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Plant Stem Cell Niches: Standing the Test of Time

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Similar to animal stem cells, plant stem cells require special niche microenvironments to continuously generate the tissues that constitute the plant body. Recent work using computer modeling and live imaging is helping to elucidate some of the mechanisms responsible for the specification and maintenance of stem cells in the root and shoot.

Plants have nowhere to run when times get tough, so they must rely on an indeterminate body plan (that is, one in which the number of organs is not predefined) to generate developmental responses to environmental changes. Specialized proliferative tissues termed meristems present at the growing tips of roots and shoots continuously generate new cells for the plant (see Review by K.D. Birnbaum and A. Sánchez Alvarado in this issue of *Cell*). In the shoot, these cells follow different paths depending on whether they will form lateral organs such as leaves or become part of the stem. The root meristem plays a more dedicated role, generating cells that enable continued growth of the root. Both populations of developmentally flexible and actively dividing cells are maintained through smaller collections of stem cells located at the heart of the meristem (Figures 1A and 1B) (Laux, 2003; Weigel and Jürgens, 2002). Organizing centers maintain these stem cell populations in a highly regulated microenvironment termed the stem cell niche, which displays remarkable longevity allowing some plants to grow for hundreds of years.

Properties of Plant Stem Cell Niches

Although variable in exact structure, the shoot and root stem cell niches show common characteristics that define equivalent populations of cells in animals. The root stem cell niche is morphologically well defined and thus can be used to clearly address the role of the organizing center in regulating the function of stem cells that generate specific tissue layers. In the roots of the model plant *Arabidopsis thaliana*, the organizing center is composed of four quiescent center cells that rarely undergo cell division and constitute the quiescent center (Figure 1A) (Laux, 2003; Weigel and Jürgens, 2002). These cells are surrounded by a single layer of stem cells that divide to produce two daughter cells: one daughter cell renews the stem cell and the other daughter cell will contribute to the formation of the mature tissues of the root.

Work from Ben Scheres' group has defined the signaling roles of the quiescent center in the niche through laser-assisted ablation experiments. An important finding of this work is that the quiescent center maintains stem cell identity in the immediately surrounding cell layer through direct cell-cell contacts (van den Berg et al., 1997). This short-range signaling ensures that stem cell numbers are restrained and stem cell populations do not become displaced from the growing root tip. This mechanism for controlling the size of the stem cell population is very similar to that found in the *Drosophila* germarium where direct contact of the germline stem cell with a supporting cap cell is required to maintain stem cell identity (Spradling et al., 2001; see Review by S.J. Morrison and A.C. Spradling in this issue). The daughter of the germline stem cells lacks such connections and goes on to differentiate into an egg chamber.

The other major finding of these studies is that positional information defines the quiescent center: these cells are

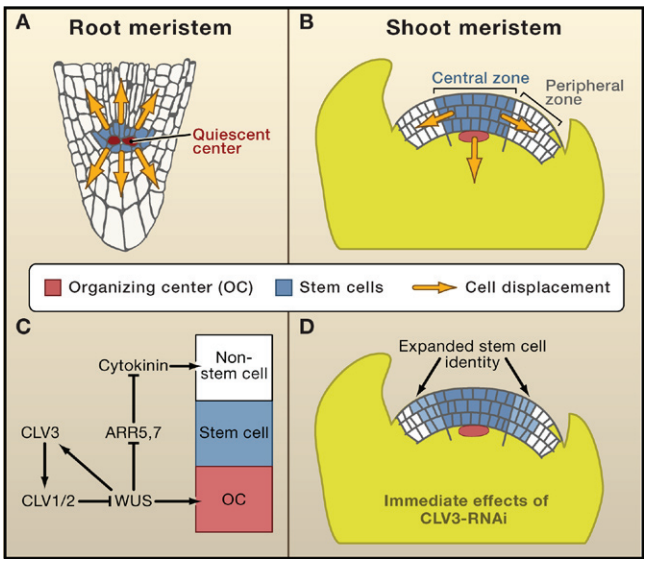

Figure 1. Plant Stem Cell Niches
Structure of the root (A) and shoot (B) stem cell niches. Both are composed of an organizing center (OC; referred to as the quiescent center in the root), which maintains stem cell identity in a neighboring population of cells.
(C) The regulatory signaling network controlling the identity of the organizing center and stem cells in the shoot meristem.
(D) The immediate effect of suppressing *CLV3* expression is the expansion of stem cell identity beyond the central zone of the shoot meristem into the surrounding peripheral zone.

not inherently unique but instead maintain their specialized function through external signaling that defines cells in that position as an organizing center (van den Berg et al., 1995). By ablating all quiescent center cells, van den Berg et al. (1995) found that cells, which had begun to assume vascular identity, instead dedifferentiated and became quiescent center cells. Thus, a long-distance signaling system must exist that can define the position and identity of the stem cell niche independent of the quiescent center. This long-range signal appears to be the plant growth hormone auxin.

The shoot stem cell niche is more spread out than that of the root and is composed of several layers of cells that overlay a smaller pool of cells comprising the organizing center (Figure 1B) (Laux, 2003; Weigel and Jürgens, 2002). Thus, direct contact with the organizing center is not required to acquire or maintain stem cell identity as it is in the root. The WUSCHEL (WUS) transcription factor is exclusively expressed in the organizing center and is required for induction of stem cell identity in overlying tissue layers (Figure 1C) (Mayer et al., 1998). WUS, in part, performs this function by directly repressing the expression of the ARABIDOPSIS RESPONSE REGULATOR (ARR), a two-component factor that suppresses signaling by the plant hormone cytokinin (Leibfried et al., 2005 and references therein). Many classic studies have demonstrated the important role that this plant hormone plays in promoting cell-cycle activity, growth, and shoot identity. In plants that constitutively express a hyperactive version of ARR7, the stem cell population is depleted (Leibfried et al., 2005), whereas mutations in the maize ARR homolog, ABPH1, lead to an increase in meristem size (Giulini et al., 2004).

Homeostasis of Plant Stem Cell Populations

Just as animals need to keep stem cells in check to prevent the formation of tumors, careful maintenance of stem cell populations in plants is necessary to avoid overproliferation. In the shoot, a second important function of *WUS* is to achieve this by activating the expression of a signaling network that forms a negative feedback loop repressing *WUS* expression (Figure 1C) (Brand et al., 2000; Schoof et al., 2000 and references therein). *WUS* promotes the expression of a small secreted ligand, CLAVATA3 (CLV3), in a region of the meristem termed the central zone. The CLAVATA1/2 receptor complex—which is expressed in a region that overlaps with the organizing center—directly perceives this stem cell-dependent signal, which ultimately leads to the downregulation of *WUS* expression. Loss of function in any CLV component results in an enlarged meristem comprising extra stem cells in the central zone, although the underlying mechanism that results in this mutant phenotype remained unclear. For example, increased cell division rates, prolonged stem cell maintenance, or the expansion of signals that confer stem cell identity could all lead to the same outcome.

To mechanistically understand the effects of perturbing CLV signaling, Reddy and Meyerowitz used live imaging to track changes in meristem structure over time (Reddy and Meyerowitz, 2005). Using a chemically inducible RNA interference system and a *CLV3* reporter line, these authors were able to suppress *CLV3* expression in a timecourse experiment and hence track the effects of this suppression on stem cell identity. Interestingly, expansion of stem cell identity occurred prior to any changes in meristem size in response to the inhibition of *CLV3* expression (Figure 1D). The investigators then used a surface marker to track cell division rates and found that *CLV3* suppression also led to an increase in cell division rates outside of the stem cell population, but at a later time point. Thus, the timecourse analysis allowed Reddy and Meyerowitz to distinguish between the immediate and late effects of CLV3 signaling. The observation that the region in which *CLV3* is expressed is more limited than the region of the meristem affected by reductions in CLV3 activity suggests that CLV3 may suppress the production of a diffusible signal that promotes stem cell identity in the central zone as opposed to suppressing the response to such a signal. It will be interesting to determine in future live-imaging studies whether this signal is dependent upon WUS activity.

In roots, a direct relationship between regulation of the size of the root stem cell niche and control of cell differentiation in animal models has been established with the characterization of the *RETINOBLASTOMA-RELATED* (*RBR*) gene in *Arabidopsis* (Wildwater et al., 2005 and references therein). The retinoblastoma gene (*Rb*) is an oncogene first characterized in animals as a cell-cycle regulator that suppresses the transition from G1 to S phase. In the root meristem, reductions in *RBR* expression weaken the requirement for stem cells to be in direct contact with the quiescent center, leading to multiple tiers of stem cells surrounding the quiescent center. In addition to the expanded expression of stem cell marker genes and lack of differentiation markers in these cells, live imaging revealed that these ectopic layers maintained their ability to undergo cell division. Thus, in both plant and animal systems, RETINOBLASTOMA-related genes limit the proliferative nature of tissues. Interestingly, these ectopic stem cells maintain their requirement for signaling from the quiescent center because ablation of the quiescent center leads to their immediate differentiation. These data suggest that the quiescent center is not simply repressing *RBR* activity in the stem cells, and future work should reveal the interplay between *RBR* action and quiescent center signaling.

Positioning a Stem Cell Niche

Although the pathways that maintain pools of stem cells at the root and shoot apex are well characterized, less is known about how a stem cell niche is generated. Primary shoot and root stem cell niches are initiated at opposite poles during early embryogenesis (Weigel and Jürgens, 2002). Formation of the shoot organizing center coincides with the expression of *WUS* in a subapical tier of cells in the early embryo (Mayer et al., 1998); however little is understood about the upstream regulation of this event. The mechanisms that lead to the specification of the root meristem, on the other hand, are better characterized and we will focus on these events. Two independent pathways converge to specify the identity of the quiescent center and regulate the functions of the associated stem cells in the root. The *SCARECROW/SHOR-*

TROOT (*SCR/SHR*) signaling pathway provides positional information along the radial axis, whereas the plant hormone auxin provides longitudinal information.

Polar Auxin Transport in the Root

Auxin is a natural candidate for regulating patterning in plants as auxin transport proteins enable the formation of information-rich concentration gradients. The *PIN-FORMED* (*PIN*) genes encode plasma membrane-bound transporters that facilitate the export of auxin out of cells (references in Friml et al., 2003; Grieneisen et al., 2007). Because PIN transporters are often localized in a polar fashion in the cell, auxin can be readily transported from one cell to the next. Each PIN isoform displays a unique expression pattern and localizes to different surfaces of the cell depending on the cell type. These differences produce complex routes by which auxin can travel and accumulate within an organ. Adding to this complexity, the auxin "superhighway" can be remodeled during development allowing auxin to control different patterning events in the same organ.

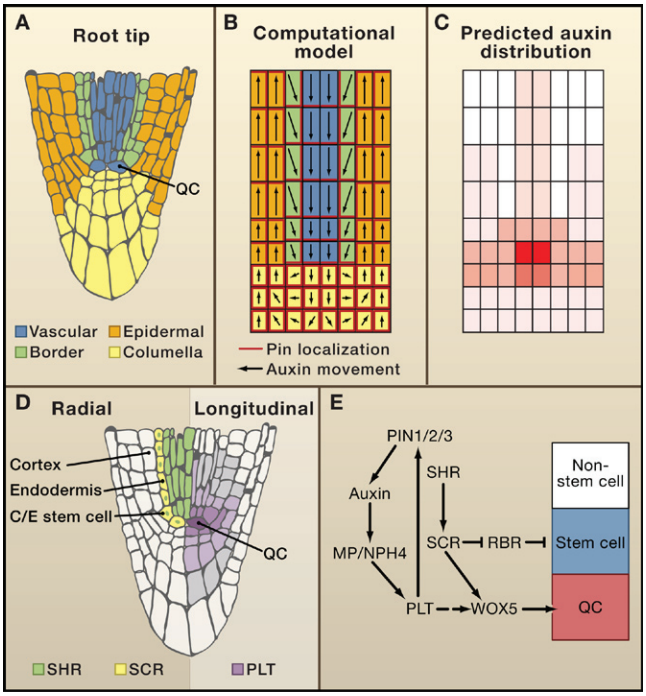

Figure 2. Positioning the Root Stem Cell Niche

(A, B, and C) Modeling polar auxin transport in the root can predict the position of the quiescent center (QC). Grieneisen et al. (2007) built a model of polar auxin transport based on the organization of cell types in the root (A). In this model the localization of the auxin transporter PIN is modeled for different tissue zones and the effect of transporter activity on the direction of auxin flow is modeled (B). The model is sufficient to predict the presence of an auxin maximum in the root tip at the approximate position of the quiescent center (C).

(D) Two pathways converge at the root tip to promote the identity of the quiescent center. The *SHR/SCR* pathway provides the radial position of the quiescent center, whereas the *PLT* pathway provides the longitudinal position.

(E) Regulatory network controlling the identity of the quiescent center and root stem cells (C/E, cortex-endodermal).

Unequal auxin accumulation is evident just after the first division of the zygote into a smaller apical cell and a larger basal cell (Friml et al., 2003). Using a reporter that marks cells with a peak auxin response, Friml et al. (2003) found that the apical cell displays a higher auxin response, which correlates with the apical localization of PIN7 in the basal cell. The apical cell of the embryo will eventually generate all cells of the shoot, hypocotyl, and embryonic root, whereas the basal cell will form the quiescent center, the columella root cap, and extraembryonic tissue. An important subsequent step during embryogenesis appears to be the relocalization of PIN transporters in the provasculature cells from the apical to the basal surface, which focuses auxin to a specific daughter of the basal cell lineage termed the hypophysis. The hypophysis subsequently divides to generate the quiescent center and root cap stem cells. Loss of function in the *PIN* genes that drive basal auxin transport or disruption of vesicular trafficking that is important for PIN localization to the plasma membrane leads to defects in hypophyseal auxin accumulation and in specification of the quiescent center.

Recent work modeling auxin flux in the root has led to a clearer understanding of how polar auxin transport generates an auxin maximum at the root tip to specify the quiescent center (Grieneisen et al., 2007; Likhoshvai et al., 2007). Grieneisen et al. (2007) created a mesoscopic model (a scale at which individual molecules do not need to be considered) for auxin transport that uses a simplified root layout composed of different cell types endowed with specific diffusion and permeability parameters for auxin (Figures 2A and 2B). Auxin influx is primarily attributed to free diffusion into the cell whereas efflux is attributed to localized PIN-transporter activity because internal stores of auxin are deprotonated and do not readily diffuse outside of the cell. The cellular nature of the model enabled Grieneisen et al. to design unique PIN localization patterns for cells in different regions of the root.

The model shows that an auxin maximum is quickly localized to a small population of cells near the root tip (Figure 2C). This maximum is generated by a combination of flux events. First, auxin is funneled to the root tip through the vascular cells; it is then redistributed in the columella region at the tip. Auxin then flows basipetally (from the tip to the more mature regions of the root) through the outer tissue layers. Very importantly, auxin is subsequently recycled back into the vascular cells through the border cells (ground tissue and pericycle). This "reverse fountain" system effectively enables the root to (1) build up and store high levels of auxin, (2) create an auxin maximum at the approximate position of the quiescent center, and (3) generate a basipetal auxin gradient. Using an auxin reporter, Grieneisen et al. (2007) found a similar distribution of auxin activity to that predicted by the model.

Overall, the model was found to be highly robust to changes in the origin of auxin. For example, simulating ubiquitous auxin production or a loss of shoot-supplied auxin had little effect on the position of the auxin maximum. This predicted robustness to changes in auxin supply was tested in plants by cutting off the shoot, which supplies most of the auxin to the root, as well as exogenously supplying auxin. Despite these dramatic perturbations, the auxin reporter maintained peak activity at the quiescent center. Thus, PIN-mediated auxin transport

in combination with topological aspects of the root and specific auxin permeability and transportation rates are sufficient to provide a plausible mechanism for the role that auxin plays in specification of the quiescent center.

Likhoshvai et al. also generated a model describing auxin distribution in a line of cells along the longitudinal axis of the root (Likhoshvai et al., 2007 and references therein). Auxin transport was modeled as a function of passive diffusion and active transport toward the tip of the root and included the ability of auxin to inhibit polar transport at high concentrations. Interestingly, although cell specialization is not considered, their model is able to replicate a peak of auxin concentration at the approximate position of the quiescent center, which can be regenerated after a simulated loss of the quiescent center. This suggests that certain aspects of auxin distribution are fundamentally encoded by simple basipetal transport with negative feedback. Notably, several studies have also modeled regulated auxin transport in the shoot focusing on the specification of lateral organ primordia and providing important examples for the use of modeling to understand patterning events (de Reuille et al., 2006; Jonsson et al., 2006; Smith et al., 2006).

The PLETHORA (PLT) class of AP2/ERF-type transcription factors plays an important role in mediating the developmental response to auxin in the root (Figures 2D and 2E) (Aida et al., 2004; Galinha et al., 2007). The strong overlap in expression between *PLT* genes and auxin reporters and their dependence on multiple *PIN* genes suggest that they may act downstream of auxin signaling (Aida et al., 2004; Blilou et al., 2005). Indeed, prolonged exposure of roots to auxin results in enhanced *PLT1/2* expression, and *PLT1/2* are dependent upon two auxin response factor-type transcription factors, MONOPTEROS (MP) and NONPHOTOTROPIC HYPOCOTYL4 (NPH4), to activate and maintain expression in the embryonic root tip (Aida et al., 2004). Consistent with the expression pattern of *PLT* genes, which peaks at the quiescent center and is dependent on auxin, loss of *PLT1/2* gene function results in reduction or loss of quiescent center identity and expansion of differentiation markers into the stem cell population. Furthermore, abrogation of *PLT*-class gene activity leads to a complete loss of all basal structures including the entire embryonic root and part of the embryonic stem (hypocotyl). In contrast, ectopic expression of *PLT1* or *PLT2* results in the proliferation of basal cell identities as well as the formation of ectopic stem cell niches. Interestingly, these ectopic stem cell populations are not marked by high auxin levels, as determined using an auxin reporter, indicating that ectopic *PLT* activity can separate auxin responses from the specification of the stem cell niche. Together these data show that *PLT* genes are both necessary and, when expressed at high levels, sufficient to specify the quiescent center and associated stem cell niche. Interestingly, four related transcription factors, PLT1,2,3 and BABYBOOM (BBM), display graded patterns of expression that extend beyond the quiescent center. Although high levels of expression of these genes are required for stem cell maintenance, lower levels also provide important patterning information and determine the size of the transit-amplifying daughter cells that make up the meristem (Galinha et al., 2007).

The regulatory relationship between auxin signaling and *PLT* gene expression may not be linear as the expression of several PIN transporters is dependent upon *PLT* activity. Thus, as temporal analysis of shoot meristem growth clarified the role of CLV3 in stem cell maintenance, temporal analysis may also be needed to distinguish between the pathways that regulate auxin distribution in the root and the pathways that are responsive to auxin. A positive feedback loop between these two components may tighten auxin transport routes at the root tip, where they are most critical.

Radial Patterning in the Root

Parallel to the auxin/PLT signaling pathway, specification of the quiescent center and stem cell functions are also regulated by two related transcription factors, SCARECROW (SCR) and SHORTROOT (SHR) (Cui et al., 2007; Levesque et al., 2006 and references therein; Figures 2D and 2E). SHR acts at the top of the hierarchy and is expressed in the central stele tissue where the vasculature is housed. The SHR protein subsequently moves into the surrounding tissue layer to directly activate *SCR* expression. SCR forms a heterodimer with SHR and is itself necessary for SHR to bind to the *SCR* promoter and activate expression (Cui et al., 2007; Levesque et al., 2006). This feedback loop is thought to enable the rapid upregulation of *SCR* expression and sequester the SHR protein in the nucleus, which limits movement to a single cell layer adjacent to the stele. Interestingly, this is accompanied by the differential activity of two zinc-finger proteins, MAGPIE (MGP) and JACKDAW (JKD), that either activate or restrain the activity of SHR and SCR (Welch et al., 2007).

The tissue layer to which the SHR protein moves and activates *SCR* expression forms several different cell types. In combination with peak *PLT* activity centered at the root tip, *SCR* and *SHR* promote quiescent center identity (Aida et al., 2004). Immediately adjacent to the quiescent center, *SCR* and *SHR* promote an asymmetric cell division in the ground tissue stem cell daughter to generate the cortex and endodermal cell layers. *SHR* plays an additional role in specifying endodermal cell identity as well. *SHR* may also play an important role in other stem cells in the stele as vascular patterning is disrupted in *shr* mutants.

Is the Shoot a Root with a View?

In studying developmental processes, the common themes that arise in diverse organs or organisms are striking. Sometimes these similarities are based on a common evolutionary origin. If, as the evidence suggests, the root was derived from a shoot-like progenitor during the evolution of land plants, then some of the molecular components controlling shoot development may control similar processes in the root. Recently, such a connection has been discovered through the characterization of a close relative of *WUS*, called *WOX5* (Sarkar et al., 2007). Similar to the specific expression of *WUS* in the organizing center, *WOX5* is exclusively expressed in the root quiescent center. Consistently, Sarkar et al. (2007) found that *WOX5* is necessary and sufficient to promote stem cell identity in the root cap. Expression analysis of *WOX5* places it downstream of *SCR*/*SHR*, *MP*/*BODENLOS* pathways and potentially of

PLT genes (Figure 2E). Genetic analyses, however, indicate that it may have functions independent of these pathways as well. Interestingly, Sarkar et al. found that *WOX5* and *WUS* are functionally interchangeable, suggesting that their roles in the root and shoot stem cell niche may be distinguished primarily on the basis of expression pattern. Although WOX5/WUS represent the first pair of related root/shoot transcriptional regulators, previous studies have suggested that components of the CLV signaling complex are also involved in regulating meristem size and cell-fate decisions in the root (Fiers et al., 2005). Thus, an ancestral WUS/CLV pathway may have been adapted to regulate stem cell proliferation during root evolution. It will be fascinating to determine how deeply the homology runs between the root and shoot pathways; such studies will provide a model for how stem cell regulation evolves to suit new functions.

Similarities between developmental systems may also represent the outcome of convergent evolution. Plants and animals are thought to have evolved multicellularity independently, thus stem cell niches are likely to be controlled in these two systems by analogous processes (see Review by K.D. Birnbaum and A. Sánchez Alvarado). Nevertheless, observed commonalities may represent the effects of constraints common to all cellular organisms. The maintenance of stem cells in highly regulated microenvironments combined with the necessity of non-stem cells to generate the niche environment ensures that stem cell organizing centers do not autonomously proliferate. The maintenance of stem cell identity in the *Drosophila* ovariole, for example, depends on signaling mediated by the morphogen decapentaplegic (Dpp); however, Dpp expression is limited to several somatic cell types near the stem cells (Spradling et al., 2001). In the same way, auxin transport driven by the PIN transporters ensures the accumulation of sufficient levels of auxin to promote the formation of the stem cell niche without the necessity that the stem cells contribute to such a signal. It will be interesting in the future to determine if the shoot organizing center is patterned and maintained by similar nonautonomous mechanisms.

Plants come in many sizes, from the smallest flowering plant, duckweed, to the largest living organism on earth, the giant sequoia. An important future direction in understanding plant stem cells will be to determine what mechanisms lead to differences in the longevity of the niche microenvironment. A greater understanding of this process may enable researchers to improve plant biomass production for the generation of biofuels.

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