthetic pathway is adenosine phosphate-isopentenyltransferases (IPTs). The IPTs are believed to catalyze the transfer of an isopentenyl group from dimethylallyl diphosphate to an adenine nucleotide (ATP, ADP, or AMP) (Kakimoto, 2001; Takei et al., 2001). Another landmark is the identification of two cytochrome P450 monooxygenases, CYP735A1 and CYP735A2, which catalyze the hydroxylation at the prenyl side chain of the iP-nucleotides to synthesize tZ-nucleotides (Takei et al., 2004). Furthermore, a cytokinin-activating enzyme in rice, LONELY GUY (LOG), has been recently identified to catalyze the last step of cytokinin biosynthesis. This step is involved in converting cytokinin-nucleotides produced by IPTs and CYP735As to the free-base form (Kurakawa et al., 2007). Besides biosynthesis, cytokinin homeostasis is also controlled by its catabolism process through CYTOKININ OXIDASE/DEHYDROGENASEs (CKXs) (Werner et al., 2003).

In contrast to auxin, cytokinin is perceived in plants through a multi-step phosphorelay pathway similar to the bacterial two-component signaling system (Kakimoto, 2003; To and Kieber, 2008). In *Arabidopsis*, three transmembrane histidine kinases have been identified as cytokinin receptors, the ARABIDOPSIS HIS KINASE 2 (AHK2), AHK3 and CYTOKININ RESPONSE1 (CRE1)/AHK4 (Hwang and Sheen, 2001; Inoue et al., 2001; Riefler et al., 2006; To and Kieber, 2008). Recent analyses on these three receptors have revealed a largely overlap expression pattern and partially redundant functions in cytokinin perception (Higuchi et al., 2004; Nishimura et al., 2004). Following the initial cytokinin perception, AHKs autophosphorylate themselves and transfer the phosphate group to members of the ARABIDOPSIS HISTIDINE PHOSPHOTRANSFER PROTEINs (AHPs) family. AHPs subsequently translocate to the nucleus to phosphorylate the ARABIDOPSIS RESPONSE REGULATORs (ARRs) proteins of either type-A or type-B (Heyl and Schmülling, 2003; Kakimoto, 2003; Ferreira and Kieber, 2005). Based on the analysis of loss- and gain-of-function mutants, at least six of 11 type-B ARRs (ARR1, ARR2, ARR10–ARR12, and ARR18) have overlapping functions in the positive regulation of the cytokinin response (Mason et al., 2005; Yokoyama et al., 2007). Furthermore, the *arr1*, *arr10*, *arr12* triple mutants show a strong reduction in cytokinin induction of multiple type-A ARR transcripts (Mason et al., 2005). Mutation analysis has indicated that at least eight of the 10 type-A ARRs are negative regulators of cytokinin signaling again with overlapping functions (To et al., 2004; To and Kieber, 2008).

For the past few years, genetic and molecular evidence has revealed the interaction between auxin and cytokinin during plant development (Dettmer et al., 2009; Růžičkaa et al., 2009; Wolters and Jürgens, 2009; Zhao et al., 2010). Many recent biochemical and genetic investigations have further confirmed that the intricate cross-talk and integration of hormone signaling are required for differentiation and maintenance of plant meristems. Hereafter, we shall focus our discussion on the interaction between auxin and cytokinin in the regulation of meristem development.

AUXIN AND CYTOKININ REGULATE MERISTEM FORMATION IN EARLY EMBRYOGENESIS

Auxin and cytokinin control events of major cell specification during embryogenesis (Müller and Sheen, 2008; Möller and Weijers, 2009). The first step of embryonic patterning is the establishment of the apical–basal axis, in which asymmetric distribution of auxin mediated by PIN proteins plays a major role. A zygote undergoes an asymmetric division to produce a smaller apical cell and a larger basal cell. The apical cell will generate the pro-embryo, while the basal cell will give rise to the suspensor (Mansfield and Briarty, 1991; Laux and Jürgens, 1997). At this two-cell embryo stage, PIN7 is expressed in the basal cell to transport auxin to the apical cell (Friml et al., 2003). After two more rounds of cell division, PIN7 localizes to the apical membrane of suspensor cells, resulting in the accumulation of auxin in the whole pro-embryos (Friml et al., 2003; Jenik and Barton, 2005). At the 16-cell globular stage, *WUSCHEL* (*WUS*) is switched on in the four inner cells of the pro-embryo, and it is an early molecular marker that represents initiation of the shoot apical meristem (SAM) in the embryo (Weigel and Jürgens, 2002). This *WUS* induction might be related to auxin accumulation. However, PIN7 polarity is reversed at the 32-cell stage, resulting in the transport of auxin towards the suspensor cells (Friml et al., 2003; Jenik et al., 2007). The transported auxin accumulates in the uppermost cell of the suspensor to form the hypophysis, the founder of the stem-cell niche of the embryonic root (Friml et al., 2003). At a later transition stage of the embryo, auxin is directed towards the center of the cotyledon primordia in the apical domain to establish the cotyledons. This early heart-stage embryo shows a cleft where the SAM will form (Weigel and Jürgens, 2002). Therefore, auxin transport is critical for the maintenance of the polar axis and the formation of two types of meristems in the embryo.

MONOPTEROS (*MP*)/*ARF5* and *BODENLOS* (*BDL*)/*IAA12* are central to auxin response during embryogenesis in *Arabidopsis*

(Hamann et al., 2002; Weijers et al., 2006). *MP* has been proposed to mediate the establishment of the embryonic axial pattern by modulating TARGET OF MP 5 (TMO5) and TMO7 functions in response to auxin signals (Schlereth et al., 2010). Loss-of-function of *MP* or gain-of-function of *BDL* causes the aberrant specification of the apical cell, and prevented the formation of the embryonic root (Weijers et al., 2006). MP promotes the expression of *PIN1* in pro-vascular cells of the globular embryos, leading to auxin accumulation at the basal pole of pro-embryos (Wolters and Jürgens, 2009). Embryos of the *mp* mutants are abnormal at their early globular stages. In addition, heart-stage *mp* embryos lack the central pro-vascular cylinder. As a result, no hypocotyl and primary root meristems are formed in *mp* mutants (Berleth and Jürgens, 1993). The gain-of-function *bdl* mutants show milder defects than *mp* mutants and have a reduced vascular system and a hypocotyl of variable length without primary root meristem (Hamann et al., 2002; Mattsson et al., 2003). Therefore, the primary auxin response mediated by MP and BDL is essential for root meristem initiation. MP and BDL are also known factors for activating quiescent centre (QC)-specific *WUSCHEL RELATED HOMEOBOX5 (WOX5)* and auxin-responsive *PLETHORA* (*PLT*) genes (Wolters and Jürgens, 2009). The double mutants *wox8 wox9* displayed abnormal *PIN1* expression pattern and abnormal auxin response in the embryo. WOX2 and WOX8 act redundantly with MP to promote *PIN1* expression and to regulate localized auxin gradients (Breuninger et al., 2008). Therefore, auxin transport and response are required to trigger the specification of the root meristem founder cell.

Even though auxin has been known for a long time to play a crucial role in specification of root stem-cell during embryogenesis, the function of cytokinin in early embryogenesis is recently suggested for a transient and antagonistic interaction between auxin and cytokinin (Müller and Sheen, 2008). Cytokinin signaling components are first detected in the hypophysis, the founder cell of the root meristem at the early globular stage of the embryo. After the first division, the apical daughter cell of the hypophysis remains to maintain the phophorelay activity of cytokinin signaling and is the precursor of the QC, whereas the basal daughter cell represses cytokinin signaling. Interestingly, in the basal cell of the hypophysis, auxin antagonizes cytokinin signaling by directly activating the repressors of cytokinin signaling, *ARR7* and *ARR15* (Figure 1) (Müller and Sheen, 2008). Thus, to sustain the activity of the embryonic root stem-cell niche, auxin mediates the suppression of cytokinin signaling in the basal cell of the hypophysis.

CYTOKININ–AUXIN CROSS-TALK CONTROLS SHOOT MERISTEM DEVELOPMENT

The SAM arises during embryogenesis and generates almost all of the aerial parts of a plant. It can be subdivided into different regions, including the central zone, peripheral zone, and rib zone (Fletcher and Meyerowitz, 2000; Clark, 2001; Sablowski, 2007). The central zone is located in the center and at the summit of the meristem in which cells divide more slowly. It provides cells to both the peripheral zone and the rib zone. The surrounding peripheral zone has a higher cell division rate and gives rise to lateral organs. The rib zone is below the central zone from which the tissues of the stem are generated. Leaf primordia originate from a group of cells in the peripheral zone of the SAM, which is then replenished by cell division in both the peripheral zone itself and the central zone. Active cell division and cell differentiation occur in SAM (Clark, 2001; Williams and Fletcher, 2005; Reddy, 2008). Thus, balance

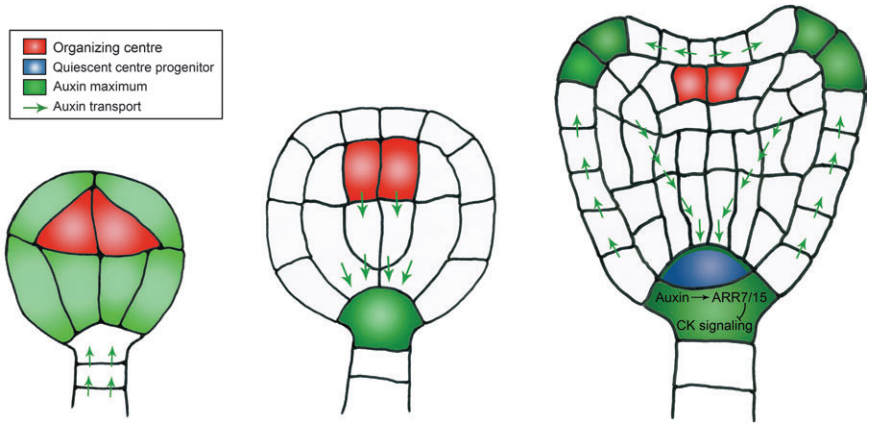

Figure 1. Interaction of Auxin and Cytokinin in Embryogenesis.

At the 16-cell stage of pro-embryo, auxin is transported from suspensor cells to the apical pro-embryo and accumulates in whole pro-embryo, in which *WUS* is initially expressed in four inner cells of this early-globular embryo. In the late-globular embryo, directional auxin transport is reversed towards the suspensor cells, leading to the accumulation of auxin in the uppermost cell of the suspensor to form the hypophysis. By the transition stage, the hypophysis has undergone asymmetrical cell division to result in the formation of the upper small cell and the large basal daughter cell. The upper cell maintains phosphorelay activity of cytokinin signaling. In the large basal cell, auxin represses cytokinin signaling through an ARR7/15-dependent pathway. This antagonistic interaction between auxin and cytokinin in both cells controls the establishment of the embryonic root stem-cell niche.

between cell division and cell differentiation is necessary to control the size and structure of SAM. The size of SAM is maintained by a negative feedback loop involving *WUS* and *CLAVATA3* (*CLV3*) in *Arabidopsis* (Brand et al., 2000; Schoof et al., 2000). *WUS*-expressing cells of the organizing center (OC) maintain the overlying cells as the stem cells within the central zone (Mayer et al., 1998; Schoof et al., 2000). *SHOOTSTEMLESS* (*STM*) is also required for the maintenance of stem cells in the meristem, and its expression in the whole shoot meristem prevents stem cells from switching to organ-specific cells (Clark et al., 1996; Endrizzi et al., 1996; Long et al., 1996).

It has been known for a long time that cytokinin plays a major role in regulating meristem function. Studies on the classical chemical regulation of growth and organ formation in *in vitro* plant tissues have indicated that excess of cytokinin over auxin promotes shoot formation from callus (Skoog and Miller, 1957). This suggests a positive action of cytokinin on SAM activity. Recent studies have shown that cytokinin deficiency reduced shoot meristem size and activity (Werner et al., 2003; Higuchi et al., 2004; Werner and Schmülling, 2009). Similarly, mutations in *AtIPTs* also cause reduction of the SAM size, further supporting the positive role of cytokinin in controlling shoot meristem activity (Miyawaki et al., 2006). These observations are consistent with the role of cytokinin in meristem development.

The mutual regulation of cytokinin and stem-cell-related genes can explain the regulation of cytokinin production and signaling in the SAM (Figure 2A). STM, a member of Class I KNOTTED-like homeobox (KNOXI) proteins, induces cytokinin biosynthesis through activating *AtIPT7* in *Arabidopsis* (Jasinski et al., 2005; Yanai et al., 2005). Cytokinin triggers a rapid increase in mRNA levels of the *KNOXI* genes (Rupp et al., 1999), indicating a positive feedback loop between *STM* and cytokinin signaling (Figure 2A) (Wolters and Jürgens, 2009). KNOX transcription factors repress the biosynthesis of the growth regulator gibberellin (GA) to maintain normal meristem function. Cytokinin also stimulates the expression of genes involved in GA catabolism to reinforce the low-GA levels established by the KNOX proteins within the SAM (Jasinski et al., 2005; Wolters and Jürgens, 2009). Thus, KNOX proteins are essential for meristem development as the key growth-regulators by simultaneously activating CK and repressing GA biosynthesis. In addition, many other studies have indicated that type-A *ARRs* (*ARR7*/*15*), the main response genes of cytokinin signaling, are required for *CLV3* expression (Zhao et al., 2010). Since CLV3 limits the expression of *WUS*, which, in turn, down-regulates the expression of *ARR5*, *ARR7*, and *ARR15* (Leibfried et al., 2005), a negative regulation loop exists between type-A *ARRs* and *WUS* in the shoot apical meristem. Recent studies suggest that cytokinin signaling partially regulates *WUS* expression through this CLV-dependent pathway (Gordon et al., 2009). The induction of *WUS* transcripts in the *clv* mutants after cytokinin treatment reveals a CLV-independent mechanism of cytokinin-induced *WUS* expression. This investigation demonstrates that cytokinin-induced increase of *WUS* transcripts is mediated primarily through an AHK2/AHK4-dependent pathway (Figure 2A) (Gordon et al., 2009).

Auxin also plays a critical role in the maintenance of shoot meristem. Auxin produced by *YUCCA* genes accumulates in the central zone of meristem at an optimal level to stimulate downstream auxin-induced genes through the Aux/IAA–ARF signaling pathway (Zhao, 2008). Interestingly, a dramatic increase in *ARR7* and *ARR15* expression was observed in the SAM of the *yucca* mutants, the *pin1* mutants, the *pinoid* mutants, as well as plants treated with N-1-naphthylphthalamic acid (NPA), indicating that *ARR7* and *ARR15* activation can be directly induced by the loss of local auxin accumulation (Zhao et al., 2010). Because auxin suppresses the expression of *STM* that promotes cytokinin biosynthesis in the shoot meristem (Heisler et al., 2005; Jasinski et al., 2005; Yanai et al., 2005), auxin might function on the repression of *ARR7* and *ARR15* through the STM-mediated pathway. Another pathway has been identified through the analysis of *ARR7* and *ARR15* expression in the *mp* mutants. *ARR7* and *ARR15* are ectopically expressed in the central zone as well as in the peripheral zone in *mp* mutants, but absent in wild-type (Zhao et al., 2010). This suggests that *MP* limits the *ARR7* and *ARR15* expression both in the central zone and the peripheral zone of the meristem. Therefore, *ARR7* and *ARR15* act not only as suppressors of cytokinin signaling, but also as targets of *MP*-mediated auxin signaling in the central zone. These results also suggest that auxin and cytokinin signaling converge on *ARR7* and *ARR15* in the central zone of meristem during the development of shoot apical meristem (Figure 2A). In contrast to cytokinin, auxin accumulates at a relatively high level in the peripheral zone of the shoot meristem to trigger organ initiation (Benková et al., 2003; Reinhardt et al., 2003). Auxin has recently been shown to rapidly down-regulate cytokinin biosynthesis in the shoot (Nordström et al., 2004). In addition, auxin accumulation, facilitated by the efflux or influx carriers to various organ initiation sites, suppresses the expression of *STM*, which acts as an inhibitor of stem-cell differentiation (Furutani et al., 2004; Heisler et al., 2005). Because *STM* has been proved to be a positive factor of cytokinin biosynthesis (Jasinski et al., 2005; Yanai et al., 2005), a model is proposed that auxin antagonizes cytokinin for organ initiation in the peripheral zone of the meristem (Figure 2A).

Cytokinin also functions on the organ initiation (Shani et al., 2006; Perilli et al., 2010). At the site of organ initiation both in the embryonic SAM and in the inflorescence meristem, cytokinin levels are reduced by auxin (Wolters and Jürgens, 2009). Thus, cytokinin responses are also negatively regulated by auxin. High levels of cytokinin are required for the maintenance of stem cells in the meristem, but not for organ initiation. Similarly to *ARR7* in *Arabidopsis*, *ABERRANT PHYLLOTAXY1* (*ABPH1*), encoding a type-A ARR, is expressed in a specific pattern in the maize SAM. Mutation in *ABPH1* leads to significantly larger SAM and an altered phyllotaxy (Giulini et al., 2004). These phenotypes suggest that ABPH1 restricts the size of the central zone of shoot meristem through negatively regulating cytokinin signaling. Since *PIN1* is significantly down-regulated in the *abph1* mutants, ABPH1 may be a convergent point of auxin signaling and cytokinin signaling

to define the position of leaf primordia in maize (Lee et al., 2009). In addition, cytokinin–auxin interaction is also involved in the predominant shoot apex growth, which inhibits the outgrowth of axillary bud. In pea, it is demonstrated that auxin flows basipetally, mediated by *PsPIN*s from the shoot apex to repress *PsIPT* expression, which is the gene for cytokinin biosynthesis (Figure 2B) (Shimizu-Sato et al., 2009). Consequently, the reduced levels of cytokinin increase apical dominance and inhibit axillary bud growth. MicroRNAs (miRNAs), which are post-transcriptional negative regulators in plants, are thought to play important roles in regulating shoot branching. The regulation of shoot branch production by miRNAs might correlate with the activities of auxin and cytokinin (Wang et al., 2010).

AUXIN–CYTOKININ INTERACTION REGULATES ROOT MERISTEM DEVELOPMENT

Post-embryonic root tip growth is sustained by the root meristem, which is divided into the proximal meristem (PM), the

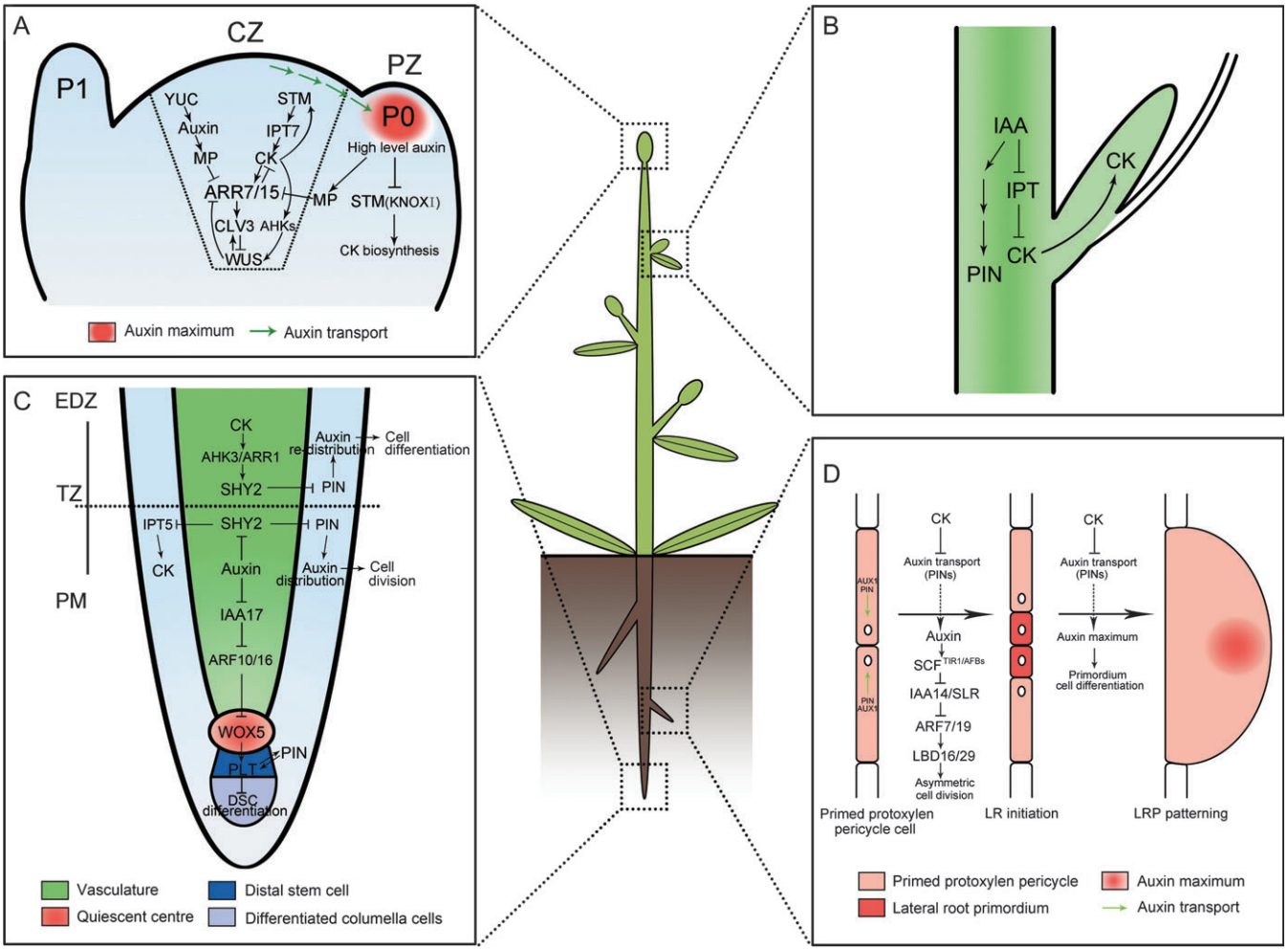

Figure 2. Molecular Mechanisms of Auxin and Cytokinin Interaction in the Regulation of Plant Meristem Development.

(A) In CZ of shoot meristem, ARR7 and ARR15 act as integrative factors in auxin and cytokinin signaling pathways. Auxin represses the expression of *ARR7* and *ARR15* while cytokinin promotes their expression through a STM-dependent pathway. Both of them regulate the expression of *WUS* in a negative feedback loop, critical for stem-cell formation. During the formation of lateral organ primordia, a high level of auxin transported from CZ blocks the biosynthesis of cytokinin by suppressing KNOXI function in PZ. CZ, central zone; PZ, peripheral zone; P0/P1, organ primordia.

(B) Auxin is transported from the shoot apex to repress cytokinin biosynthesis, leading to the inhibition of axillary bud growth.

(C) In the root meristem, auxin promotes the expression of *PIN*s through the degradation of SHY2 proteins, resulting in the maintenance of an auxin gradients and cell division. In contrast, cytokinin impedes the expression of *PIN*s by stimulating the expression of *SHY2*, leading to auxin redistribution and cell differentiation. Auxin also plays an important role in the differentiation of root DSC by mediating the expression of *WOX5* and *PLT*. PM, proximal meristem; EDZ, elongation differentiation zone; TZ, transition zone; DSC, distal stem cell.

(D) In certain xylempole pericycle cells, the transport and perception of auxin trigger an asymmetric cell division critical for the LR initiation and LRP patterning. By contrast, cytokinin negatively regulates the LR initiation and LRP patterning by inhibiting the expression of *PIN*s and the auxin distribution gradients. LR, lateral roots; LRP, lateral root primordia.

elongation differentiation zone (EDZ), and the transition zone (TZ) (Dello Ioio et al., 2007). QC is located in the region at the tip of PM, and gives rise to stem cells. Root stem cells generate daughter cells that subsequently undergo division in the PM or differentiation deviating from the PM to become the members of TZ cells. Therefore, a balance between the rate of cell division and differentiation is crucial for the maintenance of root meristem. Studies have shown that WOX5 (expressed in the QC cells) and PLTs (expressed in the stem cells surrounding the QC), major regulators of stem-cell formation and maintenance, act downstream of auxin signaling for the maintenance of distal stem-cell activity. Indeed, auxin signaling for the fate of the distal stem cells requires IAA17/AUXIN RESISTANT3 (AXR3) as well as auxin response factors (ARF10 and ARF16). Both ARF10 and ARF16 negatively regulate *WOX5* transcription and restrict *WOX5* transcripts to the QC center, thereby suppressing *PLT* gene expression and mediating the differentiation of distal stem cells in roots (Figure 2C) (Ding and Friml, 2010).

The auxin–cytokinin cross-talk also controls the root meristem development (Werner et al., 2003; Dello Ioio et al., 2007). Several studies have shown that cytokinin and auxin mutually regulate their signaling pathways or their metabolisms through certain integrators, which are the basis of interaction between these two hormones to determine a specific developmental output in root meristem (Dello Ioio et al., 2008; Moubayidin et al., 2009; Růžičkaa et al., 2009). Recently, a genetic framework has shown that antagonistic interaction between cytokinin and auxin is responsible for the control of cell division and cell differentiation in the root meristem (Dello Ioio et al., 2008; Moubayidin et al., 2009). This antagonistic interaction has been demonstrated to occur through a simple regulatory circuit via the SHORT HYPOCOTYL 2 (SHY2/IAA3), which is a member of the Aux/IAA gene family (Tian et al., 2003; Dello Ioio et al., 2008). In the wild-type roots, transcription of *SHY2* in the vascular tissues of root meristem TZ is enhanced by cytokinin application. ARR1, a member of cytokinin signaling regulators, directly binds to the promoter of *SHY2* (Dello Ioio et al., 2008). Moreover, no up-regulation of *SHY2* expression in the roots of *arr1* after cytokinin treatment suggests an ARR1-mediated cytokinin-positive regulation of *SHY2* in the vascular tissues of TZ. Furthermore, the *SHY2* gain-of-function or loss-of-function mutants show smaller or larger root meristems, respectively, confirming that *SHY2* is necessary for the control of cytokinin over the size of root meristem (Dello Ioio et al., 2008). Activation of *SHY2* results in repression of *PIN* genes expressed in the TZ, leading to the redistribution of auxin for cell differentiation (Figure 2C) (Dello Ioio et al., 2008; Moubayidin et al., 2009). Conversely, auxin mediates degradation of SHY2 protein, which is necessary for the transcription of *PIN* genes (Tian et al., 2003; Dello Ioio et al., 2008). PIN proteins collectively mediate auxin optimal distribution to regulate cell division and cell expansion in the root meristem, through a positive regulatory loop with the major regulators of stem-cell activity genes, *PLTs* (Blilou et al., 2005; Galinha et al., 2007; Grieneisen et al., 2007). Thus, this pathway mediated by PINs positively regulates cell division in the TZ to maintain the root meristem size.

Thus, cytokinin and auxin interact antagonistically to control the balance of cell division and differentiation mainly in the vascular tissue of TZ (Dello Ioio et al., 2008; Moubayidin et al., 2009). SHY2 seems to be an integrating factor that mediates not only the regulation of cytokinin-to-cell differentiation, but also the regulation of auxin-to-cell division (Figure 2C). Other results demonstrate that cytokinin regulates root meristem through the modulation on the *PIN* expression (Růžičkaa et al., 2009). It has been shown that cytokinin controls meristem size and *PIN1* expression through the AHK-mediated cytokinin signaling (Růžičkaa et al., 2009). Additionally, SHY2 also represses the activation of *IPT5* gene in the root meristem (Dello Ioio et al., 2008).

An antagonistic interaction between auxin and cytokinin biosynthesis has also been suggested in both developing roots and shoots. A recent study has shown that ectopic cytokinin results in a rapid increase in auxin biosynthesis in young roots and shoots (Jones et al., 2010). In contrast, the reduced cytokinin level represses auxin biosynthesis. This phenomenon indicates a cytokinin-mediated positive regulation of auxin synthesis. Together with the previous results, a model has been proposed for a homeostatic feedback regulatory loop involving in auxin and cytokinin signaling in developing roots and shoots tissue (Jones et al., 2010). According to this model, cytokinin functions as a positive regulator of auxin biosynthesis and auxin, however, represses cytokinin biosynthesis.

Studies on *Arabidopsis* and other plant species have revealed the roles of auxin and cytokinin in the formation of lateral roots (LR). Physiological and genetic data have demonstrated that auxin promotes LR initiation and lateral root primordium (LRP) development (Fukaki and Tasaka, 2009; Peret et al., 2009). First, auxin signals are transported by AUX1 and PINs to the protoxylem pericycle cells (Ditengou et al., 2008), and such signals are perceived by the F-box auxin receptors, TIR1 and AFBs (AFB1, AFB2, and AFB3) (Dharmasiri et al., 2005; Pérez-Torres et al., 2008). Second, the perception of auxin results in the degradation of Aux/IAA repressor proteins IAA14/ SOLITARY-ROOT (SLR) through $SCF^{TIR1/AFBs}$ complexes and 26S proteasomes (Fukaki et al., 2002). The de-repression of the ARF protein activity (ARF7/ARF19) activates the target genes LATERAL ORGAN BOUNDARIES-DOMAIN 16 (LBD16)/ASYMMETRIC LEAVES2-LIKE 18 (ASL18), LBD29/ASL16, and other targets required for asymmetric cell division during LR initiation (Wilmoth et al., 2005; Okushima et al., 2007; Fukaki and Tasaka, 2009; Peret et al., 2009). In contrast, cytokinin negatively regulates LR formation, probably through inhibiting auxin-induced expression of *PIN* genes and perturbing the establishment of an auxin gradient for LR initiation (Figure 2D) (Laplaze et al., 2007; Fukaki and Tasaka, 2009). Laplaze et al. (2007) have also shown that exogenous cytokinin inhibits the expression of several *PIN*s in LRP, suggesting that cytokinin prevents the PIN-mediated

auxin accumulation required for normal LRP patterning (Figure 2D).

AUXIN–CYTOKININ INTERACTION REGULATES *IN VITRO* ORGANOGENESIS

Regeneration of a patterned multi-cellular organism from the adult somatic tissue is a well-known phenomenon. Compared with animals, plants have a profound capacity to regenerate organs from their differentiated somatic tissues through the manipulation of plant hormones. The pioneering work has shown that a high auxin/cytokinin ratio induces root regeneration, whereas a low ratio promotes shoot induction (Skoog and Miller, 1957). This indicates that auxin and cytokinin might have a cross-talk during *in vitro* organogenesis. So far, the molecular mechanism of such interaction between auxin and cytokinin in the *in vitro* formation of meristem remains mostly unknown.

During *in vitro* establishment of the shoot meristem from the cultured root explants, cytokinin triggers the induction of ectopic *WUS* expression within the callus, as *WUS* expression is sufficient for inducing shoot regeneration (Gordon et al., 2009). Auxin pretreatment of *Arabidopsis* root explants leads to the up-regulation of a cytokinin receptor gene *AHK4* expression during the callus formation. The increased *AHK4* transcription is required for *WUS* activation during the shoot induction (Gordon et al., 2009). Buechel et al. (2009) have characterized the roles of ARR7 and ARR15 in shoot regeneration. They have found that overexpression of *ARR7* and *ARR15* suppresses shoot regeneration, whereas loss-of-function of these two genes strongly stimulates callus formation on auxin-rich callus-inducing medium (CIM) and promotes shoot induction on cytokinin-rich shoot-inducing medium (SIM) (Buechel et al., 2009). Take together, the previous studies indicate that exogenous auxin up-regulates the expression of *AHK4* on CIM. Then, AHK4 enhances the response to exogenous cytokinin when callus is transferred to SIM. High cytokinin response promotes *WUS* expression, which is essential to induce the *de novo* formation of shoot meristem.

Another study has shown the role of cytokinin in the auxin-induced organ formation using hypocotyl explants (Pernisová et al., 2009). Based on this study, treatment with exogenous auxin triggers the organogenic processes, accompanied by the production of endogenous cytokinin and tissue-specific activation of cytokinin signaling. Endogenous cytokinin modulates this auxin-induced organogenesis via a negative regulation on the expression of PIN proteins, leading to an optimal auxin distribution for the induction of root-like organ (Pernisová et al., 2009). This study suggests that auxin is capable of inducing organogenesis in hypocotyl explants. Cytokinin modulates auxin distribution through its regulation on auxin efflux during this type of organogenesis.

We have reported the effect of the cytokinin/auxin ratio on *WUS* expression in inflorescence formation from *Arabidopsis* pistil explants (Cheng et al., 2010). Our results suggest that a high ratio of cytokinin/auxin promotes shoot regeneration through the induction on *WUS* expression. Our recent studies have also shown that the local establishment of auxin gradients mediated by the tissue-specific auxin biosynthesis and the dynamic changes of PIN proteins is important for shoot regeneration within callus (unpublished data). Moreover, the local biosynthesis of cytokinin in this regeneration system is required for the regional distribution of cytokinin and *WUS* expression. Thus, it is critical to identify the crucial signaling components that integrate the auxin and cytokinin signaling for callus induction and shoot regeneration. Auxin-regulated *WUS* expression is necessary for SAM formation during somatic embryogenesis (Su et al., 2009). The establishment of auxin gradients is correlated with induced *WUS* expression and subsequent SAM formation. These results might shed new light on the cross-talk between the two hormones in regulating shoot and root stem-cell formation during somatic embryogenesis.

CONCLUDING REMARKS AND PERSPECTIVES

Understanding of the auxin and cytokinin interaction in the regulation of plant development and organogenesis has advanced considerably over more than a half-century. Many early experiments have revealed the essential roles of both hormones in the cell proliferation and new organ regeneration. It is amazing that the decision of cell fate in specific tissues depends on the ratio between auxin and cytokinin, which maintains the cell proliferation or stimulates cell differentiation to form new organs, such as shoots or roots. Studies on hormones over the past two decades have been mainly focused on analyzing the mutants of genes involved in hormone synthesis and catabolism, and those encoding for receptors and signaling components. Owing to the extensive functional redundancy among gene family members, analyzing the feedback regulation of both hormone pathways is still complicated work to do. Recently, research has been geared to identifying the key factors involved in the interaction of these two hormones to control specific aspects of plant development. The cross-talk between cytokinin and auxin in the shoot and root meristem is highlighted in Figure 2. In the root meristem, auxin induces the meristematic cell division, whereas cytokinin promotes the cell to switch from the meristematic to differentiated state through inhibiting auxin signaling. In contrast, in the shoot meristem, cytokinin promotes stem-cell proliferation and inhibits stem-cell differentiation, whereas auxin triggers organ primordium initiation through repressing cytokinin biosynthesis. Thus, the antagonistic interactions are the key regulators for the cell differentiation and maintenance in the root meristem transition zone or shoot meristem organ initiation sites.

The models proposed in this review are still not sufficient to fully understand the mechanisms of hormonal interaction between auxin and cytokinin. Because most regulators involved in auxin and cytokinin signaling function in a tissue-specific

manner, we may use micro-dissection or other techniques to harvest individual cells from specific tissue sections to aid such studies. By analyses of genome-wide profiling for epigenetics, transcriptomics, and proteomics in the harvested cells of different tissues, new information should be provided about tissue-specific gene regulatory networks involved in the hormonal cross-talk. Further studies on the structural analysis of the key integrators will be also critical to reveal how transcription factors in the auxin signaling pathway potentially interface with those in cytokinin signaling through protein–protein or protein–DNA interactions. For example, the crystal structure of auxin receptor TIR1 has been presented to explain how this protein is in complexes with auxin and the domain II region of an Aux/IAA protein (Tan et al., 2007). Additionally, the *in vitro* organogenesis may be a powerful system to study the mechanisms of hormonal cross-talk during plant organogenesis, because the regenerated organs can be induced by the known ratio of auxin and cytokinin (Skoog and Miller, 1957). Also, the single type of organs, such as shoots or roots, can be regenerated from the differentiated tissues to investigate the mechanisms involved in hormone-regulated organ regeneration.

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