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REVIEW

Root Development in *Arabidopsis thaliana*: Attraction from Underground

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In recent decades, the *Arabidopsis* root system has been extensively used to dissect developmental processes because of its simplicity and the predictability of cell fate. Its root meristem is composed of concentric layers of epidermis, cortex, and endodermis encircling the stele that contains the vascular system. Each cell file can be traced to the initials and a mitotically less-active quiescent center (QC), which serves as the stem cell niche. The combination of PLETHORA, SCARECROW, and SHORT-ROOT transcription factors, as well as local auxin distribution, are essential for correct positioning of the QC and maintenance of that niche. In the epidermis, fates for the root hair-bearing (H) and non-hair (N) cells are determined by their positions relative to neighboring cells. Recent molecular genetics studies have provided insights into the molecular mechanisms for this specification of position-dependent cell fate and regulatory circuits in the *Arabidopsis* epidermis. Taken together, such positional information plays a key role in dictating the stem cell niche and cell fates.

Keywords: Arabidopsis, cell fate specification, positional information, radial patterning, root development, stem cell niche

ROOT AS A MODEL SYSTEM FOR STUDYES ON PATTERNING AND CELL FATE SPECIFICATION

The root system plays an important role in the acquisition of water and nutrients from the soil, anchoring of that plant, and its communication with environment. Primary roots are organized during embryogenesis, and the pattern formation of roots perpetuates into post-embryonic developmental stages. Roots can be divided into three regions: meristematic, elongating, and differentiating regions from the tip along the longitudinal axis. Typically, dicot plants have concentric layers of epidermis, cortex, endodermis, and stele that contains the vascular system (outer to inner). Some roots also possess an exodermis, which is lignified at the outer sub-epidermal layers of the cortex in a radial axis. Developmental biologists have sought to answer the fundamental questions of how cell fates are specified, and how cells differentiate. Over the past decades, *Arabidopsis thaliana* has been extensively used to dissect developmental processes by molecular and genetic analyses. In particular, these roots are suitable for studying patterning and cell fate specification because the root meristems exhibit predictable cell division patterns during both embryonic and post-embryonic development (Dolan et al., 1993; Scheres et al., 1994). In addition, locally and temporally different cell files are arranged along the longitudinal axis. Several important regulatory genes have been isolated and characterized in past few years. With the complete genome sequence information and diverse tools, including microarrays and reverse genetics, we should be able to identify more players in these processes, thereby providing insights into the development of *Arabidopsis* roots. Here, we review recent advances in our understanding on radial patterning of the ground tissue, as well as specification of the stem cell niche and cell fate in the root epidermis.

RADIAL PATTERNING OF THE CORTEX AND ENDODERMIS CELL LINEAGE

In transverse sections, *Arabidopsis* roots display a stereotypical radial arrangement, with concentric rings of epidermis, cortex, and endodermis encircling the stele (from outside to inside) (Fig. 1). Such a pattern of each tissue layer stems from a set of initials that surround a small group of mitotically less-active cells, the quiescent center (QC), in the root meristem (Fig. 1A). These initials undergo asymmetric cell divisions to regenerate the initials themselves and give rise to daughter cells that will differentiate (Dolan et al., 1993).

In particular, the radial pattern formation of ground tissue (endodermis and cortex) is a well-characterized example of the role of asymmetric divisions for correct tissue patterning, as is apparent with the cortex/endodermis initial (Dolan et al., 1993; Scheres et al., 1994). The first transverse division produces the initial itself and a daughter cell, and, subsequently, the daughter cell divides longitudinally to generate the endodermis and cortex lineages (Dolan et al., 1993; Scheres et al., 1994). Although the organization of tissue layers in the root meristem has seemingly tight clonal relationships, experiments with laser ablation have shown that positional signals from more mature cells guide that patterning (van den Berg et al., 1995, 1997). Furthermore, when three adjacent daughter cells of the cortex/endodermis initials are ablated, the initial just beneath those ablated cells can generate only a daughter cell with no asymmetric divisions for the cortex/endodermis lineage (van den Berg et al., 1995). This demonstrates that information specifying cell fate in the radial axis is directed toward the tip (from top to bottom) through an individual cell layer (van den Berg et al., 1995).

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Abbreviations: ARFs, auxin response factors; H cell, hair cell; N cell, non-hair cell; QC, quiescent center; trichostatin A, TSA

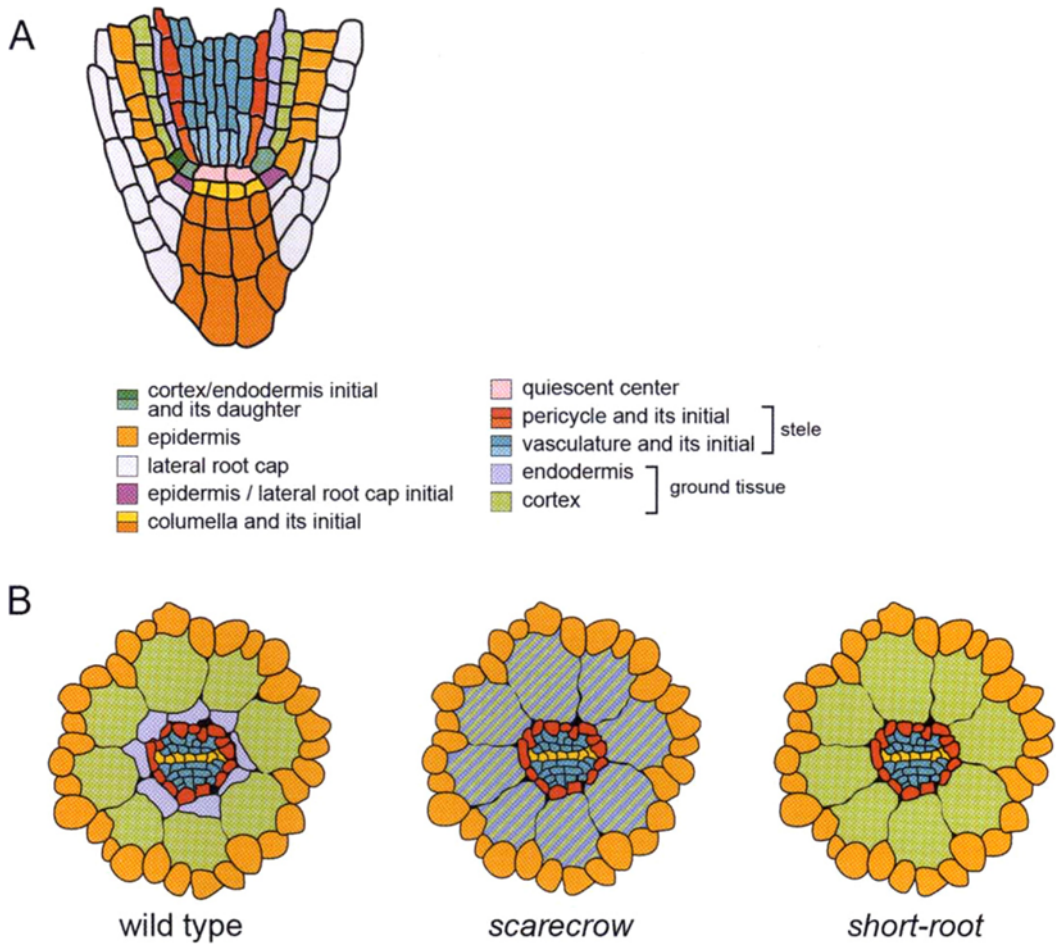

Figure 1. Organization of tissue layers in the *Arabidopsis* root. (**A**) Longitudinal section. (**B**) Transverse root sections from wild-type, *scarecrow* (*scr*), and *short-root* (*shr*) plants depicting concentric layers of epidermis, cortex, endodermis, and stele that contains pericycle, phloem, and xylem. Root of *scr* mutant has only single ground layer with mixed attributes of cortex and endodermis, whereas *shr* mutant root lacks endodermis layer.

Mutations in either *SCARECROW* (*SCR*) or *SHORT-ROOT* (*SHR*) result in aberrant radial pattern formation of the ground tissue. Interestingly, *scr* mutants have only a single layer, which exhibits differentiated characteristics of both cortex and endodermis (Fig. 1B), while *shr* mutants have a single layer that is typical of the cortex (Benfey et al., 1993; Scheres et al., 1995). The phenotype of *scr shr* double mutants is identical to that of *shr* single mutants, indicating that *SHR* is epistatic to *SCR*. Molecular and genetics studies have revealed that *SCR* is essential for asymmetric cell division but not specification of the endodermis, whereas *SHR* is required for both actions (Benfey et al., 1993; Scheres et al., 1995; Di Laurenzio et al., 1996; Helariutta et al., 2000). Both *SCR* and *SHR* encode plant-specific transcription factors of the GRAS family (Di Laurenzio et al., 1996; Pysh et al., 1999; Helariutta et al., 2000). As expected, *SCR* transcripts are detected in the endodermis, and SCR fused with GFP (Green Fluorescence Protein) is found only in the nucleus of the endodermis lineage (Di Laurenzio et al., 1996; Gallagher et al., 2004). In comparison, *SHR* transcripts are detected not in the endodermis, but in the stele (Helariutta et al., 2000). Intriguingly, SHR-GFP is localized in the nucleus of the endodermis lineage, as well as in the nucleus and cytoplasm of stele cells (Nakajima et al, 2001; Gallagher et al., 2004; Sena et al., 2004). SHR movement into the adjacent cell layer, which includes the QC, the cortex/endodermis initial, and the endodermis, plays an important regulatory role in radial patterning. In the endodermis lineage, the direct transcriptional target of SHR is *SCR*. In fact, SHR binds to the *SCR* promoter, its localization is correlated with *SCR* expression, and ectopic SHR expression induces *SCR* expression, leading to the ectopic production of tissue layers characteristic of the endodermis (Nakajima et al, 2001; Sena et al., 2004; Levesque et al., 2006). This SHR movement is tightly regulated in tissue-specific ways. For instance, SHR-GFP under the promoters of *SCR* (endodermis-specific), *SUC2* (phloem companion cell-specific), *GL2*, and *WER* (epidermis-specific) cannot move to the adjacent layer (Gallagher et al., 2004; Montiel et al., 2004; Sena et al., 2004). In addition, SHR fused to a nuclear-localized version of GFP (nlsGFP) under its native promoter shows a lack of movement, indicating that cytoplasmic localization of SHR in the stele is necessary for its movement to the adjacent cell layers (Gallagher et al., 2004).

SCR expression is also highly regulated in time and space; its induction or elimination can be controlled by using a

CRE/*lox*-based recombination system (Heidstra et al., 2004). Once *SCR* expression is induced by SHR, it is then maintained by autoregulation. In addition, transient SCR function is sufficient for asymmetric division to separate the cortex and endodermis lineage (Heidstra et al., 2004).

In summary, patterning processes for distinguishing the fates of the cortex and endodermis require the functioning of both SHR and SCR. Once *SCR* expression is induced by the translocated SHR protein, it is under autoregulation. SCR also restricts SHR movement, and mediates rapid separation of the cortex and endodermis lineage as well as the segregation of factors necessary for executing that asymmetric division (Gallagher et al., 2004; Heidstra et al., 2004; Sena et al., 2004). Microarray data analysis and the binding of SHR to the promoter regions of putative targets have confirmed the fact that *SCR* is a direct target of SHR, leading Levesque et al. (2006) to predict a possible new role for SHR in the development of vascular tissue. In addition, putative candidates that interact with SHR have been isolated and characterized in the context of patterning processes (Lee and Lim, unpublished data). Future analysis of these players will provide better understanding of the molecular mechanisms for cell fate specification and cell differentiation along the radial axis.

UNDERGROUND GROUP OF STEM CELLS

Stem cells are self-renewal, and produce daughter cells that can further differentiate. Typically, stem cells have the potential to generate the entire spectrum of cell types in an organism (Weigel and Jürgens, 2002). In the *Arabidopsis* root meristem, the initials are the stem cells that surround the QC, which consists of four mitotically less-active cells (Dolan et al., 1993). Laser ablation experiments have demonstrated that a non-autonomous signal from the QC regulates the stemness of the surrounding initial cells (van den Berg et al., 1995, 1997). Besides its role in radial patterning, *SCR* functions in the specification of the stem cell niche (i.e., the QC and the surrounding initials) (Sabatini et al., 2003). In *scr* mutants, that niche is of aberrant shape and root growth ultimately is terminated. When *SCR* is expressed in the QC but not in the cortex/endodermis initial or its derivatives in *scr-1*, the only phenotype that can be rescued is that of the early termination of root growth. In contrast, when *SCR* is expressed in the endodermis and its initial but not in the QC, only the radial-patterning defect of *scr-1* can be recovered. Thus, *SCR* is required for both radial patterning and maintenance of the QC. SHR also provides a positional signal for the specification of the QC and stem cell niche because SHR movement to the QC activates *SCR* (Nakajima et al., 2001; Sabatini et al., 2003; Vernoux and Benfey, 2005).

PLETHORA (*PLT*)*1* and *2*, which encode AP2 class transcription factors, are key regulators of stem cell patterning in the root (Aida et al., 2004). Both are redundantly required for the establishment and maintenance of the root stem cell niche. The *PLT* genes are expressed in the QC and sur-

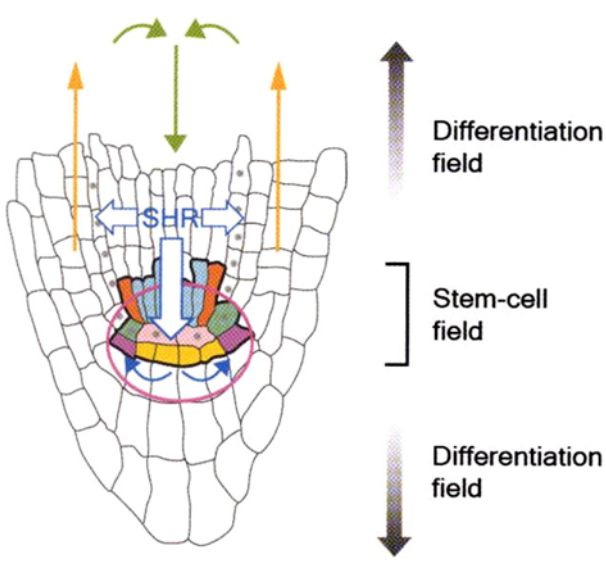

Figure 2. Model for specification of stem cell niche in the *Arabidopsis* root meristem. Circulating arrows indicate local auxin distribution, and pink circle represents *PLT* expression domain. Gray dots indicate accumulation of SHR and SCR in nuclei. Cell differentiation occurs away from stem cell niche. Combination of transcription factors (PLTs, SCR, and SHR) and local auxin distribution determines precise positioning of QC and niche specification.

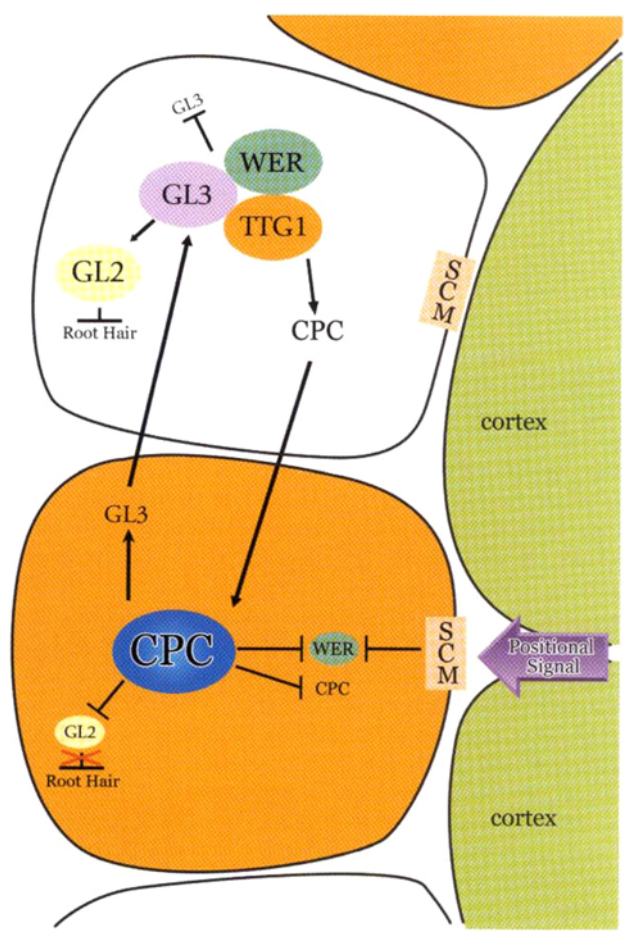

Figure 3. Model for cell fate specification in the *Arabidopsis* root epidermis. Arrows indicate positive regulation, or protein movement in the case of arrows spanning two cells; blunt-end lines indicate negative regulation.

rounding stem cells. Their ectopic expression induces root formation from shoot tissues (Aida et al., 2004). Triple-mutant analysis of *scr plt1 plt2* and *shr plt1 plt2* has indicated that the *PLT* genes act in parallel with the SCR/SHR pathway to specify the stem cell niche. In addition, at the stage when that niche is specified, embryonic expression is severely reduced in double-mutant embryos of *monopteros* (*mp*) and *non-phototropic hypocotyl4* (*nph4*), which are the most homologous members in the ARF (Auxin Response Factor) family (Aida et al., 2004). Correct perception and localization of an auxin maximum is required for the establishment and maintenance of the root meristem (Sabatini et al., 1999). This local maximum is interpreted by the *PLT* genes through PIN proteins, auxin efflux facilitators (Blilou et al., 2005). Several studies of auxin and plant pattern formation with regard to PINs have shed light on how the local distribution of auxin regulates the specification of root stem cells (Leyser, 2005; Paponov et al., 2005). When the QC is laser-ablated, the auxin maximum shifts from distal to proximal in the root tip, with no changes to PIN polarity (Xu et al., 2006). When the QC is ablated in *scr* and *shr* mutants, the polarity of PIN4 is reversed in the proximal to the ablated QC during the regeneration of a new QC (Xu et al., 2006). It is interesting to note that PIN4 attains the correct polarity only after renewed cell specification, rather than directly in response to changes in auxin distribution. This suggests that the transcription factors (PLTs, SCR, and SHR) required for the specification of cell fate can affect alterations in PIN polarity within the root (Xu et al., 2006).

In summary, SHR and SCR function in positioning the QC along the radial axis, whereas the *PLT* genes through PIN proteins are necessary for the specification of the QC and stem cells along the longitudinal axis (Aida et al., 2004; Blilou et al., 2005; Scheres, 2005). While the stem cell niche is being regenerated following laser ablation of the QC, the auxin maximum is shifted from distal to proximal in the root tip (Xu et al., 2006). Expression of the *PLT* genes is induced in response to local alterations in auxin distribution, and, consequently, SHR is localized in the nucleus of a single provascular layer, which induces *SCR* expression and new QC specification. Only after cells adopt their new fates is the correct polarity of PIN proteins set and local auxin distribution reconstituted in the root tip (Xu et al., 2006). Taken together, this combination of PLT, SCR, and SHR transcription factors plus local distribution of auxin is essential for the precise positioning of the QC and specification of the stem cell niche (Fig. 2).

CELL FATE SPECIFICATION IN THE ROOT EPIDERMIS

Three main types of epidermal cell patterns

The root epidermis comprises two types of cells: hair-bearing cell (H cell) and non-hair cell (N cell). Root hairs, the tubular extensions of epidermal cells, are well-adapted to the efficient uptake of water and nutrients. Three main types of cellular organization are found in the epidermis (Clowes, 2000; Dolan and Costa, 2001; Pemberton et al., 2001). One is the alternate pattern that results from asymmetric cell divisions, in which a large cell from an asymmetric transverse division along the axis develops into an N cell, while a small cell becomes an H cell. This pattern can be found in most monocots and some dicots (Clowes, 2000). The second pattern is position-dependent, in which the types are determined by the positioning of the epidermal cells relative to the underlying cortical cells, where H cells and N cells are distributed in rows along the axis. This pattern occurs in some eudicots, including the Brassicales, Caryophyllales, and Boraginaceae (Clowes, 2000). The third pattern is random, in which every epidermal cell has the potential to develop into H cells, and root hair development depends on environmental factors (Cormack, 1947; Clowes, 2000). Because it is predictable, the epidermis with the position-dependent pattern has been a good model system for studying cell fate specification.

Position-dependent epidermal cell patterning in the Arabidopsis root

The *Arabidopsis* root epidermis shows position-dependent cell fate specification and, therefore, is well-suited for examination of this mechanism. In the epidermis, fate relies on relative positioning. Cells that are located on the periclinal wall of the underlying cortical cell (N position) and make contact with a single cortical cell will develop into N cells, while those on the anticlinal wall of underlying cortical cells (H position), and which have contact with two cortical cells, will develop into H cells (Dolan et al., 1994; Galway et al., 1994). When the N-position cells in the middle of the division zone are laser-ablated, the neighboring H-position cells invade the free N position and manifest characteristics of the N-cell fate. In the same context, ablation of the H-position cells causes an invasion of some of the neighboring N-position cells, so that they adopt the H-cell fate (Berger et al., 1998a). Although the root epidermis generally undergoes transverse division to make cell files along the axis, longitudinal divisions take place infrequently in the division zone, and the resultant daughter cell undergoes successive transverse divisions to make one more cell file along the axis, called the T-clone. This longitudinal division mainly occurs at the H-position cell, thereby locating one cell at the H position and the other at the N position. Despite their origin, these new N-position cells show characteristics of the N-cell fate and develop into N cells (Berger, 1998b). All of these experiments have confirmed that positional information is important to cell fate specification in the root epidermis, and that the fate is not fixed until differentiation occurs.

Although the plant hormones, ethylene and auxin, and some environmental factors can also affect cell fate in the root epidermis (Wilson et al., 1990; Dolan et al., 1994; Masucci and Schiefelbein, 1997; Cao et al., 1999; Raghothama, 1999; Schikora and Schmidt, 2001; Knox et al., 2003; Müller and Schmidt, 2004), none of these seems to act within the developmental pathway. Therefore, we will focus on the factors that regulate fate in a position-dependent manner and within a developmental program.

Genes that regulate cell fate in the Arabidopsis root epidermis

Molecular genetics approaches have revealed several

genes involved in cell fate specification in the *Arabidopsis* root epidermis, and their regulatory network. For example, the *GLABRA2* (*GL2*) gene encodes an HD-Zip transcription factor (Rerie et al., 1994; Masucci et al., 1996). In *gl2* mutants, the position-dependent cell fate specification of the root epidermis is impaired, and most epidermal cells adopt the H-cell fate regardless of their position relative to the underlying cortical cells. The *GL2* gene is expressed in developing N-position epidermal cells in the meristematic region (Masucci et al., 1996). GL2 represses phospholipase Dζ1 (*AtPLDζ1*) expression, and ectopic expression of *AtPLDζ1* creates bulges in all root epidermal cells (Ohashi et al., 2003). These reports suggest that GL2 induces the N-cell fate in N-position cells through the repression of *AtPLDζ1* expression. A R2R3 MYB gene, *WEREWOLF* (*WER*), also induces the N-cell fate (Lee and Schiefelbein, 1999). The *wer* mutation abolishes *GL2* expression in the N-position cell, and causes almost every epidermal cell to develop into an H cell. *WER* is expressed in the epidermis and lateral root cap from the epidermis/lateral root cap initials. In the epidermis, it is preferentially expressed in the N-position cells. *TRANSPARENT TESTA GLABRA1* (*TTG1*), encoding a WD40 protein, is also involved in cell fate specification (Walker et al., 1999). In *ttg1* mutants, most root epidermal cells adopt the H-cell fate (Galway et al., 1994). The expression pattern of this gene in the root is not clear, even though *TTG1* transcripts accumulate in the root tip (Baudry et al., 2004). It has long been supposed that a bHLH gene is involved in this specification. This is based on findings that overexpression of the maize *R* gene, a bHLH gene, suppresses the *ttg1* mutant phenotype to cause most epidermal cells to adopt the N-cell fate (Lloyd et al., 1992; Galway et al., 1994). Two closely related bHLH genes in the *Arabidopsis* genome – *GLABRA3* (*GL3*) and *ENHANCER OF GLABRA3* (*EGL3*) – are involved in cell fate specification in the root epidermis (Bernhardt et al., 2003). Whereas *gl3 egl3* double mutants do not specify the N-cell fate in the root epidermis, overexpression of those genes makes most of the root epidermal cells develop into N cells. This implies that *GL3* and *EGL3* act redundantly to induce an N-cell fate in the N-position cells of the root epidermis.

A few genes involved in H-cell fate specification have been reported (Wada et al., 1997; Schellmann et al., 2002; Kirik et al., 2004). For example, mutations of *CAPRICE* (*CPC*), a gene that encodes a single MYB repeat protein, result in reduced frequency of the H-cell fate while overexpression is associated with increased frequency of the H-cell fate (Wada et al., 1997). Because CPC protein lacks any discernible activation domain it has been proposed that this protein counteracts the WER protein as a transcriptional repressor. The *Arabidopsis* genome contains four *CPC*-like genes, and mutations in two of the *CPC*-like genes, *TRY* and *ENHANCER OF TRY AND CPC1* (*ETC1*), dramatically enhance the *cpc* mutant phenotype in the root epidermis (Schellmann et al., 2002; Kirik et al., 2004). However, mutations in *TRY* and *ETC1* genes do not show any significant phenotype in the root epidermis without the *cpc* mutant background. These results suggest that *CPC* plays a major role and that two other *CPC*-like genes – *TRY* and *ETC1* – have only supplementary functions in specifying the H-cell fate (Schellmann et al., 2002; Kirik et al., 2004).

Except for *TTG1*, most of the aforementioned genes encode transcription regulators. However, it has long been a fundamental and important question as to how the epidermal cells know their positions. Recently, a leucine-rich repeat (LRR) receptor-like kinase, SCRAMBLED (SCM), has been demonstrated to regulate position-dependent cell fate specification in the *Arabidopsis* root epidermis, thereby providing an answer for how cells perceive the positional signal (Kwak et al., 2005). In *scm* mutants, this specification is partially disrupted, so that the distribution of H and N cells is not strictly correlated with their position relative to the underlying cortical cell, even though there is no difference in the proportion of cell types between the *scm* and wild-type epidermis.

Model for cell fate specification in the Arabidopsis root epidermis

GL2 is expressed in the N-position cells in the meristem, and induces the N-cell fate by regulating the expression of target genes, including *AtPLDζ1* (Masucci et al., 1996; Ohashi et al., 2003). However, cells that do not express the *GL2* gene develop into H cells. In mutants that are impaired in their N-cell fate specification, e.g., *ttg1*, *wer*, and *gl3 egl3*, the level of *GL2* expression is greatly reduced. However, in *ttg1* and *gl3 egl3* mutants, this position-dependent expression pattern is similar to that found in the wild-type epidermis (Hung et al., 1998; Bernhardt et al., 2003). In contrast, for *wer* mutants, the position-dependent expression pattern of *GL2* is disrupted and its expression is also greatly reduced (Lee and Schiefelbein, 1999). Only a few cells express *GL2*; they can be found at either position. These results, together with the preferential expression of *WER* in the N-position cells, suggest that WER specifies N-cell fate by inducing *GL2* expression in a position-dependent manner, and that TTG1 and GL3/EGL3 amplify this WER-induced expression. In contrast, CPC negatively regulates *GL2* and *WER* expression. Transcript levels for those genes are elevated in the *cpc* mutant due to *GL2* and *WER* expression in the H-position cells that normally do not strongly express *GL2* and *WER* in the wild type (Lee and Schiefelbein, 2002; Wada et al., 2002).

Interestingly, *CPC* transcripts are not accumulated in the H-position cells but they are in the N-position cells (Lee and Schiefelbein, 2002; Wada et al., 2002). However, CPC protein accumulates more in the nuclei of the H-position cells, which implies that CPC moves from there to the neighboring N-position cells (Kurata et al., 2005). This expression pattern and the movement of CPC protein demonstrate the importance of cell-cell communication, and both features imply that N-position cells expressing the *CPC* gene play an important role in specifying the H-cell fate of neighboring cells. Unexpectedly, *CPC* expression in the N-position cells is abolished in the *wer* mutant root epidermis (Lee and Schiefelbein, 2002). Recently, Ryu et al. (2005) have shown that WER induces *CPC* transcription directly, via the glucocorticoid receptor-inducible system. WER can bind directly to the *CPC* promoter (WBSI and WBSII) to activate *CPC* expression in the N-position cells (Koshino-Kimura et al.,

2005; Ryu et al., 2005). For cell fate specification in the root epidermis, the role of WER is not restricted to the N cells, but that gene also functions in specifying H-cell fate by regulating *CPC* expression (Lee and Schiefelbein, 2002; Ryu et al., 2005). Therefore, WER can be considered as a master regulator in epidermal-cell patterning, not just as a positive or a negative regulator of a specific fate. This idea is supported by reports of *WER* overexpression, which does not prompt all the epidermal cells to adopt the same fate but instead prompts a randomized pattern, especially in the *wer* mutant (Lee and Schiefelbein, 2002; Ryu et al., 2005). This is also consistent with the concept that positional information flows through WER, not through GL3/EGL3 or TTG1.

GL3 and EGL3 induce *GL2* expression in the N-position cells and influence N-cell fate in a redundant manner (Bernhardt et al., 2003). For *gl3 egl3* double mutants, *CPC* expression in the N-position is also reduced (Bernhardt et al., 2003). GL3 and EGL3 interact with WER in yeast, suggesting that an activating complex, composed of WER and GL3/EGL3, induces *GL2* and *CPC* expression (Bernhardt et al., 2003). Interestingly, *GL3/EGL3* transcripts accumulate in the H-position cells, with this transcription being positively regulated by CPC (Bernhardt et al., 2005). The *GL3* gene is transcribed in H-position cells and the protein moves into N-position cells where it seems to constitute an activating complex with WER (Bernhardt et al., 2005). TTG1 may be a possible component in that complex, for three reasons: 1) it can interact with GL3/EGL3 in yeast, 2) most cells in the *ttg1* mutant root epidermis adopt the H-cell fate, and 3) overexpression of *GL3* can suppress the *ttg1* mutant phenotype (Bernhardt et al., 2003).

The position-dependent expression of *WER* is disrupted in *scm* mutants and *WER*-expressing cells and non-expressing cells are found at both positions (Kwak et al., 2005). As discussed for *wer* mutants, a few epidermal cells express *GL2*, and their positions are not restricted to the N position (Lee and Schiefelbein, 1999). In addition, overexpression of *WER* randomizes the pattern of epidermal cell types (Lee and Schiefelbein, 1999, 2002). Taken together, it is likely that SCM in the epidermal cell perceives positional information from the underlying cortical cells and transduces that information into the epidermal cells, such that *WER* expression is regulated in a position-dependent manner. *WER* is negatively regulated by SCM, a conclusion based on an analysis of the *scm* mutant and an *SCM*-overexpressing line (Kwak and Schiefelbein, 2007). There, the steady-state level of *WER* transcripts is higher in the *scm* mutant root than in the wild type. In addition, overexpression of *SCM* leads to a reduction in the steady-state level of *WER* transcripts in the root.

These regulatory circuits provide a model for cell fate specification in the *Arabidopsis* root epidermis (Fig. 3). At the early developmental stage, cells at both positions, N and H, are equally competent to both fates, and they express fate regulators at similar levels. A positional signal from beneath is perceived by SCM in the H-position cell. Consequently, SCM inhibits *WER* expression in those H-position cells, thereby disrupting the balance of lateral inhibition mediated by CPC from the H-position cells to the N-position cells and from the N-position cells to the H-position cells. Slightly weaker lateral inhibition from the H-position cells to the N-position cells enhances the difference in *WER* expression in two position cells, subsequently increasing the difference in *CPC* expression. Greater accumulations of *CPC* transcripts in the N-position cells make lateral inhibition to the H-position cells more prominent, such that these adopt the H-cell fate. In addition, the H-position cells show higher expression of the *GL3/EGL3* genes than do the N-position cells because more CPC is detected in the H-position cells moving from the neighboring N-position cells. GL3 then moves to the neighboring H-position cell, causing this cell to adopt the N-cell fate through activation of *GL2* expression. The regulatory circuit, which includes a lateral inhibition mechanism, establishes this position-dependent patterning of epidermal cells.

Chromatin structure and cell fate in the Arabidopsis root epidermis

The chromatin structure provides epigenetic control of gene expression, playing an important role in plant development and responses to various environmental stimuli. Vernalization is an excellent example for the conversion of such a structure. Cold treatment in vernalization promotes flowering time in winter-annual accessions by repressing the expression of *FLOWERING LOCUS C* (*FLC*) (Michaels and Amasino, 1999; Sheldon et al., 1999). Furthermore, *VERNALIZATION1* (*VRN1*), *VERNALIZATION2* (*VRN2*), and *VERNALIZATION INSENSITIVE3* (*VIN3*) modify the chromatin around the *FLC* locus, an action that is required for this repression (Gendall et al., 2001; Levy et al., 2002; Sung and Amasino, 2004). Change in that structure is also essential to cell fate specification in the *Arabidopsis* root epidermis. For example, histone acetylation affects the patterning of the epidermis (Xu et al., 2005). A specific inhibitor of histone deacetylase – trichostatin A (TSA) – can modulate this patterning in a concentration-dependent manner. TSA treatment causes the epidermal cells to adopt the H-cell fate regardless of their relative position. Moreover, levels of H3 and H4 acetylation at the *CPC*, *GL2*, and *WER* loci are increased by exposure to TSA. Similar treatment also causes increased *CPC* and *GL2* expression, but reduces that of *WER*, disrupting their position-dependent expression patterns. Likewise, *hda18* mutants (defective in At5g61070 encoding histone deacetylase) show increased root hair densities and alterations in the cellular patterns of their root epidermis similar to that noted from TSA treatment. This provides complementary support for the involvement of histone acetylation in cell fate specification. Differences have been reported in the chromatin status around the *GL2* locus at the N-position and H-position cells (Costa and Shaw, 2006). In the former type, *GL2* is fully accessible to a GL2 probe in three-dimensional fluorescence *in situ* hybridization (FISH). This indicates the existence of an open chromatin conformation at the *GL2* locus. In comparison, no signal is detected in the H-position cells, demonstrating that the chromatin state is closed so that the *GL2* probe cannot access that region. In *wer* and *cpc* mutants, a strong *GL2* FISH signal is measured in every cell regardless of its position, implying that CPC is required for a closed chromatin

state in the *GL2* region. In addition, for *fasciata2* (*fas2*), defective in the activity of one of three subunits for chromatin assembly factor-1 (CAF-1), both chromatin states in the *GL2* region can be found at the N-position and at the H-position. Ectopic *GL2* expression and a reduction in the number of H cells in this mutant indicate that correct assembly of chromatin is required if the cell is to link positional signalling to cell fate specification. In T-clones, cells in two files with the same clonal origin do not inherit the chromatin state of the original cell, but their own states in the *GL2* region respond to a new position and are remodeled.

CONCLUDING REMARKS

Because of it simplicity and predictability, the *Arabidopsis* root is a good model system for studies of stem-cell positioning and maintenance, as well as for research into cell fate specification. Several major advances in this field have provided important pieces that fit the complex puzzle of how those two phenomena are regulated.

Analysis of its functioning has demonstrated a dual role for SCR in the radial patterning and specification of the quiescent center (Heidstra et al., 2004). Furthermore, interactions among PLTs, SCR, SHR, and auxin provide an attractive model for the specification of the stem cell niche (Aida et al., 2004; Blilou et al., 2005; Scheres, 2005; Xu et al., 2006) (Fig. 2). However, that model is still missing some pieces, including the identity of signals that emanate from the QC to prevent the initials from differentiating, as well as the interaction of other PLTs, SCR, and SHR partners for stem cell specification. Microarray data analysis has now identified a set of new target genes for SHR, and has demonstrated its binding to the promoter regions of those potential targets (Levesque et al., 2006). In addition, putative candidates that interact with SHR have been isolated, and their characterization will provide better understanding of the molecular mechanisms for cell fate specification and differentiation along the radial axis (Lee and Lim, unpublished data).

SCM is believed to be localized in the cell membrane, supposedly perceiving a positional signal from beneath to regulate *WER* expression in the nucleus. Therefore, it is reasonable to presume that some additional factors relay that signal from the cell membrane to the nucleus. LRR receptor-like kinases, e.g., *TOO MANY MOUTH* (*TMM*), *ERECTA* (*ER*), *ERECTA LIKE1* and *2* (*ERL1* and *ERL2*), *mitogen-activated protein kinase kinase kinase* (*MAPKKK*), *YODA* (*YDA*), and two R2R3 MYBs – *FOUR LIPS* (*FLP*) and *AtMYB88* – have been reported to be involved in stomatal patterning (Nadeau and Sack, 2002; Bergmann et al., 2004; Lai et al., 2005; Shpak et al., 2005). Mitogen-activated protein kinase cascades link extracellular stimuli to appropriate cellular responses. These are composed of three layers of phosphorylation events via MAPKKKs, mitogen-activated protein kinase kinases (MAPKKs), and mitogen-activated protein kinases (MAPKs) (Ichimura et al., 2002). For example, the MAPKK4/MAPKK5-MAPK3/MAPK6 signaling module acts downstream of YDA in stomatal patterning before guard mother cell specification (Wang et al., 2007). It will be interesting to learn whether this kind of cascade functions in relaying the signal from the cell membrane to the nucleus to achieve cell fate specification in the *Arabidopsis* root epidermis.

Although a similar set of genes regulates epidermal cell patterning during embryogenesis and post-embryonic phases (Hung et al., 1998; Lin and Schiefelbein, 2001; Bernhardt et al., 2003), *SCM* is not required for this action during embryonic development (Kwak and Schiefelbein, 2007). Functionally redundant genes may possibly act mainly during embryogenesis, or else a completely different mechanism might be involved in epidermal cell patterning during embryogenesis. However, Kwak and Schiefelbein (2007) have now reported that mutations in two genes closely related to *SCM* (*srf1* and *srf3*) show no significant defects in such patterning, either alone or in combination. This implies that functionally redundant genes may not be responsible. If so, it will be valuable to determine which gene, other than *SCM*, is responsible during embryogenesis.

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