Dispatches

Root Development: Cytokinin Transport Matters, Too!

Unlike the plant hormone auxin, the mechanism and function of cytokinin transport is poorly characterised. Two new studies now demonstrate that cytokinins transported from shoot to roots via the phloem are critical for creating mutually exclusive auxin and cytokinin signalling domains that control root vascular patterning.

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The hormone signals auxin and cytokinin often interact antagonistically during plant development. Skoog and Miller first described this relationship in 1957 [1]. Cross-regulation between auxin and cytokinin and their signaling and synthesis pathways are now recognized to be essential for numerous developmental processes including shoot branching [2], controlling the size of the root apical meristem [3,4], and lateral root patterning [5]. Auxin transport is frequently a target for regulation during auxin–cytokinin interactions [2–5]. In contrast, the role of cytokinin transport remains unclear since little is currently known about this process. Putative cytokinin transporters identified to date include the equilibrative nucleoside transporter family (ENT) [6,7] and the purine permease family (PUP) [8,9]. Nevertheless, the functional importance of these putative cytokinin transporters to plant growth and development remains to be proven.

In this issue of *Current Biology*, Bishopp *et al.* [10] elegantly demonstrate that cytokinins are translocated from the shoot to the root via the phloem and that this transport pathway controls vascular patterning in the root apex. The authors used a fluorescent image analyzer to visualize ^{14}C-labeled cytokinin applied on the hypocotyl (a shoot tissue) accumulating at the wild-type root apex. In contrast, transport of labeled cytokinin was disrupted in the *apl* mutant (which lacks phloem), and in the inducible *pAPL::XVE>>cals3 m* transgenic line expressing callose synthase in phloem (which blocks symplastic connections such as sieve plates and plasmadesmata). Hence, cytokinin is transported to root tips via the phloem rather than employing a specialized polar transport system like auxin (reviewed in [11]).

Phloem-derived cytokinins are likely to form a gradient across vascular tissues. Indeed, the cytokinin-responsive gene *PIN7* is expressed in two domains of procambial cells adjacent to both phloem poles that flank the xylem axis [10] (Figure 1). *PIN7* encodes an auxin efflux carrier [12]. Its cytokinin-induced expression domain is likely to cause auxin to accumulate in the xylem axis, triggering auxin-inducible markers like AHP6 that control protoxylem cell differentiation (Figure 1). How important is the phloem cytokinin source in defining these distinct hormone response domains? Bishopp *et al.* [10] observed that blocking the phloem transport pathway (using the *pAPL::XVE>>cals3 m* line) caused the precise location of *PIN7* and *AHP6* expression domains to become more variable, resulting in ectopic protoxylem formation. To confirm that this effect was due to blocking cytokinin transport (rather than other phloem-borne signals such as auxin), the authors either targeted expression of the cytokinin-degrading enzyme CKX to the root phloem or grafted shoots over-expressing CKX onto reporter rootstocks. The *AHP6* expression domain expanded in both cases, elegantly confirming that shoot-derived cytokinins transported via the phloem impact hormone-responsive expression domains in root vascular tissues.

In a second paper in this issue of *Current Biology* [13], Bishopp *et al.* report that the auxin and cytokinin interaction specifying mutually exclusive protoxylem and procambial signaling domains involves an inhibitory feedback loop. The authors initially analyzed the expression of several auxin- and cytokinin-regulated genes (respectively *AHP6*, *IAA2*, *DR5*, and *ARR5*, *TCS*). Their responses fell into two bisymmetric, complementary domains: the xylem axis for auxin-regulated genes, and the intervening procambial cells flanking the xylem axis for cytokinin-responsive genes. Changes in cytokinin levels induced strong effects in vascular patterning and were always coupled with changes in the distribution of auxin reporters, consistent with cytokinin functioning to position the auxin maximum that specifies vascular pattern.

The procambial expression of auxin efflux carriers like PIN7 (Figure 1) appears necessary for auxin to accumulate in the xylem axis. Mutating *PIN7* and *PIN3* results in an unstable auxin maximum [13]. Similarly, ectopically expressing *PIN7* throughout vascular tissues also disrupts the formation of an auxin maximum in the xylem axis. Hence, vascular patterning appears to require that *PIN7* is expressed in a bisymmetric pattern. So, what stops both *PIN7* expression domains from merging together? The cytokinin response inhibitor AHP6 appears to provide the key (Figure 1). Mutating *AHP6* causes the *PIN7* expression domain to expand into the xylem axis, including the protoxylem pole [13]. Hence, the auxin-inducible AHP6 protein is required to disrupt cytokinin signaling in the xylem axis and block the expression of cytokinin-responsive genes like *PIN7*. This mutually inhibitory mechanism successfully explains how competing hormonal inputs are propagated into complementary expression domains specifying the bilateral pattern of the vasculature.

Can this patterning mechanism explain more complex vascular organizations (with more than two xylem poles) in roots of other plant species? Intriguingly, chemically inducing increased vascular cell numbers resulted in increased numbers of xylem poles expressing *AHP6* and a switch from two to four xylem poles [13]. Conversely, reducing vascular

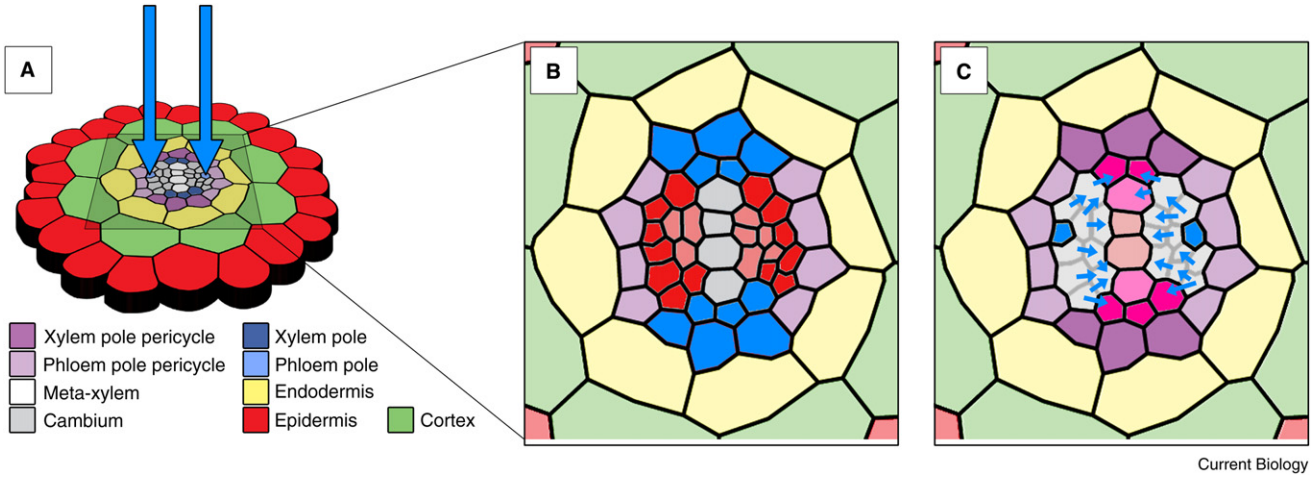

Figure 1. Cytokinin transported via the phloem is critical for creating mutually exclusive auxin and cytokinin signaling domains that control root vascular patterning.

(A) Schematic of a radial section through an *Arabidopsis* root to reveal the individual cell types and bisymmetric vascular morphology. Each root cell type is color coded as denoted in the key. Blue arrow denotes the apical–basal flow of signals like auxin and cytokinin via both phloem poles. (B) Enlarged image of pericycle and vascular tissues color-coded to denote expression domains for cytokinin- and auxin-responsive genes *PIN7* (red) and *AHP6* (blue), respectively. (C) PIN7-dependent hormone transport (denoted with blue arrows) results in auxin accumulating at the meta-xylem and proto-xylem poles (denoted in pink).

cylinder size genetically results in the formation of a single vascular strand [14]. Hence, the initial bi-symmetry conferred by the cotyledons can be over-ridden by varying vascular cell number. Currently, this vascular patterning mechanism has only been demonstrated to function in *Arabidopsis* roots. It will be fascinating to test whether this mechanism also serves to pattern vascular tissues in other organs like leaves and cambium (wood).

How is bilateral symmetry of *Arabidopsis* root vascular tissues initially established? Once again, cytokinin and AHP6 appear to provide the key [13]. During the mid to late heart stage of embryogenesis, two domains of *AHP6* expression are detected migrating from the pair of cotyledons into the pro (immature) vasculature. Cytokinin signaling appears necessary for this bisymmetric *AHP6* expression pattern to form since it is blocked in the cytokinin receptor mutant *wol* [13]. Following germination, this bisymmetric *AHP6* expression pattern continues, presumably being maintained through phloem cytokinin transport, which patterns xylem formation. Intriguingly, cytokinin in the proto (immature) phloem does not appear to be important for this process, despite its proximity to other provasculature cell types in the root apex. Unlike mature phloem, targeting the expression of the cytokinin-degrading enzyme CKX in the proto-phloem does not disrupt xylem patterning or differentiation. Hence, cytokinin sourced from the mature phloem in the basal region of the root apical meristem appears to be more important for vascular patterning than the immature phloem.

Two other developmentally important auxin–cytokinin interactions also occur in the basal region of the root apical meristem that control the size of the root apical meristem [3,4] and position of lateral root initiation [5,15]. This proximity raises an important question, how does the radial patterning mechanism integrate with auxin–cytokinin crosstalk along the apical–basal axis that determines root meristem size? Bishopp *et al.* [10] observed that the number of dividing cortical cells in the transgenic line expressing CKX in the mature phloem was equivalent to wild type, suggesting that the phloem-derived cytokinin source does not control root meristem size. The authors suggest that this may reflect differences in ligand specificity for the CRE1 and AHK3 cytokinin receptors that control root vascular and meristem size, respectively. AHK3 preferentially binds the tZ versus iP class of cytokinin [16] that is synthesized in the root and shoot, respectively [17]. Hence, iP transported from the shoot via the phloem would be expected to impact vascular patterning rather than meristem size.

In summary, Bishopp *et al.* [10,13] have elegantly demonstrated that cytokinin transport via the phloem provides an important source of positional signal required to maintain mutually exclusive auxin and cytokinin signaling domains that control root vascular patterning. This clearly illustrates that during root development, in parallel with auxin, cytokinin transport matters, too!

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Neuroanatomy: Uninhibited Connectivity in Neocortex?

The mouse neocortex is now the focus of research using twenty-first century techniques of circuit analyses, which are revealing different wiring strategies for excitatory and inhibitory connections and providing important insights into the possible computations of cortical circuits.

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"The connectivity diagram of neocortical circuits is still unknown…"

With this opening statement of their report on 'Dense inhibitory connections in neocortex', Fino and Yuste [1] pitch their readers into icy water. Surely they cannot be serious that after 121 years of research on the fine structure of cortical neurons and their connections [2], we are still so far from dry land? It seems they are serious, for they finish their first sentence with the equally bone-chilling assertion that we cannot even be sure whether cortical circuits are wired specifically or randomly.

This preamble is their justification for a conceptually simple experiment in which they mapped the distribution of somatostatin-expressing neurons that inhibit single pyramidal cells in

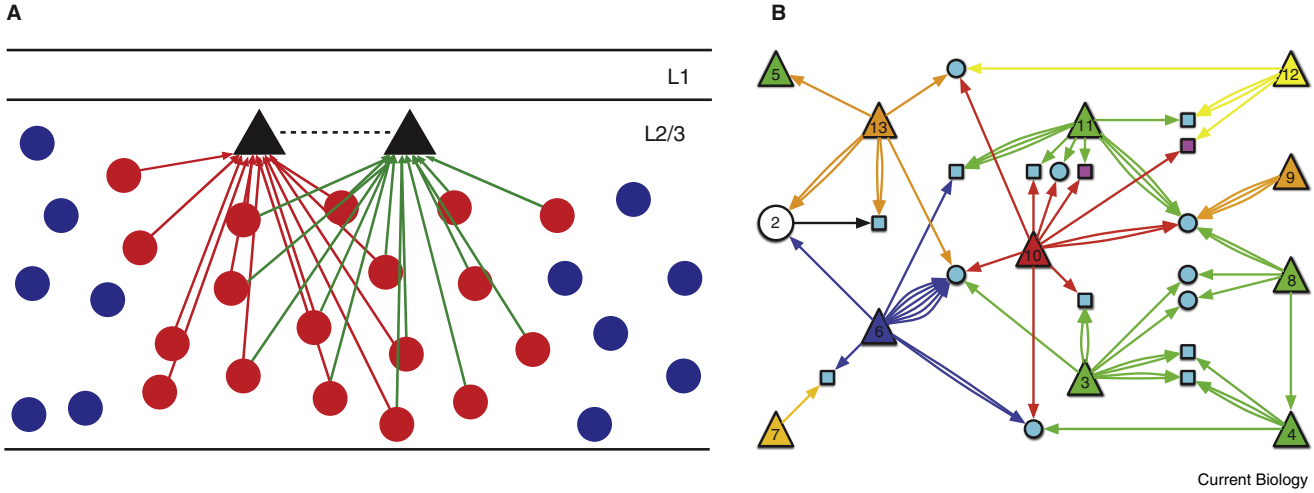

Figure 1. Models of cortical connectivity.

(A) Hypothetical circuit model showing a dense connectivity of inhibitory inputs from somatostatin-expressing GFP inhibitory neurons (filled circles) to pyramidal cells (black triangles) in cortical layer 2/3 (L2/3). Within a local region, the connectivity may be complete (connections from red circles). The dashed line between the two pyramidal cells illustrates that the same model applies whether or not the pyramidal cells are also connected with each other [1]. Adapted with permission from [1]. (B) Directed network graph of the functionally characterized cells and their targets established by Bock *et al.* [3]. Presynaptic pyramidal cells indicated as triangles, coloured according to their varied orientation preferences. Smooth (inhibitory) neuron indicated as open circle. Postsynaptic excitatory (magenta) and inhibitory (cyan) targets with cell bodies contained within the EM volume are drawn as circles. Other postsynaptic targets (dendritic fragments) are drawn as squares. Adapted with permission from [1].