Plant Physiol. (1998) 117: 73–84

A Common Position-Dependent Mechanism Controls Cell-Type Patterning and *GLABRA2* Regulation in the Root and Hypocotyl Epidermis of Arabidopsis[1]

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A position-dependent pattern of epidermal cell types is produced during root development in *Arabidopsis thaliana*. This pattern is reflected in the expression pattern of *GLABRA2* (*GL2*), a homeobox gene that regulates cell differentiation in the root epidermis. *GL2* promoter::*GUS* fusions were used to show that the *TTG* gene, a regulator of root epidermis development, is necessary for maximal *GL2* activity but is not required for the pattern of *GL2* expression. Furthermore, *GL2*-promoter activity is influenced by expression of the *myc*-like maize *R* gene (*35S::R*) in Arabidopsis but is not affected by *gl2* mutations. A position-dependent pattern of cell differentiation and *GL2*-promoter activity was also discovered in the hypocotyl epidermis that was analogous to the pattern in the root. Non-*GL2*-expressing cell files in the hypocotyl epidermis located outside anticlinal cortical cell walls exhibit reduced cell length and form stomata. Like the root, the hypocotyl *GL2* activity was shown to be influenced by *ttg* and *35S::R* but not by *gl2*. The parallel pattern of cell differentiation in the root and hypocotyl indicates that *TTG* and *GL2* participate in a common position-dependent mechanism to control cell-type patterning throughout the apical-basal axis of the Arabidopsis seedling.

A fundamental problem in developmental biology is understanding how diverse cell types are specified in multicellular organisms. The formation of the root epidermis provides a simple model for investigating this problem in plants. In many species, two distinct cell types are formed during root epidermis development, root-hair cells and hairless cells (Cormack, 1949; Bunning, 1951; Cutter, 1978). The specification of cell fate in the root epidermis (i.e. the process that is responsible for causing a newly generated epidermal cell to differentiate into a root-hair cell or a hairless cell) varies in different plant species. In many monocots, cell specification is associated with an asymmetric cell division; the smaller daughter cell differentiates into a root-hair cell and the larger one becomes a mature, hairless cell (Sinnot and Bloch, 1939; Avers, 1963). In crucifers (e.g. Arabidopsis), cell specification is associated with the relative position of the epidermal cells. Immature epidermal cells located outside an anticlinal cortical cell wall (i.e. in contact with two underlying cortical cells) differentiate into root-hair cells, whereas cells located outside a periclinal cortical cell wall (i.e. in contact with a single cortical cell) differentiate into mature, hairless cells (Bunning, 1951; Cutter, 1978).

In Arabidopsis the differentiating epidermal cells, like many other cells of the root, are organized into columns (or files), with the newly formed cells located near the apex (in the meristematic region) and the older cells located farther from the apex (Dolan et al., 1993; Schiefelbein et al., 1997). Therefore, the differentiating epidermal cell files resemble an "assembly line" of cells, with each cell more developmentally advanced than the one before it, enabling an accurate assessment of the developmental fate of each cell. Furthermore, the precursors to the root-hair and hairless cells can be accurately identified prior to cell maturity. The developing root-hair cells (trichoblasts) possess more densely staining cytoplasm and reduced vacuolation relative to the developing hairless cells (atrichoblasts; Dolan et al., 1994; Galway et al., 1994).

The correlation between cell position and cell type in the root epidermis of Arabidopsis implies that cell signaling plays an important role in cell specification. However, the molecular events involved in the presumed cellular signaling and the subsequent cell differentiation are unknown. To define the molecules controlling cell specification in the Arabidopsis root epidermis, genetic studies have been used to identify loci that affect the normal pattern of root-hair and hairless cells (Dolan et al., 1994; Galway et al., 1994; Masucci and Schiefelbein, 1994; DiCristina et al., 1996; Masucci et al., 1996; Schneider et al., 1997; Wada et al., 1997). The analysis of two of these loci, *TRANSPARENT TESTA GLABRA* (*TTG*) and *GLABRA2* (*GL2*), suggest that they encode negative regulators of root-hair-cell differentiation or, alternatively, positive regulators of hairless cell differentiation (Galway et al., 1994; Masucci et al., 1996). *TTG* and *GL2* are also required for the appropriate formation of

[1] This research was supported by a National Science Foundation grant to J.S. (no. IBN-9724149) and a U.S. Department of Agriculture grant to M.D.M. (no. 95-37304-2219). C.-Y.H. and M.Z. were partially supported by funds from the University of Michigan Rackham Graduate School (Ann Arbor).

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Abbreviations: bHLH, basic helix-loop-helix; 4-MUG, 4-methylumbelliferyl β-D-glucuronic acid; X-Gluc, 5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid.

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trichomes and the production of seed-coat mucilage (Koornneef, 1981; Koornneef et al., 1982; Rerie et al., 1994).

In the Arabidopsis root the recessive *ttg* mutations cause nearly all root epidermal cells, regardless of their position, to differentiate as root-hair cells (Galway et al., 1994). Expression of the maize *R* (*Lc*) cDNA under control of the cauliflower mosaic virus 35S promoter in Arabidopsis suppresses the *ttg* defects and causes an excessive number of root epidermal cells to differentiate as hairless cells (Galway et al., 1994). Together with the ability of the *35S::R* transgene to suppress all of the other *ttg* defects, these results suggest that an R homolog (a *myc*-like bHLH transcriptional activator; Ludwig et al., 1989) in Arabidopsis may act at the same point or downstream from the TTG product to control these various processes (Lloyd et al., 1992; Galway et al., 1994).

Like the *ttg* mutations, mutations in the *gl2* gene cause root hairs to form on essentially every root epidermal cell (Masucci et al., 1996). However, the ectopic root-hair cells in the *gl2* mutant differ from those in the *ttg* mutant because they retain the cellular characteristics of wild-type hairless cells during their formation, including differences in cell vacuolation, cytoplasmic density, and cell length (Masucci et al., 1996). The *GL2* gene encodes a homeodomain protein of the HD-Zip class and is expressed preferentially in the differentiating hairless epidermal cells within the meristematic and elongation regions of the root, which implies that *GL2* acts as a cell-position-dependent transcriptional regulator to repress root-hair formation (Rerie et al., 1994; DiCristina et al., 1996; Masucci et al., 1996). Because *GL2* affects a subset of the processes controlled by *TTG* (Masucci et al., 1996), and because the steady-state level of *GL2* RNA is reduced in the *ttg* mutant (DiCristina et al., 1996), *TTG* may be a positive regulator of *GL2*.

In the present study the regulation of the *GL2* homeobox gene and its role in epidermis development were analyzed. One goal of this research was to define genes/proteins that influence the spatial and/or quantitative regulation of *GL2* during root development. To accomplish this, a series of *GL2* promoter::*GUS* reporter gene fusions were constructed and used as sensitive, in vivo reporters of *GL2*-promoter activity. These led to the identification of a promoter region necessary for the cell-position-dependent expression of *GL2* during root development. In addition, we show that maximal *GL2*-promoter activity in the root is *TTG* dependent and is influenced by the *35S::R* transgene but not by *gl2* mutations. Furthermore, these studies led to the discovery of a cell-position-dependent pattern of *GL2*-promoter activity and cell differentiation within the developing hypocotyl of Arabidopsis. Our results indicate that *TTG* and *GL2* participate in a common mechanism to specify cell-type patterning during the development of the root and hypocotyl epidermis.

MATERIALS AND METHODS

Genetic Stocks and Growth Conditions

Arabidopsis thaliana stocks *ttg-1* (no. 89) and *gl2–1* (no. 65) were obtained from the Arabidopsis Biological Resource Center at The Ohio State University (Columbus). The *ttg-w* line was obtained from M. Koornneef (Agricultural University, Wageningen, The Netherlands). The *ttg-398* line was isolated from an ethyl methanesulfonate-mutagenized population in the Wassilewskija genetic background.

Two transgenic lines (1439 and 1434) bearing the *35S::R* construct and displaying similar phenotypic effects were used in these studies; their root defects were originally described by Galway et al. (1994).

The *ttg gl2* double mutant was constructed by crossing single-mutant plants and examining F_2 progeny for the *ttg* phenotype. These plants were subsequently test crossed with the *gl2* mutant to identify the *ttg gl2* double mutant. The *35S::R gl2* line was generated by crossing *35S::R* plants with the *gl2* mutant, backcrossing the F_1 to the *gl2* mutant, and testing the progeny to identify individuals bearing the *35S::R* construct and homozygous for the *gl2* mutation.

Unless otherwise noted, seedlings were grown on vertically oriented Petri dishes on agarose-solidified medium containing 1% Suc, 0.6% agarose, and mineral nutrients under continuous illumination (Estelle and Somerville, 1987) following a 2-d chilling period, as previously described (Schiefelbein and Somerville, 1990).

Microscopy

To determine the number of root-hair and hairless cells in the root epidermis, 5-d-old seedlings were mounted in artificial pond water (Schiefelbein et al., 1992) and viewed with differential interference contrast optics. Two to six trials were performed for each line. A cell was scored as a root-hair cell if any protrusion was present, regardless of its length.

To determine the location of root-hair-bearing cells, 5-d-old seedlings were submerged in molten 3% agarose. After the agarose solidified, transverse root sections were hand-cut with a double-edged razor blade and stained with a solution of 0.002% toluidine blue dye in artificial pond water. The location of root-hair cells relative to the underlying cortical cells was determined by viewing sections from at least five roots from each line.

The agarose-embedding technique was also used to analyze GUS staining within the hypocotyl of 3-d-old seedlings. To observe both the histochemical GUS staining and the position of hypocotyl cells, the cell walls of these sections were subsequently stained with FB28, as described previously (Galway et al., 1994), and viewed simultaneously under fluorescent and visible light.

The cellular characteristics of the hypocotyl epidermis were determined by GUS staining, clearing with 95% ethanol, and staining with toluidine blue dye. The relative cell length and stomatal formation in *GL2::GUS*-expressing and -nonexpressing files were measured from 10 cells of each file type from each of four independent sets of five seedlings grown for 5 d on the agarose-solidified medium described above. Cells in the stomatal complexes were not included in the cell-length calculations.

Plastic transverse sections were obtained from roots embedded in resin (JB-4, Polysciences, Warrington, PA)

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stained with 0.05% toluidine blue O, as previously described (Masucci and Schiefelbein, 1996).

Gene Fusions and Transgenic Plants

The construction of the 4-kb *GL2 promoter::GUS* reporter gene fusion was previously described (Masucci et al., 1996), and the set of *GL2*-promoter deletions is described elsewhere (D. Szymanski and M.D. Marks, unpublished data). At least two independent transgenic lines were generated and used in the qualitative and quantitative GUS assays for each of the *GL2::GUS* constructs.

GUS-Reporter Gene Assays

For histochemical analysis of GUS expression, 2- to 7-d-old seedlings harboring a transgene were assayed for GUS activity according to established methods (Gallagher, 1992) for 1 to 24 h of incubation (depending on the particular *GL2::GUS* construct and mutant background), cleared in 95% ethanol, and examined by light microscopy. Transverse sections of root apices were obtained from 4-d-old seedlings as previously described (Masucci et al., 1996).

Quantitative analysis of GUS activity in seedlings was performed essentially as described previously (Gallagher, 1992). Groups of 10 to 15 seedling root apices (excised 0.5 cm from the tips of 3- to 5-d-old seedlings) or hypocotyl/cotyledon segments (excised at the root-hypocotyl junction from 3-d-old seedlings) were ground with a plastic pestle in a microfuge tube in 200 μL of extraction buffer composed of 50 mM $NaPO_4$, 1 mM EDTA, 0.1% sodium lauryl sarcosine, 0.1% Triton X-100, and 10 mM DTT. The homogenized sample was centrifuged for 10 min, and 100 μL of the supernatant was assayed for GUS activity in a 30-min reaction at 37°C with 1 mM 4-MUG and 20% methanol. The reaction was terminated by the addition of an equal volume of 0.2 M Na_2CO_3, and fluorescence from the product was measured with a fluorometer (model TKO 100, Hoefer, Piscataway, NJ).

To accurately assess the effect of mutations and constructs on the activity of a particular *GL2::GUS* transgene, groups of seedlings used in the quantitative GUS assays were selected, based on their phenotype, from an F_2 pool derived from a cross between the mutant or construct line with the *GL2::GUS* line. The relative effect of a mutation or construct on GUS activity was determined by comparing the GUS activity (expressed as millimoles of product per milligram of protein) in the mutant seedlings with that of the wild-type seedlings within the F_2 pool. To ensure that the *GL2::GUS* construct was equally distributed in each phenotypic class within an F_2 pool, seedlings were tested for the presence of the construct. At least three assays were conducted for each mutant/transgene combination, and two independent lines containing a particular *GL2* promoter::*GUS* transgene were tested for each combination.

RESULTS

Analysis of Cell-Position-Specific Activity of the *GL2* Promoter in Roots

In a previous study the 4-kb DNA segment 5′ to the *GL2* transcriptional unit (hereafter called the *GL2* promoter) was shown to be sufficient to direct expression of the GUS-reporter gene in a cell-position-dependent pattern during root epidermis development that reflects *GL2* RNA accumulation (Masucci et al., 1996). To begin to define the *GL2*-promoter region(s) required for the cell-position-specific expression of *GL2*, a series of deletions of the *GL2* promoter were generated and fused to the GUS-reporter gene, as shown in Figure 1. Transgenic Arabidopsis plants possessing these constructs were produced and tested for GUS expression in the developing roots by histochemical staining with the X-Gluc substrate.

The results of this analysis indicate that a 500-bp *Eco*RV/*Xba*I DNA fragment located between position −840 and −1340 in the *GL2* promoter is necessary to direct the appropriate pattern of GUS expression in differentiating hairless epidermal cells (Fig. 1). In plants bearing each of the constructs containing this fragment, GUS activity in the root was limited to epidermal cells located outside a periclinal cortical cell wall (i.e. the normal *GL2* root-expression pattern; Masucci et al., 1996). No GUS activity could be detected in the roots of plants bearing the ΔMHp construct, which lacks this fragment. Likewise, plants containing the ΔMR construct did not exhibit detectable staining of differentiating epidermal cells, although they did display a trace of GUS staining in emerging secondary roots.

Effect of *ttg* and *35S::R* on *GL2*-Promoter Activity in Roots

Qualitative GUS Assay

In a prior study the steady-state level of *GL2* mRNA in roots was shown to be reduced in the *ttg* mutant (DiCristina et al., 1996). To determine whether this was due to an effect of *ttg* on *GL2*-promoter activity, we introduced the 4-kb *GL2*-promoter::*GUS*-reporter gene fusion into the *ttg-1* mutant background by genetic crosses. The roots of these *ttg-1 GL2::GUS* seedlings exhibited a significant reduction in *GUS* activity compared with roots from the *GL2::GUS* seedlings in a wild-type background (Fig. 2, A–D). To determine the spatial pattern of GUS expression in the *ttg-1 GL2::GUS* roots, transverse root sections were prepared and analyzed. Because of the reduced GUS activity in the *ttg-1 GL2::GUS* roots, no GUS staining was detected in thin, plastic sections, but thick agarose sections from the *ttg-1 GL2::GUS* roots showed that, like the wild type, GUS activity was limited to the differentiating epidermal cells located outside the periclinal cortical cell walls (Fig. 2, G, H, and J). Similar results were obtained when the *GL2::GUS* construct was introduced into two other *ttg* mutant allele backgrounds (*ttg-w* and *ttg-398*, data not shown). Therefore, the *ttg* mutant appears to affect the level of *GL2*-promoter activity but not its cell-position-dependent pattern during root development.

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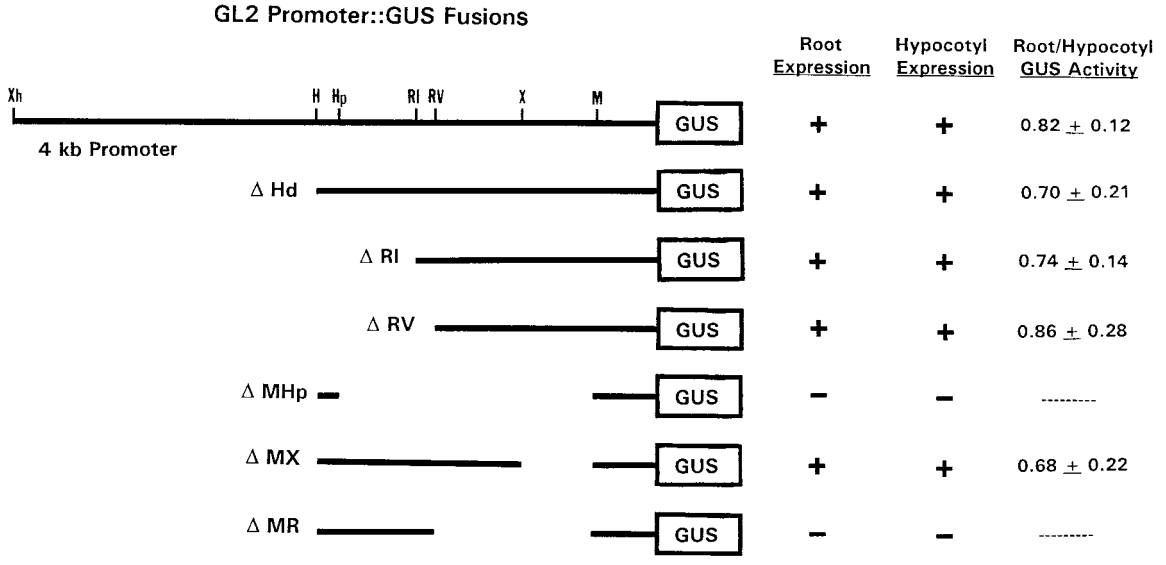

Figure 1. Effect of *GL2*-promoter fragments on GUS-reporter expression in roots and hypocotyls of Arabidopsis seedlings. The *GL2*-promoter fragments fused to the GUS-reporter gene are shown on the left. The ability of these constructs to drive GUS expression in a cell-position-dependent manner in the seedling root and/or hypocotyl was determined by histochemical staining with the X-Gluc substrate. +, Typical GUS expression pattern detected; −, abnormal/no GUS expression detected. The relative root/hypocotyl GUS-activity value was determined by comparing the GUS activity (calculated as millimoles of product per milligram of protein per minute) in root versus hypocotyl extracts from a common set of 3-d-old seedlings bearing the indicated transgene. Values are means ± SD. Xh, *Xho*I; H, *Hin*dIII; Hp, *Hpa*I; RI, *Eco*RI; RV, *Eco*RV; X, *Xba*I; M, *Msc*I; and △, deletion.

To further investigate the role of the *TTG* pathway on *GL2* expression in roots, we tested the effect of the *35S::R* construct, which is able to suppress *ttg* mutant root defects (Galway et al., 1994). Seedling roots containing both the *35S::R* and the *GL2::GUS* constructs displayed ectopic GUS activity compared with the wild-type *GL2::GUS* pattern (Fig. 2, E and F). Transverse sections from the *35S::R GL2::GUS* roots showed that GUS activity accumulated throughout the epidermis (indicating ectopic expression in the differentiating root-hair cells), in lateral root cap cells, and in cortical cells (Fig. 2I). Therefore, the expression of the *myc*-like *R* gene by the *35S* promoter is sufficient to induce ectopic *GL2*-promoter activity during root development.

To further analyze GUS expression in the *35S::R GL2::GUS* line, GUS activity in a *35S::GUS* transgenic line was examined and compared with *GL2::GUS* and *35S::R GL2::GUS* roots (Fig. 2, K–M). This analysis is useful because the *35S::GUS* provides an indication of the likely location of R protein accumulation in the *35S::R* line. The comparison showed that the strong *35S*-promoter activity in the region of the root meristem initials and lateral root cap coincides with ectopic expression of *GL2::GUS* in these regions in the *35S::R GL2::GUS* line. However, despite *35S*-promoter activity in columella root cap cells and vascular tissue, the *35S::R GL2::GUS* roots lack detectable GUS activity in these regions (Fig. 2, K–M). Therefore, ectopic *GL2* expression occurs in some but not all of the cells that are likely to accumulate the *R* protein, which implies that additional factor(s) are necessary to activate the *GL2* promoter in these root tissues.

Quantitative GUS Assay

The possibility of reduced GUS accumulation in the *ttg* mutant and abnormal GUS accumulation in the *35S::R* background led us to examine the quantitative effect of these factors on *GL2*-promoter activity. To accurately assess the relative effect of these factors, pools of F_2 seedlings (derived from crosses between the mutant and the *GL2::GUS* containing line) were used and the amount of GUS activity present in mutant and wild-type seedling roots within these common genetic backgrounds were compared using the 4-MUG substrate in a fluorometric assay (Gallagher, 1992). This quantitative analysis showed that roots of *ttg-1* mutants with the *GL2::GUS* transgene possess approximately 30% of the GUS activity present in *GL2::GUS* plants in a wild-type background (Table I). Accordingly, plants homozygous for the *ttg-w* and *ttg-398* mutant alleles possess 59 and 36%, respectively, of the GUS activity of their corresponding wild-type seedling roots (Table I). These results confirm the GUS staining results and support the view that *TTG* is required for the appropriate level of *GL2*-promoter activity in roots.

The quantitative GUS analysis of seedlings possessing the *35S::R* and *GL2::GUS* constructs showed that the *35S::R* roots have a slight reduction in GUS activity relative to the wild type (Table I). This result was somewhat unexpected, because ectopic GUS expression is present in the *35S::R GL2::GUS* roots (Fig. 2), which would be expected to lead to an increase in the overall root GUS activity. Apparently, the quantitative effect of *35S::R* on root GUS expression is due to a reduction in GUS expression in the cells that

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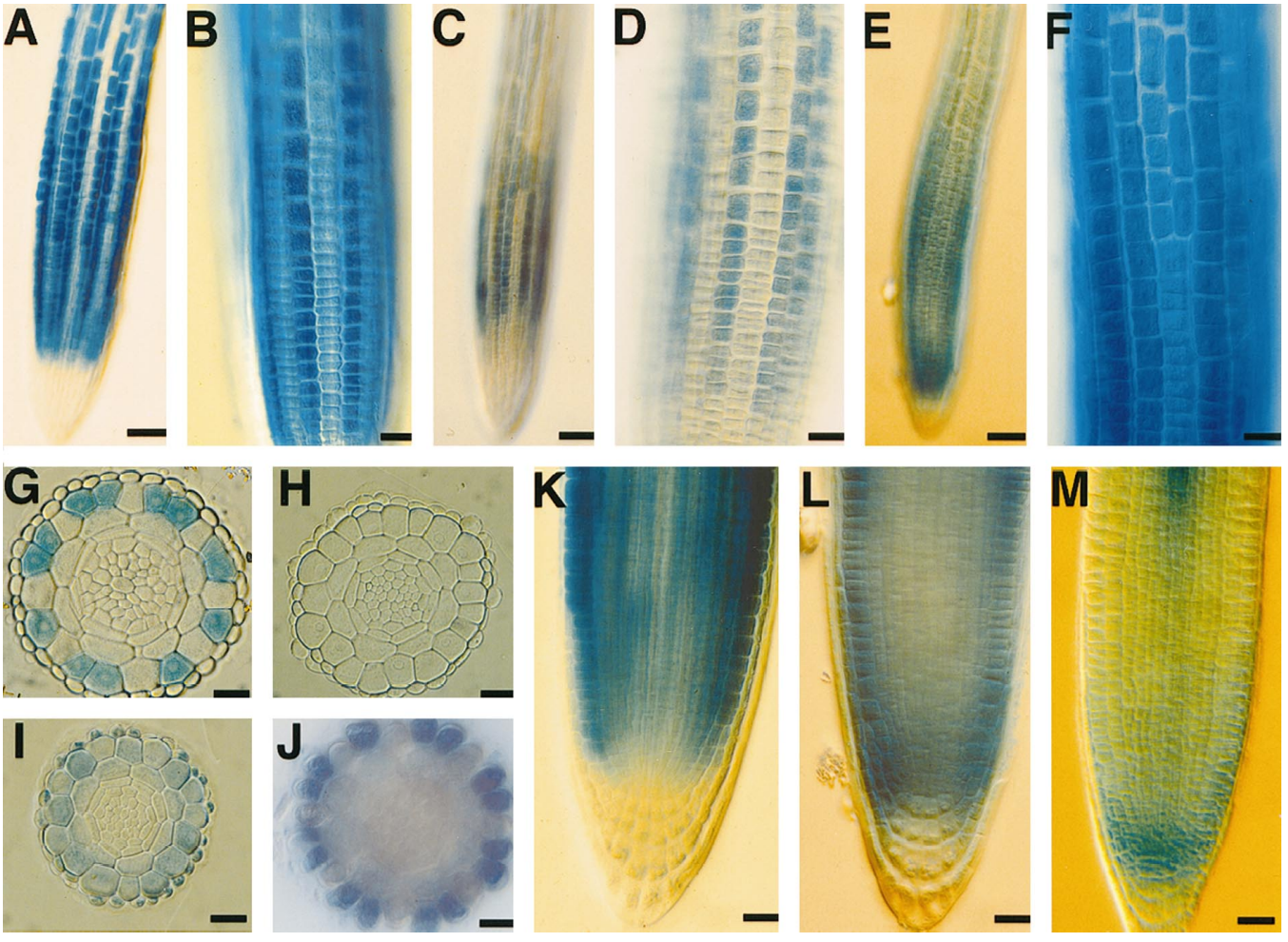

Figure 2. Spatial expression pattern of *GL2::GUS*-reporter-gene fusion construct during root development in Arabidopsis seedlings. Four-day-old seedlings were assayed for GUS activity by histochemical staining with the X-Gluc substrate. A, Wild-type root containing the *GL2::GUS* transgene. Bar = 50 μm. B, Wild-type root containing the *GL2::GUS* transgene. GUS-expressing cells are preferentially located in specific epidermal cell files. Bar = 20 μm. C, *ttg-1* mutant root containing the *GL2::GUS* transgene. Bar = 50 μm. D, *ttg-1* mutant root containing the *GL2::GUS* transgene. GUS-expressing cells are preferentially located in specific epidermal cell files. Bar = 20 μm. E, *35S::R* root containing the *GL2::GUS* transgene. Bar = 50 μm. F, *35S::R* root apex containing the *GL2::GUS* transgene. GUS-expressing cells are not clearly located in specific epidermal cell files. Bar = 20 μm. G, Wild-type root containing the *GL2::GUS* transgene; transverse plastic section taken from the late meristematic region. GUS expression is limited to epidermal cells located outside periclinal cortical cell walls (i.e. in contact with a single cortical cell). At this developmental stage, a single layer of lateral root cap cells surrounds the epidermis. Bar = 20 μm. H, *ttg-1* mutant root containing the *GL2::GUS* transgene; transverse plastic section taken from the late meristematic region. No GUS expression is observed. At this developmental stage, a single layer of lateral root cap cells surrounds the epidermis. Bar = 20 μm. I, *35S::R* root containing the *GL2::GUS* transgene; transverse plastic section taken from the late meristematic region. GUS expression is observed throughout the epidermis, cortex, and lateral root cap. At this developmental stage, a single layer of lateral root cap cells surrounds the epidermis. Bar = 20 μm. J, *ttg-1* mutant root containing the *GL2::GUS* transgene; thick transverse section from agarose-embedded root. GUS expression is observed in epidermal cells located outside periclinal cortical cell walls. Bar = 20 μm. K, Wild-type root apex containing the *GL2::GUS* transgene. Whole-mount root preparation showing GUS expression near the meristem initials but not within the lateral or columella root cap cells. The dense staining visible in the upper portion of this root is due to GUS-expressing epidermal cells above and below the plane of focus. Bar = 20 μm. L, *35S::R* root apex containing the *GL2::GUS* transgene. Whole-mount root preparation showing GUS expression throughout region containing meristem initials and within the lateral root cap cells but not within the columella root cap cells. The dense staining visible in the upper root is due to GUS-expressing cells above and below the plane of focus. Bar = 20 μm. M, *35S::GUS* root apex. Whole-mount root preparation showing preferential GUS expression throughout region containing meristem initials, the root cap, and the developing vascular tissue. Bar = 20 μm. These *GL2::GUS* seedlings all contain the full-length 4-kb *GL2* promoter::GUS transgene. Note that root hairs are not visible in these images near the root apex; hairs form on epidermal cells at a later developmental age, just beyond the field of view shown in A, C, and E.

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Table I. *Root-hair formation and GL2-promoter activity in roots and hypocotyls of mutant and transgenic Arabidopsis seedlings*

Genotype	Hair Formation[a]	Ectopic Root-Hair Cells[b]	Proportion of Wild-Type GUS Activity[c]	
			Root	Hypocotyl
	no. mm^{-1}	%		
Landsberg (wild type)	61 ± 6	4	–	–
ttg-1	124 ± 12	47	0.29 ± 0.08	0.09 ± 0.06
ttg-w	94 ± 11	28	0.59 ± 0.15	0.25 ± 0.08
ttg-398	116 ± 19	44	0.36 ± 0.10	0.16 ± 0.09
35S::R	15 ± 6	6	0.78 ± 0.24	1.05 ± 0.18
gl2–1	132 ± 19	52	0.94 ± 0.20	1.21 ± 0.15
ttg-1 gl2–1	136 ± 20	56	n.d.[d]	n.d.
gl2–1 35S::R	110 ± 25	41	n.d.	n.d.

[a] Means ± SD. [b] Percentage of the root-hair-bearing cells that are located over a periclinal cortical cell wall (ectopic position). [c] GUS activity per milligram of protein in the mutant relative to the wild type within a common pool of F_2 seedlings containing the full-length 4-kb *GL2* promoter::GUS fusion. Values are means ± SD. [d] n.d., Not determined.

normally express *GL2* (differentiating hairless cells), as well as the induction of GUS expression in cells that normally do not express *GL2*.

To define the *GL2*-promoter region required for the *ttg* and *35S::R* effects on *GL2* expression, the *GL2*-promoter deletion::GUS lines (Fig. 1) were introduced into the *ttg* and *35S::R* genetic backgrounds and each combination was tested for GUS activity. As shown in Table II, the *ttg* mutant was found to impart the same relative effect on each *GL2::GUS* construct; the amount of GUS activity and the intensity of GUS staining was reduced (but the cell-position expression pattern was not affected) in each line that exhibited GUS expression. This shows that the DNA fragment in the *GL2* promoter responsible for the *ttg* effect could not be separated from the 500-bp *Eco*RV/*Xba*I DNA fragment responsible for the cell-position-dependent root expression (Fig. 1). The analysis of *GL2::GUS* lines bearing the *35S::R* construct generated a similar outcome; the amount of GUS activity was slightly reduced (relative to the wild type) and ectopic GUS expression was observed (by X-Gluc staining) in the roots from each line (Table II). The correlation between the *GL2*-promoter region required for the cell-position-dependent expression (determined in Fig. 1) and the region required for the *ttg* and *35S::R* effects suggests that the *TTG* and/or an *R* homolog influence *GL2*-promoter activity at or near the same site required for cell-position-dependent promoter activity.

Effect of the *gl2* Mutant on *GL2*-Promoter Activity

To determine whether the GL2 homeodomain protein may regulate the activity of its own promoter, we examined the effect of the *gl2–1* mutation on the expression of the *GL2::GUS* transgene. The analysis of *gl2–1 GL2::GUS* seedling roots stained for GUS activity did not reveal any significant qualitative difference in GUS accumulation compared with wild-type *GL2::GUS* roots (data not shown). Similarly, quantitative GUS assays showed that the *GL2-GUS* lines homozygous for the *gl2–1* mutation do not display a difference in total GUS accumulation in the roots relative to the wild type (Table I). Therefore, a functional GL2 protein is not required for normal *GL2*-promoter regulation during root development.

Genetic Analysis of *TTG, 35S::R,* and *GL2* during Root Development

To further examine the relationship among *TTG*, *35S::R*, and *GL2* during root epidermis development, two genetic experiments were conducted. In one experiment, the *35S::R* construct was introduced (by genetic crosses) into the *gl2–1* mutant background to determine whether a functional GL2 protein is required for hairless cells to be induced by *35S::R*. The *gl2–1 35S::R* roots were found to produce an excessive number of root hairs, including a significant frequency of ectopic root-hair cells, which resembled the *gl2–1* mutant phenotype (Table I). This result shows that the effect of the *35S::R* transgene is *GL2* dependent and suggests that GL2 acts downstream or independently from the *R* product to influence root epidermis development.

In a second experiment, the *ttg gl2* double mutant was constructed and analyzed. The root epidermis in *ttg-1 gl2–1* roots was indistinguishable from either single mutant with respect to the production of root-hair and hairless cells (Table I), and no synergistic effect on root development

Table II. *Effect of the ttg mutation and 35S::R transgene on GUS activity in roots of GL2::GUS lines containing GL2-promoter deletions*

GL2::GUS Transgene[a]	GUS Expression[b]		Proportion of Wild-Type GUS Activity[c]	
	ttg-1	*35S::R*	*ttg-1*	*35S::R*
4-kb *GL2* promoter	+[d]	+	0.29 ± 0.08	0.78 ± 0.24
ΔHd	+	+	0.19 ± 0.05	0.61 ± 0.20
ΔRI	+	+	0.21 ± 0.11	0.47 ± 0.23
ΔRV	+	+	0.24 ± 0.13	0.64 ± 0.13
ΔMHp	−[e]	−	–	–
ΔMX	+	+	0.23 ± 0.11	0.94 ± 0.23
ΔMR	−	−	–	–

[a] *GL2::GUS* transgenes defined in Figure 1. [b] GUS expression in roots, as assessed by histochemical staining. [c] GUS activity per milligram of protein in the mutant relative to the wild type within a common pool of F_2 seedlings containing the indicated *GL2* promoter::GUS fusion. Values are means ± SD. [d] +, Typical qualitative pattern of GUS expression detected. [e] −, No GUS expression detected.

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was detected. This indicates that *TTG* and *GL2* are not likely to possess redundant functions in root development and is consistent with the notion that *TTG* and *GL2* act within the same pathway.

GL2-Promoter Activity during Hypocotyl Development

During the course of our studies of *GL2* expression in root development, GUS activity was detected in the hypocotyl of Arabidopsis seedlings bearing the *GL2::GUS* transgene. The detailed examination of these hypocotyls showed that GUS activity is present in an epidermis-cell-file-specific pattern that mirrors the *GL2::GUS* pattern in the root epidermis (Fig. 3, A and B). Transverse hypocotyl sections showed that GUS activity preferentially accumulates in epidermal cells located over a periclinal cortical cell wall, a location that is analogous to the location of *GL2::GUS*-expressing cells in the root (Fig. 3, C and D). It is interesting to note that this pattern is maintained in both tissues even though the hypocotyl contains two layers of cortical cells and the root contains a single layer (Scheres et al., 1994). Therefore, the *GL2* promoter is active in the same position-dependent manner in epidermal cells throughout the root-hypocotyl axis during seedling development.

Two additional features of the *GL2::GUS* expression pattern were observed. First, although no GUS expression was detected within the cotyledon proper, the *GL2::GUS* seedlings displayed GUS activity in a ring of cells around the circumference of the cotyledons (Fig. 3, G and H). These GUS-expressing cells at the cotyledon margin appeared to be epidermal cells located outside a periclinal cortical cell wall (Fig. 3I). Second, the analysis of GUS accumulation in hypocotyls of *GL2::GUS* seedlings at different developmental stages showed that the intensity of the hypocotyl GUS staining was greatest in 2- or 3-d-old seedlings and becomes diminished and largely localized to the upper portion of the hypocotyl at later stages of development (Fig. 3, A and J). This suggests that *GL2*-promoter activity in the hypocotyl may be correlated with the differentiation of the hypocotyl epidermal cells.

To define the *GL2*-promoter region(s) involved in directing the hypocotyl-epidermis expression, GUS accumulation was examined by X-Gluc staining in 3-d-old seedlings containing the *GL2* promoter::GUS constructs illustrated in Figure 1. This analysis showed that the 500-bp *Eco*RV/*Xba*I fragment at −840 to −1340 is necessary for the cell-file-specific expression of GUS in hypocotyls (Fig. 1). Arabidopsis seedlings bearing *GL2::GUS* transgenes lacking this 500-bp fragment exhibited no detectable GUS activity in the hypocotyls. This result shows that the region of the *GL2* promoter necessary for position-dependent expression in the root is also required for proper expression in the hypocotyl epidermis.

Although the same *GL2*-promoter region is required for the root- and hypocotyl-expression pattern, other promoter elements may exist that exert different quantitative effects on the root and hypocotyl expression. To test this possibility, the ratio of root to hypocotyl GUS activity was determined for each of the *GL2::GUS* lines by conducting quantitative GUS assays on extracts from the root and hypocotyl of a common set of 3-d-old seedlings. If a *GL2*-promoter element exists that differentially affects root versus hypocotyl expression, then a *GL2::GUS* transgene that lacks this element would be expected to display an altered ratio of root to hypocotyl GUS activity. The results of this experiment showed that the root to hypocotyl GUS activity ratio was similar in each of the *GL2::GUS* transgene lines (Fig. 1), which indicates that *GL2*-promoter activity is not differentially regulated in the root and hypocotyl in a quantitative manner by promoter elements located outside the 500-bp *Eco*RV/*Xba*I fragment.

Position-Dependent Cell Differentiation in the Hypocotyl Epidermis

The detection of a position-dependent pattern of *GL2* expression in the hypocotyl epidermis led us to examine the characteristics of the epidermal cells to determine whether a corresponding pattern of cell differentiation exists in the hypocotyl epidermis. Although, unlike root epidermal cells, hypocotyl epidermal cells do not produce hairs, cellular differences were detected when the *GL2::GUS*-expressing cells were compared with the non-*GL2::GUS*-expressing cells in 5-d-old hypocotyls. First, the GUS-expressing hypocotyl cells are longer (by approximately 40%) than their non-GUS-expressing neighbors (Fig. 3E; Table III). In addition, stomata were preferentially observed in the non-GUS-expressing epidermal cell files (Fig. 3F; Table III). Because of the observed correlation among cell position, GL2 expression, and cellular characteristics, these results indicate that the hypocotyl epidermis of Arabidopsis undergoes a position-dependent pattern of cell differentiation.

Effect of Mutations on *GL2*-Promoter Activity in the Hypocotyl

To investigate the possibility that hypocotyl *GL2* expression may be influenced by factors controlling root *GL2* expression, GUS accumulation was analyzed in the hypocotyls of 3-d-old seedlings bearing the *GL2::GUS* transgene and the *ttg*, *35S::R*, or *gl2* mutations.

Compared with hypocotyls bearing the *GL2::GUS* in a wild-type background, *ttg-1 GL2::GUS* hypocotyls exhibit a greatly diminished level of GUS activity, as assessed by X-Gluc staining (Fig. 3, K and L) and quantitative 4-MUG GUS assays (Table I). The small amount of hypocotyl GUS activity was in epidermal cells located outside a periclinal cortical cell wall, indicating that the spatial pattern of *GL2::GUS* expression was unaltered (Fig. 3M).

In the *35S::R GL2::GUS* hypocotyls, GUS activity is detected in all epidermal cells and is particularly intense near the upper end of the hypocotyl (Fig. 3, N–P). In addition, ectopic GUS expression was observed extending beyond the margins of the cotyledons in these seedlings (Fig. 3O; data not shown). Nonetheless, the overall amount of hypocotyl GUS activity in the *35S::R GL2::GUS* seedlings was not significantly different from the wild-type *GL2::GUS* (Table I).

The qualitative and quantitative analysis of GUS activity in hypocotyls of the *gl2–1 GL2::GUS* seedlings did not

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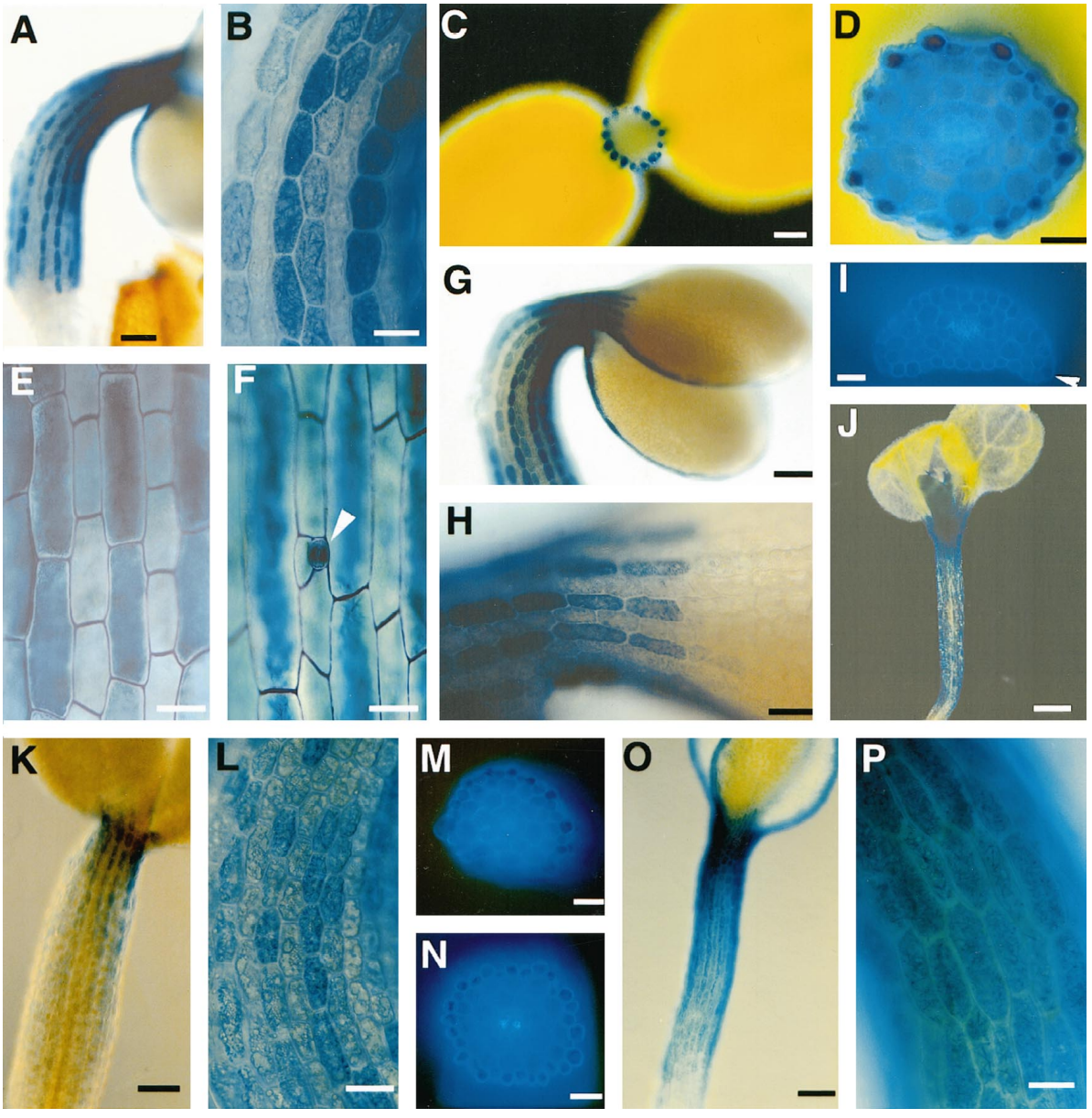

Figure 3. The spatial-expression pattern of the *GL2-GUS*-reporter-gene fusion construct during hypocotyl development. Seedlings harboring the 4-kb *GL2* promoter*::GUS* transgene were stained for GUS activity using X-Gluc. A, Wild-type hypocotyl from 3-d-old seedling. Bar = 100 μm. B, Wild-type hypocotyl epidermis from 3-d-old seedling. GUS-expressing cells are located within specific epidermal cell files. Bar = 50 μm. C, Wild-type 3-d-old seedling sectioned through the hypocotyl. A ring of GUS-expressing hypocotyl epidermal cells is visible. Bar = 100 μm. D, Wild-type hypocotyl from 3-d-old seedling; transverse agarose section. GUS expression is present in epidermal cells located outside a periclinal cortical cell wall. E, Wild-type hypocotyl from 5-d-old seedling. Note that cells in the *GL2::GUS*-expressing files are longer than cells in the nonexpressing files. Bar = 40 μm. F, Wild-type hypocotyl from 5-d-old seedling. Stomatal development (arrowhead) occurs in non-*GL2::GUS*-expressing cell files. Bar = 40 μm. G, Wild-type hypocotyl and cotyledons from 3-d-old seedling. GUS-expressing cells are visible at the margin of cotyledons. Bar = 100 μm. H, Wild-type hypocotyl/cotyledon junction region from 3-d-old seedling. Bar = 50 μm. I, Wild-type cotyledon; transverse section taken near cotyledon apex (3-d-old). Arrowhead indicates a GUS-staining epidermal cell. Bar = 50 μm. J, Wild-type hypocotyl from 7-d-old seedling. GUS expression is visible in the developing leaf primordia and trichomes. Bar = 200 μm. K, *ttg-1* mutant hypocotyl from 3-d-old seedling. Bar = 100 μm. L, *ttg-1* mutant hypocotyl from 3-d-old seedling. GUS-expressing cells appear to be located within specific epidermal cell files. Bar = 50 μm. M, *ttg-1* mutant hypocotyl from 3-d-old seedling; transverse agarose section. GUS expression is present in epidermal cells located outside a periclinal cortical cell wall (i.e. in contact with a single cortical cell). Bar = 50 μm. N, *35S::R* hypocotyl from 3-d-old seedling; transverse agarose section. GUS expression is present in cells located throughout the epidermis. Bar = 50 μm. O, *35S::R* hypocotyl from 3-d-old seedling. Bar = 100 μm. P, *35S::R* hypocotyl from 3-d-old seedling. GUS-expressing cells are located throughout the epidermis. Bar = 50 μm.

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Table III. *Epidermal cell differentiation in hypocotyls of wild-type and mutant Arabidopsis seedlings*

Genotype	Relative Cell Length[a]	Stomatal Development[b]
Landsberg (wild type)	1.42 ± 0.12	0.95 ± 0.05 (146)
ttg-1	1.29 ± 0.15	0.74 ± 0.05 (121)
gl2–1	1.39 ± 0.11	0.79 ± 0.07 (119)

[a] Values represent the mean ratios (± SD) of the length of cells in files exhibiting *GL2::GUS* expression compared with the length of cells in non-*GL2::GUS*-expressing files in the hypocotyl epidermis. [b] Values represent the mean proportions (± SD) of stomata located in non-*GL2::GUS*-expressing cell files. Total number of stomata analyzed are shown in parentheses.

indicate a significant difference compared with the wild-type *GL2::GUS* activity (Table I; data not shown).

Taken together, analysis of the *ttg*, *35S::R*, and *gl2* showed that these factors exert a similar effect on the hypocotyl *GL2*-promoter activity as they do on the root *GL2* activity, which suggests that the same regulatory mechanism is responsible for controlling root and hypocotyl *GL2* expression.

Effect of Mutations on Cell Differentiation in the Hypocotyl Epidermis

To determine whether mutations that alter position-dependent root epidermis development also affect hypocotyl cell differentiation, the hypocotyl epidermis of the *gl2* and *ttg* mutants were examined using the *GL2::GUS* transgene as a marker of cell position. It was not possible to analyze the effect of the *35S::R* on hypocotyl cell differentiation in this manner because the *35S::R GL2::GUS* hypocotyls do not exhibit position-dependent GUS expression.

In *gl2–1 GL2::GUS* hypocotyls, a significantly greater proportion of stomata were found in GUS-expressing cells (21% ectopic stomata in *gl2* versus 5% in the wild type), although the length of the GUS-expressing cells relative to the non-GUS-expressing cells was similar to the wild type (Table III). Likewise, *ttg-1 GL2::GUS* hypocotyls possessed a greater proportion of ectopic stomata than the wild type but did not exhibit a statistically significant deviation in cell length (Table III). These alterations in stomatal patterning indicate that *TTG* and *GL2* are required to restrict stomatal development to the epidermal cells located over the anticlinal cortical cell walls of the hypocotyl.

DISCUSSION

Control of *GL2* in Root Development

A major goal of this study was to identify regulators of the homeobox gene *GL2* during root development. Using *GL2::GUS* fusions as sensitive reporters of *GL2*-promoter activity in vivo, we found that the *ttg* mutations and the *35S::R* transgene, but not the *gl2* mutations, influence *GL2* expression in the root. In the *ttg-1* and *ttg-398* mutants, a 71 and 64% reduction, respectively, in *GL2*-promoter activity was observed (Table I), which shows that maximal *GL2* expression in the root requires a functional *TTG* product. This result is consistent with the findings of a previous study showing a reduction in steady-state *GL2* RNA level in the *ttg* mutant (DiCristina et al., 1996) and provides evidence for a role for *TTG* (either directly or indirectly) in the transcriptional regulation of *GL2* during root development. Despite the reduction in *GL2*-promoter activity by *ttg* mutations, the appropriate pattern of *GL2* expression is retained in each of the three *ttg* mutant backgrounds tested (i.e. *GL2* expression occurs in differentiating epidermal cells in contact with a single cortical cell). This suggests that *TTG* is not required to specify the spatial pattern of *GL2* expression in the root, which implies that other, as-yet-unidentified patterning elements may exist.

Although the *ttg-1* mutant exhibits 29% of the *GL2*-promoter activity present in wild-type roots, this level of *GL2* expression is apparently inadequate to cause hairless cell differentiation, because root hairs are produced on essentially every root epidermal cell in the *ttg-1* mutant (Table I; Galway et al., 1994). These results imply that the inhibition of root-hair formation in atrichoblasts is sensitive to the quantity of the GL2 homeodomain protein. This supposition is supported by the previously reported finding that the *gl2* mutation displays a dosage effect in the roots; with *gl2*/+ seedlings possessing a significant proportion of ectopic root-hair cells (Masucci et al., 1996). Therefore, a major factor in appropriate epidermal cell specification in the root may be sufficient induction of *GL2* expression in atrichoblasts (by TTG and probably other genes) to ensure that hair formation is inhibited.

The GUS-expression pattern in *35S::R GL2::GUS* roots shows that the *R*-gene product is sufficient to induce expression of the *GL2* promoter in cells that normally do not exhibit detectable *GL2* activity. In particular, *GL2* expression is greatly enhanced in epidermal cells located outside anticlinal cortical cell walls (Fig. 2I), which is correlated with a change in the developmental fate of these cells (Table I; Galway et al., 1994). This implies that, in at least some cells, a protein related to the *myc*-like R product (or a protein controlled by an R-like protein) may be the limiting factor controlling *GL2* expression and root epidermal cell fate. In this regard, it is interesting that GUS activity accumulates in lateral root cap cells of the *35S::R GL2::GUS* roots, because the cells of the epidermis and lateral root cap are derived from a common initial cell in the root meristem (Dolan et al., 1993; Scheres et al., 1994). Thus, regulation of the activity of an R-like protein may be the critical factor responsible for differential activation of *GL2* in the daughter cells of the epidermis/lateral root cap initial and, more specifically, within a particular subset of epidermal cells. Additional support for the view that an R-like protein acts through *GL2* comes from the phenotype of the *35S::R gl2* line (Table I), which shows that *35S::R* requires a functional GL2 protein for its effects on root epidermis development.

It is interesting to note that a slight decrease in overall GUS activity is observed in the *35S::R GL2::GUS* roots, despite some ectopic GUS accumulation. This suggests that the cells exhibiting high levels of *GL2* expression in the wild type (differentiating hairless cells) are inhibited to some extent in their *GL2*-promoter activity in the *35S::R* background. This may be due to the titration of a transcrip-

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tion factor partner(s) required for *GL2*-promoter activity by the high-level expression of the *myc*-like bHLH maize R protein.

The analysis of the *GL2*-promoter region shows that an approximately 500-bp region at −840 to −1340 is necessary for cell-position-dependent promoter activity in the root epidermis. In addition, the effects of the *ttg* and *35S::R* factors on *GL2*-promoter activity are also dependent on the presence of this 500-bp fragment. This fragment contains a putative *myb*-binding site (TACTAACAGTATA), which opens the possibility that the TTG and/or an R-like protein may interact with (or control the activity of) a *myb*-like protein to regulate *GL2* in this region. It is interesting to note that the same 500-bp region important for *GL2*-promoter activity in the root/hypocotyl epidermis has also been found to be important for trichome and leaf primordia expression of *GL2* in the Arabidopsis shoot (D. Szymanski and M.D. Marks, unpublished data).

Control of Epidermis Development in the Arabidopsis Hypocotyl

A major finding in the present study was the discovery of position-dependent *GL2*-promoter activity and cell differentiation in the developing hypocotyl of Arabidopsis. The *GL2*-promoter preferentially directs expression to the differentiating and expanding hypocotyl cells in contact with a single cortical cell, which is the same relative position occupied by *GL2*-expressing cells in the root epidermis. Furthermore, the control of the hypocotyl *GL2*-promoter activity is similar to the control of *GL2* in the root: (a) the same 500-bp region is necessary for cell-position-specific expression (Fig. 1); (b) *GL2*-promoter activity is diminished but its spatial pattern is not affected by the *ttg* mutations (Fig. 3, K–M; Table I); (c) ectopic *GL2*-promoter activity is induced by the *35S-R* transgene (Fig. 3, N–P; Table I); and (d) *GL2*-promoter activity is not affected by the *gl2* mutations (Table I). Together, these results suggest that the expression of *GL2* is regulated in a common manner within the developing root and hypocotyl of the Arabidopsis seedling.

The position-dependent pattern of *GL2*-promoter activity is correlated with a position-dependent pattern of hypocotyl epidermal cell differentiation. Our results show that hypocotyl epidermal cells located over an anticlinal cortical cell wall (non-*GL2::GUS*-expressing cells) differ from epidermal cells located over periclinal cortical cell walls (*GL2::GUS*-expressing cells) because they are of reduced length and preferentially develop stomata (Table III). In previous studies of cell differentiation in the light-grown Arabidopsis hypocotyl epidermis, two morphologically distinct cell types were identified, with protruding epidermal cell files located outside periclinal cortical cell walls and burrowed cell files located outside anticlinal cortical cell walls (Wei et al., 1994; Gendreau et al., 1997). Together, these results show that cells of the hypocotyl epidermis, like the root epidermis, undergo position-dependent cell differentiation, which generates a common pattern of epidermal cell types in the root and hypocotyl. The identification of a common patterning mechanism for cell types within the root and hypocotyl epidermis, together with the previous finding of common radial patterning of tissue types in the root and hypocotyl (Scheres et al., 1995), provides evidence for patterning events that control development throughout the apical-basal axis of the Arabidopsis seedling.

Although stomata have been observed in the Arabidopsis hypocotyl (Reed et al., 1993; Wei et al., 1994) and the patterning of stomata in the leaf epidermis has been described (Larkin et al., 1997), the results from our study provide evidence for a previously unreported (to our knowledge) cell-position-dependent pattern of stomatal development in the hypocotyl. These results show that stomata preferentially develop in cell files located outside an anticlinal cortical cell wall (Table III). One possible reason for this pattern is that it may enable more efficient gas exchange through the apoplast. Furthermore, we find that *ttg* and *gl2* mutations significantly alter stomatal patterning in the hypocotyl, enabling a greater proportion of cells located outside periclinal cell walls to produce stomata (approximately 25% ectopic stomata in each mutant; Table III). Therefore, *TTG* and *GL2* are required to ensure that cells located outside a periclinal cortical cell wall differentiate into hairless cells in the root epidermis and differentiate into nonstomatal cells in the hypocotyl epidermis.

The difference in cell length that we have identified between hypocotyl epidermal cells in adjacent files indicates that there is a position-dependent difference in the extent of cell division during hypocotyl development. Specifically, this implies that developing hypocotyl cells located outside anticlinal cortical cell walls undergo a greater number of cell divisions than developing cells located outside periclinal cortical cell walls. This is similar to the position-dependent difference in root epidermal cell division recently reported (Berger et al., 1998) and provides further support for a close relationship between the epidermal developmental mechanisms operating in the root and hypocotyl. Because the *gl2* and *ttg* mutations do not appear to significantly alter the relative hypocotyl cell length present in the wild type (Table III), it is likely that other genes are involved in regulating this cell characteristic.

A Model for Epidermal Cell Differentiation in the Root and Hypocotyl

The similar pattern of cell types and regulation of *GL2*-promoter activity in the root and hypocotyl indicates that a common mechanism exists to influence cell differentiation in both tissues. Our results suggest a possible gene pathway for this mechanism, as illustrated in Figure 4. In this pathway, the TTG product (and, likely, other, as-yet-unidentified proteins) is proposed to be required for the activation of an Arabidopsis *R* homolog in a cell-position-dependent manner. The *TTG* gene has recently been cloned and shown to encode a WD40 repeat protein, which may mean that TTG acts by facilitating protein interactions or by activating a signal-transduction pathway that leads to transcriptional regulation (A. Walker, P. Davison, C. James, J. Esch, M.D. Marks, and J. Gray, unpublished data). The

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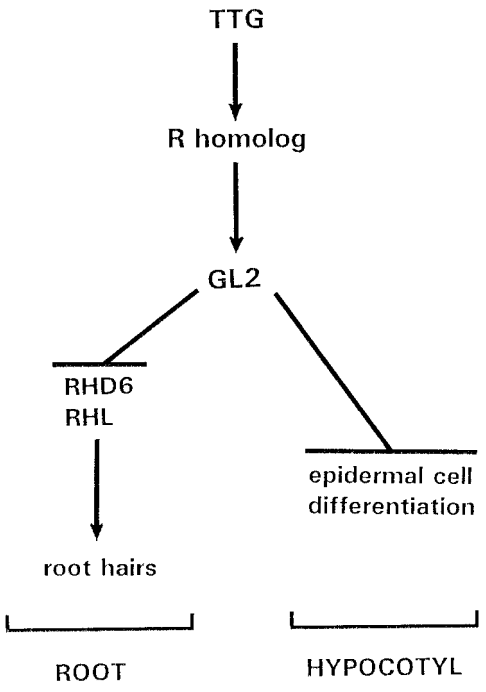

Figure 4. Proposed pathway for the regulation of cell differentiation in the root and hypocotyl of Arabidopsis. The TTG is proposed to activate an R-like bHLH protein that positively controls the transcription of *GL2*. The GL2 homeodomain protein is proposed to control root and hypocotyl epidermal cell differentiation. See text for additional discussion. Arrows indicate positive action; blunted lines indicate negative regulation. RHD6, Root hair defective 6; and RHL, root hairless.

myc-like R homolog is proposed to be a positive regulator of *GL2* transcription (Fig. 4). Subsequent cell differentiation, controlled in part by GL2, is proposed to be achieved by parallel activities in the root and hypocotyl. The GL2 homeodomain protein is required for inhibition of root-hair formation in the root epidermis, perhaps by negatively regulating the transcription of hair-promoting genes such as the *RHD6* (Masucci and Schiefelbein, 1996) and *RHL* (Schneider et al., 1997) loci (Fig. 4). In the hypocotyl epidermis, GL2 is proposed to negatively regulate genes controlling stomatal development (Fig. 4).

In the future, this proposed gene pathway is likely to be refined by the analysis of additional loci that have recently been shown to influence root epidermis development, such as the *CPC*, *ERH*, and *RHL* loci (Schneider et al., 1997; Wada et al., 1997). In particular, genetic evidence indicates that the *CPC* gene product, which contains a *myb*-like DNA-binding domain, may act as a negative regulator of *GL2* (Wada et al., 1997).

In addition to providing insight into the components and arrangement of the pathway that controls cell differentiation in the seedling epidermis, the results from this study provide clues regarding the regulation of this pathway. Because the *GL2*-promoter activity is present in a similar pattern throughout the apical-basal axis in the Arabidopsis seedling, including specific cells of the root, hypocotyl, and cotyledon, the proposed pathway may be initiated during embryo development. It is possible that a "prepattern" of *TTG/R/GL2* expression may be established at an early stage of embryogenesis when the radial pattern of cells is organized (Scheres et al., 1994), such that epidermal cells located outside periclinal cortical cell walls throughout the apical-basal axis initiate expression of this pathway.

An interesting developmental issue that is amenable to analysis in this system is the manner in which common regulatory genes are used to direct the development of different cells or tissues in a multicellular organism. As described here, the developing root and hypocotyl use common genetic components to generate a similar pattern of cell types and gene activity. In addition, the *TTG* and *GL2* genes are used to regulate trichome formation and seed coat mucilage, two other epidermal developmental processes (Koornneef, 1981; Koornneef et al., 1982; Marks, 1997). Despite these similarities, there is also evidence of variation in the control of the *TTG/GL2* pathway in different tissues. For example, the hypocotyl *GL2* expression is reduced approximately 3-fold compared with root *GL2* expression in the *ttg-1* mutant background (Table I). Therefore, despite some common components, the regulation of cell differentiation in different tissues is likely to depend on differences in the organization of the pathway, on redundancy for some of the factors in different tissues, and/or on differences in the degree of the influence of the common components. The continued analysis of the *TTG/GL2* pathway in different Arabidopsis tissues is expected to lead to a better understanding of the complex regulation of cell specification in plants.

ACKNOWLEDGMENTS

We thank James Masucci, Haiyang Wang, Ben Scheres, Fred Berger, Katharina Schneider, Liam Dolan, and Takuji Wada for helpful discussions. We acknowledge the contribution of genetic stocks by the Arabidopsis Biological Resource Center (Ohio State University, Columbus).

Received November 14, 1997; accepted January 26, 1998.
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