

# Cell Pattern in the Arabidopsis Root Epidermis Determined by Lateral Inhibition with Feedback

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**In the root epidermis of Arabidopsis, hair and nonhair cell types are specified in a distinct position-dependent pattern. Here, we show that transcriptional feedback loops between the *WEREWOLF* (*WER*), *CAPRICE* (*CPC*), and *GLABRA2* (*GL2*) genes help to establish this pattern. Positional cues bias the expression of the *WER* MYB gene, leading to the induction of *CPC* and *GL2* in cells located in a particular position (N) and adoption of the nonhair fate. The truncated MYB encoded by *CPC* mediates a lateral inhibition mechanism to negatively regulate *WER*, *GL2*, and its own gene in the alternative position (H) to induce the hair fate. These results provide a molecular genetic framework for understanding the determination of a cell-type pattern in plants.**

## INTRODUCTION

The appropriate specification and patterning of distinct cell types is essential for the proper development of multicellular organisms. In plants, a simple case of cell patterning occurs during development of the root epidermis in Brassicaceae species (e.g., Arabidopsis), in which the distribution of the root hair and nonhair cell types is determined by a position-dependent mechanism (Cormack, 1935; Bunning, 1951; Berger et al., 1998a). Epidermal cells in contact with two underlying cortical cells (the H position) adopt the root hair cell fate, whereas cells located outside a single cortical cell (the N position) adopt the nonhair fate. This pattern implies that positional information is responsible for guiding the specification of the epidermal cell types.

In Arabidopsis, several genes have been implicated in the establishment of the root epidermal pattern, including *GLABRA2* (*GL2*), *WEREWOLF* (*WER*), and *CAPRICE* (*CPC*). The *GL2* gene encodes a homeodomain transcription factor protein (Rerie et al., 1994) that is required to specify the nonhair cell type (DiCristina et al., 1996; Masucci et al., 1996). Accordingly, *GL2* is expressed preferentially in the N cell position during epidermis development, beginning in the early embryo (Masucci et al., 1996; Lin and Schiefelbein, 2001). The *WER* gene encodes a MYB transcription factor required for the specification of the nonhair cell type, and *WER* expression occurs preferentially in the developing epidermal

cells in the N position of the root (Lee and Schiefelbein, 1999). Furthermore, *WER* is a positive regulator of *GL2* expression (Lee and Schiefelbein, 1999). The *CPC* gene encodes a small MYB protein without a putative transcriptional activation domain that is required for hair cell specification (Wada et al., 1997), although recent findings show that *CPC* is expressed preferentially in the N cell position (T. Wada and K. Okada, personal communication).

The opposing effects of the *WER* and *CPC* genes on root epidermal cell fate have been proposed to be responsible for the establishment of the cell-type pattern, perhaps as a result of competition between these MYB proteins for access to partner proteins or promoter targets (Lee and Schiefelbein, 1999). To further understand the roles of these genes in root epidermal patterning, we wished to examine their possible regulatory interactions to determine whether negative transcriptional regulation may contribute to cell patterning. Here, we report that transcriptional feedback loops exist between the *WER*, *CPC*, and *GL2* genes and that they are used as part of a lateral inhibition pathway to define the cell-type pattern in the root epidermis.

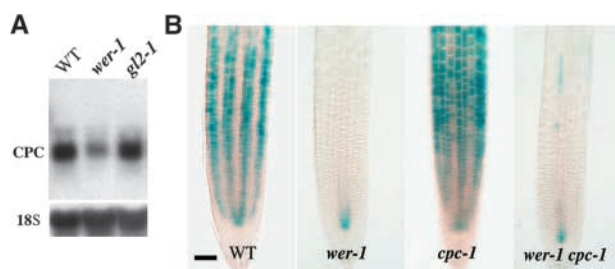
## RESULTS

### *WER* Is a Positive Regulator of the *CPC* Gene

To define the possible regulatory interactions between *WER*, *GL2*, and *CPC*, we first examined whether *WER* regulates *CPC* gene expression. Using RNA gel blot analysis, we found that root tips of the *wer-1* mutant accumulate significantly less *CPC* RNA than do wild-type roots (Figure 1A).

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**Figure 1.** *CPC* Gene Expression Is Regulated by *WER* and *CPC*.

**(A)** RNA gel blot hybridization analysis using a *CPC*-specific gene probe. RNA was isolated from the root tips of seedlings from the genotypes indicated. The same blot was rehybridized with an 18S rRNA probe as a loading control.

**(B)** Expression of the *CPC::GUS* reporter in the developing root epidermis of 4-day-old seedlings. This reporter also is expressed in the developing stele near the root tip, but this expression is not associated with the role of *CPC* in epidermal development (M.M. Lee and J. Schiefelbein, unpublished observations).

WT, wild type. Bar in **(B)** = 50  $\mu$ m.

In addition, we used a *CPC:: $\beta$ -glucuronidase* (*GUS*) reporter fusion that displays an N cell-specific expression pattern and has been found, in unpublished work, to correspond to the pattern of *CPC* RNA accumulation (T. Wada and K. Okada, personal communication). This *CPC::GUS* construct was introduced into the *wer-1* mutant by crossing, and we discovered that the N cell-specific expression of *CPC* was diminished greatly in this background (Figure 1B). Rare *GUS*-expressing epidermal cells in the *wer-1* *CPC::GUS* roots were found in both the N and H cell positions (data not shown), indicating that the *wer-1* mutation abolishes the position-dependent expression of *CPC*. Interestingly, these effects of *wer-1* on *CPC* expression are analogous to the effects of *wer-1* on *GL2* expression (Figures 2A and 2B) (Lee and Schiefelbein, 1999). These results show that despite the known role of *WER* in defining the nonhair cell fate (Lee and Schiefelbein, 1999), *WER* also is a positive regulator of the hair fate-promoting gene *CPC*.

There is abundant root hair production in the epidermis of the *wer-1* mutant, which lacks *CPC* expression (Table 1, Figure 1), and in the *wer-1 cpc-1* double mutant, which lacks a functional *CPC* gene (Table 1) (Lee and Schiefelbein, 1999). These results suggest that *CPC* is not required for epidermal cells to be specified as root hair cells. Furthermore, a comparison of cell specification in wild-type, *cpc-1*, and *wer-1 cpc-1* lines shows that the production of nonhair cells in the H position of *cpc-1* is mediated by *WER* (Table 1). This finding suggests that in wild-type roots, *CPC* promotes the hair cell fate by preventing *WER*-directed nonhair cell specification in the H position. Given the preferential expression of *WER* in N cells (Lee and Schiefelbein, 1999) and

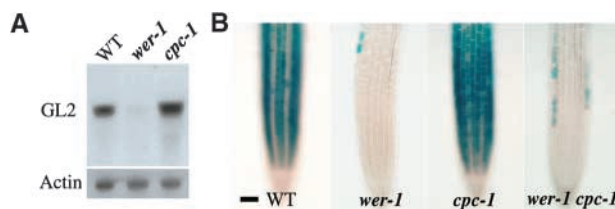
the positive regulation of *CPC* by *WER* (Figure 1), the simplest interpretation is that *CPC* mediates a lateral inhibition pathway that originates in the N cells and prevents neighboring cells in the H position from adopting the nonhair cell fate.

### ***CPC* Is a Negative Regulator of the *WER*, *GL2*, and *CPC* Genes**

If the hypothesis described above is correct, *CPC* might be expected to inhibit the expression of *GL2* and/or *WER* in the H cell position. To test this possibility, we first examined the effect of the *cpc-1* mutation on *GL2* RNA accumulation and reporter gene expression. An increased amount of *GL2* RNA was detected in root tips of the *cpc-1* mutant (Figure 2A), and ectopic *GUS* expression in the H cell position was observed in *cpc-1* *GL2::GUS* roots (Figure 2B). We also generated a *wer-1 cpc-1* *GL2::GUS* line and found that the ectopic *GL2::GUS* expression is *WER* dependent (Figure 2B). Furthermore, by examining mature epidermal cells, we found that the infrequent non-*GUS*-expressing cells in the *cpc-1* *GL2::GUS* line correspond to root hair cells (M.M. Lee and J. Schiefelbein, unpublished observations). These results show that *CPC*-dependent root hair formation is linked to the repression of *GL2* transcription in the H cell position.

In a similar way, we examined the effect of the *cpc-1* mutation on *WER* gene expression. We found that the *cpc-1* mutant had an increased amount of *WER* RNA in the root (Figure 3A) and significant ectopic *WER::green fluorescent protein* (*GFP*) reporter expression in the H cell position during root epidermis development (Figure 3B). Thus, *CPC* acts as a negative regulator of both *GL2* and *WER* expression in the H cell position.

We next considered the possibility that the *CPC*-depen-



**Figure 2.** *GL2* Gene Expression Is Regulated by *WER* and *CPC*.

**(A)** RNA gel blot hybridization analysis using a *GL2*-specific gene probe. RNA was isolated from root tips from the genotypes indicated and subjected to RNA gel blot analysis. The same blot was rehybridized with an actin probe as a loading control.

**(B)** Expression of the *GL2::GUS* reporter in the developing root epidermis of 4-day-old seedlings. The *wer-1* and *wer-1 cpc-1* plants were stained overnight, whereas the other lines were stained for 3 hr. WT, wild type. Bar in **(B)** = 50  $\mu$ m.

**Table 1.** Specification of Cell Types in the Root Epidermis

Genotype	Hair Cells in Epidermis (%)	H Cell Position		N Cell Position	
		Hair Cells (%)	Nonhair Cells (%)	Hair Cells (%)	Nonhair Cells (%)
Wild type (Columbia)	41.3 ± 6.6	93.8 ± 3.4	6.2 ± 3.4	2.2 ± 3.8	97.8 ± 3.8
<i>wer-1</i>	93.2 ± 5.0	95.8 ± 7.2	4.2 ± 7.2	91.3 ± 9.2	8.7 ± 9.2
<i>cpc-1</i>	8.7 ± 3.9	18.9 ± 5.1	81.1 ± 5.1	0 ± 0	100 ± 0
<i>wer-1 cpc-1</i>	83.6 ± 6.1	94.0 ± 5.3	6.0 ± 5.3	81.3 ± 6.4	18.7 ± 6.4
35S:: <i>WER</i>	42.5 ± 5.0	92.9 ± 3.4	7.1 ± 3.4	1.6 ± 3.3	98.4 ± 3.3
35S:: <i>WER wer-1</i>	59.8 ± 5.5	78.6 ± 10.2	21.4 ± 10.2	37.4 ± 5.1	62.6 ± 5.1
35S:: <i>WER wer-1 cpc-1</i>	41.2 ± 6.4	61.0 ± 8.8	39.0 ± 8.8	37.2 ± 10.5	62.8 ± 10.5
35S:: <i>CPC</i>	86.5 ± 6.3	94.4 ± 3.8	5.6 ± 3.8	82.2 ± 3.8	17.8 ± 3.8
35S:: <i>CPC cpc-1</i>	91.0 ± 5.9	98.3 ± 1.9	1.7 ± 1.9	83.0 ± 6.7	17.0 ± 6.7
35S:: <i>CPC wer-1</i>	87.5 ± 5.1	97.3 ± 0.8	2.7 ± 0.8	91.3 ± 5.8	8.7 ± 5.8

At least 10 5-day-old seedlings were examined for each strain. Values represent means ± SD. In all strains, ~40% of epidermal cells were in the H position.

dent lateral inhibition mechanism also might reduce the capacity of H cells to produce CPC. To test this possibility, we examined the expression of the *CPC* gene in the *cpc-1* mutant background. We found that *CPC::GUS* reporter expression was increased significantly in the H cell position of *cpc-1* relative to that of the wild type (Figure 1B). Furthermore, we generated a *cpc-1 wer-1 CPC::GUS* line and found that the ectopic *CPC* expression in the *cpc-1* background is *WER* dependent (Figure 1B). Together with the results described above, this shows that *CPC* participates in feedback loops to negatively regulate the expression of its own gene and the *WER* gene in the H cells.

### GL2 Does Not Regulate *WER* or *CPC* Expression

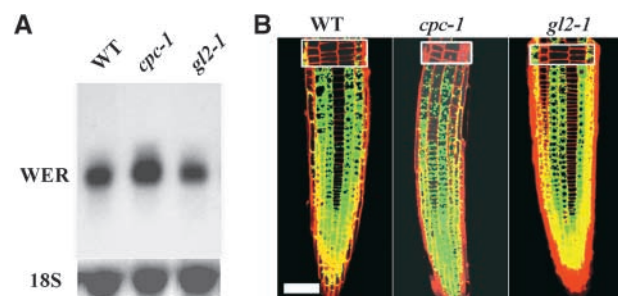
We also tested the possibility that the homeodomain protein GL2 might participate in the *WER*-*CPC* feedback loops. However, we found no significant difference in *WER* RNA accumulation and *WER::GFP* expression in the *gl2-1* mutant compared with that of the wild type (Figures 3A and 3B). Likewise, a normal level of *CPC* RNA and a normal pattern of *CPC::GUS* reporter expression were observed in *gl2-1* roots (Figure 1A) (M.M. Lee and J. Schiefelbein, unpublished data). Thus, GL2 is not required for the appropriate regulation of *WER* or *CPC*.

### Spatial Expression of *WER* Determines the Cell Pattern

Together, the results described above suggest that *WER* influences both the nonhair cell fate (by positively regulating *GL2*, which represses hair cell differentiation) and the hair cell fate (by positively regulating *CPC*, which mediates the lateral inhibition of the nonhair fate and thereby promotes hair cell differentiation). Thus, *WER* may be a critical director

of the epidermal cell pattern, as illustrated in Figure 4. To test this possibility, we examined the effect of expressing the *WER* gene throughout the epidermis using the 35S promoter of *Cauliflower mosaic virus* (35S). We employed two lines, 35S::*WER* and 35S::*WER wer-1*, that had been generated previously (Lee and Schiefelbein, 1999) and characterized them in greater detail.

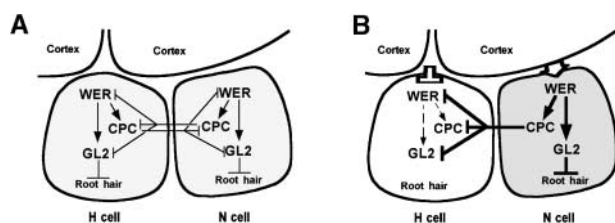
In plants harboring the 35S::*WER* construct, a normal pattern of epidermal cell types is formed (Table 1), although there is increased *GL2* expression in the H cell position

**Figure 3.** *WER* Gene Expression Is Regulated by *CPC*.

(A) RNA gel blot hybridization analysis using a *WER*-specific gene probe. RNA was isolated from root tips from the genotypes indicated and subjected to RNA gel blot analysis. The same blot was re-hybridized with an 18S rRNA probe.

(B) Expression of the *WER::GFP* reporter in the developing root epidermis of 4-day-old seedlings analyzed by confocal fluorescence microscopy. Propidium iodide dye (red signal) was included to visualize cell boundaries. The insets are focused on the cortical cell layer and therefore indicate the positions of the epidermal cell files.

WT, wild type. Bar in (B) = 50  $\mu$ m.



**Figure 4.** Model Showing the Regulatory Interactions between *WER*, *CPC*, and *GL2* That Define Cell Pattern in the Arabidopsis Epidermis.

**(A)** Interactions that occur in cells with equivalent developmental potentials. In the absence of positional cues, all epidermal cells express *WER*, *CPC*, and *GL2* and use *CPC* to inhibit their neighbors from expressing these genes.

**(B)** Positional cues generate a bias in *WER* expression as a result of induction in N cells and/or inhibition in H cells (open symbols). Feedback loops enhance this bias to generate a high level (thick lines) of *WER*, *CPC*, and *GL2* expression (and the nonhair cell fate) in N cells and a low level (dotted lines) of *WER*, *CPC*, and *GL2* expression (and the hair cell fate) in H cells.

(Figure 5A), as reported previously (Lee and Schiefelbein, 1999). The lack of a major effect of this construct may be attributable to the influence of the endogenous *WER* gene, which the *35S::WER* construct cannot overcome. Therefore, we examined the effect of *35S::WER* in the *wer-1* mutant background. Rather than generating a single-cell fate and a similar level of *GL2* expression throughout the epidermis, *35S::WER wer-1* roots produced a nonuniform distribution of epidermal cell types (Table 1) and *GL2::GUS* expression (Figure 5A). In particular, we found that both N and H cells were able to adopt the nonhair or hair fate (Table 1), and a similar frequency of cells at each position were able to establish *GL2* expression (Table 2). Thus, in the absence of an endogenous *WER* gene, the expression of *WER* throughout the epidermis generates a pattern of cell fates that is not determined strictly by cell position. This finding indicates that *WER* gene regulation is critical for proper interpretation of the positional cues that define the normal cell pattern.

The novel cell-type pattern in the *35S::WER wer-1* line may arise because stochastic fluctuations cause some developing epidermal cells to accumulate slightly more *CPC* than do their neighbors. Subsequently, as a result of the *CPC*-dependent feedback mechanism (Figure 4), these cells may establish a relatively high level of *CPC* and *GL2* expression and adopt the nonhair fate while inhibiting their neighbors from expressing *CPC* and *GL2* and forcing them to adopt the hair cell fate. If this interpretation is correct, then we would expect (1) that the pattern of *CPC* expression is altered (like that of *GL2*) in these lines and (2) that the novel cell-type pattern is dependent on *CPC* function.

To test the first of these expectations, we introduced the *CPC::GUS* reporter into the *35S::WER* and *35S::WER wer-1* lines by crossing. The *CPC::GUS* reporter was in fact ex-

pressed in some root epidermal cells at each position in the *35S::WER wer-1* background, whereas *35S::WER CPC::GUS* plants possessed a relatively normal pattern of *CPC* expression (Figure 5B). To directly test the possibility that *GL2* and *CPC* are expressed in the same set of epidermal cells, we used an available *GL2::GFP* reporter fusion (Lin and Schiefelbein, 2001) to generate *35S::WER wer-1 GL2::GFP CPC::GUS* plants. By analyzing both GFP and GUS expression in the same plants, we determined that *GL2* and *CPC* are expressed coordinately in a subset of root epidermal cells in this line (Figure 5C).

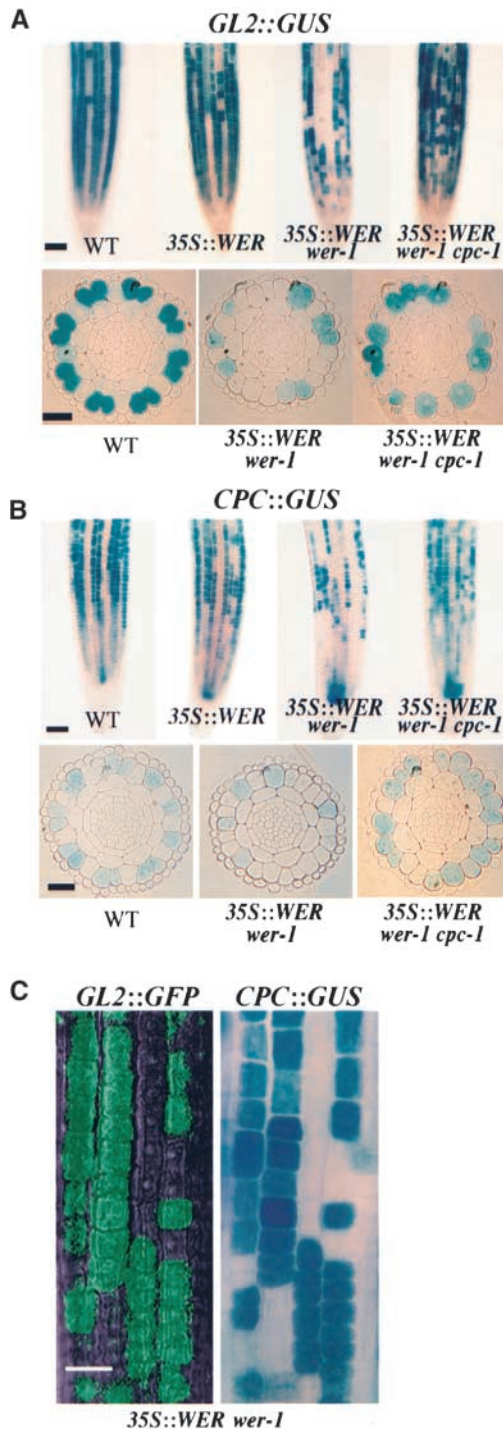
To test the second expectation, we introduced the *cpc-1* mutation into the *35S::WER wer-1* mutant by crossing and analyzed its cell-type pattern. Indeed, the cell pattern produced by *35S::WER wer-1 cpc-1* plants was significantly different from that produced by *35S::WER wer-1* plants. A reduced number of root hair cells was formed (Table 1), and an increase in the frequency of *GL2::GUS*-expressing cells was observed (Figure 5A, Table 2). These results indicate that *CPC* is required, at least in part, for the novel *35S::WER wer-1* pattern.

### Effect of *CPC*-Dependent Lateral Inhibition

Although the model illustrated in Figure 4 shows *CPC* regulating all three genes (*GL2*, *WER*, and *CPC*) in the H position, an alternative is that *CPC* inhibits only the expression of *WER* and that *GL2* and *CPC* are affected indirectly through their dependence on *WER*. We were able to exclude this possibility by analyzing *35S::WER wer-1* and *35S::WER wer-1 cpc-1* plants. As described above, we observed *CPC*-dependent inhibition of nonhair cell fate and *GL2* expression in these lines. Furthermore, we introduced the *CPC::GUS* reporter into the *35S::WER wer-1 cpc-1* line and discovered an increased frequency of GUS-expressing cells relative to the *35S::WER wer-1 CPC::GUS* line (Figure 5B). Thus, *CPC*-dependent lateral inhibition is able to operate in the absence of a wild-type *WER* gene, which means that *CPC* does not control *GL2* and *CPC* expression in the H cell position merely by regulating *WER*.

To further define the role of *CPC*, we analyzed plants containing a *35S::CPC* construct (Wada et al., 1997). In both *35S::CPC* and *35S::CPC cpc-1* roots, nearly all epidermal cells adopted the hair cell fate (Table 1) and exhibited reduced *GL2* expression (Figure 6). This finding indicates that the expression of *CPC* alone throughout the epidermis is sufficient to inhibit cells in both the N and H positions from adopting the nonhair fate. Furthermore, the inability of the *35S::CPC* construct to generate a nonuniform pattern of cell types in the *cpc-1* background (as in the analogous *35S::WER wer-1* line) is consistent with Figure 4 and suggests that the *CPC* pathway does not regulate a secondary feedback loop. Finally, to determine whether a *WER*-dependent factor might contribute to the effect of *35S::CPC*, we generated and examined *35S::CPC wer-1 GL2::GUS* plants.





**Figure 5.** 35S::WER Alters the Epidermal Cell Pattern.

**(A)** Expression of the *GL2::GUS* reporter in the developing root epidermis of 4-day-old seedlings. The top row contains surface views, and the bottom row shows transverse sections from the meristematic region. Note that a single layer of lateral root cap cells surrounds the epidermis in the transverse sections. All lines were stained for the same period of time (3 hr).

These roots possessed an epidermal cell pattern (Table 1) and a *GL2* expression pattern (Figure 6) similar to that of 35S::CPC, implying that *WER* gene function is not required for the 35S::CPC effect.

## DISCUSSION

Lateral inhibition is a type of cell-to-cell interaction whereby a cell adopting a particular fate prevents its neighbors from developing in the same way (Wigglesworth, 1940). Lateral inhibition is employed widely during animal development (Goriely et al., 1991; Heitzler and Simpson, 1991; Sternberg, 1993), and it has been invoked on several occasions to explain the formation of cell patterns in plants (Barlow and Carr, 1984; Korn, 1993; Hulskamp et al., 1994; Szymanski et al., 2000). However, to date, no molecular component, or its regulation, has been defined in any lateral inhibition pathway in plants.

In this study, we identified two fundamental features of the patterning mechanism responsible for the position-dependent distribution of epidermal cell types in the Arabidopsis root. First, we showed that lateral inhibition is involved in the patterning process and that two MYB-related proteins, *WER* and *CPC*, are components of this pathway. We found that this lateral inhibition pathway acts to prevent cells in a specific location (the H position) from adopting the primary cell fate (the nonhair fate). Second, we discovered regulatory feedback loops between *WER* and *CPC* that are used to establish differential expression of the downstream transcription factor gene *GL2* and position-dependent cell fate. Specifically, we showed that *WER* is a positive regulator of *CPC* at the N position and that *CPC* is a negative regulator of the *WER*, *GL2*, and *CPC* genes at the H position (Figure 4). Together, these results provide a new framework for understanding the molecular determination of cell fate in plants.

This work highlights the important role of the *WER* gene in

**(B)** Expression of the *CPC::GUS* reporter in the developing root epidermis of 4-day-old seedlings. The top row contains surface views, and the bottom row shows transverse sections from the meristematic region.

**(C)** Coordinate expression of *GL2* and *CPC* in root epidermal cells. The accumulation of GFP and GUS is shown in a 5-day-old seedling root bearing the genotype 35S::WER *wer-1* *GL2::GFP* *CPC::GUS*. An image was first recorded using fluorescence microscopy (to detect *GL2::GFP* expression); then, GUS activity was assessed (to detect *CPC::GUS* expression).

WT, wild type. Bars in **(A)** and **(B)**, top rows = 50  $\mu$ m; bars in **(A)** and **(B)**, bottom rows = 25  $\mu$ m; bar in **(C)** = 20  $\mu$ m.

**Table 2.** Location of *GL2::GUS* Expression in the Developing Root Epidermis

Genotype	H Cell position		N Cell Position	
	Non-GUS-Expressing Cells (%)	GUS-Expressing Cells (%)	Non-GUS-Expressing Cells (%)	GUS-Expressing Cells (%)
Wild type Columbia <i>GL2::GUS</i>	85.3 ± 5.6	14.7 ± 5.6	0 ± 0	100 ± 0
35S:: <i>WER wer-1 GL2::GUS</i>	71.9 ± 6.8	28.1 ± 6.8	65.4 ± 7.7	34.6 ± 7.7
35S:: <i>WER wer-1 cpc-1 GL2::GUS</i>	56.2 ± 4.8	43.8 ± 4.8	47.6 ± 5.5	52.4 ± 5.5

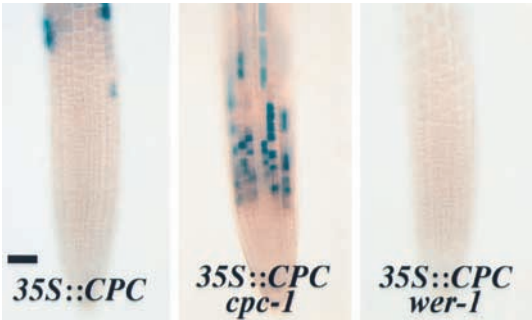
A series of transverse sections from the meristematic region of at least five 4-day-old roots was examined for each strain. Values represent means ± SD.

root epidermal patterning. *WER* contributes to the specification of both the nonhair cell fate, by inducing *GL2*, and the hair cell fate, by inducing *CPC*. Accordingly, proper spatial regulation of the *WER* gene is crucial for the normal cell-type pattern to arise. We have shown that expressing the *WER* gene throughout the epidermis, using the 35S::*WER wer-1* line, leads to an abnormal pattern of *GL2* gene expression (Figure 5, Table 2) and epidermal cell types (Table 1) that is not linked to cell position. Thus, *WER* is likely to be a target of positional cues, which may direct the cell-type pattern by inducing *WER* expression in the N position and/or inhibiting *WER* expression in the H position (Figure 4). Furthermore, it is likely that differential *WER* expression is initiated early in Arabidopsis embryogenesis, because the epidermal pattern is known to be established by the heart stage of embryogenesis (Lin and Schiefelbein, 2001).

Although we have found that *CPC* is an essential component of an N cell-specific lateral inhibition pathway, the precise mechanism of *CPC* action is unclear. It is conceivable that *CPC* moves from N cells into H cells via plasmodesmata and directly affects the transcription of the *GL2*, *CPC*, and *WER* genes. The cell-to-cell movement of a variety of

plant proteins has been documented previously (Lucas et al., 1995; Perbal et al., 1996; Sessions et al., 2000; Nakajima et al., 2001), and developing epidermal cells are known to be coupled symplastically (Duckett et al., 1994). Furthermore, this possibility is consistent with our previous model for *CPC* and *WER* action, which proposes that the relative abundance of these proteins in the N and H positions is critical for establishing the cell pattern, because the truncated MYB protein encoded by *CPC* can interfere with *WER*-dependent transcriptional activation (Lee and Schiefelbein, 1999). An alternative possibility is that *CPC* indirectly influences transcription in the H cells by activating an as yet unidentified cell-to-cell signaling pathway in the N cells.

These results show that reciprocal regulation of the *WER* and *CPC* MYB genes, biased by positional cues, generates the position-dependent pattern of cell types in the Arabidopsis root epidermis (Figure 4). Numerous examples of cell-type patterns influenced by positional cues have been described in plants (Barlow and Carr, 1984; Berger et al., 1998b; Hung et al., 1998; Geisler et al., 2000), and these may be governed by a mechanism similar to the one described here. Furthermore, because we have shown that this same machinery can generate a position-independent cell pattern if its linkage to positional cues is severed (e.g., the pattern in the 35S::*WER wer-1* line), a common mechanism involving lateral inhibition with feedback may be employed to produce both position-dependent and position-independent patterns of cell types in plants. For example, the nonrandom distribution of trichome cells in Arabidopsis may be generated by similar patterning elements, particularly given that some of the same components described here are used in trichome specification, such as the *GL2* gene (Koomneef, 1981; Rerie et al., 1994) and a MYB gene (*GLABROUS1*; Oppenheimer et al., 1991) that is functionally equivalent to *WER* (Lee and Schiefelbein, 2001). Finally, a conceptually similar mechanism of lateral inhibition with feedback operates to pattern some animal cell types (e.g., the Notch signaling system; Artavanis-Tsakonas et al., 1999), which implies that despite their independent evolutionary origin, multicellular plants and animals may have adopted fundamentally similar strategies to generate defined patterns of cell fates.



**Figure 6.** 35S::*CPC* Alters the Epidermal Cell Pattern.

Expression of the *GL2::GUS* reporter in the developing root epidermis of 4-day-old seedlings. 35S::*CPC* and 35S::*CPC wer-1* plants were stained overnight, whereas the 35S::*CPC cpc-1* line was stained for 3 hr. Bar = 50 μm.

## METHODS

### Plant Materials and Growth Conditions

The mutant alleles of *Arabidopsis thaliana* used in this study were isolated previously: *cpc-1* (Wada et al., 1997), *gl2-1* (Koornneef, 1981), and *wer-1* (Masucci et al., 1996), and these are likely to represent loss-of-function alleles. The *GL2::β-glucuronidase (GUS)*, *WER::green fluorescent protein (GFP)*, *35S::CPC*, and *35S::WER* lines have been reported previously (Masucci et al., 1996; Wada et al., 1997; Lee and Schiefelbein, 1999). The *CPC::GUS* line was a kind gift from T. Wada and K. Okada (Kyoto University, Kyoto, Japan) and includes a 1.2-kb *CPC* promoter region sufficient to complement the *cpc-1* mutation (Wada et al., 1997). The *GL2::GFP* line has been described (Lin and Schiefelbein, 2001). Lines with multiple mutations and/or various transgene combinations were generated by crossing, and their genotypes were confirmed by backcrossing to single mutants, reporter gene expression, and/or polymerase chain reaction-based tests. Seedlings were grown in vertically oriented Petri dishes on agarose-solidified medium containing mineral nutrients as described previously (Schiefelbein and Somerville, 1990).

### Microscopy

The pattern of epidermal cell types was determined by staining 5-day-old roots with propidium iodide, followed by examination with a fluorescence microscope to determine the cell type and relative locations of epidermal cells. The proportion of hair and nonhair cell types in the root epidermis was determined by examining at least 10 5-day-old seedlings from each strain. An epidermal cell was scored as a root hair cell if any protrusion was visible, regardless of its length.

The *GUS* constructs were introduced into the indicated mutant and transgene lines by genetic crosses, and histochemical analysis of *GUS* activity was performed essentially as described (Masucci et al., 1996). The spatial expression of *GL2::GUS* was assessed in a series of transverse sections from the meristematic region of 4-day-old roots that were fixed and embedded in JB-4 resin after *GUS* staining, as described (Masucci et al., 1996). The proportion of cells expressing *GL2::GUS* was determined by examining sections from at least five 4-day-old seedlings from each strain.

The *WER::GFP* and *GL2::GFP* constructs were crossed into the various mutant and transgenic lines. GFP accumulation was visualized in 5-day-old roots after propidium iodide staining (10 μg/mL) using a Zeiss (Jena, Germany) LSM510 confocal microscope.

To assess the expression of both GFP and *GUS* in the same roots, 5-day-old seedlings were analyzed by fluorescence microscopy with a supporting cover slip and then subjected immediately to *GUS* assay by infusing 5-bromo-4-chloro-3-indolyl-β-glucuronic acid solution under the cover slip and staining for 3 hr under previously published conditions (Masucci et al., 1996).

### Molecular Biology Methods

RNA was isolated from root tips from the genotypes indicated in the figures and the text and subjected to RNA gel blot analysis as described previously (Masucci et al., 1996). The gene-specific probes used here have been described for *GL2* (Masucci et al., 1996) and *WER* (Lee and Schiefelbein, 1999). A 300-bp fragment including

most of the *CPC* gene was found to be *CPC* specific (data not shown) and was used in the *CPC* RNA gel blot experiments.

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# Cell Pattern in the Arabidopsis Root Epidermis Determined by Lateral Inhibition with Feedback

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