Cytokinin and *WUSCHEL* tie the knot around plant stem cells

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Once upon a time, plant development was all about hormones. Darwin (1) wrote nearly 130 years ago that "some influence," later shown to be the phytohormone auxin, moved down the shoot to control the elongation of oat seedlings. In the 1950s, Skoog and Miller (2) showed that auxin and cytokinin control shoot regeneration in vitro, a technique that is extensively used to this day. With the rise of developmental genetics at the end of the 20th century, however, plant development became focused on transcription factors and their exquisite expression patterns. More recently, both views have converged to explain how plants develop intricate structures with precise expression patterns superimposed on cells that are constantly displaced by growth. This convergence was first exemplified by the role of auxin in patterning the growing root tip (3). In this issue of PNAS, Gordon et al. (4) shed light on the interplay of regulatory genes and cytokinin in the dynamic patterning of the opposite end of the plant, the shoot meristem.

The shoot meristem contains a small population of stem cells that constantly renews itself while providing precursor cells to build all aerial parts of the plant (5, 6). In *Arabidopsis*, maintenance of these stem cells requires a signal produced by a small group of underlying cells that express the *WUS* gene (7) (Fig. 1*B*). Because division and growth constantly displace cells in the meristem, the location of the *WUS*-expressing domain has to be frequently updated. The best-characterized signal that controls *WUS* expression is the CLV3 peptide, which is produced by the stem cells, diffuses to the underlying cell layers, and activates a receptor containing the CLV1 polypeptide to repress *WUS*. This negative signal limits the size of the stem cell population; however, it does not explain how *WUS* expression is maintained in a sharp domain in the center of the meristem, at a predictable distance from the epidermal layer. Many other genes regulate *WUS* directly or indirectly (5), but none of these inputs have explained how *WUS* expression can be transient in individual cells yet stable within the meristem.

Gordon et al. (4) now implicate cytokinin in the dynamic pattern of *WUS* expression. Cytokinin is an adenine-like phytohormone that is sensed by a family of *Arabidopsis* histidine kinase (AHK) receptors, which pass on the signal to two types of transcription factors: type B *Arabidopsis* response regulators (ARRs) activate cytokinin-induced genes, and type A ARRs function in a negative feedback loop to dampen cytokinin responses (8). Cytokinin had previously been implicated in shoot meristem function. In maize, the *abnormal phylotaxy 1 (abphyl1)* mutation disrupts a type A ARR and increases meristem size (9). Consistent with type A ARRs inhibiting meristem function, *WUS* directly represses their expression (10). In rice, the cytokinin biosynthesis gene *LONELY GUY* (*LOG*) is expressed in the apical region of the shoot meristem and is required for meristem maintenance (11). All of these studies implicated cytokinin in controlling meristem size, but did not place cytokinin at the core of the mechanism that patterns the meristem. Gordon et al. (4) took advantage of a recently developed marker gene that reports cytokinin signaling (12) to show that cytokinin responses are not uniform within the meristem: the reporter was expressed most strongly in the center of the meristem, in the same region as *WUS*. They also found that the cytokinin signal and *WUS* reinforce each other through multiple feedback loops. External cytokinin activated *WUS* directly and independently of the CLV pathway; at the same time, repression of *CLV1* by cytokinin (13) further facilitated *WUS* expression. Considering that *WUS* represses type A ARRs that normally act as a break on cytokinin signaling (10), cytokinin signaling and *WUS* should reinforce each other in a positive feedback loop (Fig. 1*A*).

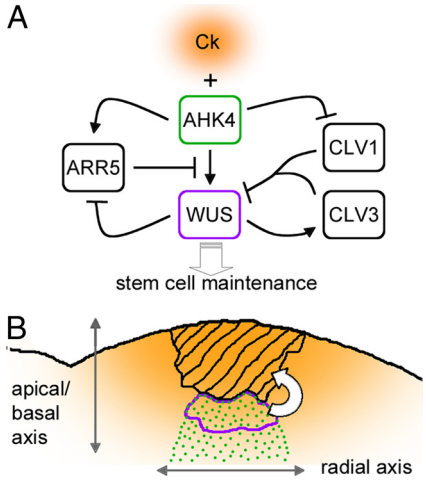

Fig. 1. Role of cytokinin in patterning the shoot stem cell niche. (*A*) Regulatory network described by Gordon et al. (4). Ck is cytokinin; blunted lines and black arrows indicate repression and activation, respectively, and the white arrow represents the maintenance signal induced by *WUS*. (*B*) Speculative model of how cytokinin signaling establishes the spatial pattern of *WUS* expression. Graded orange represents an apical-basal gradient of cytokinin produced in the meristem region where stem cells are located (hatched region); green dots mark the region where *AHK4* is expressed in response to cues along the radial axis of the shoot. Where a minumum level of cytokinin reaches the AHK4 receptor, the network represented in *A* translates the graded cytokinin signal into the sharp boundaries of the WUS-expressing domain (surrounded by the purple line). This region then produces the signal (white arrow) that maintains the shoot stem cells.

The behavior of a system with multiple feedback loops (Fig. 1*A*) is not easy to understand intuitively, but the importance of these results was made clear by computer simulations of the meristem regulatory network. In a network where cytokinin simultaneously activated *WUS* and repressed *CLV1*, *WUS* expression increased very steeply above a critical cytokinin concentration. No sharp increase was seen in simulations where cytokinin controlled only *WUS* or *CLV1*. Spatially, this steep increase in *WUS* expression could be translated into a sharp expression boundary within a field of cells with increasing cytokinin signaling (Fig. 1*B*). This binary-like behavior would also be a mechanism to increase robustness of *WUS* expression to fluctuations in the underlying cytokinin signal.

Because of the positive feedback loop between cytokinin signaling and *WUS*, the cytokinin reporter alone could not tell whether the higher levels of signaling in the center of the meristem were a cause or a consequence of *WUS* expression. Here, a crucial piece of information was that the cytokinin input into *WUS* and *CLV1* regulation was medi-

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ated by the AHK2 and AHK4 receptors and that at least the latter was spatially restricted, with higher expression in the center of the meristem. According to published expression array data, *AHK2* and *AHK4* are not activated by *WUS* (10). Therefore, the feedback loops that activate and stabilize *WUS* expression seem to take spatial cues from an underlying, independently established expression pattern of cytokinin receptors (Fig. 1*B*). What sets up this pattern? Throughout *Arabidopsis* development, *AHK4* (also known as *WOODEN LEG*) is expressed in the vascular cylinder (14), which is the innermost region of the concentric arrangement of tissues seen in plant organs. So *AHK4* might link the mechanism that establishes the radial pattern of the plant to the mechanism that specifies the shoot stem cell niche. The radial pattern of the shoot is controlled by a family of HD-ZIP transcription factors (15); combined mutation of three of these HD-ZIP factors (CORONA, PHABULOSA, and PHAVOLUTA) caused not only an enlargement of the vascular cylinder of the stem, but also an increase in meristem size very similar to that seen in *clv* mutants (16). It will be interesting to see whether this occurs at least in part through misregulation of the AHK4 receptor leading to an increased *WUS* expression domain.

How does all of this explain the dynamic pattern of *WUS* expression? A compelling model emerges in the light of recent evidence suggesting that the stem cells overlying the WUS domain are a source of cytokinin (11, 17, 18)

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(Fig. 1*B*). So in addition to the (still unknown) signal from *WUS*-expressing cells that specifies stem cells, a signal may be sent back by the stem cells to specify the WUS domain. Distance from the source of cytokinin would determine how deep the WUS domain is; overlap with the preexisting radial pattern of the AHK4 receptor would locate expression to the center of the meristem. The multiple feedback loops connecting cytokinin and *WUS* would translate a graded cytokinin signal into the sharp expression boundaries of *WUS*. The specification of the stem cell niche by the overlap of radial and apical-basal patterns would be reminiscent of what happens in the root meristem, where an apical-basal auxin gradient overlaps with the radial expression pattern of *SCARECROW* and *SHORT ROOT* to specify the position of the root stem cell niche (3).

Another important implication of Gordon et al. (4) relates back to the classical experiments on shoot regeneration in vitro. Shoot formation from root explants in vitro is triggered by high levels of cytokinin through ectopic activation of *WUS*, which is sufficient to specify new shoot stem cell niches (19, 20). Gordon et al. (4) show that activation of *WUS* during in vitro regeneration also correlates with *AHK4* expression; pretreatment with auxin, which facilitates subsequent shoot regeneration, also causes broad activation of *AHK4*. So the study of *WUS* regulation looped back to classic in vitro culture experiments; the auxin-*AHK4*-cytokinin-*WUS* connection clarifies the link between the long-known role of hormones during shoot regeneration in vitro and their normal role in the shoot meristem. The circular interactions between regulatory genes and plant hormones do not make it easier to understand regulatory networks, but studies like that of Gordon et al. (4) show that these loops exist for a good reason.

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