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of *Arabidopsis thaliana*

Fred Berger,*[,1,2] Chen-Yi Hung,†[,3] Liam Dolan,* and John Schiefelbein†

**John Innes Centre, Colney, Norwich, NR4 7UH, United Kingdom;*
and †Department of Biology, University of Michigan,
Ann Arbor, Michigan 48109

The formation of the root epidermis in *Arabidopsis thaliana* provides a simple model to study mechanisms underlying patterning in plants. In this paper we have analyzed the relationships between cell fate specification and the pattern of cell division that occur in the root epidermis. Using clonal analysis, the two cell types of the developing root epidermis, trichoblasts and atrichoblasts, were distinguished by different rates of cell division, highest in trichoblasts. This character appears to be dependent on *TTG* which controls epidermal cell fate specification. The ability of epidermal cells to undergo longitudinal divisions which are involved in the control of the radial symmetry was shown to be controlled in a cell-specific manner by *TTG.* The control of the rate and the orientation of cell division in the root meristem epidermal layer thus appear to be under the control of cell fate specification mechanisms. © 1998 Academic Press

Key Words: **Meristem; root; *Arabidopsis thaliana; ttg; gl2;* cell division.**

INTRODUCTION

Meristems are centers for the production of new cells throughout the plant life. Cells generated by meristems adopt appropriate fates and ultimately differentiate. A balance between cell production and cell differentiation can be, at least partially, achieved by controlling the rate of cell division in meristematic cells. If such controls are perturbed, the meristem may not maintain itself. This is illustrated by mutants affected for the function of the shoot apical meristem (SAM), *wuschel* (Laux *et al.,* 1996), *shoot-meristemless* (Long *et al.,* 1996) and *clavata* (Clark *et al.,* 1995, 1996; Leyser and Furner, 1992). Although the SAM and the root meristem accomplish similar functions, none of the mutants which affect SAM function affect root meristem functions (Meyerowitz, 1996). The root meristem of *Arabidopsis thaliana* presents a highly organized architecture (Dolan *et al.,* 1993). In the embryonic and young primary root meristems, concentric rings of initials generate cell files through series of anticlinal (i.e., perpendicular to the surface of the root) transverse cell divisions. Periclinal divisions, parallel to the surface of the root, create additional cell layers and are restricted to the meristematic initials. For example, epidermis and root cap layers originate from common initials (Dolan *et al.,* 1994; Scheres *et al.,* 1994) as typical for most dycotyledons (Clowes, 1994). Later in development this simple structure alters to assume a spiral pattern (Baum and Rost, 1996). Typically most divisions of daughters of the initials in each file are transverse to the main longitudinal axis of the root.

The developing root epidermis comprises two cell types, trichoblasts which form hair-bearing epidermal cells and atrichoblasts which form mature hairless cells. Those two cell types can be organized in three types of patterns among plant species (Dolan, 1996). In *Arabidopsis thaliana,* trichoblasts and atrichoblasts are organized in interspersed cell files which originate from transverse divisions of 16 alternating trichoblast and atrichoblast initials (Dolan *et al.,* 1993; Dolan and Roberts, 1995). Maintaining this simple architecture is likely to involve a tight control of the orientation and rate of cell division. It has been reported that trichoblasts are shorter cells than atrichoblasts (Dolan *et al.,* 1993) which gave us an indication that the number of cells in each file is different, which in turn suggested that the rate of cell cycle in each cell type is different. In addition, a limited number of anticlinal longitudinal divisions occur in the developing epidermis and generate more cell files (Baum and Rost, 1996). We show that these divisions

[1] To whom correspondence should be addressed at present address: RDP, ENS-Lyon, 46 allée d'Italie, 69364 Lyon cedex 07, France. Fax: 33472728600. E-mail: frederic.berger@ens-lyon.fr.
[2,3] These authors have contributed equally to this work.

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create clones of adjacent trichoblasts and atrichoblasts files. The analysis of such clones allowed us to determine that each cell type does not divide at the same rate and that the orientation of the cell plane is differentially regulated between the two cell types. The mutants *transparent testa glabra* (*ttg*) and *glabra2* (*gl2*) have been shown to form root hairs in ectopic position (Galway *et al.,* 1994; Masucci *et al.,* 1996) and it has been proposed that both genes control cell fate specification of trichoblasts versus atrichoblasts. *TTG* is epistatic to *GL2* (Korneef, 1981) and *TTG* but not *GL2* was reported to affect early differential characters of epidermal cells such as cytoplasmic density and cell length (Galway *et al.,* 1994; Masucci *et al.,* 1996). Moreover, *ttg* mutation causes in some roots some disruption of the root meristem (Galway *et al.,* 1994). We have shown, using clonal analysis, that *TTG* but not *GL2* controls the rate and the orientation of cell division in the root meristem epidermis. Those results provide a link between cell fate specification and the control of cell division in plants.

MATERIALS AND METHODS

Plant Material

The wild-type (WT) *A. thaliana* used were Columbia, Landsberg *erecta,* and WS. The *gl2* and *ttg* mutants in Landsberg *erecta* genetic background were obtained from the *Arabidopsis* Biological Resource Center (Ohio State University) and the mutant *ttg* and *35S::R* transformant lines in WS genetic background have been described earlier (Galway *et al.,* 1994). The transgenic line containing the construct p*GL2–GUS* was obtained as previously described (Masucci *et al.,* 1996).

Plant Growth Conditions

Seeds were sterilized in 5% sodium hypochlorite and vernalized on growth medium at 4°C in the dark for 2–3 days. Growth medium was 0.3% gelrite, 1% sucrose in half strength Murashige and Skoog medium at pH 5.8. Measurements were made at 3 to 4 days after germination. Plants were grown under light under sterile conditions in small chambers made from 35-mm petri dishes in the top of which a hole was bored and filled with a coverslip. Roots were growing at 45° through the culture medium and along the coverslip and were amenable to microscopic observations without disturbance.

Root Epidermis Cell Mapping by Confocal Microscopy

Seedlings were incubated for 60 min in 10 μg/ml propidium iodide (Sigma) solution in growth medium to stain the intercellular space (van den Berg *et al.,* 1995). Optical sections were obtained on live roots using a confocal microscope (Bio-Rad, MRC 1000) with the 488-nm excitation line and a 585LP barrier filter. Images were processed using NIH image and Photoshop (Adobe) software. The identity of cell files in the meristem was established by their position relative to the cortical anticlinal wall. Cell files overlying this latter position were deemed trichoblasts files and the other files were deemed atrichoblasts files.

Root Epidermis Cell Mapping Using GL2 Promoter GUS Fusions

Seedlings from the line transformed with the *pGL2–GUS* construct were stained for β-glucuronidase as described earlier (Masucci *et al.,* 1996) and viewed using DIC optics with a Zeiss Axiophot microscope.

Clonal Analysis

Longitudinal anticlinal divisions are relatively rare events in the root epidermis and lead to clones of epidermal cells which are easily recognized on confocal sections of the root epidermis stained with propidium iodide (Fig. 2). Clones were individually drawn and the position of each file relative to the anticlinal cortical cell wall (ACCW) was determined. The number of cells in the designated trichoblast and atrichoblast files was measured. The probability of occurrence of a longitudinal division (pL) was established as follows. On average, four cell files per root were observed. There are more atrichoblast files than trichoblast files and we estimated the proportion of each in the various backgrounds. There are 8 trichoblast files and on average 10, 12, and 14 atrichoblast files respectively in WS, Landsberg *erecta,* and Columbia backgrounds (Galway *et al.,* 1994; Massuci *et al.,* 1996). Hence we could estimate the total number of files of each cell type for the total number of roots that we had observed. We measured the average total number of trichoblasts and atrichoblasts per file in the elongation and division zones in each background (Table 1). Multiplied by the total number of files, this gave an estimate of the total population of cells which was screened for the clonal analysis. Each clone originated from a longitudinal anticlinal division. The total number of epidermal clones which were detected corresponded to the total number of longitudinal anticlinal divisions which had taken place in the total population of H and N cells that we observed. An estimate of the probability pL of the occurrence of such division is the ratio between the total number of longitudinal anticlinal divisions ant the total number of cells.

RESULTS

Arabidopsis Trichoblast Files Contain More Cells Than Atrichoblast Files

Using confocal sections obtained from live *Arabidopsis* roots stained with the fluorescent dye propidium iodide, precise cellular maps of the epidermis were obtained. Four developmental regions were defined in WT seedling roots: the division zone, the slow and fast elongation zones, and the differentiation zone (Fig. 1, Table 1). Epidermal initials were recognized as cells terminating epidermal cell files. Those cells were longer than wide and were more elongated than their daughters. Above the initial and for the most part lying under the root cap layers was a region of small cells which were wider (23.8 μm; SEM = 2.57; n = 30) than long (9.4 μm; SEM = 0.29; n = 70) and were dividing actively. This zone defined as the ‘‘division zone’’ contained 15 to

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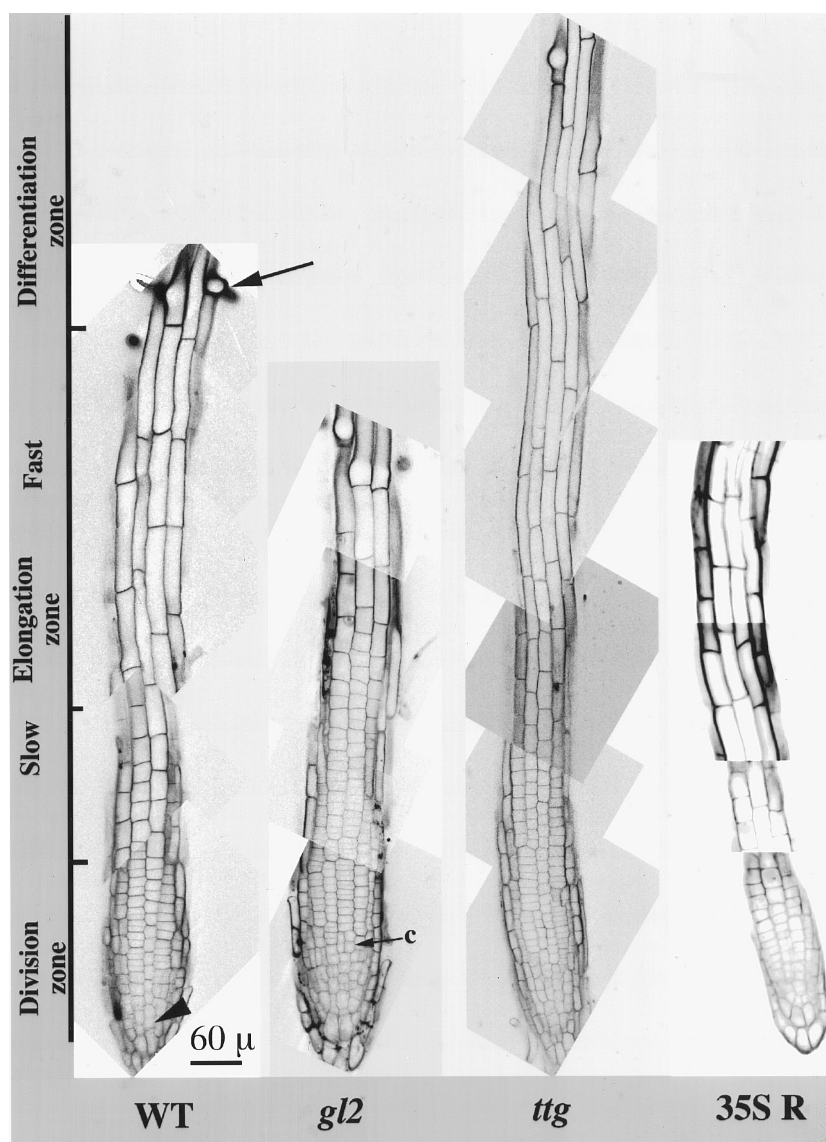

FIG. 1. Longitudinal structure of the epidermis of the root tip in wild type and mutants altered in epidermal cell patterning. Montage of confocal sections of epidermis in WT, mutants *ttg* and *gl2,* and the transformant *35S*::*R.* The WT root is divided into four developmental zones (left). Trichoblasts file can be followed from the differentiation zone where root hairs are formed (arrow). Epidermal files terminate at the position of epidermal initials (arrowhead). Clones of epidermal cells resulting from a longitudinal anticlinal division can be observed (c).

25 trichoblasts depending on the wild-type ecotype. The division zone was shown to be followed by a zone where cell length increases discontinuously. We observed that epidermal cells experienced an initial small increase in cell length in a zone that we defined as the ''slow elongation zone'' comprising 5 to 8 trichoblasts and further elongated rapidly in the ''fast elongation zone'' comprising 4 to 6 trichoblasts. Although we did not performed a direct measurement of cell elongation rate, we thought that the measurement of static cell shape gave a reliable estimate of the differential speed of events. Eventually elongation terminated and epidermal cells reached the ''differentiation zone'' where trichoblasts differentiated root hairs. This longitudinal organization of the root tip was determined for 3- to 4-day-old primary roots and was well conserved under our growth conditions.

Root epidermis cell files in *Arabidopsis* comprise either trichoblasts or atrichoblasts and regularly alternate. It has been shown that hair cell files overly the longitudinal anticlinal cell walls of the underlying cortical cells (Bünning, 1951; Dolan *et al.,* 1993). This was used in this study as a criterion to define trichoblasts in wild-type and mutant

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TABLE 1
Longitudinal Organization of the Epidermis in the Root Tip of Various Genetic Backgrounds

Genotype	WT Columbia	WT L. *erecta*	WT WS	*gl2* (L. *erecta*)	*ttg* (L. *erecta*)	*35S::R* (WS)
Ratio T/A	1.32	1.36	1.30	1.26	1.09	1.07
SEM	0.02	0.02	0.04	0.04	0.01	0.01
n (T/A)	70	105	51	60	59	212
Relative length of the cell division zone (%)	58.2	64.4	72.0	71.5	68.8	66.8
Relative length fo the slow elongation zone (%)	25.3	20.3	14.0	21.7	15.6	19.3
Relative length of the fast elongation zone (%)	16.5	15.2	14.0	6.8	15.6	13.9
Total length	28.5	31.5	35.7	38.2	37.2	30.1
(SEM)	1.6	2.0	2.0	1.8	2.5	4.5
n (mapping)	35	28	31	28	36	30

Note. The ratio T/A corresponds to the total number of trichoblasts in the area below the first differentiated cell and above the initial divided by the total number of atrichoblasts in the same area. The lengths are determined in number of cells and the contribution of each developmental zone was determined as a relative number.

backgrounds. The total number of cells present between the differentiation zone and the initials was determined in trichoblast (T) files and in atrichoblast (A) files. In all three wild-type ecotypes examined, the ratio of cell number trichoblasts to atrichoblasts (T/A) was approximately 1.3 (Table 1). This showed that T files contain more cells than A files and suggested that the ratio T/A is a marker of the relative fate specification between trichoblasts and atrichoblasts.

Measurement of the ratio T/A was also performed in defined consecutive segments along the root tip in WS wild type (Table 2). The ratio was relatively invariant in the different parts of the root though markedly lower in the most-basal segment near the initials. This suggested that the control of T/A ratio is exerted early in development and then maintained until cell differentiation.

The Role of TTG and GL2 on the Ratio T/A

To assess the genetic control of the ratio T/A we measured this ratio in mutants known to affect epidermal cell fate identity. The mutations *ttg* and *gl2* cause a higher density of root hairs and the development of ectopic root hair cells (not positioned over the cortical anticlinal cell wall) (Galway *et al.*, 1994; Masucci *et al.*, 1996). *GL2* encodes an homeodomain protein (Reerie *et al.*, 1994), whereas *TTG* has not been cloned. However, *ttg* mutants can be rescued by the transformation with the gene *R* from maize under the control of the 35S promoter (Lloyd *et al.*, 1992; Galway *et al.*, 1994). Although it is likely that *TTG* does not encode an *R*-like protein, 35S::*R* transformants have roots deprived of root hairs and further analysis has shown that early fate specification is altered in trichoblasts files (Galway *et al.*, 1994). Thus those plants could be analyzed as overexpressers of TTG function. None of the genetic backgrounds, *gl2, ttg,* and *35S*::*R* were shown to affect the relative length of each meristematic zones (Fig. 1; Table 1). Remarkably, the ratio T/A was much reduced in *ttg* mutants and *35S::R* transformed plants but not in *gl2* mutants (Table 1).

The ratio T/A is directly related to the relative cell length between the two cell types. The alteration in relative cell length present in the *ttg* mutant could be compensated by

TABLE 2
Ratio of the Cell Number T/A in Consecutive Segments along the Root Tip

Developmental zones	Fast elongation	Slow elongation	Cell division				Initials
Segment Boundaries (relative cell number, %)	100	85.2	64.3	51.2	34.6	17.1	0
Ratio T/A	1.36	1.36	1.26	1.50	1.44	1.16	—
(SEM)	0.20	0.20	0.23	0.32	0.29	0.21	

Note. The boundaries of each segment are given as a relative length expressed in number of epidermal cells.

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TABLE 3
Geometrical Characteristics of Root Epidermal Cells in WT and *ttg*

	Relative cross-sectional area T/A	Relative cell length T/A	Relative cell volume T/A
Wild-type WS	1.32	0.68	0.90
SEM	0.08	0.11	
ttg (WS)	1.29	0.86	1.11
SEM	0.12	0.12	

Note. The relative area of cross-sectional area of each cell type in the division zone was measured together with the relative cell length which yielded the relative volume between trichoblasts and atrichoblasts.

changes in another dimension to maintain a constant cell volume. To test this possibility we measured average cross-section areas and cell lengths in the division zone in *ttg* mutant and corresponding wild-type roots (Table 3). In *ttg* mutants, the average cell length in the division zone was very similar for cells present at positions corresponding to trichoblast and atrichoblast fate (respectively 9.1 μm; SEM =0.3; $n = 101$ and 10.5 μm; SEM = 0.3; $n = 95$) and was comparable to the length of trichoblasts in the wild type (10.95 μm; SEM = 0.2; $n = 87$; atrichoblast length is 16.5 μm; SEM = 0.6; $n = 92$) (Fig. 1). The relative cross-sectional areas between trichoblasts and atrichoblasts were of similar values between the wild type and *ttg* (Table 3). Hence *ttg* was shown to affect specifically the relative cell length, i.e., the relative difference in cell number between the two cell types which is expressed by the ratio T/A. This character thus appeared to be a marker for cell fate specification in the root epidermis.

In conclusion, global measurements of cell numbers showed that trichoblast files contain more cells than atrichoblast files and that this relative difference as measured by the ratio T/A is under the control of *TTG.* This suggests that cell division times in A and T cells are different. However, since the cell number measurements included elongating cells which have stopped their cell division, we could not precisely calculate the difference in cell division rate. To address this issue, clones of epidermal cells were detected and used to precisely monitor this parameter during root development in various genetic backgrounds.

Epidermal Cell Fate Specification Involves a Differential Control of the Cell Division

Root epidermal cells typically undergo several rounds of anticlinal transverse cell divisions; i. e., divisions are both transverse to the long axis of the root and perpendicular to its surface. However, some anticlinal longitudinal divisions were occasionally observed on confocal sections taken in the division zone (Figs. 1 and 2A). When followed, both daughter cells were observed to divide transversely, hence creating two files of cells of clonal origin. Those clones could be readily recognized on confocal sections (Figs. 1 and 2B–2E). Because trichoblasts are always positioned over anticlinal cell walls (ACCW) of the underlying cortex layer, longitudinal anticlinal divisions in trichoblasts would generate one daughter cell located over the ACCW and another daughter cell over the periclinal cortical cell wall (PCCW). We observed that in such clones, the cell overlying the ACCW would generate a file which contains more cells than the neighbor cell file located over the PCCW. As shown by measurements on entire cell files this relative higher cell number was characteristic of trichoblasts. Hence, each clone comprised one T file and one A file. The relative number of cells in each file was the direct consequence of the number of transverse cell divisions undergone by each cell initially separated by a longitudinal anticlinal division. Hence, the ratio T/A would reflect directly the difference in rates of cell division between the two epidermal cell types.

A total of 200 to 250 clones were analyzed in WT roots in Columbia, WS, and Landsberg backgrounds. Clones were divided into classes corresponding to the maximum number of cells in trichoblast position expected for a given number of cell divisions including the initial longitudinal division. For example, the class 4 corresponded to clones containing 5 to 8 trichoblasts (Fig. 2D). The longest clones observed in the WS and Columbia genetic backgrounds contained 32 trichoblasts. The Landsberg *erecta* background presented occasional clones containing up to 48 trichoblasts (class 7).

For each clone the ratio T/A was calculated. The average ratio T/A for all clones classes was less than 1.5, similar to the average ratio T/A measured for whole roots (Table 1) and no clones exhibited T/A > 2. This meant that the difference in the rate of cell division between trichoblasts and atrichoblasts is not large enough to be expressed in all clones by a difference in cell number after one round of cell division. We observed that as clone size increased, the ratio T/A increased (Fig. 3) together with the percentage of clones exhibiting a different number of cells (Fig. 4). When more than 3 cell divisions had taken place in the trichoblast file, an unequal cell number was observed in most clones between the two files (Figs. 2 and 4). At this stage, the ratio T/A reached an average maximum value of approximately 1.5 and remained stable during further divisions (Fig. 3). This showed that, on average, trichoblasts accomplish their cell division 1.5 times faster than atrichoblasts and that, within clones, this difference is expressed after more than 3 cell divisions.

The Relationships between the Control of Cell Division, TTG, and GL2

A comparative analysis of epidermal clones was carried out in the mutants *gl2* and *ttg* and in the transformants *35S::R* to define precisely how those genes control the cell division rate. In all genetic backgrounds 3 cell divisions were necessary to observe a maximal ratio T/A. The ratio

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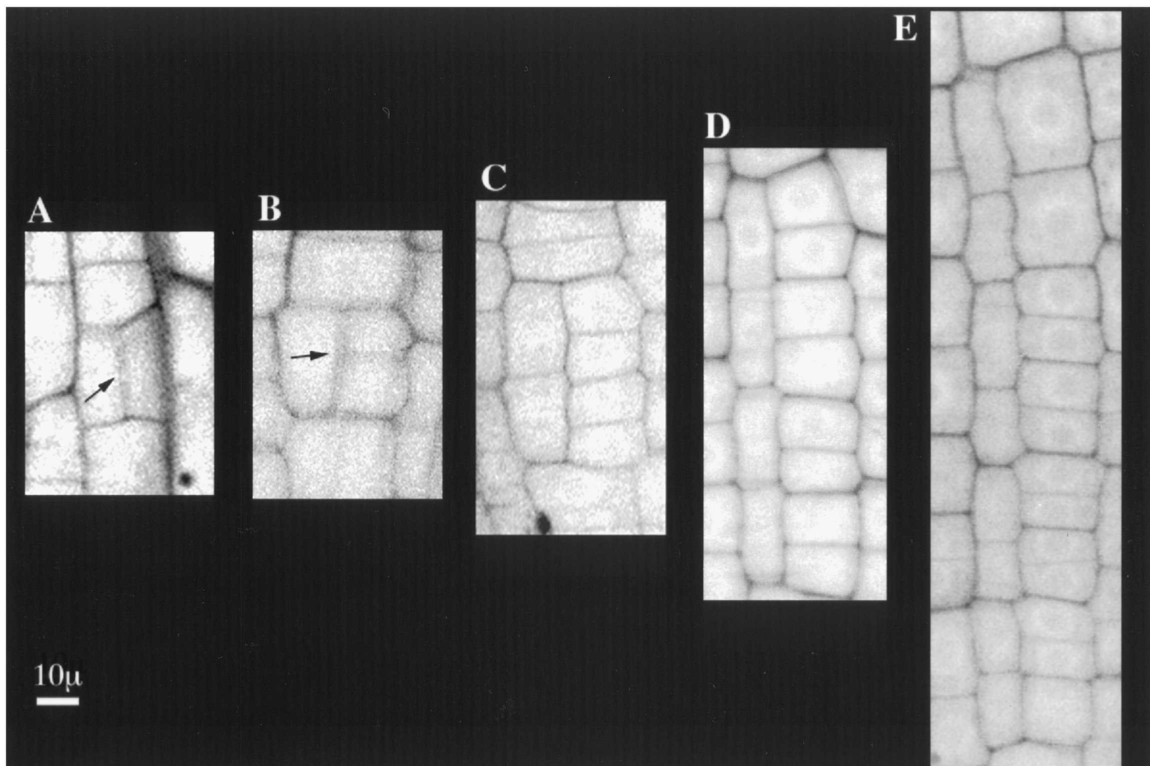

FIG. 2. Clones of epidermal cells. Those clones originate from a trichoblast which divides longitudinally (A, arrow) and undergoes further transverse divisions (B, arrow; C–E). As a result, the number of cells in each file is different (B–E). Clones presented in A–E belong to classes 1 to 5, respectively.

T/A measured for clones belonging to the largest classes 4–6 was not affected in *gl2* mutants but was much reduced in both *ttg* mutants and *35S*::*R* transformants (Fig. 3). Thus, TTG but not *GL2* appears to control the rate of cell division in epidermal cells.

To further examine the relationship of the *GL2* gene to cell division control, GL2 expression was followed using a line transformed with the gene coding for β-glucuronidase

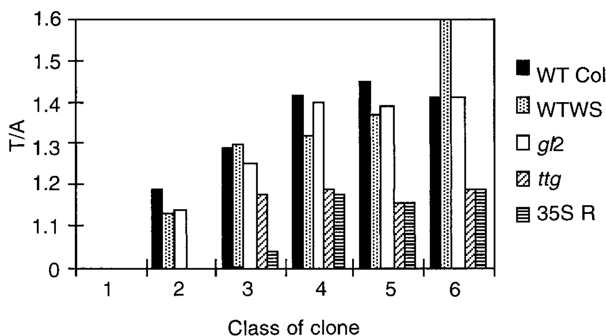

FIG. 3. Expression of the fate marker T/A during development of epidermal clones. The average ratio between the number of trichoblasts and atrichoblasts (T/A) was calculated for each class of epidermal clones sampled in roots of wild types Columbia and WS, mutants *gl2* and *ttg,* and *35S*::*R* transformants. The number for each class corresponds to the maximum number of cell divisions experienced by the trichoblast (the mother cell of the clone).

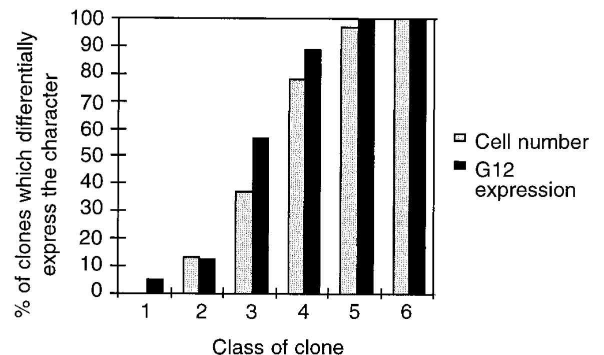

FIG. 4. Comparative expression of two markers of epidermal fate in epidermal clones: the differential cell number and the expression of *GL2* monitored with a *GUS* reporter gene. Each marker was scored for individual clones belonging to different classes.

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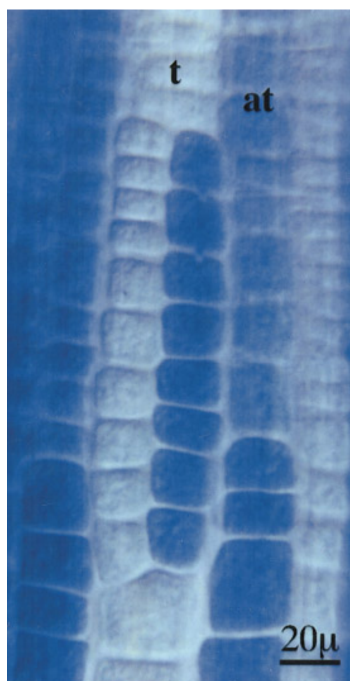

FIG. 5. Differential expression of the *GL2* in an epidermal clone. The expression of a *GUS* reporter gene under the control of the *GL2* promoter shows that, in an epidermal clone, *GL2* is expressed in the file which contains the smaller number of cells.

under the control of the *GL2* promoter (Masucci *et al.,* 1996). It has been shown previously that *GL2* is preferentially expressed in atrichoblasts rather than in trichoblasts and the difference in expression between trichoblast and atrichoblast files is noticeable as early as the cell at position 3 above the initial (Masucci *et al.,* 1996). *GL2* expression as reported by GUS expression was recorded in WT clones produced in trichoblast files. GUS staining was not observed in cells belonging to the trichoblast position in any of those clones (Fig. 5, Table 5). GUS expression was observed in atrichoblast files for 64% of clones including all clones belonging to classes 5 and 6 (Fig. 4).

To compare the control of *GL2* expression with the regulation of cell division between trichoblasts and atrichoblasts we determined the percentage of clones with differential GUS expression (under the control of the *GL2* promoter) together with the percentage of clones exhibiting different cell number in both files (Fig. 4). Differential GUS expression was observed in a higher proportion of clones than differential cell number. This suggested that the control of *GL2* expression was taking place or was expressed relatively earlier than the control of the cell division rate. We searched for clones exhibiting the same cell number in both files (T/A = 1) and a differential GUS stain. We found 18 clones belonging to classes 2 to 4 which exhibited such characteristics (Table 4). Moreover two class 1 clones with differential *GL2* expression were observed. In contrast, clones with a ratio T/A superior to 1 but exhibiting no differential *GL2* expression were rare (Table 4) and most of them were located within cells close to the initial where staining for GUS in normal files was not to be always observed. This suggested that the control of cell cycle rate and the control of GL2 expression are independent of each other and that GL2 differential expression takes place relatively earlier.

The Orientation of Cell Division Is Cell Specific and Is under the Control of TTG but Not of GL2

We have shown that the fate of root epidermal cells is linked to the control of the rate of transverse anticlinal cell divisions by *TTG.* We investigated whether this gene also controlled the orientation of the cell division plane. The large number of clones analyzed allowed us to estimate the probability of occurrence of a longitudinal anticlinal division per cell (pL). In WT ecotypes approximately 90% of the clones were observed in trichoblast files. The probability pL showed that approximately 1 of 30 trichoblasts underwent a longitudinal division (Table 5), whereas this event is up to 20 times less frequent in atrichoblasts. This was reflected by the ratio of probabilities (pLT/pLA). These probabilities were not affected by the *gl2* mutation (Table 5). In contrast, pLT/pLA was much reduced in *ttg* mutants and *35S::R* transformants which indicates that pL is a characteristic of fate specification between the two cell types. This also indicates that *TTG* but not *GL2* controls the occurrence of longitudinal divisions, which are characteristically more frequent in trichoblasts.

DISCUSSION

Plant meristematic activity is considered to be iterative since meristems reproduce continuously the same pattern. In those areas where there is an intense activity of cell production, the structure and division patterns must be conserved. In root meristems as precisely organized as *Arabidopsis,* this implies that the rate of cell division and the orientation of the cell plate are tightly controlled. Such controls have been shown to be associated with the definition of appropriate cell fate in the root meristem initials (van den Berg *et al.,* 1995; Di Laurenzio *et al.,* 1996). We report that cell division rate and orientation is tightly controlled in the epidermis and that this control is dependent on *TTG,* a gene which has been shown previously to be involved in cell fate specification (Galway *et al.,* 1994). This illustrates how in plants cell division can be part of a cell fate specification program and not instrumental in its definition.

The Regulation of Transverse Cell Division Is Not Responsible for Cell Fate Specification but Accounts for the Control of Cytological Differentiation of Epidermal Cells

We have demonstrated that the cell division rate accounts for differences in size between trichoblasts and atrichoblasts

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TABLE 4
The Relative Position of the Fate Markers T/A and Differential *GL2* Expression in Epidermal Clones

	Clones exhibiting differential expression of *GL2* and T/A > 1	Clones exhibiting differential expression of *GL2* and T/A = 1	Clones which do not exhibit differential expression of *GL2* and T/A > 1
Total number	163	20	8
Distribution among size classes	2 to 5	1 to 4	2 to 3

which had been previously reported (Dolan *et al.,* 1994). The two cell types have been distinguished by other characters. Once differentiated they possess different ornaments in their cell walls (Dolan *et al.,* 1994). Earlier in development, atrichoblast cell walls are specifically recognized by the monoclonal antibody CCMC-M2 directed against an RG-1 epitope (Freshour *et al.,* 1996). Moreover, in the division zone, the two cell types are readily recognizable by inversely correlated degrees of vacuolation and cytoplasmic density (Dolan *et al.,* 1994). Trichoblasts have the densest cytoplasm and the highest rate of cell division. Those two parameters are probably linked since vacuolation develops as soon as elongation starts. Another character recently recognized is the differential organization of the splicing machinery between the two cell types. Coiled bodies are nuclear organelles where splicing factors concentrate (Lamond and Carmo-Fontseca, 1993). Trichoblasts appear to contain more and smaller coiled bodies than atrichoblasts (Boudonck *et al.,* 1997). This could be directly correlated to the difference in cell division rate and could also reflect differential transcriptional activities. In conclusion, the difference in cell division rate between the two epidermal cell types may control a number of cytological characters typical of cell fate differentiation.

The control of the cell division appears to be a relatively late character since it takes on average three divisions until a difference in cell number is observed in clones. Therefore, this criterion may simply represent a consequence of cell fate specification rather than a cause. This interpretation is in agreement with the results concerning the control of the ratio T/A by *GL2* and *TTG.*

The expression of *GL2* is typical of atrichoblast fate (Masucci *et al.,* 1996) and we wanted to establish the relationships between the control of the cell division and *GL2* and *TTG* functions. The observation of clones formed in trichoblasts enabled us to show that, on average, differential *GL2* expression is observed earlier than the difference in cell division rate. In *gl2* mutants, however, the ratio T/A is not affected on the whole root nor in clones which shows that cell division rate is not under the control of *GL2.* Together those results demonstrate the independence of the controls of *GL2* expression and of the cell division rate. This is in agreement with earlier observations showing that vacuolation and cell size of root epidermal cells are not affected in *gl2* mutants (Masucci *et al.,* 1996). In contrast, *ttg* mutations affect those characters (Galway *et al.,* 1994). We have shown that trichoblasts accomplish their cell division more rapidly than atrichoblasts and this difference is *TTG* dependent. *TTG* thus is involved in controlling the rate of cell division in the root epidermis which appears to be one of many characters of trichoblast/atrichoblast fate specification.

TTG Is Involved in the Control of the Circumferential Architecture of the Root Epidermis

Arabidopsis radial structure depends on the maintenance of longitudinal periclinal division of the endodermis/cortex

TABLE 5
Distribution and Probabilities of Occurrence of a Longitudinal Anticlinal Division Which Generate an Epidermal Clone

Genotype	WT Columbia	WT WS	*gl2*	*ttg*	*35S::R*
Number of clones analyzed	240	201	181	215	201
% clones in A files	6.2	10.2	9.9	21.9	17.4
Probability of occurrence of a longitudinal division in a T cell (pLT)	0.042	0.033	0.028	0.031	0.013
Probability of occurrence of a longitudinal division in a A cell (pLA)	0.0021	0.0038	0.0029	0.0093	0.0030
pLT/pLA	20.0	8.7	9.6	3.4	4.3

Note. Measurements were carried out in wild-type Columbia and WS, and in *ttg, gl2,* and *35S::R* mutants in a WS genetic background where the identity of epidermal cells is not correctly specified.

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and of the epidermis/root cap initials. The orientation of this type of division appears to be specifically controlled. When the control breaks down, as in the mutant *scarecrow* where the periclinal division of the endodermis/cortex initial does not take place, a single cell layer is formed with characteristics of both endodermis and cortex cell types (Di Laurenzio *et al.,* 1996). The root epidermis offers the unique opportunity to study the control of longitudinal anticlinal divisions. Those divisions control the number of cell files per cell layer in the mature root. In the embryo those divisions separate the 8 epidermal initial precursor cells into 16 cells positioned alternatively over the anticlinal and periclinal cortical cell walls (Scheres *et al.,* 1994, 1995) which have been shown to be linked with trichoblast/atrichoblast fate specification (Dolan *et al.,* 1993). We have observed in this study that supernumerary longitudinal anticlinal divisions create clones of two cell files which adopt different fate. Anticlinal longitudinal divisions which cause file duplication have been reported earlier (Baum and Rost, 1996). In the differentiation area 18 to 22 epidermal cell files are usually observed. This implies that file duplication occurs and can be accounted for by anticlinal longitudinal divisions forming clones of cells. An increase of the number of epidermal initials (from 16 to 22) has also been reported in 2-week-old roots (Baum and Rost, 1996).

The probability of occurrence of longitudinal anticlinal divisions is relatively low but markedly higher in trichoblasts than in atrichoblasts. This shows that most clones and file duplications originate from trichoblasts. This is altered in *ttg* and *35S::R* backgrounds which respectively cause the development of ectopic trichoblasts and atrichoblasts. It is difficult to explain why trichoblasts tend to divide longitudinally more frequently than atrichoblasts. The former are wider and shorter than the latter and an explanation based on the geometry and physical constraints can be proposed. However, in *ttg* mutants, although pL is altered, the relative cross-sectional area between the two cell types does not change. Thus, the production of the longitudinal walls is not associated with physical constraints linked to the geometry of the cells and more specific controls must be invoked. *TTG* is thus a gene required for a specific cell division to occur in a specific cell type.

TTG Is a Master Gene Involved in the Control of Root Epidermal Development

In *ttg* mutants, trichoblasts divide at the same rate as in wild type and their pL is not affected, whereas atrichoblasts divide more quickly and have a higher pL than in wild type. Conversely, when the regulatory pathway controlled by *TTG* is strongly activated in *35S::R* background, atrichoblasts are not affected and trichoblasts exhibit more atrichoblast-like characters. If *TTG* were an activator of atrichoblast fate, *35S::R* transformants should be characterized by a much shorter division zone and atrichoblasts should present a pL inferior to normal values. This was not observed and therefore our data demonstrate that *TTG* negatively controls trichoblast cell fate in atrichoblasts. Consequently, it can be hypothesized that trichoblast fate represents a default state.

TTG thus appears to be the earliest point of control of root epidermis fate specification known to date. This correlates with its role in the control of trichome development (Hülskamp *et al.,* 1994; Larkin *et al.,* 1994, 1996, 1997) where it appears to occupy the top of a cascade of events controlled by various genes.

In conclusion, we propose the following model for the action of *TTG* and *GL2* in root epidermis development. *TTG* negatively controls trichoblast fate and the integrity of the structure of the root cap/epidermis lineage. *TTG* function leads to a slower cell division rate in atrichoblasts which in turn causes a lower number of coiled bodies, a higher degree of vacuolation, and an increase in cell length. In parallel, *TTG* would antagonize the production of anticlinal longitudinal production which is responsible for cell file duplication. Independently of its control on cell division, *TTG* would positively regulate *GL2* expression. *gl2* mutants are characterized by the absence of trichome development and it has been postulated that *GL2* is involved in the control of morphogenesis (Hülskamp *et al.,* 1994). In the root epidermis, *GL2* is preferentially expressed in atrichoblasts where it would repress root hair morphogenesis and control cell wall composition.

Cell Division and Cell Fate Specification in Plants

Cell division and cell fate have been reported to be relatively independent from each other in plants. *Arabidopsis* transformants which overexpress ectopically the mitotic cyclin cyc1At contain more cells than wild types but do not present a significant alteration in cell size and overall structure of root meristems (Doerner *et al.,* 1996) and dominant negative transformants for the cyclin kinase CDC2A which causes a slower cell division rate have larger cells but present an overall normal organization of tissues (Hemerly *et al.,* 1995). This suggests that cell division does not control cell fate in plants. Rather, domains of positional information may be established and divided into smaller units by cell divisions. This hypothesis is consistent with the phenotypes observed in the mutants *fass* and *tangled.* The *Arabidopsis* mutant *fass* where cytokinesis is disturbed exhibits a correct organization of the radial and apical–basal axes in the embryo (Torres-Ruiz and Jürgens, 1994). Leaf development is normal in *tangled* maize leaves in which the plane of cell division is altered (Smith *et al.,* 1996).

However, in specific cases, cell division has been shown to be a key feature in the control of cell fate specification. Mutations in the genes *SCARECROW* (Di Laurenzio *et al.,* 1996) and *SHORTROOT* (Scheres *et al.,* 1995) are characterized by the absence of the division which separates the initials for the cortical and the endodermis layers in the root. Roots in *scarecrow* possess only one ground tissue layer which expresses both markers for cortical and endodermal identity. Those markers can be segregated when a

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FASS mutation which results in supernumerary cell layers is introduced (Scheres *et al.,* 1995). This suggests that *SCARECROW* specifically controls the production of the longitudinal division in the cortex/endodermis initial in the root meristem and that this division allows the correct segregation of cell fate. This however might represent a rather exceptional case.

In our study we show an example of the third class of possible relationships where cell fate specification controls the rate and orientation of cell division which is a fairly general mechanism in animal development (Follette and O'Farrell, 1997; Cohen, 1996; Schnabel, 1996). In the root epidermis, this is achieved at least by *TTG.* Studies of the *STM* and *CLAVATA* mutants (Clark *et al.,* 1996, Meyerowitz, 1996) have provided examples of controls of cell proliferation in the shoot apical meristem. This type of relationship between fate specification and cell proliferation may be characteristic of meristems in plants. This idea is in agreement with the fact that mutants for meristematic activity present an embryonic phenotype. Thus the basis of meristematic activity is likely to be laid down during embryogenesis. The mechanisms involved in those processes remain very poorly defined and are likely to involve positional information. The clonal analysis performed in this study strongly suggests that the fate of the cells in each newly created file in a clone is dictated by positional information. This is expressed by the differential expression of *GL2* and by the difference in division rate between each cell file of a clone. We are currently studying further the role and origin of positional information in root epidermis development using clonal analysis and cell ablation experiments.

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REFERENCES

Barlow, P. W. (1976). Towards an understanding of the behaviour of root meristems. *J. Theor. Biol.* **57,** 433–451.

Baum, S. F., and Rost, T. L. (1996). Root apical organization in *Arabidopsis thaliana.* 1. Root cap and protoderm. *Protoplasma* **192,** 178–188.

Boudonck, K. J., Shaw, P. J., and Dolan, L. (1997). Cell differentiation in the *Arabidopsis* root epidermis is accompanied by changes in number and size of coiled bodies. In press.

Bünning, E. (1951). Uber die Differenzierungsvorgänge in der Cruciferenwurzel. *Planta* **39,** 126–153.

Clark, S. E., Jacobsen, S. E., Levin, J. Z., and Meyerowitz, E. M. (1996). The *CLAVATA* and *SHOOT MERISTEMLESS* loci competitively regulate meristem activity in *Arabidopsis. Development* **122,** 1567–1575.

Clark, S. E., Running, M. P., and Meyerowitz, E. M. (1995). *CLAVATA3* is a specific regulator of shoot and floral meristem development affecting the same processes as *CLAVATA1. Development* **121,** 2057–2067.

Clowes, F. A. L. (1994). Origin of the epidermis in root meristems. *New Phytol.* **127,** 335–347.

Cohen, S. M. (1996). Controlling growth of the wing: Vestigial integrates signals from the compartment boundaries. *BioEssays* **18,** 855–858.

Di Laurenzio, L., Wysocha-Diller, J., Malamy, J. E., Pysh, L., Helariutta, Y., Freshour, G., Hahn, M. G., Feldmann, K. A., and Benfey, P. N. (1996). The *SCARECROW* gene regulates an asymmetric cell division that is essential for generating the radial organization of the *Arabidopsis* root. *Cell* **86,** 423–433.

Doerner, P., Jorgensen, J. E., You, R., Steppuhn, J., and Lamb, C. (1996). Control of root growth and development by cyclin expression. *Nature* **380,** 520–523.

Dolan, L. (1996). Pattern in the root epidermis: an interplay of diffusible signals and cellular geometry. *Ann. Bot.* **77,** 547–553.

Dolan, L., Duckett, C. M., Grierson, C., Linstead, P., Schneider, K., Lawson, E., Dean, C., Poethig, S., and Roberts, K. (1994). Clonal relationships and cell patterning in the root epidermis of *Arabidopsis. Development* **120,** 2465–2474.

Dolan, L., Janmaat, K., Willemsen, V., Linstead, P., Poethig, S., Roberts, K., and Scheres, B. (1993). Cellular organisation of the *Arabidopsis thaliana* root. *Development* **119,** 71–84.

Dolan, L., and Roberts, K. (1995). The development of cell pattern in the root epidermis. *Philos. Trans. R. Soc. London Ser. B Biol. Sci.* **350,** 95–99.

Follette, P. J., and O'Farrell, P. H. (1997). Connecting cell behaviour to patterning: lessons from the cell cycle. *Cell* **88,** 309–314.

Freshour, G., Clay, R. P., Fuller, M. S., Albersheim, P., Darvill, A. G., and Hahn, M. G. (1996). Developmental and tissue-specific alterations of the cell wall polysaccharides of *Arabidopsis thaliana* roots. *Plant Physiol.* **110,** 1413–1429.

Galway, M. E., Masucci, J. D., Lloyd, A. M., Walbot, V., Davis, R. W., and Schiefelbein, J. W. (1994). The *TTG* gene is required to specify epidermal cell fate and cell patterning in the *Arabidopsis* root. *Dev. Biol.* **166,** 740–754.

Hemerly, A., de Almeida Engler, J., Bergounioux, C., Van Montagu, M., Engler, G., Inzé, D., and Ferreira, P. (1995). Dominant negative mutants of the Cdc2 kinase uncouple cell division from iterative plant development. *EMBO J.* **14,** 3925–3936.

Hülskamp, M., Misra, S., and Jurgens, G. (1994). Genetic dissection of trichome cell development in *Arabidopsis. Cell* **76,** 555–66.

Jürgens, G. (1995). Axis formation in plant embryogenesis: cues and clues. *Cell* **81,** 467–470.

Korneef, M. (1981). The complex syndrome of *ttg* mutants. *Arabid. Inf. Serv.* **18,** 45–51.

Lamond, A. I., and Carmo-Fontseca, M.(1993). The coiled body. *Trends Cell Biol.* **3,** 198–204.

Larkin, J. C., Marks, M. D., Nadeau, J., and Sacks, F. (1997). Epidermal cell fate and patterning in leaves. *Plant Cell* **9,** 1109–1120.

Larkin, J. C., Oppenheimer, D. G., Lloyd, A. M., Paparozzi, E. T., and Marks, M. D. (1994). Roles of the *GLABROUS1* and *TRANSPARENT TESTA GLABRA* genes in *Arabidopsis* trichome development. *Plant Cell* **6,** 1065–1076.

Larkin, J. C., Young, N., Prigge, M., and Marks, M. D. (1996). The control of trichome spacing and number in *Arabidopsis. Development* **122,** 997–1005.

Laux, T., Mayer, K. F., Berger, J., and Jürgens, G. (1996). The *WUSCHEL* gene is required for shoot and floral meristem integrity in *Arabidopsis. Development* **122,** 87–96.

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Leyser, H. M. O., and Furner, I. J. (1992). Characterization of three shoot apical meristem mutants of *Arabidopsis thaliana. Development* **116,** 397–403.

Lloyd, A. M., Walbot, V., and Davis, R. W. (1992). *Arabidopsis* and *Nicotiana* anthocyanin production activated by maize regulators R and C1. *Science* **258,** 1773–1775.

Long, J. A., Moan, E. I., Medford, J. I., and Barton, M. K. (1996). A member of the KNOTTED class of homeodomain proteins encoded by the *STM* gene of *Arabidopsis. Nature* **379,** 66–69.

Masucci, J. D., Rerie, W. G., Foreman, D. R., Zhang, M., Galway, M. E., Marks, M. D., and Schiefelbein, J. W. (1996). The homeobox gene *GLABRA2* is required for position-dependent cell differentiation in the root epidermis of *Arabidopsis thaliana. Development* **122,** 1253–1260.

Meyerowitz, E. M. (1996). Plant development: local control, global patterning. *Curr. Opin. Gen. Dev.* **6,** 475–479.

Reerie, W. G., Feldmann, K. A., and Marks, M. D. (1994). The *GLABRA2* gene encodes an homeodomain protein required for normal trichome development in *Arabidopsis. Genes Dev.* **8,** 1388–1399.

Scheres, B., Di Laurenzio, L., Willemsen, V., Hauser, M-T., Jaanmat, K., Weisbeek, P., and Benfey, P. N. (1995). Mutations affecting the radial organisation of the *Arabidopsis* root display specific defect throughout the embryonic axis. *Development* **121,** 53–62.

Scheres, B., Wolkenfelt, H., Willemsen, V., Terlouw, M., Lawson, E., Dean, C., and Weisbeek, P. (1994). Embryonic origin of the *Arabidopsis* primary root and root meristem initials. *Development* **120,** 2475–2487.

Schnabel, R. (1996). Pattern formation: regional specification in the early *C. elegans* embryo. *BioEssays* **18,** 591–594.

Smith, L. G., Hake, S., and Sylvester, A. W. (1996). The *tangled-1* mutation alters cell division orientations throughout maize leaf development without altering leaf shape. *Development* **122,** 481–489.

Torres-Ruiz, R. A., and Jürgens, G. (1994). Mutation in the *FASS* gene uncouples pattern formation and morphogenesis in *Arabidopsis* development. *Development* **120,** 2967–2978.

van den Berg, C., Willemsen, V., Hage, W., Weisbeek, P., and Scheres, B. (1995). Cell fate in the *Arabidopsis* root meristem determined by directional signalling. *Nature* **378,** 62–65.

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