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The Cytokinin Side Chain Commands Shooting

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Functional distinction between structurally diverse cytokinins as essential plant hormones has remained enigmatic for decades. In this issue of *Developmental Cell*, Kiba et al. (2013) provide compelling evidence for the central role of CYP735A1/2 in synthesizing *trans*-hydroxylated cytokinins, which specify shoot growth, vital for energy and biomass production.

Complex land plants have evolved different organs for specialized and complementary functions to support a sessile and photoautotrophic life strategy. Photosynthetic leaves and inflorescence stems bearing flowers, fruits, and seeds are especially vital organs for the mission of sustainable energy acquisition and reproduction, whereas the root system anchors and supports plants for water and inorganic nutrient uptake. All plant organs originate from the shoot apical meristem (SAM) and the root apical meristem (RAM) at the shoot and root tips, respectively, during postembryonic development. The plant growth hormone cytokinins are known to play essential roles in maintaining the size and activity of the SAM and RAM (Hwang et al., 2012). However, classical bioassays with exogenous cytokinins or genetic manipulation of cytokinin degradation by cytokinin oxidases (CKXs) or cytokinin synthesis and signaling have documented opposing roles of cytokinins: they promote shoot growth but inhibit the root system, suggesting the biological complexity of multifaceted cytokinin actions (Hwang et al., 2012; Miyawaki et al., 2006; Werner et al., 2003).

Structurally, cytokinins are adenine derivatives carrying either an isoprene-derived or an aromatic side chain at the N^6 terminus. The isoprenoid cytokinins, N^6-(Δ^2-isopentenyl)adenine(iP)-type and *trans*-zeatin(*tZ*)-type, are abundant in most plants. Small side-chain variations, e.g., hydroxyl groups and their stereoisomeric position, distinguish different cytokinins (Figure 1), but the biological significance of different variants is poorly understood (Hirose et al., 2008). Although variable side chains may direct differential perception, degradation, and transport of cytokinins (Choi et al., 2012; Hirose et al., 2008; Stolz et al., 2011; Werner et al., 2003), whether structurally distinct cytokinins specify distinct regulatory roles in diverse processes of plant growth and development remained unresolved. In this issue of *Developmental Cell*, Kiba et al. (2013) now unequivocally define the physiological functions of cytokinin side-chain modification.

Although *tZ* was first isolated in 1963, Kiba et al. (2013) are the first to demonstrate that the *Arabidopsis* cytochrome P450 monooxygenases, CYP735A1 and CYP735A2, provide specific activities for *trans*-hydroxylation of iP riboside 5′-phosphates (iPRPs) and generation of *tZ*-type cytokinins (Figure 1). Although single mutants grow normally, *cyp735a1/cyp735a2* double mutants (*cypDM*) reveal surprisingly specific roles of *tZ*-type cytokinins in shoot growth promotion, defining the size of rosette leaves, petioles, the SAM, inflorescence stems, and the number of leaf epidermal cells, flowers, and siliques (Figure 1). Interestingly, these *cypDM* mutants exhibit undetectable perturbation in the root system, suggesting that *tZ* is dispensable for normal root growth and development. Exogenous application of *tZ*, but not 10-fold excess iP, fully complements the shoot growth in *cypDM* mutants. *CYP735A2* overexpression enhances *tZ*-type cytokinins, leaf expansion, and shoot growth without overt root phenotypes. Because *CYP735A* orthologs do not exist in algae, moss, and lycophyte, these comprehensive and exciting findings provide the first conclusive evidence for differential regulation of more elaborate shoot and root developmental programs by distinct cytokinins in higher plants (Figure 1).

Because different cytokinin receptors display differential ligand binding affinity and shoot versus root function and expression (Choi et al., 2012; Stolz et al., 2011), the roles of each of the three *Arabidopsis* histidine kinase (AHK2, AHK3, and AHK4) cytokinin receptors were examined in the *cypDM* mutant background. Although AHK3 and AHK2 play more prominent roles than AHK4 in shoot growth, all cytokinin receptors contribute to *tZ* perception and signaling in shoots. Thus, the differential shoot responses to *tZ* cannot be fully explained by differential expression patterns and ligand sensitivity of different cytokinin receptors (Kiba et al., 2013). It also appears that *tZ*-type cytokinins are critical for maximal leaf growth but not leaf senescence, two different developmental processes in the same organ (Hwang et al., 2012; Kiba et al., 2013). Because *CYP735A1/A2* are predominantly expressed in the root vasculature and *tZ*-type cytokinins are mainly detected in the xylem sap in *Arabidopsis* plants (Hirose et al., 2008), it has been postulated that *tZ*-type cytokinins are mainly synthesized in roots for directional transport to shoots. However, grafting experiments uncovered previously unrecognized flexibility in both shoot-to-root and root-to-shoot communication that compensates for CYP735A deficiency in either roots or shoots for normal growth in the whole plant. Thus, the synthesis sites and long-distance transport of cytokinins are much less confined and restricted than expected (Hirose et al., 2008; Kiba et al., 2013). These studies provide insight into the remarkable capacity in the regulation of cytokinin

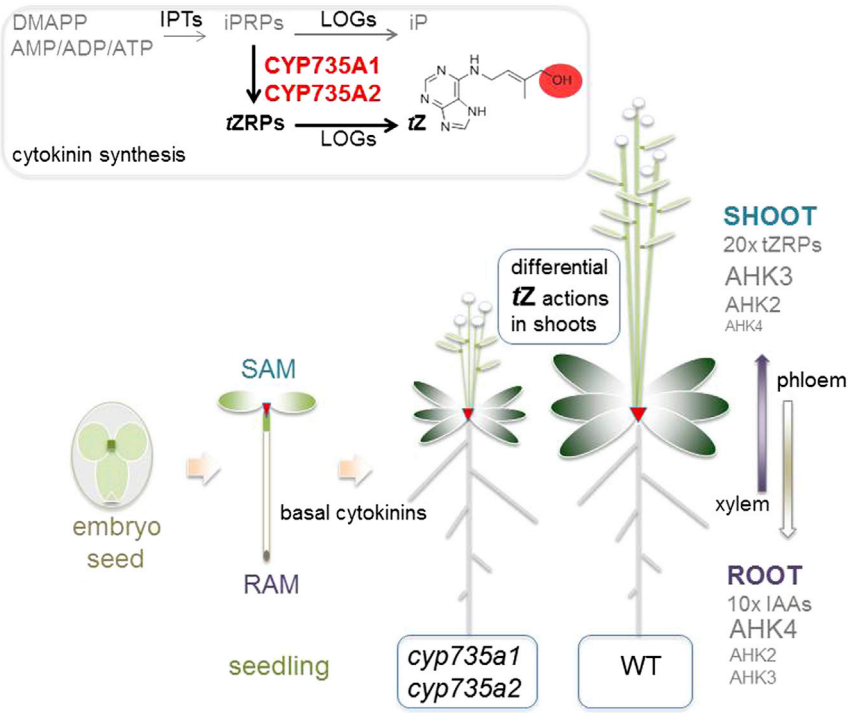

Figure 1. *trans*-Zeatin Specifies Shoot Growth Regulation in *Arabidopsis thaliana*
Basal cytokinin levels are quantitatively essential for SAM and RAM activity and maintenance, as well as basic shoot and root development. High-level *tZ* is dispensable for root growth and development but specifically promotes shoot growth. Bidirectional transport systems via xylem and phloem likely support the predominant shoot destination of *tZ*, *tZR*, or *tZRPs* originated from either shoots or roots. Inset: The cytokinin synthesis pathway is initiated by adenosine phosphate-isopentenyltransferases (IPTs) using dimethylallyl diphosphate (DMAPP) and ATP, ADP, or AMP to generate iPRPs. CYP735A1/A2 convert iPRPs to *tZRPs*. LOGs release active cytokinins, N^6-(Δ^2-isopentenyl)adenine (iP), and *trans*-zeatin (*tZ*).

homeostasis by functional coordination of distinct organs and the utilization of bidirectional long-distance transport systems (Figure 1), and they highlight the plasticity in plant growth and developmental programs modulated by mobile and systemic regulatory signals.

These discoveries raise important questions about how plant shoots and roots manage and differentially respond to different cytokinin signals at the cellular and molecular levels in different biological processes. It is conceivable that differential cytokinin synthesis, transport, retention, sequestration, degradation, perception, and signaling are influenced by different factors in the organ-, tissue-, cell-type-, and subcellular-specific environments and nutrients (Hirose et al., 2008; Hwang et al., 2012; Moubayidin et al., 2013). Experimental innovations have unraveled the core cytokinin signaling circuitry, which employs a large repertoire of genes with overlapping and specific functions to modulate a large set of direct and indirect target genes and cellular activities (Hwang et al., 2012). Kiba and colleagues (2013) find that the reduced cell numbers in leaves, the SAM, and inflorescence stems is the most prominent feature of the *cypDM* shoot. The loss of sensitive and quantitative activation of the cell-cycle regulator *CYCD3* due to exceedingly low *tZ* levels may partially explain the differentially reduced shoot growth vigor and size (Hwang et al., 2012; Riou-Khamlichi et al., 1999). Consistently, quantitative measurement of cytokinins reveals 20-fold higher level of *tZRPs* in the shoot than in the root, which is readily converted to *tZ* by local LONELY GUYs (LOGs) (Figure 1) to support cell proliferation in the SAM, leaf primordia, and young leaves and inflorescence stems in wild-type but not *cypDM* plants (Hirose et al., 2008; Kiba et al., 2013; Tokunaga et al., 2012). It will be exciting to learn how the shoot destination of *tZ*, *tZR*, and *tZRPs* is controlled. Curiously, shoots accumulate 10-fold lower auxins than the roots (Kiba et al., 2013), which can also significantly influence shoot versus root responses to *tZ* and iP cytokinins through complex molecular interactions between cytokinins and auxin, as well as other signal transduction pathways in different cellular contexts (Hwang et al., 2012; Moubayidin et al., 2013).

Given plants' vast capacity in chemical synthesis and signaling, whether chemical distinctions also contribute to specific actions of other plant hormones, such as auxin and gibberellin, existing in multiple active forms is a fascinating question.

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