

## REVIEW

# A novel regulatory circuit specifies cell fate in the *Arabidopsis* root epidermis

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The specification of distinct cell fates in multicellular organisms is a fundamental process in developmental biology. The *Arabidopsis* root epidermis, which consists of root-hair cells and non-hair cells, provides a useful model system for studying cell fate specification. In this tissue, the cell fates are determined by their relative position to the underlying cortical cells, and many genes have been identified that regulate this position-dependent cell fate specification. Recent studies using genetic, molecular, and biochemical approaches have shed new light on this process and revealed a complex network of interacting and interdependent components. In particular, a novel regulatory circuit has recently been identified, which includes a lateral inhibition pathway and a feedback loop that enables intercellular communication and ensures that two distinct cell types arise in an appropriate pattern. This regulatory circuit is also influenced by a positional signaling pathway which includes the SCRAMBLED leucine-rich repeat receptor kinase. The studies of cell fate specification in the *Arabidopsis* root epidermis provide new insights into the molecular strategies used to define distinct cell types in plants.

**Introduction**

In the field of developmental biology, a fundamental and challenging problem is to understand how particular cell fates are specified during the development of multicellular organisms. In general, this process is thought to depend on the establishment of differential gene expression because of the influence of internal and external factors, but the molecular mechanisms involved are poorly understood. In plants, the development of the *Arabidopsis* root epidermis has been used for many years as a good model system to study cell fate specification. The *Arabidopsis* root epidermis is composed of only two types of cells, root-hair cells (which

possess a long tubular extension) and non-hair cells. Furthermore, these two cell types are specified in a predictable position-dependent pattern, which is advantageous, because it enables one to reliably predict the fate of a cell before it has differentiated. A cell that is located against the periclinal cell wall of the underlying cortex and in contact with a single cortical cell (designated the N position) consistently adopts the non-hair cell fate, while a cell located on the anticlinal cell wall of the underlying cortex and thereby contacts two cortical cells (designated the H position) adopts the hair cell fate (Dolan et al. 1994, Galway et al. 1994). Because root hairs are easy to observe at the seedling stage of *Arabidopsis* development, many mutants that

**Abbreviations** – CPC, CAPRICE; EGL3, ENHANCER OF GLABRA3; ETC1, ENHANCER OF TRY and CPC1; GL1, GLABROUS1; GL2, GLABRA2; GL3, GLABRA3; LRR, leucine-rich repeat; SCM, SCRAMBLED; TRY, TRIPTYCHON; TTG1, TRANSPARENT TESTA GLABRA1; WER, WEREWOLF.

alter the number or arrangement of hair/non-hair cells have been identified, and the corresponding genes have been analyzed. These molecular genetic studies are providing an emerging picture of a complex network of regulatory factors that specifies the epidermal cell fates. In this review, we discuss these studies and describe the regulatory circuit that is responsible for directing the establishment of the two cell types in the *Arabidopsis* root epidermis.

### Genes that regulate cell fate in the *Arabidopsis* root epidermis

During the past decade, molecular genetic studies have uncovered several genes that affect the cell fate decision in the *Arabidopsis* root epidermis (Table 1). One of the first genes identified is *GLABRA2* (*GL2*). The *gl2* mutant was found to have a “hairy” root phenotype, because almost all of the root epidermal cells differentiate into root-hair cells regardless of their relative positions to the underlying cortical cells (Masucci et al. 1996). The *GL2* gene encodes a homeodomain-Zip-related transcription factor (Rerie et al. 1994) and is expressed in the N-position cells in the root epidermis within the meristematic region (Masucci et al. 1996). These results imply that *GL2* induces the non-hair cell fate in the root epidermis. It has long been thought that *GL2* acts by promoting the transcription of target genes to induce the non-hair cell fate. A phospholipase  $D\zeta 1$  (*AtPLD $\zeta$ 1*) was reported as a direct target gene of *GL2* (Ohashi et al. 2003). Interestingly, *AtPLD $\zeta$ 1* expression is repressed by *GL2* rather than induced, and ectopic expression of this gene leads to abnormalities in root-hair development including hair initiation (bulges) in all cell files

(Ohashi et al. 2003). Together, these suggest that *GL2* induces the non-hair cell fate through repression of the genes involved in hair cell differentiation, including *AtPLD $\zeta$ 1*. This interpretation is consistent with the view, supported by surgical studies in other *Brassicaceae* species (Bunning 1951), that the hair-cell fate is the default fate for a root epidermal cell.

*TRANSPARENT TESTA GLABRA1* (*TTG1*) is another gene involved in root epidermal cell fate specification. Like the *gl2* mutant, the *ttg1* mutant produces root-hair cells in place of non-hair cells (Galway et al. 1994). The *TTG1* gene encodes a protein containing four WD40 repeats, which are believed to function in protein–protein interactions (Walker et al. 1999). However, it is not clear yet how this protein mechanistically acts to induce the non-hair cell fate nor is the *TTG1* expression pattern known. Nevertheless, because *TTG1* is required to specify the non-hair cell fate, it seems likely that *TTG1* acts in the N-position epidermal cells.

Overexpression of the maize *R* gene, which encodes a basic helix-loop-helix (bHLH)-type transcription factor, is able to rescue the *ttg1* mutant phenotype and causes most of the epidermal cells to adopt the non-hair cell fate regardless of their relative position (Galway et al. 1994). Based on this result, it has been assumed that an *R* homolog in *Arabidopsis* is involved in cell fate specification and acts downstream of *TTG1*. Indeed, two closely related bHLH genes, *GLABRA3* (*GL3*) and *ENHANCER OF GLABRA3* (*EGL3*) were shown to be required to specify the non-hair cell fate, because the *gl3 egl3* double mutant lacks the non-hair cell type, and overexpression of either gene causes nearly all of the epidermal cells to adopt the non-hair cell fate (Bernhardt et al. 2003). These findings imply that *GL3*

**Table 1.** Genes that regulate cell fate in the *Arabidopsis* root epidermis

Gene name	Function in the root epidermis	References
<i>GL2</i>	Suppresses the hair cell fate in the N-position cell, suppresses the expression of <i>AtPLD<math>\zeta</math>1</i> expression, is expressed preferentially in the N-position cell	Masucci et al. (1996) Ohashi et al. (2003)
<i>TTG1</i>	Suppresses the hair cell fate in the N-position cell, increases the expression level of <i>GL2</i> gene	Galway et al. (1994)
<i>GL3</i>	Suppresses the hair cell fate in the N-position cell, acts redundantly with <i>EGL3</i> , increases the expression level of <i>GL2</i> gene	Bernhardt et al. (2003)
<i>EGL3</i>	Suppresses the hair cell fate in the N-position cell, acts redundantly with <i>GL3</i> , increases the expression level of <i>GL2</i> gene	Bernhardt et al. (2003)
<i>WER</i>	Suppresses the hair cell fate in the N-position cell, regulates the position-dependent expression pattern of <i>GL2</i> , regulates the position-dependent expression pattern of <i>CPC</i>	Lee and Schiefelbein (1999) Lee and Schiefelbein (2002)
<i>CPC</i>	Induces the hair cell fate in the H-position cell	Wada et al. (1997)
<i>TRY</i>	Induces the hair cell fate in the H-position cell, shows partial redundancy with <i>CPC</i>	Schellmann et al. (2002)
<i>ETC1</i>	Induces the hair cell fate in the H-position cell, shows partial redundancy with <i>CPC</i>	Kirik et al. (2004)
<i>SCM</i>	Is involved in the position-dependent cell patterning, regulates the position-dependent expression pattern of <i>WER</i> .	Kwak et al. (2005)

and *EGL3* act redundantly to induce the non-hair cell fate in the N-position cells during development.

An R2R3 MYB-type transcription factor gene, *WEREWOLF* (*WER*), also induces the non-hair cell fate (Lee and Schiefelbein 1999). In the *wer* mutant, almost all the root epidermal cells differentiate into hair cells. Accordingly, the *WER* gene is expressed preferentially in the N-position cells from the epidermis/lateral root cap initial cells (Lee and Schiefelbein 1999).

While several genes are known to help specify the non-hair cell fate, there are also a few genes with a role in the specification of the hair cell fate. The *CAPRICE* (*CPC*) gene was identified as a positive regulator of the hair cell fate, because mutation of this gene causes cells in the H position to adopt the non-hair cell fate, and *CPC* overexpression converts N-position cells into root-hair cells (Wada et al. 1997). *CPC* encodes a single MYB-repeat protein lacking any discernible activation domain; hence, it has been suggested to act as a transcriptional repressor by counteracting the effect of *WER* (Lee and Schiefelbein 1999). The *Arabidopsis* genome has four *CPC*-related genes including *TRIPTYCHON* (*TRY*). Although the *try* mutants do not show any significant phenotype in the root epidermis, they are able to significantly enhance the *cpc* mutant phenotype so that, in the *try cpc* double mutant, essentially all cells adopt the non-hair cell fate (Schellmann et al. 2002). Mutations in another *CPC*-like gene, *ENHANCER OF TRY AND CPC1* (*ETC1*), are also able to enhance the *cpc* mutant phenotype in the root epidermis, even though the single mutant does not show any phenotype (Kirik et al. 2004). Together, these results suggest that *CPC* plays a major role, and the two *CPC*-like genes, *TRY* and *ETC1*, play a supplementary function in hair cell fate specification.

### **A regulatory circuit controlling cell fate specification**

The regulatory relationships between the genes described above have been elucidated by investigating the expression and localization of the genes and gene products. As mentioned above, the *GL2* gene is expressed in the N-position cells in the meristematic region (Masucci et al. 1996). In the *ttg1* mutant, the expression level of *GL2* is reduced significantly, but its expression pattern (in the N-position cells) is not affected (DiCristina et al. 1996, Hung et al. 1998). This suggests that *TTG1* induces the non-hair cell fate by increasing the expression level of the *GL2* gene but does not control its position-dependent pattern. Similarly, *GL3* and *EGL3* seem to induce the non-hair cell fate by upregulating *GL2* expression, because the

level of *GL2* expression is greatly reduced in the *gl3 eg/3* double mutant, but the position-dependent expression pattern is not disrupted (Bernhardt et al. 2003). On the other hand, the *wer* mutants nearly abolish *GL2* expression in the root epidermis, and the remaining *GL2* expression is not present in a position-dependent pattern (Lee and Schiefelbein 1999). Therefore, *WER* appears to specify the non-hair cell fate by inducing expression of *GL2* in the N-position cells. In other words, the positional information that specifies the cell fate pattern appears to flow through *WER* rather than *TTG1*, *GL3*, or *EGL3*.

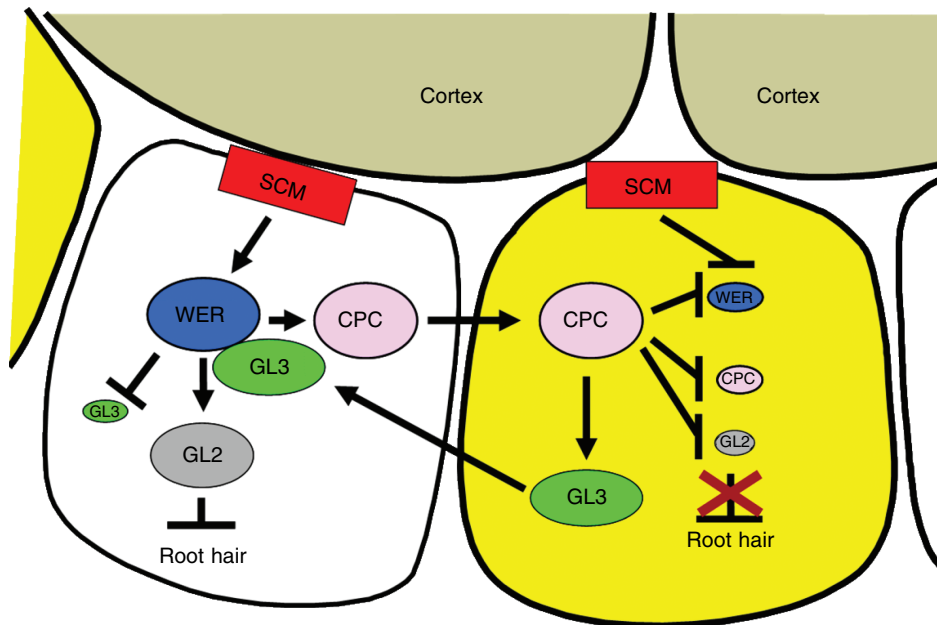
The *CPC* gene also regulates *GL2* expression during root epidermis development. In the *cpc* mutant (which produces non-hair cells in the H position), the expression level of *GL2* is increased, and there is significant *GL2* expression in the H-position cells (Lee and Schiefelbein 2002, Wada et al. 2002). In plants overexpressing the *CPC* gene (*35S::CPC*), *GL2* expression is effectively abolished in the root epidermis. Furthermore, the *cpc gl2* double mutant shows a similar phenotype as the *gl2* single mutant; that is, most of the epidermal cells differentiate into the root-hair cell type (Wada et al. 1997). These findings indicate that *CPC* induces the hair cell fate by inhibiting *GL2* expression in the H-position cells. However, in what was a surprising result at the time, the promoter activity and RNA accumulation of the *CPC* gene are preferentially detected in the N-position cells rather than the H-position cells, and the *CPC* protein (as determined from a *CPC*-GFP fusion protein) is found in all the epidermal cells regardless of their positions (Wada et al. 2002). These observations imply that the *CPC* gene is transcribed and translated in the N-position cells, and then, the *CPC* protein moves into the neighboring H-position cell where it induces the hair cell fate by inhibiting *GL2* expression. Thus, *CPC* formally acts in a lateral inhibition process, because it inhibits neighboring cells from adopting the same fate as the cell in which it is produced. Although *CPC* protein is also present in the N-position cells, these cells do not adopt the hair cell fate, perhaps because *WER* is expressed strongly in these cells and can overcome the *CPC* effect and induce *GL2* expression (Lee and Schiefelbein 1999, Ryu et al. 2005).

Because *WER* and *CPC* are expressed in the same cells (the N-cell position), it seemed likely that both of them may be regulated by the same transcription factor(s) or that one of the two genes may regulate the other. Indeed, *CPC* expression was found to be positively regulated by *WER*, because in the *wer* mutant, *CPC* expression in the root epidermis is abolished almost completely (Lee and Schiefelbein 2002). The fact that a positive regulator of the non-hair cell fate,

WER, can induce the expression a positive regulator of the hair cell fate, CPC, suggests that WER is involved in specifying both cell fates. The overexpression of *WER* supports this notion. In the root epidermis of plants overexpressing *WER* in the *wer* mutant background (*35S::WER wer*), there is a partially randomized pattern of cell fates rather than the production of the non-hair cell fate throughout the root epidermis (Lee and Schiefelbein 2002). Also, when the chimeric protein WER fused to VP16, a strong activation domain (Triezenberg et al. 1988), is over-expressed in the *wer* mutant, it exhibited the same phenotype (Ryu et al. 2005). This implies that the randomized pattern is not an intermediate phenotype caused by insufficient WER activity; but it is due to the uniform production of a factor (WER) that directs the specification of both of the cell fates. Furthermore, overexpressing *WER* in the *cpc wer* double mutant causes more cells to adopt the non-hair cell fate than overexpressing *WER* in the *wer* single mutant (Lee and Schiefelbein 2002), suggesting that *CPC* mediates partly the hair cell fate specification by *WER* (Fig. 1).

Recently, the expression patterns of *GL3* and *EGL3* were reported, and unexpectedly, their promoter activity and RNA accumulation are preferentially detected in the H-position cells of the meristematic region (Bernhardt et al. 2005). The *GL3* protein, however, is

localized to the nuclei of the N-position cells (based on the analysis of a *GL3*-YFP fusion), which implies that these bHLH proteins move from the H cell to the N cells (Bernhardt et al. 2005). Interestingly, *GL3* and *EGL3* expression are found throughout the root epidermis in the *wer* mutant and is decreased in the *cpc* mutant in the H-position cells (Bernhardt et al. 2005). That is, in the wild-type root, the WER protein inhibits the expression of *GL3* and *EGL3* in the N-position cells, and the *CPC* protein is required to induce their expression in the H-position cells. Because *CPC* expression is induced by WER (Lee and Schiefelbein 2002) and *GL3/EGL3* (Bernhardt et al. 2003), there exists a regulatory circuit in this cell fate specification mechanism (Fig. 1). In summary, *WER* expression is induced in the N-position cells, the WER protein induces *CPC* expression in the N-position cells, the *CPC* protein moves to the neighboring H-position cells, the *CPC* protein inhibits *GL2* expression but induces *GL3/EGL3* expression in the H-position cells, the *GL3* (and probably *EGL3*) protein moves to the N-position cells, and finally, the *GL3/EGL3* proteins work together with the WER in the N-position cells to induce *GL2* expression and *CPC* expression. It is likely that this regulatory circuit helps to establish the distinct gene expression patterns by ensuring that neighboring cells assist one another to adopt alternative fates.



**Fig. 1.** A regulatory circuit controls cell fate specification in the *Arabidopsis* root epidermis. Arrows indicate positive regulation or protein movement in the case of *CPC* and *GL3*. Blunt-end lines indicate negative regulation. In this model, *EGL3*, *TRY* and *ETC1* are not included for simplicity; the *EGL3* seems to act like the *GL3*, and the *TRY* and *ETC1* seem to act like *CPC*. The *TTG1* is not included in this model because its expression pattern and regulation are not known yet.

## Evidence to support the proposed model

Although the model for root epidermal cell specification outlined in Fig. 1 fits the experimental results so far, it will be necessary to further test and refine the model with additional experiments. In particular, because this model is based primarily on genetic studies, direct biochemical evidence to support this model will be very helpful. Recently, studies of this type are beginning to be reported. It has now been shown, using the glucocorticoid receptor inducible system, that the WER protein induces *CPC* transcription directly (Ryu et al. 2005). Also, there are two reports that show WER protein binding to the *CPC* promoter (Koshino-Kimura et al. 2005, Ryu et al. 2005). In each report, two WER-binding sites were identified; one site from each report is the same, but the other sites in each report are different. Mutations at any of these sites affect the expression of *CPC* and abolish it almost completely in the root epidermis. Therefore, the WER protein appears to bind to three regions in the *CPC* promoter. In animals, MYB proteins bind to the consensus DNA sequence, YAACKG (Biedenkapp et al. 1988, Golay et al. 1994, Howe and Watson 1991). In plants, there are many more MYB proteins, and they show different binding specificity among themselves. For example, maize C1 and P bind to the DNA sequence, CC(T/A)ACC (Sainz et al. 1997), AtMYB2, which functions in abscisic acid signaling in *Arabidopsis* (Abe et al. 2003), binds to the DNA sequence, TGGTTAG (Abe et al. 1997), and MYB.Ph3 from petunia shows DNA-binding specificity to the two DNA sequences, aaaAaaC(G/C)GTTA and aaaAGTTAGTTA (Solano et al. 1995). It appears that the WER protein is also able to bind multiple sites; two related DNA sequences CTCCAACCTG and ACAACCGC, and the other DNA sequence AGTAGTTA (Koshino-Kimura et al. 2005, Ryu et al. 2005). Together, these findings provide direct evidence that WER acts, at least in part, through its ability to regulate *CPC* transcription.

Many transcription factors physically interact with other transcription factors and work together, including many MYB proteins which can interact with bHLH proteins. For example, the maize R protein mentioned above interacts with a maize MYB protein, C1, to regulate anthocyanin production (Goff et al. 1992, Grotewold et al. 1994) and an *Arabidopsis* MYB protein, AtMYB2, interacts with AtMYC2, a bHLH protein, to regulate abscisic acid signaling (Abe et al. 2003). One of the predictions of the model for root epidermal cell specification is that the WER protein might physically interact with a bHLH protein to induce *GL2* expression in the N-position cell (Lee and Schiefelbein 1999). It has

been shown that WER can indeed interact with the GL3 and EGL3 in yeast and that *WER* acts together with *GL3* (or *EGL3*) to specify the non-hair cell fate (Bernhardt et al. 2003). Also, the GL3 was shown to interact with the TTG1 protein using yeast two-hybrid assay (Payne et al. 2000). As a group, the WER, GL3 (or EGL3), and TTG1 appear to interact to make an activating complex to induce *GL2* expression in the N-position cell. Interestingly, the CPC protein was also shown to interact with the maize R protein (Wada et al. 2002). This prompted the view that an activating complex (WER, GL3, and TTG1) acts in the N-position cells to specify the non-hair cell fate by inducing *GL2* expression, and an inhibiting complex (CPC, GL3, and TTG1) acts in H-position cells to specify the hair cell fate by failing to activate *GL2* expression. However, the GL3 protein is now known to move to the N-position cells and accumulate in the nucleus (Bernhardt et al. 2005), which implies that the proposed inhibiting complex cannot be formed in the H-position cell. Therefore, it may be that an activating complex induces *GL2* expression in the N-position cell, and the absence of the activating complex and/or presence of the CPC protein in the H-position cell may result in the lack of *GL2* gene expression (Fig. 1).

## Perception of the positional cue

From the beginning, investigators studying root epidermal cell specification have focused attention on the nature of the position-dependent control of the cell types. That the cell fates are indeed defined by positional cues rather than by lineage-dependent factors has been demonstrated by several convincing lines of experiments. First, using a different *Brassicaceae* species, it has been shown that separating the epidermis from the underlying cells causes all of the epidermal cells to adopt the hair cell fate (Bunning 1951), which implies that the cell fate is not fixed at an early stage. In a study of *Arabidopsis*, the fate of cells derived from rare anticlinal epidermal cell divisions (which places daughter cells in different relative positions) was followed, and the daughter cells consistently adopted fates appropriate for their positions (Berger et al. 1998a). Finally, a laser ablation method was employed in *Arabidopsis* to destroy one epidermal cell and allow a neighboring cell to invade the position occupied previously by the ablated cell. In these cases, the fates of the cells were changed according to their new position (Berger et al. 1998b). Together, these studies show that positional cues exist to guide the fate of the undifferentiated epidermal cells and cause them to adopt a fate that is

dependent on their positions relative to the underlying cortical cells.

Currently, there is great interest in understanding the molecular basis of the positional cues and their perception by the epidermal cells. In animal systems, position-dependent cell behavior is generally considered to be due to the release of a diffusible signal molecule, the formation of a concentration gradient of the molecule, and the perception of the molecule by the cell via a receptor. In plant systems, there is evidence for the role of leucine-rich repeat (LRR) receptor-like kinases in many developmental processes where patterning is involved, such as shoot meristem organization and stomata spacing (Clark et al. 1997, Masle et al. 2005, Nadeau and Sack 2002, Shpak et al. 2005). Recently, a LRR receptor-like kinase, SCRAMBLED (SCM), was reported to regulate the position-dependent cell fate specification in the *Arabidopsis* root epidermis, which provides a possible answer to how cells perceive the positional signal (Kwak et al. 2005). In the *scm* mutant, the overall root hair density is not different from the wild-type, but epidermal cell fate is not strictly dependent on position, so that root-hair cells and non-hair cells can be found at both N and H positions. In addition, the *scm* mutants disrupt the position-dependent expression of *GL2*, *WER*, *CPC*, and *EGL3*, causing them to exhibit a "patchy" distribution rather than a file-specific pattern (Kwak et al. 2005). As we discussed earlier, *WER* is a master regulator of both cell fates. Because *SCM* is necessary for the position-dependent pattern of *WER* expression and the cell fates in the root epidermis, it is likely that the SCM receptor perceives the positional signal, transduces the signal inside the cell, and then regulates *WER* expression, so that it is greater in the N position than in the H position. Following this SCM-dependent bias in *WER* expression, the subsequent events in the regulatory circuit can act to establish the epidermal cell pattern (Fig. 1).

## Conclusions and future directions

As described here, we now have a molecular genetic framework for the regulatory events responsible for cell fate specification in the *Arabidopsis* root epidermis (Fig. 1). In brief, the SCM receptor kinase is likely to perceive a positional cue and generate differential *WER* expression in the N and H position cells. Then, the *WER* protein establishes the distinct gene expression patterns in the two cell positions through a regulatory circuit which includes a CPC-dependent lateral inhibition pathway. Ultimately, this results in the differential expression of the *GL2* gene, which determines whether a cell will or will not express the hair cell differentiation genes.

Despite this framework of understanding, there are still many points that need to be elucidated. In particular, we know nothing about the identity of the positional cue which presumably acts as a ligand for the SCM receptor. Similarly, we do not know what the relay molecules might be between the SCM receptor and *WER* gene expression. There is also a need for additional biochemical-based tests of the model, including analysis of *GL2* and *GL3* gene regulation by *WER* and *CPC*. Furthermore, we know little about the mechanistic role of *TTG1* in this system; does it act as a transcriptional co-factor or does it perform some other function? Also, to understand how the early specification network leads to appropriate changes in cell morphogenesis, it will be necessary to define genes which act downstream of these fate regulators. Finally, because epidermal cell differentiation can be affected by some environmental factors and by hormones, including phosphorous- or iron-deficient conditions (Muller and Schmidt 2004, Raghothama 1999, Schikora and Schmidt 2001) and ethylene or auxin production (Cao et al. 1999, Dolan et al. 1994, Knox et al. 2003, Masucci and Schiefelbein 1996, Wilson et al. 1990), studies aimed at this aspect will be needed to define the molecular basis for environmental modification of the patterning program.

Another interesting feature of this system is that many of the genes known to regulate root epidermal cell fate also play a role in the patterning of other epidermal cell types in the *Arabidopsis* plant (Larkin et al. 2003). For example, the *GL3/EGL3* (Zhang et al. 2003), *TTG1* (Koornneef et al. 1982), and *GL2* (Rerie et al. 1994) genes are all required to specify trichomes. In this regard, it is notable that the *WER* gene does not have a role in trichome specification. Instead, a *WER* homolog, *GLABROUS1* (*GL1*) is required for trichome development (Oppenheimer et al. 1991). The fact that *GL1* is expressed in the above-ground tissues while *WER* is expressed in the under-ground tissues suggests a paralogous function of these two genes in epidermal cell differentiation at different places. Indeed, it has been shown that *WER* and *GL1* gene products can substitute for each other if their expression is controlled by the promoter of the other (Lee and Schiefelbein 2001). Given the likely involvement of the root patterning network in multiple developmental contexts, it seems that this system should provide attractive opportunities for understanding the evolution of developmental mechanisms in plants.

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