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# HOW DO CELLS KNOW WHAT THEY WANT TO BE WHEN THEY GROW UP? Lessons from Epidermal Patterning in Arabidopsis

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■ **Abstract** Because the plant epidermis is readily accessible and consists of few cell types on most organs, the epidermis has become a well-studied model for cell differentiation and cell patterning in plants. Recent advances in our understanding of the development of three epidermal cell types, trichomes, root hairs, and stomata, allow a comparison of the underlying patterning mechanisms. In Arabidopsis, trichome development and root epidermal patterning use a common mechanism involving closely related cell fate transcription factors and a similar lateral inhibition signaling pathway. Yet the resulting patterns differ substantially, primarily due to the influence of a prepattern derived from subepidermal cortical cells in root epidermal patterning. Stomatal patterning uses a contrasting mechanism based primarily on control of the orientation of cell divisions that also involves an inhibitory signaling pathway. This review focuses on comparing and contrasting these patterning pathways to identify and illustrate general themes that may be broadly applicable to other systems. Where these pathways occur in the same tissue, interaction and competition between these pathways is also discussed.

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## INTRODUCTION

Plants, like other multicellular eukaryotes, develop from a single-celled zygote that ultimately gives rise to the many specialized cell types of the adult organism. Cell patterning is when cells are guided to their appropriate differentiated fate at the correct time and place in the developing organism. Although patterning mechanisms are very heterogeneous in detail, some common themes emerge repeatedly in a wide variety of cell types and organisms. First, commitment to a specific cell fate is typically regulated by the combinatorial interaction of several transcription factors. Second, the timing and location of differentiation is often regulated by both long-range hormonal signals and local cell-cell signaling. Third, differentiating cells often either stop cycling mitotically or enter a modified cell cycle.

The plant epidermis is an excellent tissue for studying cell patterning. In both shoots and roots, the epidermis consists of few cell types (20). The epidermis is also readily accessible to observation and developmental manipulation. In genetically well-studied plants such as *Arabidopsis thaliana*, numerous mutants affecting epidermal cell types have been isolated and studied. This review focuses on recent work that provides significant insight into the mechanisms involved in the patterning of three epidermal cell types in Arabidopsis: trichomes, root hairs, and stomatal guard cells. Work on other cell types and plant species is discussed where appropriate. Individually, these cell types have been the focus of numerous recent reviews

(17, 67, 82, 91). However, this review focuses on comparing and contrasting these patterning pathways in an attempt to identify and illustrate general themes that may be broadly applicable to other systems. Where these pathways occur in the same tissue, we also discuss the interaction and competition between them.

## THE EPIDERMIS

The migration of plants onto land in the Ordovician resulted in new selective pressures acting on the surface tissues of early land plants (78). The requirements for gas exchange were changed, and both dehydration and UV damage became severe threats. Soon, defenses were needed against new types of herbivores and pathogens, and new opportunities arose for the dispersal of spores and gametes. In response, a wide variety of epidermal specializations arose, including a waxy cuticle and many different specialized types of cells. These specialized cells include stomatal guard cells, root hairs, trichomes (shoot hairs), various secretory cells of glands and nectaries, the enlarged bulliform cells of monocot leaves, and ordinary epidermal pavement cells, among many others (20).

The epidermis of angiosperms has three different developmental origins. The epidermis of the embryonic organs, the cotyledons and the hypocotyl, originates directly from the divisions establishing the embryo tissues (20). After germination, the shoot's epidermis originates from the outer or L1 layer of the shoot apical meristem (20, 87), and the root's epidermis originates from the root apical meristem as well as from the meristems of lateral roots. At the root tips, the meristem and developing epidermis (protoderm) are covered by the root cap, which is also produced by the root meristem. The shoot and root meristems differ significantly in the way tissues derived from the meristem are produced, and lateral organs (leaves and lateral roots) are produced much differently in the shoot and root (20). As a consequence of these differences, the root protoderm typically has a more orderly relationship with the underlying cortical tissue than the leaf protoderm has with the underlying mesophyll. These differences have interesting consequences for the patterning of shoot and root epidermal cell types (see below).

## TRICHOMES

Trichomes (shoot epidermal hairs) are present on the aerial surfaces of most plants (trachaeophytes), ranging from ferns to angiosperms. The term trichome is derived from trichos, the Greek root meaning hair. Trichomes exist in a wide variety of morphologies, from single celled to multicellular, and include both glandular secretory hairs and nonglandular hairs (20, 95). A wide variety of functions have been ascribed to trichomes in various plants, including resisting insect herbivores, reducing transpiration, increasing freezing tolerance, and protecting plants from UV light (40). In addition to their roles in the plant, some types of trichomes have significant economic importance; for instance, cotton fibers are trichomes isolated from the epidermis of *Gossypium* ovules.

One of the most thoroughly studied plant cell differentiation pathways is the development of Arabidopsis trichomes (91). Ecological studies suggest that Arabidopsis trichomes protect plants from insect herbivores (60). These trichomes are single cells that protrude from the epidermis of leaves and stems. On leaves, these cells have an unusual branched shape resulting from a dramatic program of cellular morphogenesis. On stems, trichomes are generally unbranched. The single nucleus of a wild-type trichome continues to replicate its genomic DNA during differentiation, reaching average nuclear DNA levels of 20C–32C (34, 63), a process known as either endoreplication or endoreduplication (19). Endoreplication is a common variant of the cell cycle in which mitosis and cytokinesis are suppressed, but cycles of DNA replication continue. The ready accessibility of trichomes on the leaves of Arabidopsis has facilitated the isolation of mutations identifying genes involved in all stages of trichome development (56).

Trichome development begins near the distal tips of leaves when they are approximately 100-$\mu$m long, and proceeds basipetally (48). Trichomes are found adjacent to one another much less frequently than would be expected by chance, suggesting that an active mechanism exists to govern trichome spacing (34, 48). Epidermal cells that have entered the trichome pathway can first be identified by an increase in cell and nuclear volume. The nuclear enlargement in developing trichomes is correlated with the start of endoreplication (34, 104). The trichome then begins to expand out of the plane of the epidermis and branching ensues, followed by elaboration of the thickened secondary cell wall of the mature trichome.

## Cell Fate Transcription Factors

Several genes that directly control trichome initiation and development have been identified. Null alleles of the *GLABRA1* (*GL1*) gene result in plants with no or very few trichomes. *GL1* encodes an R2-R3 MYB transcription factor with two repeats of the MYB DNA-binding domain (72), typical of a large family of plant MYBs (43). The GL1 protein is localized to the nucleus (89). The highest levels of *GL1* transcripts are found in early stages of developing trichomes, but low levels of *GL1* expression are found throughout the developing epidermis (46). The increase in *GL1* expression appears to precede detectable expansion of trichome precursor cells. This observation suggests that commitment to the trichome fate involves a positive feedback loop regulating *GL1* expression.

Null alleles of *TRANSPARENT TESTA GLABRA* (*TTG*) result in a hairless phenotype as well as reduced anthocyanin pigmentation, absence of seed coat mucilage, and an increase in the number of root hairs (22, 42). The various aspects of the pleiotropic *ttg* mutant result from a defect in a single biochemical function, rather than from multiple independent functional domains in the protein (47). *TTG* encodes a small protein with four or five WD repeats (103). WD repeats appear to function as protein interaction domains in a wide variety of processes, and no WD-repeat protein has either enzymatic activity or a DNA-binding domain (68).

A third gene that plays a central role in trichome initiation is *GLABRA3* (*GL3*). Loss-of-function *gl3* mutants produce a reduced number of trichomes that are smaller and have fewer branches than wild type (34). The nuclear DNA content

of *gl3* mutant trichomes is also reduced (34). The recent demonstration that *GL3* encodes a basic helix-loop-helix (bHLH) protein closely related to the maize *R* gene (74) clears up a lingering mystery. The maize *R* gene was previously shown to suppress the mutant defects of *ttg* when constitutively expressed in Arabidopsis, as well as cause ectopic production of trichomes, especially when coexpressed with *GL1*, but until the identification of *GL3*, no Arabidopsis gene encoding a bHLH protein had been implicated in trichome development (45, 54). Payne et al. (72) also shed light on why even apparent *gl3* null alleles produce some trichomes. They showed that the Arabidopsis genome contains a close homolog of *GL3* that may be functionally redundant with *GL3*.

Recent work suggests that the *GLABRA2* (*GL2*) gene may also play a role in trichome initiation (36, 71). Loss-of-function *gl2* mutants produce small trichomes with reduced branching and aberrant expansion, in addition to the pleiotropic phenotypes of extra root hairs and absence of seed coat mucilage (16, 79). *GL2* encodes a homeodomain-leucine zipper protein (16, 79) that is expressed at high levels in developing trichomes and is localized to the nucleus of developing trichomes (79, 89). Because altered cell expansion is the primary effect of *gl2* mutations on trichome development, *GL2* has been proposed to regulate polar cell expansion of developing trichomes (34, 79). Constitutive overexpression of *GL2* under control of the C*auliflower mosaic virus* 35S promoter appears to be lethal (71). However, viable transgenic lines exhibiting moderate overexpression of *GL2* were identified, and these plants produce increased numbers of trichomes, as well as increased numbers of trichomes adjacent to another trichome (trichome clusters), strongly suggesting a role for *GL2* in trichome initiation (71).

Several other genes have been implicated in the initiation of trichome development. The *REDUCED TRICHOME NUMBER* (*RTN*) locus was discovered as a quantitative trait locus (QTL) variant between the Columbia and Landsberg *erecta* ecotypes of *Arabidopsis* (48). This locus affects the number of trichomes produced on leaves by controlling the time during leaf development that the initiation of new trichomes ends. This study also described two other potential QTLs that may influence the trichome density on leaves (48). The recently described *TRANSPARENT TESTA-GLABRA2* (*TTG2*) gene, which encodes a WRKY transcription factor, may also play a role in trichome initiation (39). Finally, several genes regulate trichome development via local or hormonal signaling; the roles of these genes are described below in separate sections. Two other loci, *FIDDLEHEAD* (*FDL*) (113) and *INCREASED CHALCONE SYNTHASE EXPRESSION* (*ICX*) (37, 101) also affect the number of trichomes per leaf.

## Interactions Among Cell Fate Transcription Factors

Initial experiments in which *GL1* and the maize *R* gene were expressed constitutively from the *Cauliflower mosaic virus* 35S promoter suggested that *GL1* was likely to function at the same genetic step in trichome development as an Arabidopsis *R* homolog (44, 45). With the demonstration that *GL3* encodes a homolog of *R*, these interactions were confirmed using the endogenous Arabidopsis genes (74). Indeed, co-overexpression of *GL1* and *GL3* results in increased numbers of

trichomes and ectopic trichomes, and can bypass the need for *TTG* function. The latter result suggests that *TTG* is functionally upstream of *GL1* and *GL3*.

There is strong evidence that the products of these three genes interact based primarily on a careful yeast two-hybrid study (Figure 1*a*) (74). First, both GL1 and GL3 contain transcription activation domains, GL1 in its C terminus and GL3 in its N terminus. The GL1 activation domain may correspond to an essential region of 34 amino acids near the C terminus that is rich in acidic amino acids (21, 31). Second, GL3 can homodimerize via a C-terminal region containing the bHLH domain. Third, GL1 and GL3 interact strongly via the N-terminal MYB domain repeats of GL1, and at least the first 96 amino acids of GL3 (74). GL1 and the maize R protein also interact in vitro, although in these experiments the N terminus of GL1 alone was not sufficient for interaction with R (89). Interaction between GL1 and GL3 may explain the curious observation that overexpression of *GL1* inhibits trichome formation (45), if it is assumed that GL3 is limiting, and that GL1 that is not bound to GL3 inhibits transcription from downstream promoters. Consistent with this hypothesis, overexpression of both *GL1* and *GL3* together rescues the inhibitory effect of *GL1* overexpression (74).

Finally, Payne et al. (74) demonstrated that, in yeast, TTG interacts with the N-terminal region of GL3 distinct from that to which GL1 binds. TTG does not bind to GL1. Although genetic interactions between *gl1* and *ttg* alleles were initially interpreted as evidence that GL1 and TTG interact directly, these genetic interactions are also consistent with these two proteins being part of the same complex, but not in physical contact (47). Although TTG interacts with GL3, it is unclear whether TTG actually enters the nucleus. A closely related homolog from petunia, AN11, appears to be cytoplasmic (15), and *AN11* can rescue *ttg* mutations when expressed in Arabidopsis (74). At this point, while TTG may be part of the transcription complex with GL1 and GL3 (Figure 1*a*), it may also act solely in the cytoplasm to stabilize homo- or heterodimer formation, or be involved in activating the GL1/GL3 complex in some way, perhaps by stabilizing its interaction with a modifying enzyme such as a protein kinase.

The only candidate gene so far identified as a likely target regulated by GL1 and GL3 is *GL2* (Figure 1*a*). Overexpression of *GL1* together with the maize *R* gene results in ectopic expression of a *GL2* reporter construct throughout the plant, including tissues that normally do not express *GL2* (89), although it has not been determined whether this expression is independent of new protein synthesis, as predicted for a direct transcriptional target (102). This ectopic expression was dependent on a region of the *GL2* promoter required for *GL2* expression in developing trichomes. It is also unlikely that *GL2* is the only target of this transcription complex.

## Local Signaling in Trichome Development

As noted above, at the time of initiation, trichomes are spaced nonrandomly, with an average distance of three cells between developing trichomes (34, 48). This minimum-distance spacing pattern (111) cannot be explained by a specialized cell lineage based on a stereotyped pattern of cell divisions (48), suggesting that the

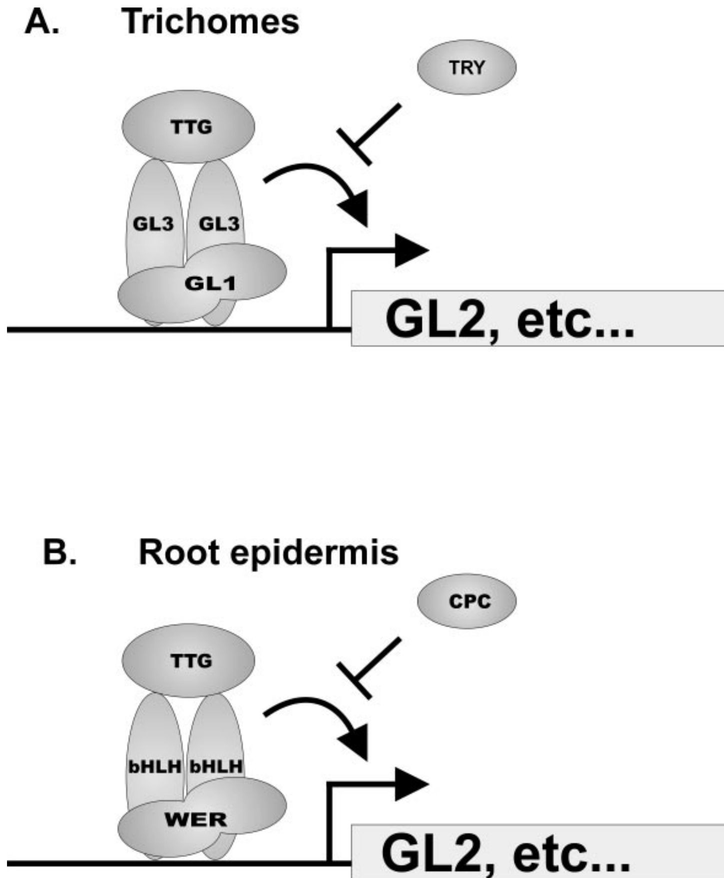


**Figure 1** Transcription factor interactions involved in trichome and root epidermal patterning. (*A*) Interactions during trichome development. Although *TRANSPARENT TESTA GLABRA* (*TTG*) is shown here as part of the transcription complex, it is unclear whether TTG enters the nucleus (see text). *CAPRICE* (CPC) omitted for clarity. (*B*) Proposed interactions during root epidermal patterning. WER and TTG are assumed to interact with a GL3-like bHLH protein. TRY has been omitted for clarity.

spacing pattern may be generated by an inhibitory signal, analogous to the lateral inhibition patterning mechanisms seen in other systems (2, 26, 88, 108, 109). The *TRIPTYCHON* (*TRY*) gene encodes a key component of the trichome lateral inhibition system. In *try* mutants, trichomes arise adjacent to other trichomes at a much higher frequency than in wild type (34), and *try* acts noncell autonomously, as predicted for a signaling molecule (84). Homozygous *try* mutants that also constitutively overexpress *GL1* and maize *R* have more trichomes than plants that just overexpress the two transcription factors (84). Thus *TRY* cannot be solely a repressor

of *GL1* transcription, but must inhibit trichome development by interfering with the ability of *GL1* and *GL3* to activate transcription of downstream genes (Figure 1*a*). Consistent with this possibility, *try* mutations can allow *GL1* overexpression to partially bypass the need for *TTG* for trichome initiation (84). Additionally, constitutive expression of TRY eliminates production of trichomes (81).

*TRY* encodes a single-repeat MYB protein with no apparent transcription activation domain (81). The nature of the predicted TRY protein is consistent with the hypothesis that it acts as a transcriptional repressor of targets of the *GL1*/*GL3* complex. The sequence of *TRY* is similar to that of the *CAPRICE* (*CPC*) gene (100), an important negative regulator of root epidermal patterning, as described below. However, *CPC* also plays at least a minor role in trichome patterning parallel to that of *TRY*. Constitutive expression of *CPC* in leaves eliminates trichome production (100), and *cpc* mutants have an increased number of trichomes, especially in combination with *try* (81). Both *TRY* and *CPC* are expressed most strongly in developing trichomes and not in other epidermal cells, even though their function is to inhibit neighboring cells from developing as trichomes (81). However, plant transcription factors can move between cells via plasmodesmata (55), and a reasonable model is that these proteins are synthesized in the developing trichome and move to neighboring cells to inhibit them from developing as trichomes.

Figure 2 shows a model for selecting trichome precursors in the observed minimum-distance spacing pattern based on a lateral inhibition mechanism. Lateral inhibition is a patterning mechanism whereby cells taking on a particular fate send an inhibitory signal to their neighbors, preventing them from adopting the same fate (2, 26, 88, 108, 109). Initially, all cells in the protoderm are competent to develop as trichomes (Figure 2*a*). These cells produce low levels of GL1 and presumably GL3 and TTG as well. Together, these proteins comprise a transcriptional activator that induces transcription of the downstream genes *GL2*, *TRY*, and *CPC*, among other genes (Figure 2*a*). The GL2 protein promotes the trichome cell fate, while TRY and CPC are transported to neighboring cells, where they inhibit expression of *GL1*, *GL2*, *TRY*, *CPC*, and perhaps *GL3* or *TTG*. At first, the cells are locked in mutual inhibition (Figure 2*a*). This state is metastable, and owing to stocastic variations in gene expression levels, some cells will have higher levels of the transcriptional acitvators (Figure 2*b*). These cells will then produce higher levels of TRY and CPC, and due to the feedback loops between cells, these cells will ultimately become committed to the trichome fate and succeed in inhibiting their neighbors from doing the same. Although direct evidence for the intercellular feedback loops postulated here has not yet been demonstrated for trichome development, such feedback loops have now been clearly demonstrated for the closely related mechanism described below that patterns the root epidermis (50).

## A Case of Transdetermination?

There is one result that does not seem to fit the proposed model based on lateral inhibition. The trichome clusters in *try cpc* double mutants are large, consisting of 20–30 adjacent trichomes (81). During the formation of these clusters, all cells

immediately adjacent to a developing trichome begin to expand from the epidermis and develop as trichomes. Their neighbors repeat this process for several rounds, resulting in a large trichome cluster. In wild type, cells adjacent to a trichome expand within the epidermal plane and become trichome accessory cells. Accessory cell differentiation is induced by a trichome late in its development. Perhaps the accessory cell program is a modification of the trichome program, and *TRY* and *CPC* act to shunt these cells into the accessory cell fate by repressing part of the trichome program. In this case, as is observed, a trichome developing on a *try cpc* mutant would induce trichome formation in its neighbors, rather than accessory cell development, and these trichomes would induce further trichomes, etc., ultimately producing a large cluster.

## Tissue-Specificity and Hormone Regulation of Trichome Production

Several genes are involved in restricting trichome expression to appropriate tissues. *TRY* has in determining the tissue-specificity of trichome formation. Overexpression of *GL1* in a *try* mutant produces large numbers of ectopic trichomes on cotyledons, the inflorescence, and floral organs (85, 92). In addition, subepidermal tissues in these plants produce trichomes (85, 92). *TRY* may also have a direct role in subepidermal tissues other than regulating trichome development, because leaves of *try* mutants have an increased number of mesodermal cells per unit of leaf area relative to wild type (92). *COTYLEDON TRICHOME1* (*COT1*) (90) and *CONSTITUTIVE PHOTOMORPHOGENESIS1* (*COP1*) are also potentially involved in regulating tissue and organ specificity of trichome formation (64). Finally, ectopic trichomes are formed on cotyledons of *leafy cotyledon* (*lec*) and *fusca3* (*fus3*) mutants due to apparent homeotic conversion of cotyledons into true leaves (62, 106).

In addition to these tissue-specific regulatory factors, trichome formation is positively regulated by long-day photoperiods and by gibberellins (GAs) (11). GA-deficient plants grown under short-day conditions lack trichomes, and application of exogenous GA restores trichome production (11). Overexpression of *GL1* and maize *R* overcomes this GA requirement, and GA regulated *GL1* expression (76). The production of trichomes on the abaxial surfaces of leaves is particularly sensitive to GA. The abaxial surfaces of the first two leaves of wild-type plants never form trichomes, and neither long days nor added GA can induce trichome formation on these surfaces (94), indicating that these abaxial tissues are not competent to respond to either signal.

## The Role of the Cell Cycle in Trichome Formation

Trichomes are the earliest differentiated cells to appear in the Arabidopsis epidermis, and at the time trichomes initiate development, the surrounding protodermal cells are rapidly dividing. Thus, the trichome cell fate decision is a choice between continued mitotic division and trichome development, rather than a choice between

one differentiated state and another. Approximately 11 rounds of cell division are required to generate the adaxial epidermis of the Arabidopsis first leaf (48). Trichome development begins during the fifth cell cycle and continues for about four cell cycles. During this time, the trichome nucleus increases its DNA content by endoreplication to approximately 20C–32C, or approximately four rounds of endoreplication (34, 63). The first recognizable event in trichome development is enlargement of the nucleus, indicative of the onset of endorepliciation (34, 104). These results suggest that one of the earliest steps in trichome development is the suppression of mitosis and a switch to an endoreplication cell cycle (endo cycle). These endo cycles might then continue to be coupled to general growth signals present in the developing leaf until cycling ceases with the terminal differentiation. Indeed, the primary purpose of endoreplication in developing trichomes may be that mitosis suppression coupled with continued cell growth allows production of a large cell suitable for modification as a defense against herbivores, rather than a direct need for an increased DNA content.

Several genes involved in the trichome cell fate decision are involved in endoreplication during trichome development. Trichomes on *gl3* mutants exhibit reduced endoreplication, as well as reduced size and branching (34). However, *try* mutant trichomes exhibit increased levels of endoreplication, as well as increased size and branching (34). The role of *GL1* is less clear; one group reported that *GL1* overexpression results in increased endoreplication in trichomes (85), whereas another group reported that *GL1* overexpression results in decreased endoreplication (92). In one author's laboratory, there was no convincing effect of *GL1* overexpression on endoreplication (J.D. Walker & J.C. Larkin, unpublished observations). Recently, mutations in the *SIAMESE* (*SIM*) gene were found to result in multicellular trichomes with a reduced DNA content per nucleus, indicating that this gene normally suppresses mitosis during the switch from mitotic cell cycles to endocycles (104). Taken together, these results suggest that among the targets of the trichome cell fate transcription factors are genes whose products promote the switch to endocycling. These downstream genes are likely to include both factors that promote S phase and factors that inhibit mitosis, as has been found for other cases of endoreplication (19, 29).

## ROOT EPIDERMAL PATTERNING

The root epidermis of most angiosperms is composed of only two cell types: cells bearing long cylindrical hairs (root-hair cells) and cells lacking these appendages (nonhair cells). Root hairs increase the surface area of the root and are likely to aid in nutrient acquisition, anchorage, and microbe interactions (33). The density of root hairs can vary dramatically in different plant species and under different environmental conditions.

The mechanisms that govern the patterning of hair and nonhair cells in angiosperms can be divided into three major categories (12). In one group of plants, every root epidermal cell appears capable of differentiating into a root-hair cell,

and the final distribution of hair and nonhair cells depends primarily on the environmental conditions. In a second group, which includes many monocots, a terminal asymmetric division generates a smaller daughter cell that becomes a root-hair cell and a larger daughter that differentiates to a nonhair cell. Some plants, including Arabidopsis and other members of the family Brassicaceae, generate a position-dependent pattern of hair/nonhair cells whereby epidermal cells located in a cleft between two underlying cortical cells develop as hair cells while epidermal cells outside a single cortical cell become mature nonhair cells (5, 8, 14, 18, 22). This latter mechanism has been studied in detail at the molecular level, and it is the focus of the remainder of this section.

The simple correlation between cell position and cell type differentiation implies that cell-cell communication events are crucial for establishing the position-dependent pattern of root epidermal cells. Furthermore, it is clear that this patterning mechanism must be operating at an early stage in epidermis development because immature epidermal cells destined to become root-hair cells (trichoblasts) can be distinguished cytologically in many ways from immature nonhair cells (atrichoblasts) prior to the formation of the hair itself (6, 18, 22, 57).

## Cell Fate Transcription Factors

Researchers have identified several mutants that possess a disrupted pattern of root epidermal cell types in Arabidopsis. Three mutants, *werewolf* (*wer*), *ttg*, and *gl2*, produce root hairs on essentially every root epidermal cell, which implies that the normal role of the *WER*, *TTG*, and *GL2* genes is either to promote nonhair cell differentiation or to repress root-hair cell differentiation (16, 22, 49, 57). These mutations differ in their specific effects on nonhair cell differentiation; the *wer* and *ttg* mutations alter all aspects of nonhair differentiation whereas the *gl2* mutations only affect the final cell morphology and do not affect the immature phenotypes (6, 22, 49, 57). This suggests that *WER* and *TTG* are required at an earlier developmental stage than *GL2*.

The *WER* gene encodes a MYB transcription factor of the R2-R3 type (49). It is preferentially expressed in developing nonhair epidermal cells, which are the same cells whose fate is mis-specified in the *wer* mutant. *WER* expression is first observed in the epidermal stem cells (initial cells) in the root core meristem and it persists through the meristematic and early elongation stages of development (49). Overexpression of the WER gene has little effect on the epidermal pattern (49), possibly due to the presence of efficient feedback loops that are not easily overcome (see below). Promoter swapping experiments have demonstrated that the WER MYB protein is functionally equivalent to the GL1 MYB protein, despite the fact that they share only 57% amino acid identity (49).

As already discussed, the *TTG* gene encodes a small protein with WD40 repeats (103). Although the protein sequence does not provide a clear mechanistic understanding of *TTG*'s role, it is known that *ttg* mutations can be functionally complemented by expression of the maize *R* cDNA (under the control of the strong *Cauliflower mosaic virus* 35S promoter) (22, 54). This may mean that *TTG*

activates an Arabidopsis homolog of the maize R, equivalent to GL3 in trichomes, to specify the nonhair cell fate.

The *GL2* gene encodes a homeodomain transcription factor protein (16, 79), and it is preferentially expressed in the differentiating nonhair epidermal cells within the meristematic and elongation regions of the root (57). Transcription of the *GL2* gene is influenced by the *WER* and *TTG* genes, with *wer* mutations effectively abolishing *GL2* promoter activity and *ttg* mutations causing a reduction in *GL2* promoter activity (35, 49, 52). Because the position-dependent *GL2* expression pattern is maintained in the *ttg* mutant, but not the *wer* mutant, it is likely that *WER* (but not *TTG*) mediates the positional *GL2* expression. Taken together, *WER*, *TTG*, and an R-like bHLH protein appear to begin to positively regulate the expression of *GL2* (and perhaps other unidentified genes) in a cell position-dependent manner to specify the nonhair cell type at an early stage in embryonic development (Figure 1*b*).

In contrast to *WER*, *TTG*, and *GL2*, two other previously mentioned Arabidopsis genes, *CPC* and *TRY*, help specify the root-hair cell fate (81, 100). Mutations in CPC reduce the hair frequency considerably, and, although *try* mutants have little effect, the *cpc try* double mutant lacks hair cells altogether (81). This implies that *CPC* and *TRY* are positive regulators of the hair cell fate. Interestingly, the *GL2* gene is ectopically expressed in *cpc* mutant roots, which suggests that *CPC* acts as a negative regulator of *GL2* transcription (50). As previously discussed, the *CPC* and *TRY* genes encode related proteins with a single MYB-like DNA binding domain but without a typical transcriptional activation domain (81, 100). Thus, CPC and TRY may promote the hair cell fate indirectly by interfering with WER-dependent activation of *GL2* transcription in the developing hair cells (Figure 1*b*).

## Local Signaling in Root Epidermal Patterning

Recently, a detailed study of the regulation of *WER*, *CPC*, and *GL2* uncovered an intercellular transcriptional feedback loop that is critical for patterning (50). The *WER* gene is required for positive regulation of *CPC* transcription in the developing nonhair cells, and CPC acts as a negative regulator of *WER*, *GL2*, and its own gene in the developing hair cells. Thus, CPC appears to act in a cell-nonautonomous fashion as part of a lateral inhibition pathway to indirectly promote the hair cell fate. In addition, Wada et al. (99a) found that while *CPC* is expressed primarily in the nonhair cells, a CPC::GUS fusion protein expressed from the *CPC* promoter is found equally in both hair and nonhair cells. These results indicate that the nonautonomous action of CPC may be mediated by movement of CPC protein from nonhair cells to hair cells.

These findings also suggest a simple model for the origin of the cell pattern (Figure 3). In this model, the specification of a particular cell type, hair or nonhair, relies on the relative activity of two competing transcription factors, WER and CPC. In heart-stage embryos, adjacent cell files are balanced in mutual inhibition (Figure *3a*). The pattern is established by positional cues from the underlying tissue that break the symmetry of the inhibition and cause greater *WER* transcription in the cells overlying a single cortical cell (Figure *3b*). This leads to a high level of *GL2* and *CPC* expression (and probably other genes) and

to the nonhair cell fate. In the alternate cell position, the CPC protein produced by the developing nonhair cell is proposed to accumulate by virtue of its cell-cell trafficking, and it represses *GL2*, *WER*, and *CPC* expression, permitting hair cell differentiation to proceed. Although TRY involvement has not been rigorously examined, it is likely that CPC and TRY act in a partially redundant fashion in the lateral inhibition mechanism.

The molecular basis of the positional cues from underlying cells that establish the root cell pattern is not understood. Several mutants exist that may help clarify this issue. These include the *roothairless* mutants *rhl1*, *rhl2*, and *rhl3*; and the *ectopic root hair* mutants *erh1*, *erh2/pom1*, and *erh3* (83); as well as the *tornado* mutants *trn1* and *trn2* (13). Each mutant alters the early differentiation features of the hair and nonhair cells, indicating that they affect cell specification rather than a later root-hair morphogenesis process.

## Prepattern and Hormone Signaling

Two different reporter gene fusions show position-dependent expression in the immature root and hypocotyl epidermis during Arabidopsis embryogenesis (5, 13, 52, 57). A *GL2* promoter::*GFP* reporter exhibits the earliest position-dependent expression, beginning at the early heart stage. Thus, it appears that positional information is provided during embryonic root development to establish a prepattern of gene activities that ultimately leads to appropriate postembryonic cell type differentiation.

The prepattern setup during embryogenesis may be modified postembryonically by hormone action. Results from numerous pharmacological and genetic experiments indicate that ethylene and auxin help promote root-hair cell differentiation in Arabidopsis. For example, aminoethoxyvinylglycine (AVG, an ethylene biosynthesis inhibitor) or $Ag^+$ (an inhibitor of ethylene perception) blocks root-hair formation (58, 93) and 1-amino-cyclopropane-1-carboxylic acid (ACC, an ethylene precursor) induces some ectopic root-hair cells in Arabidopsis (93). Although these hormones are involved in root-hair development, results from epistasis tests and reporter gene analyses show that the ethylene/auxin pathway does not regulate the *TTG*/*GL2* gene pathway (59). In addition, studies of the developmental timing of the hormone effects indicate that the ethylene and auxin pathways promote root-hair outgrowth after epidermal cell-type characteristics have developed (10, 59). Taken together, the results suggest that the initial patterning involving the transcription factor genes acts upstream of, or independently from, the ethylene/auxin pathway to define the pattern of cell types in the root epidermis.

## The Cell Cycle in Root Epidermal Patterning

Immature root-hair and nonhair cells in Arabidopsis resemble stem cells in that the fate-specifying process acts before division ceases, and each file produces multiple differentiated cells of the appropriate type. Well before morphological differentiation begins, the distinction between the two cell types is apparent in the cell division rate, with the developing hair cells displaying a higher rate of cell division than the

developing nonhair cells, as illustrated in Figure 3*b* (6). This may be due to nonhair cells exiting the cell cycle earlier or progressing more slowly through the cell cycle. However, the hair and nonhair cell types do not exhibit a significant difference in their extent of endoreplication (M.T. Hauser, personal communication).

## TRICHOMES AND ROOT HAIRS: ONE MECHANISM, TWO PATTERNS

As we have seen, trichome patterning and root epidermal patterning share a common mechanism based on lateral inhibition, and even share some of the same components. In spite of these parallels, the patterns resulting from lateral inhibition in these two systems are quite different. Trichomes obey a minimum-distance spacing pattern at the time of formation, but are otherwise randomly arranged. In contrast, root-hair and nonhair cells are arranged in orderly files. This difference is the result of differences in the role of cells underlying the epidermis in providing a prepattern. In the shoot, the only prepattern seems to be an inhibition of trichome formation in subepidermal tissues, maintained by expression of *TRY*, and lateral inhibition is the sole patterning mechanism within the epidermis (Figure 2). In the root epidermis, underlying cortical cells provide a prepattern that biases the winners of the mutual inhibition battle (Figure 3). On top of these local patterning mechanisms, there are various tissue-specific controls, as well as hormonal and environmental cues that can modify the basic pattern to suit the organism's immediate needs.

Although our current models are compelling, several aspects remain unknown or unclear. Theoretical models of lateral inhibition pathways predict that cell fate decisions triggered by the inhibitory signals acting between cells should be reinforced by positive feedback loops acting within the cells (61, 97). Although the expression patterns of *GL1* and *WER* hint at the existence of such positive feedback loops, the molecular components have not been defined. There is also no direct evidence that TRY and CPC are the actual molecules carrying the lateral inhibition signal. The nature of the signals from the root cortex that generate the root prepattern also are unknown.

## ADDITIONAL CELL TYPES AND PATHWAYS EMPLOYING MYB/bHLH PROTEINS: A CONSERVED REGULATORY MODULE?

One striking similarity of the trichome and root-hair patterning pathways is the involvement of a related set of MYB, bHLH, and WD-repeat proteins. Interestingly, other plant pathways are regulated by the combinatorial action of MYB/bHLH proteins, and in at least one case, a WD-repeat protein. Together, these suggest that a WD/MYB/bHLH protein cassette or complex has been widely employed in plant gene regulation.

A well-studied example of transcriptional control by MYB/bHLH proteins is flavonoid biosynthetic gene expression [reviewed in (65)]. In Arabidopsis, the *TT8* gene encodes a bHLH protein necessary for anthocyanin production in addition to the *TTG* WD-repeat protein (69), although no MYB partner has been defined. In *Petunia hybrida*, bHLH, MYB, and WD-repeat proteins required for regulation of anthocyanin production have all been identified (65, 86). Although there is no known cell-patterning aspect to Arabidopsis seed-coat development, the genes controlling this process are closely related to trichome and root-hair patterning genes, and include *GL2*, *TTG*, and an R2-R3 MYB gene, *MYB61* (75, 79, 107, 110). Other processes in which R2-R3 MYBs were implicated include conical cell formation on petals (3, 70, 98), formation of multicellular trichomes in *Nicotiana tobacum*, (28, 73), and the response of Arabidopsis plants to abscisic acid (1). Recently, an R2-R3 MYB gene was also implicated in a late stage of stomatal development, as described below (F. Sack, personal communication).

The Arabidopsis genome contains more than 90 genes encoding the plant-specific R2-R3 MYB protein family (43) and appears to be typical of most angiosperm genomes in this respect. An R2-R3 MYB gene was also found in a bryophyte (51). Most of the pathways controlled by the MYB/bHLH/WD-repeat module discussed here are epidermal, and all are specific to land plants. It is intriguing to speculate that diversification of the R2-R3 gene family may have been driven by the challenges faced by the first land plants.

## STOMATA

Stomata are pores on the leaf surface that regulate gas exchange between the plant and the atmosphere. They consist of two cells, called guard cells, that surround an opening in the epidermis and regulate its size (67). In many dicots, stomata appear to have no regular arrangement on the leaf surface, but stomata are almost never found next to one another on the mature leaf (9, 67, 80). This minimum-distance spacing pattern was initially proposed to be maintained by inhibitory signals (9). It was later pointed out that formation of dicot stomata typically involves a stereotypical cell-lineage pattern that might explain all or most of the pattern (41, 80). However, Geisler and coworkers showed that the major factor in generating the spacing pattern in Arabidopsis is control of the orientation of cell divisions in the stomatal lineage by inhibitory signals emanating from stomatal precursors (23).

The first event in stomatal differentiation in Arabidopsis is the asymmetric division of a protodermal cell, called a meristemoid mother cell (MMC). Asymmetric division of an MMC produces a smaller triangular meristemoid and a larger sister cell. Meristemoids can either differentiate immediately into an oval-shaped guard mother cell (GMC) or can continue to divide asymmetrically, producing another meristemoid at each division. The sister cell of a meristemoid may become an MMC and divide asymmetrically to produce a new meristemoid. These

meristemoids formed from cells that were already part of a stomatal lineage are called satellite meristemoids (44). In Arabidopsis, a majority of the stomata on the mature leaf appear to be formed from satellite meristemoids (23). There is no prohibition against MMCs forming adjacent to one another, or against meristermoids forming adjacent to one another (23). Furthermore, most stomatal complexes have at least one neighbor that is not clonally related (23). Thus, neither cell lineage nor inhibitory signals preventing MMC or meristemoid formation can be a significant patterning mechanism. Instead, the primary patterning mechanism is controlling the plane of MMC division such that a satellite meristemoid always forms on the side away from an adjacent guard cell, GMC, or meristemoid (23).

Recent advances in understanding the molecular mechanisms underlying stomatal patterning in Arabidopsis allow a fruitful comparison of this unique mechanism to the trichome and root epidermal patterning mechanisms described above. The focus will be on the way in which this comparison can illuminate issues involved in all patterning events. We will not attempt a complete review of stomatal development; recent reviews are available or in progress (44, 67).

## Initiation of Stomatal Development

Nothing is known about the regulatory mechanism by which the first MMCs become committed to the stomatal pathway, or about how this commitment is maintained through repeated cell divisions. No stomatal patterning mutant isolated to date plays a positive role in committing cells to the stomatal pattern. Only one gene identified as part of the patterning mechanism, *FOUR LIPS* (*FLP*), encodes a transcription factor (F. Sack, personal communication), and it seems to act far downstream in the process. Although stomata are likely essential for plant growth, even mutants with no stomata could probably germinate and be detected. Perhaps such genes are also involved in other pathways, and the mutants are lethal or otherwise unrecognizable. However, few alleles are available for the stomatal signaling mutants described below, and it is likely that many genes that could mutate to a stomatal patterning phenotype remain undetected.

## Local Signaling in Stomatal Development

Most current knowledge about stomatal patterning involves the signaling mechanism controlling the division plane of MMCs and the progression of cells from meristemoids to GMCs. Two genes, *TOO MANY MOUTHS* (*TMM*) and *STOMATAL DENSITY AND DISTRIBUTION1* (*SDD1*), were described that affect this aspect of stomatal patterning. A third gene, *FLP*, appears to restrict divisions of GMCs, and is discussed below.

Loss-of-function *tmm* mutants have a stomatal clustering phenotype (112). These clusters vary in shape and contain up to 26 adjacent guard cells. Loss of *TMM* function randomizes the orientation of asymmetric cell divisions that occur adjacent to preexisting stomata, or stomatal precursors (23). Positional cloning identified *TMM* as a leucine-rich repeat-containing receptor-like protein (LRR-RLP)

that is related to disease-resistance receptors and *CLAVATA2* (*CLV2*) (66). Cells expressing a *TMM* reporter construct showed the strongest expression in meristemoids, as well as expression in many cells that neighbor meristemoids, GMCs, or guard cells, but not all neighbor cells express this construct. Expression is highest in the smallest neighboring cells, which are most likely to divide asymmetrically. Thus, *TMM* appears is an early marker of commitment to the stomatal pathway.

Mutants with defects in the *SDD1* locus show increased stomatal density and a much higher frequency of adjacent stomata than wild-type (4). Almost half of the plant's stomata are in clusters and almost every epidermal cell is in contact with at least one stoma. However, individual clusters contain far fewer stomata than those of *tmm* plants. There is also a nearly twofold increase in the proportion of cells that adopt the stomatal fate compared to wild-type cells. Dental impression replicas of *sdd1* mutant epidermis made during leaf development show that all stomatal clusters come from satellite meristemoids. Thus, *SDD1* acts to properly orient the asymmetric divisions of satellite meristemoids, similar to the role of *TMM*. *SDD1* encodes a subtilisin-like serine protease (99). In situ hybridization and expression of a reporter gene construct show that *SDD1* is highly expressed in meristemoids and GMCs, but shows little, if any, expression in neighboring cells. In addition, the gene is weakly expressed in mesophyll cells of developing rosette leaves and in all layers of the SAM (99). Preliminary studies indicate that *SDD1* may be associated with the extracellular side of the plasma membrane. The *SDD1* promoter is negatively feedback regulated by *SDD1* in all cells except for meristemoids and GMCs (99). Overexpression of *SDD1* in wild-type plants caused a two- to threefold decrease in stomatal density. The *tmm* mutant phenotype was epistatic to this *SDD1* overexpression phenotype, consistent with the possibility that these two genes act in the same pathway (99). A simplified but plausible interpretation of the genetic relationships and function of *SDD* and *TMM* in stomatal development is shown in Figure 4.

The demonstration that TMM is similar to CLAVATA2, a protein involved in signaling during shoot apical meristem development (38), suggests a signaling pathway for stomatal patterning. CLAVATA2 interacts with CLAVATA1, a leucine-rich repeat receptor-like kinase (LRR-RLK). Together, they bind to the small peptide produced by the *CLAVATA3* gene and activate a signal transduction cascade in response to this ligand (96). *TMM* is expressed in meristemoids and neighbor cells that are potential MMCs, exactly the cells that need to receive a signal altering cell-division orientation. *SDD1* is expressed in meristemoids and GMCs, the same cells that must produce the signal. It is tempting to speculate that the SDD1 protein is involved in cleaving a protein that is necessary to produce the signal controlling orientation of neighbor cell divisions, and that TMM is part of the receptor for this signal. It is interesting to note that monocots have apparent *TMM* homologs (66). Although monocot stomata are typically arranged in rows, and are often assumed to be patterned by lineage, clonal analysis shows that rows of stomata in maize leaves are not clonal, consistent with the need for TMM-like signaling in maize (32).

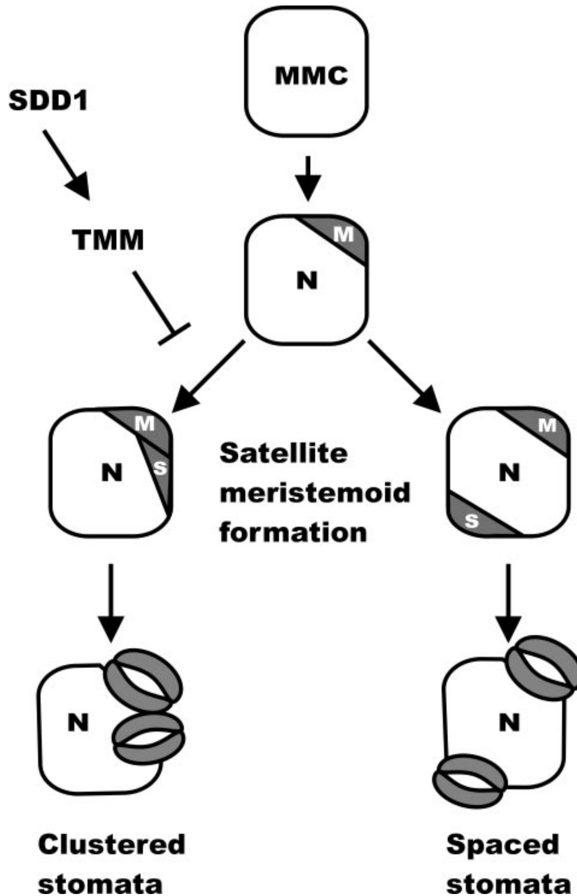


**Figure 4** Model of stomatal patterning. A meristemoid mother cell (MMC) produces a merstemoid (M) and a neighbor cell (N) by asymmetric division. SDD1 generates, and TMM receives an inhibitory signal that prevents neighbor cells from dividing to produce a satellite meristemoid (S) adjacent to M, preventing formation of clustered stomata. SDD1 and TMM are probably also involved in inhibiting cells from becoming MMCs or M cells from becoming GMCs if they are adjacent to a meristemoid.

## Tissue-Specific and Environmental Influences on Stomatal Patterning

Stomatal density varies widely on different epidermal surfaces of shoots of Arabidopsis and other plants. The phenotypes of *tmm* and *flp* mutants also vary widely among tissues (24). For example, a *tmm* mutation virtually eliminates stomata from the adaxial surface of sepals, but nearly doubles the number of stomata on the abaxial surface of sepals (24). How this can be explained by the simple signaling

model presented above (Figure 4) is not clear. In any case, this example shows that stomatal patterning responds to tissue-specific cues.

Stomatal density is also altered by environmental conditions, the best studied of which is $CO_2$ concentration. Growing wild-type plants in closed containers produces stomatal clusters similar to those found in *tmm* and *flp* mutants by growing them in closed containers, perhaps due to increased $CO_2$ or ethylene. These extra stomata come from neighbor cells from the lineage of preexisting stomata. Also, loss of HIGH CARBON DIOXIDE (HIC) function causes abnormal perception of $CO_2$ concentration, which results in increased stomatal density during times of elevated $CO_2$ (30). This is the inverse of the wild-type response to $CO_2$. HIC encodes a 3-ketoacyl coenzyme A synthase (KCS) that is involved in the synthesis of waxes, cutin, and other complex fat-containing compounds. *HIC* appears to be expressed only in guard cells (30).

## The Cell Cycle in Stomatal Patterning

The stomatal lineage resembles a stem cell population, committed to a particular cell fate but continuing to divide and amplify the cell population. In this respect, stomatal development resembles root-hair development, where cells become committed to the root-hair fate but continue to divide for some time. How this stem cell state is initiated, maintained, and terminated in plant cells is unknown. One gene that may play a role in terminating divisions in the stomatal cell lineage is *FLP*. Plants with *flp* mutations produce clusters of stomata (112), but unlike the clusters seen in *tmm* and *sdd1*, each cluster develops from a single GMC (44; F. Sack, personal communication). Thus, FLP does not act in stomatal initiation. Clusters can have unpaired guard cells and range in size from one to eight guard cells. FLP encodes an R2-R3 MYB protein (F. Sack, personal communication). The role of *FLP* is to limit GMC division, as part of the guard-cell differentiation process.

# INTERACTIONS AMONG CELL-FATE PATHWAYS

## Integration of Trichome, Stomatal, and Pavement Cell Differentiation During Leaf Development

Coordination among alternative cell-differentiation programs is essential for optimal functioning of a tissue. Although little is known about how this coordination is achieved in any system, the examination of several different cell-fate pathways gives some insight into this process in the leaf and root epidermis. The mature leaf epidermis consists of three primary cell types: trichomes, stomatal guard cells, and epidermal pavement cells. As described above, trichomes are the first differentiated cell type to develop in the leaf epidermis. Trichome development proceeds basipetally from the distal tip of the leaf. New trichomes continue to develop between the maturing trichomes, indicating that the protoderm remains competent to initiate trichome formation for a period of time. Eventually, trichome development

stops, first at the tip of the leaf and then toward the base. An experiment using a modified version of the maize R protein expressed in Arabidopsis that can be activated by the mammalian steroid hormone analog dexamethasone clearly demonstrates that beyond a certain developmental stage, protodermal cells are no longer competent to develop as trichomes (53). In this experiment, activation of R could rescue loss of *ttg* function and restore trichome formation. However, if R was activated late in leaf development, no trichomes were formed on distal regions of the leaf, and there was a sharp boundary between the cells competent to respond and those that were not competent. Stomatal development also proceeds basipetally, and it is tempting to speculate that the cells no longer competent to develop as trichomes are those that have entered the stomatal pathway. The signals that coordinate trichome development and stomatal development with the growth of the leaf are unknown. Glover et al. (28) showed that when the number of multicellular trichomes in tobacco is increased by expression of a heterologous Antirhinum MYB gene, the number of stomata formed is proportionately decreased. This suggests that, at least in tobacco, these two cell-differentiation programs can compete for the pool of uncommitted cells.

The remaining cell type in the epidermis is the epidermal pavement cell. Like trichomes, the majority of these cells undergo endoreplication of their nuclear DNA, and expand roughly in proportion to their DNA content, adopting a characteristic jigsaw-puzzle shape (63). Some pavement cells remain diploid; it has been suggested that these cells may act as stem cells allowing the epidermis to continue to divide, for example, under short-day conditions (63). Approximately 50% of leaf pavement cells, and 80% of all epidermal cells, are the product of stomatal lineages (23). It is unknown why some epidermal cells enlarge and endoreplicate more than others, but it seems possible that this decision may be controlled by inhibitory signals regulating passage through the stomatal pathway, as well as the overall growth signal regulating leaf development.

## Patterning of Stomata in Hypocotyl Uses the Root Epidermal Patterning Mechanism

The patterning of stomata in the Arabidopsis hypocotyl is similar to the patterning of the nonhair root epidermal cells. Although hypocotyl epidermal cells do not produce root hairs, there are two epidermal cell types in the Arabidopsis hypocotyl that arise in a position-dependent manner (7, 25, 35, 105). The stomatal cells are preferentially located in the cleft between underlying cortical cells, equivalent to the position of hair cells in the root epidermis. The nonstomatal cells tend to be located in a position equivalent to the nonhair root epidermal cells. This means that cells of the hypocotyl epidermis and the root epidermis undergo position-dependent cell differentiation to generate a common pattern of cell types throughout the apical-basal axis of the Arabidopsis seedling.

The similarity in cell specification in the root and hypocotyl epidermis is also apparent in the molecular components employed. The *wer*, *ttg*, and *gl2* mutations

significantly alter the patterning of the hypocotyl cell types, causing a greater proportion of ectopic stomata (stomata located outside a periclinal cell wall) (7, 35, 49). Furthermore, the *WER*, *GL2*, and J2301 enhancer-trap GFP reporter genes are preferentially expressed in epidermal cells located outside the periclinal cortical cell wall of the root and hypocotyl (7, 35, 49). The similar pattern of specialized and nonspecialized epidermal cells in the root and hypocotyl is initiated during embryogenesis, as demonstrated by similar marker gene expression beginning at the heart stage (7, 52). The parallel pattern of gene activity indicates that the WER/TTG/GL2 pathway is employed in both organs of the seedling beginning during embryogenesis to establish the similar cell-type pattern. Furthermore, although the role of CPC and TRY in the hypocotyl has not been demonstrated, it is tempting to speculate that the same lateral inhibition mechanism identified in the root epidermis is also employed to pattern these stomata.

## CONCLUSION: TOWARD A GENERAL THEORY OF CELL FATE SPECIFICATION?

Substantial information is now available about three different epidermal patterning systems in plants. Two of these systems, trichomes and root hairs, are patterned by essentially the same mechanism, but due to external factors produce different patterns of differentiated cells. The third patterning system, controlling stomatal development, uses a much different patterning mechanism based on control of asymmetric divisions. Some common themes recur in all three mechanisms. All three mechanisms involve local inhibitory signaling that regulates competition between adjacent cells for a particular cell fate. All three mechanisms involve modification of the mitotic cell cycle, although the way in which the cell cycle is altered varies in each case. All three mechanisms respond to tissue-specific and environmental or hormonal cues. Both trichome and root-hair formation are controlled by transcription factors, and it is likely that transcription factors will ultimately be identified controlling entry into the stomatal pathway.

Other general lessons pertaining to developmental biology as a whole have also emerged from these comparisons. As developmental biology has moved from discussion of fields and patterns to focusing on specific molecular details, it has sometimes seemed as if we are drowning in details and that each system must be analyzed on its own terms, without any general rules or guidelines. The examples described here make it clear that this is not so. At a basic level, the same regulatory components are often recycled by evolution to generate quite different patterns, as seen here for the two pathways controlled by the MYB/bHLH/WD-repeat module. But on a more fundamental level, developmental decisions as different as the choice between lysis and lysogeny in bacteriophage $\lambda$ (77), heterocyst differentiation in blue-green algae (109, 114), and macrochaete differentiation in Drosophila (2, 26) involve alternative positive feedback loops coupled by inhibitory pathways. This coupling of positive and negative regulatory interactions is at the heart of many

classic theoretical models of pattern formation (61, 97). Indeed, this may be one of a limited number of ways in which a biological system can be caused to choose between two alternatives; the positive feedback loops stabilize the alternative possibilities, and the inhibitory interactions render the system metastable and force a choice to be made. Furthermore, this type of mechanism is flexible enough to give rise to alternative patterns in response to different initial conditions, as we have seen for trichome and root epidermal patterning.

At least for the root-hair patterning system, some of these feedback loops have been clearly demonstrated, and while the biochemical mechanism of patterning in this system is utterly different from that used by Drosophila macrochaetes, the regulatory interactions are parallel. A careful reading of the literature on trichome and root-hair patterning reveals that investigators in these fields have been guided by analogies from Drosophila and *Caenorhabditis elegans* development for nearly a decade (34, 45, 49). Experiments based on these analogies have led directly to the discovery of a biochemically novel patterning mechanism. On the strength of this success, it is reasonable to predict that the positive feedback loops within these cell types expected on theoretical grounds will be discovered, and that mutually inhibitory feedback loops connecting neighboring cells will be detected regulating stomatal development.

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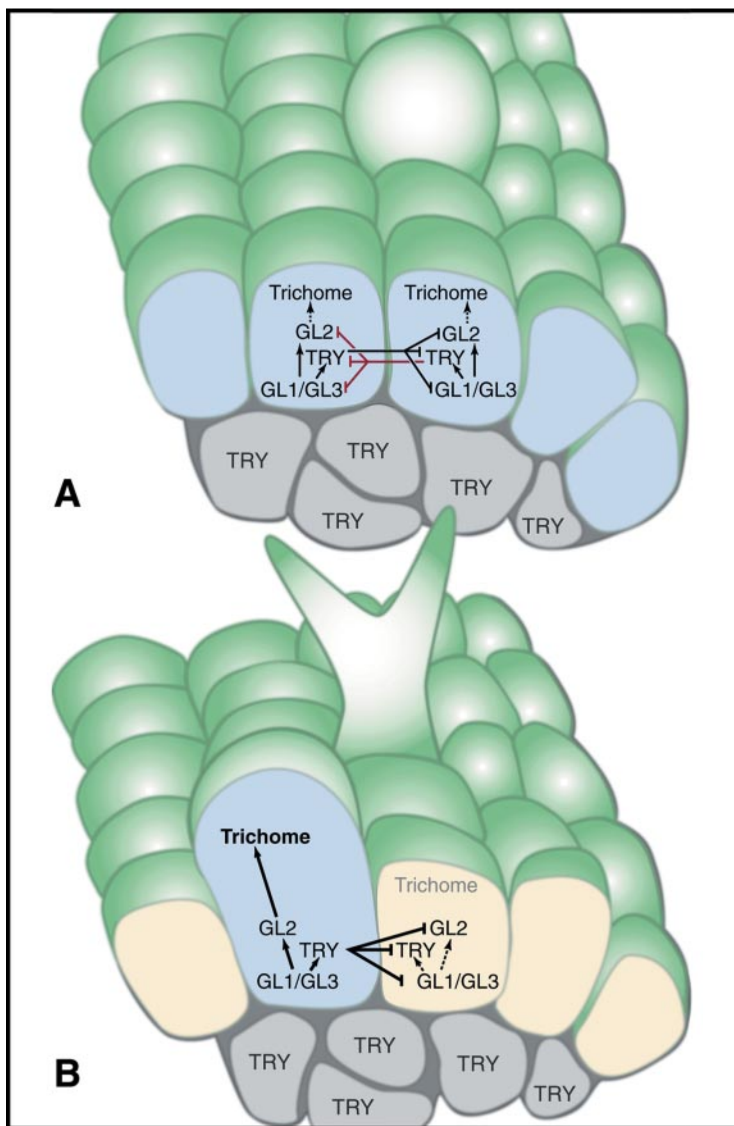


**Figure 2** Model of trichome patterning. (*A*) Mutual inhibition in epidermal cells prior to trichome initiation. All cells have equivalent developmental potential, and express GL1, GL3, and GL2, and use TRY to inhibit their neighbors from expressing these genes. (*B*) Due to stochastic fluctuation, some cells overcome TRY inhibition. Expression of GL1, GL2, and TRY increases in these developing trichomes, and increased production of TRY inhibits GL1, GL3, GL2, and TRY expression or function in adjacent epidermal cells. Constitutive low-level TRY expression in subepidermal cells prevents subepidermal trichome formation. Blue indicates potential for trichome development. CPC omitted for clarity.

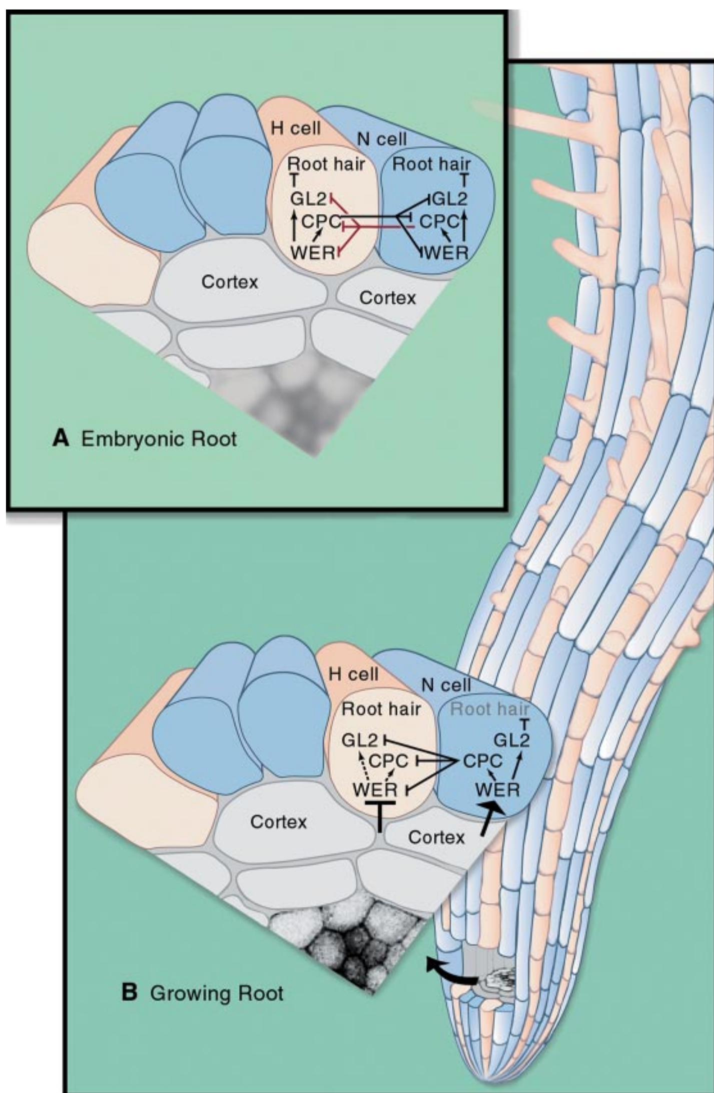


**Figure 3** Model of root epidermal patterning. (*A*) Mutual inhibition in heart-stage embryos. All cells have equivalent developmental potential, express WER and GL2, and use CPC to inhibit their neighbors from expressing these genes. A bHLH gene is probably also expressed. (*B*) Growing root. Position cues from underlying cells generate a bias in WER expression due either to inhibition of WER in H cells or activation of WER in N-cells. This bias is enhanced by increased CPC levels, which carry an inhibitory signal from N-cells to H-cells. N-cells: Cells in files that will produce nonhair cells (blue). H-cells: Cells in files that will produce hair cells (brown). Note that cells continue to divide after fate is determined, producing a file of cells that differentiate.