Positional information in root epidermis is defined during embryogenesis and acts in domains with strict boundaries

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Background: Cell position rather than cell lineage governs most aspects of development in plants. However, the nature and the origin of positional information remains elusive. Animal epidermal patterning relies in many cases on positional information provided by cell–cell communication. The epidermal layer of the *Arabidopsis* root is made of alternating files of two cell types and thus presents a simple pattern to study positional mechanisms.

Results: Clonal analysis of the root epidermis in combination with molecular and morphological markers has shown that cell fate is determined by position relative to the underlying cell layer, the cortex. The epidermal pattern appears to be organised during embryogenesis. Fate is not fixed in the developing root, though, as cells that move into a position previously occupied by neighbour cells ablated using laser microsurgery change fate. In contrast, cell fate is not altered when communication with living neighbour cells is impaired. Precise mapping of the influence of the position of extracellular cues on cell fate has shown that domains of positional information are organised with well-defined boundaries.

Conclusions: Cell-fate specification in the root epidermis relies on positional information that is organised in stable domains with sharp boundaries. The epidermal pattern is defined during embryogenesis and positional information remains active in the root until the initiation of cell morphogenesis. The origin of some positional cues might be extracellular.

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Background

Plant cell fate is dictated by cell position rather than by cell lineage. This has been shown clearly in studies of cell lineage in the shoot meristem of chimaeras. When cells from one tissue layer invade the position of the adjacent layer they tend to adopt the fate typical of their final position [1]. Domains of gene expression defining compartments for organ identity have been characterised in the floral meristem and it seems that the number of organs that develop in those domains depends on the regulation of cell proliferation [2]. The analysis of mutants defective in embryogenesis strongly suggests that positional information is defined at early stages [3,4] and early markers for basic embryonic features have been characterised [5–7]. However, the mechanisms involved in the organisation and the maintenance of positional information domains remain ill-defined.

The epidermis is the outermost layer of cells in the mature root [8]. It is composed of two cell types — atrichoblasts and trichoblasts — organised in alternate files [9]. Unlike atrichoblasts, trichoblasts form root hairs once differentiated and the two cell types can be distinguished at early stages of development by their difference in size and degree of vacuolation [10]. Three characterised mutants appear to be affected in the development of root epidermal fate: *transparent testa glabra* (*ttg*) [11], *glabra2* (*gl2*) [12] and *caprice* (*cpc*) [13]. In *Arabidopsis* and other crucifers the organisation of epidermal pattern is related to the position of the cells of the underlying cortical layer [8,14–16], suggesting that positional information is involved in patterning. To investigate this further, we carried out a clonal analysis of the root epidermis together with experiments based on laser microsurgical ablation.

The two types of epidermal cell are distinguished by their morphology once differentiated, their rate of cell division [17], the expression of β-glucuronidase (GUS) under the control of the *GLABRA2* (*GL2*) promoter [12] and the expression of the green fluorescent protein (GFP) in the marker line J2301 [18] isolated from a collection of enhancer trap lines (J.H., unpublished observations).

Results

The pattern of the root epidermis originates from positional information

Confocal sections of living roots stained with propidium iodide enabled the generation of precise maps of the root epidermis and of the underlying cortical cell layer. Both tissues are composed of monolayers of cells. We have

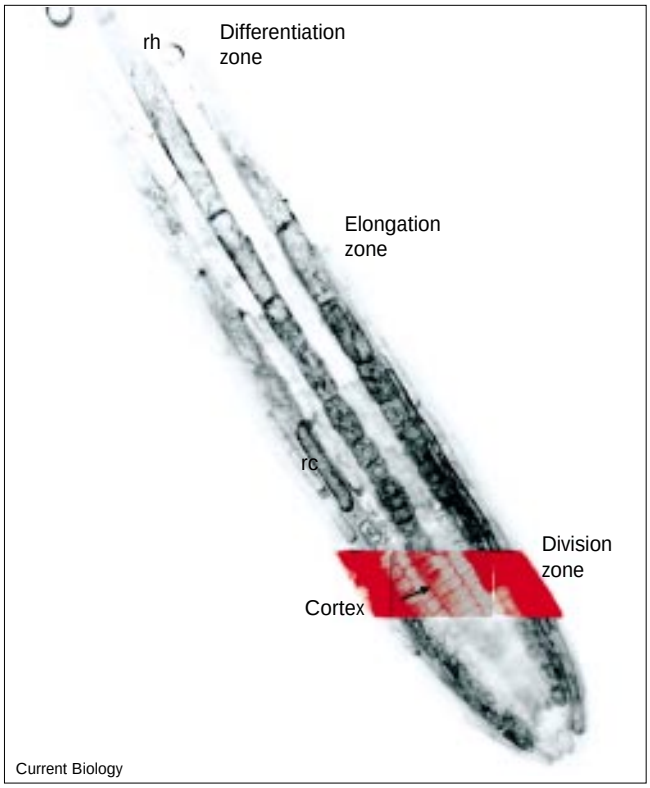
shown previously that cells are organised along the longitudinal axis of the root in distinct developmental zones [17] (Figure 1). Cells are produced at the basal region of the meristem by actively dividing initials and undergo on average five to six cell divisions before they exit the division zone. Epidermal cells later elongate and ultimately differentiate. Hence the longitudinal axis of the root is composed of three distinct zones reflecting different phases of development.

The epidermis is composed of alternating files of trichoblasts, which give rise to hair cells (H cells), and atrichoblasts, which give rise to non-hair cells (N cells). The former, but not the latter, differentiate elongated appendages (root hairs). The invariant position of the H cells over the longitudinal anticlinal walls of the underlying cortical cells suggests the existence of positional information. We have shown recently that H cells go through the cell cycle faster than N cells [17]. The two cell types have also been distinguished on the basis of expression of the gene *GL2* [12]; GUS activity can be detected in N cells in a transgenic line in which *GUS* expression is under the control of the *GL2* promoter (Figure 2c). We identified another molecular marker for N cells during a screen of a pool of enhancer-trap lines that express different patterns of modified green fluorescent protein 5 (mGFP5) [19]. Line J2301 is characterised by the preferential expression of the fluorescent marker in N-cell files, which always overlie the periclinal cortical cell walls (PCCWs) (Figure 1). This pattern was observed in 99% of the roots ($n = 310$) and the difference in level of expression can be observed as far as the epidermal initials. The expression pattern of mGFP5 can be followed in live roots and the line J2301 thus provides a good marker for experimental investigations on root epidermal development.

Epidermal cells originate from series of transverse anticlinal cell divisions that create files of cells extending up from the initial. Rarely, a longitudinal anticlinal division takes place in H cells and is followed by transverse anticlinal divisions, creating a clone of two parallel files of cells (Figure 2a). The initial longitudinal division is rarely aligned over the anticlinal cortical cell wall (ACCW) that abuts on the inner longitudinal periclinal wall of the H cell. Consequently, in most clones, each cell file occupies a different position relative to the ACCW. If this position were linked with cell-fate specification, each file of each clone should differentially express morphological, physiological and molecular markers. On the other hand, if cell fate were specified by lineage, the expression of markers should be identical in both files.

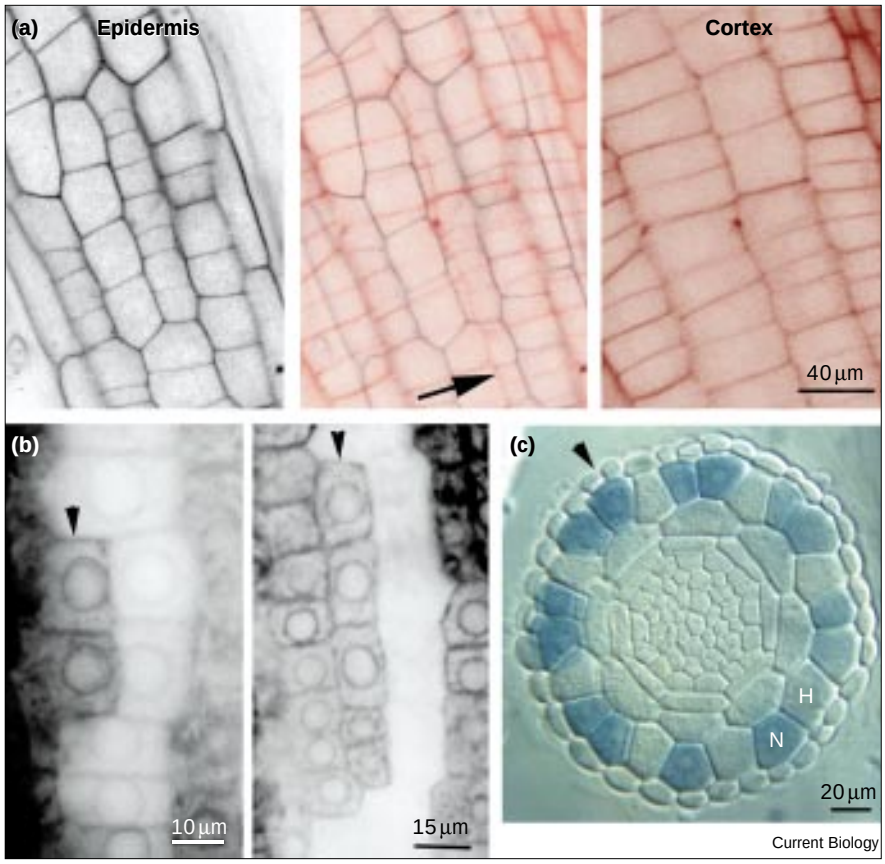
We have screened 500 root tips and found 375 epidermal clones. Five clones were detected in the differentiation zone. All contained a file with differentiated root hairs overlying the ACCW and a neighbour file where root hairs did not differentiate. The examination of confocal sections of the root epidermis in the meristem identified a large number of clones that exhibited unequal numbers of cells between the two files. In most clones (99.5%; $n = 375$) the file that contained the highest number of cells, which is typically the H-cell file, overlaid the ACCW (Figure 2a). A screen for epidermal clones in a line expressing GUS under the control of the *GL2* promoter showed that the highest GUS activity was detected ($n = 64$) in the file overlying the PCCW in all clones (Figure 2c). A survey of 96 clones in the J2301 line has shown that all cells in the position of N cells expressed mGFP5 (Figure 2b). These results showed that cells of the same epidermal clone differentially expressed four

Figure 1



General organisation of the root epidermis in the marker line J2301. A montage of confocal sections of the root epidermis (in black and white) shows its organisation at the tip of the root and its position relative to the underlying cortical cell walls (in red). Epidermal cells are organised into cell files which abut initial cells. In the marker line J2301, epidermal N cells express a GFP marker (intensities of grey) and are distinguished from H cells which develop a root hair (rh) in the differentiation zone, at the most apical pole of the tip. Before their morphological differentiation, epidermal cells elongate and divide in zones (elongation zone and division zone, respectively) that are well conserved. The difference in marker expression between the two cell types is readily visible in the initial cells. The marker is also expressed uniformly in root cap (rc) cells, which cover the epidermis in the division zone and slough off the root during elongation. H-cell files, which do not express the GFP marker, invariably overlie anticlinal cortical cell walls (arrow).

Figure 2



Differential expression of markers in epidermal clones. Most epidermal cells divide transversally to the main axis of the root. Occasional longitudinal divisions occur in the division zone and are followed by transverse division. This leads to the creation of epidermal clones that contain two files. (a) The superimposition (centre photo) of confocal sections of the epidermal (left) and of the cortical (right) cell walls shows that in such a clone formed in an H file that overlies an ACCW (arrow), only one file (the one on the right) is at this position, whereas the other one lies over the PCCW. The file over the ACCW contains eight cells whereas the other file contains four. A larger cell number is characteristic of H-cell fate. (b) The expression of the GFP marker of N-cell fate in clones occurring in the epidermis of the J2301 line shows its differential expression as soon as the two files are formed (left), together with a characteristic difference in cell number in the two files (right; 8 H cells/6 N cells); the marker is correctly expressed in the putative N-cell file (arrowheads). (c) Transverse section of a root in a line transformed to study the expression of *GUS* under the control of the *GL2* promoter. *GL2* is expressed in files that overlie the PCCW, that is, the N files. The section shows many occurrences of H-cell files separated by two N-cell files. Those are the result of multiple file duplications and show that newly formed files at the N position express their identity marker.

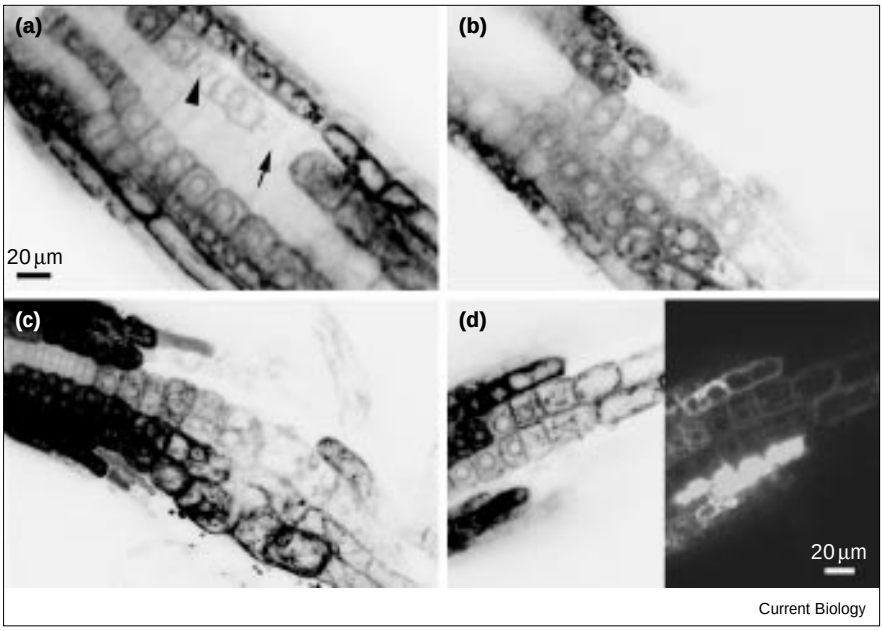
identity markers according to their final position relative to the ACCW. Hence, cell fate was specified by position, independently of the clonal origin of cells.

Positional information is active throughout epidermal development and cell fate is not fixed until differentiation

We wanted to investigate whether positional information could be experimentally manipulated in any part of the root meristem. We used cell ablation to free access to putative positional domains by non-ablated neighbour cells. This technique has proved successful in studying radial positional information in root meristematic initials [20]. Rows of four to six N cells were ablated in the wild-type Columbia ecotype and in the J2301 line. Ablations were performed in the middle of the division zone (that is eight to ten cells above the initials), above which H cells accomplish usually two to three transverse cell divisions before beginning cell elongation [17] (Figure 3a). Most H cells that invaded the position previously occupied by N cells expressed the mGFP5 characteristic of N-cell fate within 12 to 24 hours after ablation ($n = 35$; 87%; Figure 3b). In 94% of such ablated roots, H cells that occupied an N-cell position did not differentiate a root hair (Figure 3c). No change in expression of molecular markers was observed when invasion did not take place ($n = 11$; 100%; Figure 3d) and normal hair-cell differentiation occurred. The ablation of rows of six to eight H cells was also carried out but, probably because of physical constraints, was less often followed by invasion of the H position by neighbour N cells (30%; Figure 3d). The level of mGFP5 expression in N cells decreased when they invaded the space previously occupied by H cells ($n = 7$; 100%), and they eventually produced root hairs ($n = 4$). No change in fate was detected in N cells when invasion did not occur ($n = 16$). In agreement with the clonal analysis, these results showed that cell fate in the epidermis is specified by positional information in the root meristem.

In cell ablation experiments, the switch in cell fate occurred typically during the last two cell divisions before the beginning of cell elongation. This suggested that epidermal cell fate is not fixed early in development. We have observed two 2-cell clones in the differentiation zone, each with one in the H position and one in the N position. These clones result from the atypical longitudinal orientation of the last meristematic cell division. Only the cell in the N position expressed the GFP marker (Figure 4a) and the cell in the H position later differentiated a root hair

Figure 3



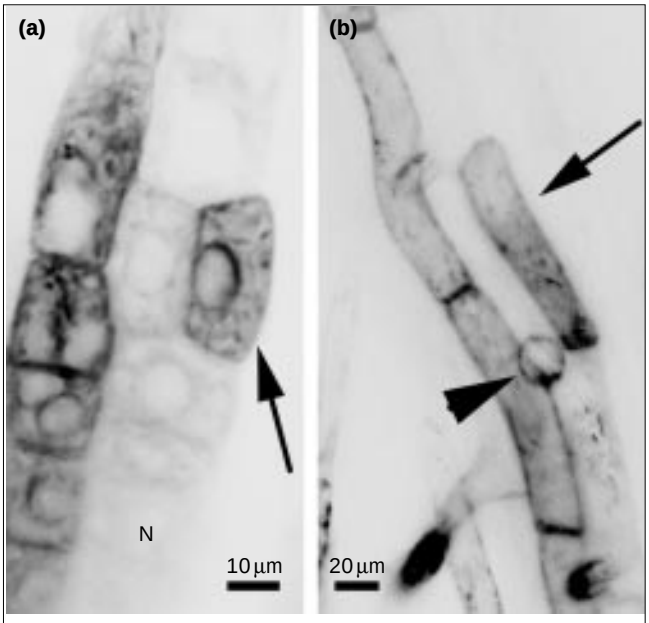
Change in fate of epidermal cells in response to changes in position provoked by cell ablations. (a) Ablation of N cells in the division zone was performed using the J2301 line. N cells express the GFP marker targeted to the endoplasmic reticulum. The nuclei and cell walls are outlined by the distribution of the endoplasmic reticulum. Immediately after laser microsurgery, nuclear structure appears disrupted (arrowhead) and 1 min later the GFP marker has disappeared (arrow). Cells are gradually pushed by their neighbour H cells which do not express the GFP marker. (b) Twenty-four hours after ablation, some H cells have invaded the vacant space and have begun to express the GFP marker. (c) When these cells reach the differentiation zone, they do not differentiate root hairs and still express the GFP marker. (d) Response to ablation of H cells. The right-hand panel shows the distribution of propidium iodide, which is confined to cell walls in live cells but invades the cytoplasm of dead cells. The left-hand panel shows the distribution of the GFP marker. There is little invasion of the space by N cells and after 24 h those cells were still expressing the GFP marker.

whereas the other cell did not (Figure 4b). This observation demonstrated that fate is flexible until the last cell division in the root epidermis.

Pattern of positional information is established during embryogenesis

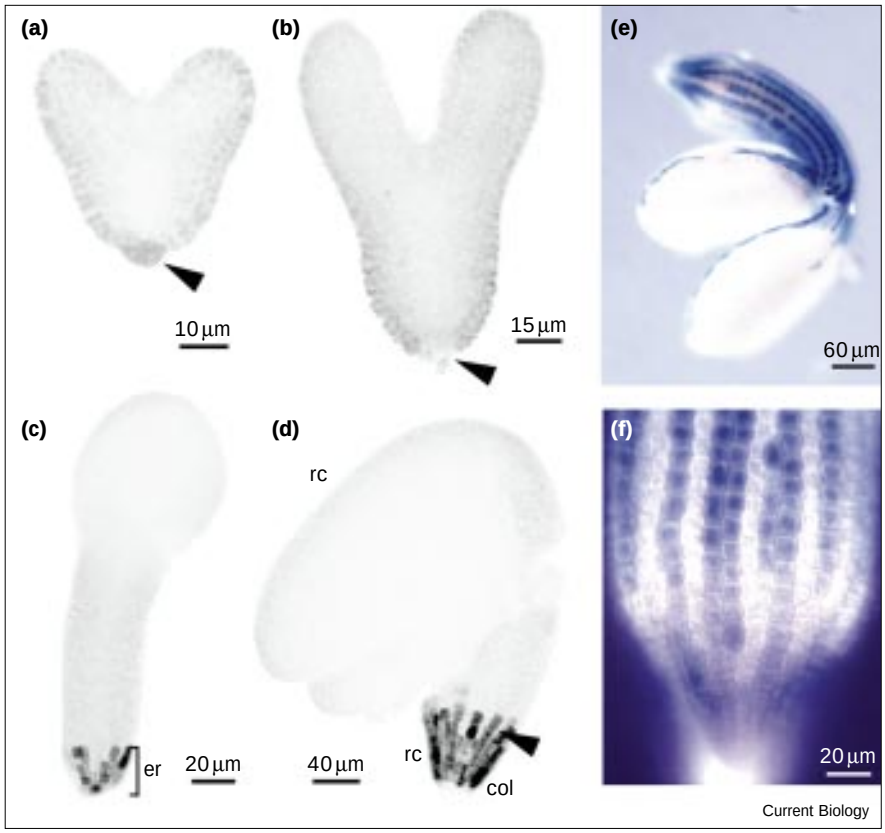
Since the J2301 line exhibits differential expression of GFP at the level of the initials (Figure 1), we investigated whether the pattern of expression of GFP was established during early development. Living *Arabidopsis* embryos were isolated from the early heart stage (stage 12) through the mature embryo stage (stage 19) [21] and the pattern of GFP expression was recorded on longitudinal confocal sections along the apical–basal axis of the embryo (Figure 5, Table 1). No expression was detected at stage 12. From mid-heart stage or stage 13 to early torpedo stage (stage 15) GFP was characteristically expressed in the derivatives of the hypophysis from which originates the lower tier of the root meristem [21,22] (Figure 5a). In parallel, the expression of the marker in the root cap/epidermal lineage was detected in some embryos as soon as stage 13 but only became prominent in all embryos by stage 16 (Figure 5b,c). At stage 15, some embryos did not express GFP at all, indicating that this stage represents a transition between the expression in the hypophysis derivatives and the root cap/epidermal lineage (Table 1). We never observed GFP expression in the hypophysis after stage 15 and it was later confined to the embryonic root epidermis and root cap precursors. By the end of the torpedo stage, an alternating

Figure 4



Late switches in cell fate. (a) A rare longitudinal division occurs in the end of the division zone, forming a two-cell clone, and the cell occupying the newly defined N-cell position (arrow) starts to express the GFP marker. (b) Ultimately, one cell of the clone differentiates as a root hair cell (arrowhead) and one as a GFP-expressing N cell (arrow) in the differentiation zone.

Figure 5



Expression of molecular markers for N-cell fate during embryogenesis. (a–d) The distribution of the GFP marker in line J2301. (a) During heart stage, the expression of the GFP marker is confined to the derivatives of the hypophysis. (b) During the transition to the torpedo stage, the marker is no longer expressed. (c) During torpedo stage, GFP expression appears again in the outer layer of cells from which the embryonic root (er) will develop. (d) In the mature embryo, all cells of the root cap (rc) express the marker and its expression is confined to half of the future epidermal cells (arrowhead). A clear embryonic epidermal pattern like the one in the mature root tip is observed. The columella (col) is devoid of marker expression. (e,f) Distribution of GUS activity in the *GL2* promoter::*GUS* line. GUS activity in mature embryos is present in half of the epidermal cells (e) and in cells at equivalent position in the hypocotyl (f).

pattern of protodermal cells expressing GFP was detected in a few embryos. This pattern was recognised clearly in embryos reaching maturity (stages 18 and 19) where files containing four to seven protodermal cells were observed to express the marker (Figure 5d). An embryonic epidermal pattern was observed also in the line expressing a GUS reporter under the control of the promoter of *GL2*, which is specifically expressed in N cells in the root meristem [12] (Figure 5e,f). Interestingly, unlike the GFP pattern, the epidermal pattern of GUS expression encompasses the future hypocotyl as well. This showed that root epidermal patterning takes place during embryogenesis.

Maintenance of the pattern of positional information does not involve communication between living cells

The pattern of positional information established during embryogenesis must be perpetuated in active primary root meristem because cell fate is not fixed at that time. A potential mechanism for maintenance may involve communication between live epidermal cells in the same file or in neighbouring files. To test this hypothesis we performed a series of ablations by which epidermal cells were isolated from their neighbours in adjacent or the same file (Table 2). We did not succeed in achieving complete isolation of cells from all their neighbours as this would have caused too much damage. Ablations were performed at the level of four to six cells above the initials, that is, at an early stage of development where fate is not fixed. Single cells were isolated and subsequently divided to form groups of cells before their differentiation. We considered for analysis only the cells that had permanently lost all contacts with cells in the files where ablations were performed. Isolation of both cell types from their neighbours in the same file did not affect the expression of the marker mGFP5 in the line J2301 (Figure 6a,c, Table 2). Differentiation of those isolated cells occurred correctly with the exception of some H cells where no hair developed although GFP was not expressed. Both N and H cells isolated from their neighbour cells in neighbour files did not change their mode of expression of GUS under the control of the *GL2* promoter, nor of mGFP5 in the line J2301, and differentiated according to their origin (Figure 6b,d, Table 2). This showed that when communication between live epidermal cells within a single file or between neighbour files was disrupted, cell fate was still maintained.

Because the cortical layer seems to have a crucial role in the definition of cell fate, we tested whether communication between the epidermal and the cortical cells had a role in the maintenance of positional domains. Cortical cells were

Table 1

Patterns of expression of the GFP marker during embryogenesis in the line J2301.

Developmental stage	Total number	Embryos expressing GFP in hypophysis derivative and columella (%)	Embryos expressing GFP in embryonic root but not columella (%)	Embryos presenting an epidermal pattern of GFP expression in alternating files (%)
13	9	55	67	0
14	6	67	100	0
15	18	11	52	0
16	27	0	100	7
17	20	0	100	80
18	11	0	100	100
19	6	0	100	100

ablated without harming the overlying epidermal N cells by focusing the laser on the ACCW. Single N cells isolated from underlying cortical cells in this way did not alter their expression of the GFP marker for at least 48 hours after ablation and did not develop root hairs (Figure 6e, Table 2). H cells were isolated successfully from cortical cells on a few occasions and gave the same result as for N cells (Figure 6e). In conclusion, the maintenance of positional information and cell fate did not depend on a single communication pathway between living epidermal cells (within or between files) nor between epidermal and cortical cells, although redundant and combinatorial use of these pathways could not be excluded.

Positional information is established as domains with strict boundaries

A previous study had shown that an epidermal clone that has experienced at least three cell divisions contains up to twice as many H cells as N cells and that this ratio is altered in the mutant *ttg*, in which the epidermal cell fate is not defined properly [17]. Thus the ratio of H cells to N cells (*H*/*N*) in a specific clone was considered to represent the degree of differentiation of fate between the two files. However, very rare clones exhibited an *H*/*N* ratio equal to one and this correlated with a symmetrical position of the clone over the ACCW. Those observations encouraged us to study the correlation between the degree of differentiation assessed by the ratio *H*/*N* and the position of the ACCW in relation to the basal epidermal cell walls of epidermal clones. It was hypothesised that the closer the ACCW was to the centre of the H cell, the lower would be the ratio *H*/*N*. Thus each clone was characterised by a positional index which was defined as the ratio of the width of the H cell (*W*) to the distance of the ACCW to the longitudinal wall at the middle of the clone (*L*) (Figure 7a). Thus *W*/*L* ranges from 2.0 — when the ACCW is farthest from the centre — to higher values — when the ACCW is closer to the centre of the clone. A large number of such clones were collected (n = 148) and a general correlation between the two parameters *W*/*L* and

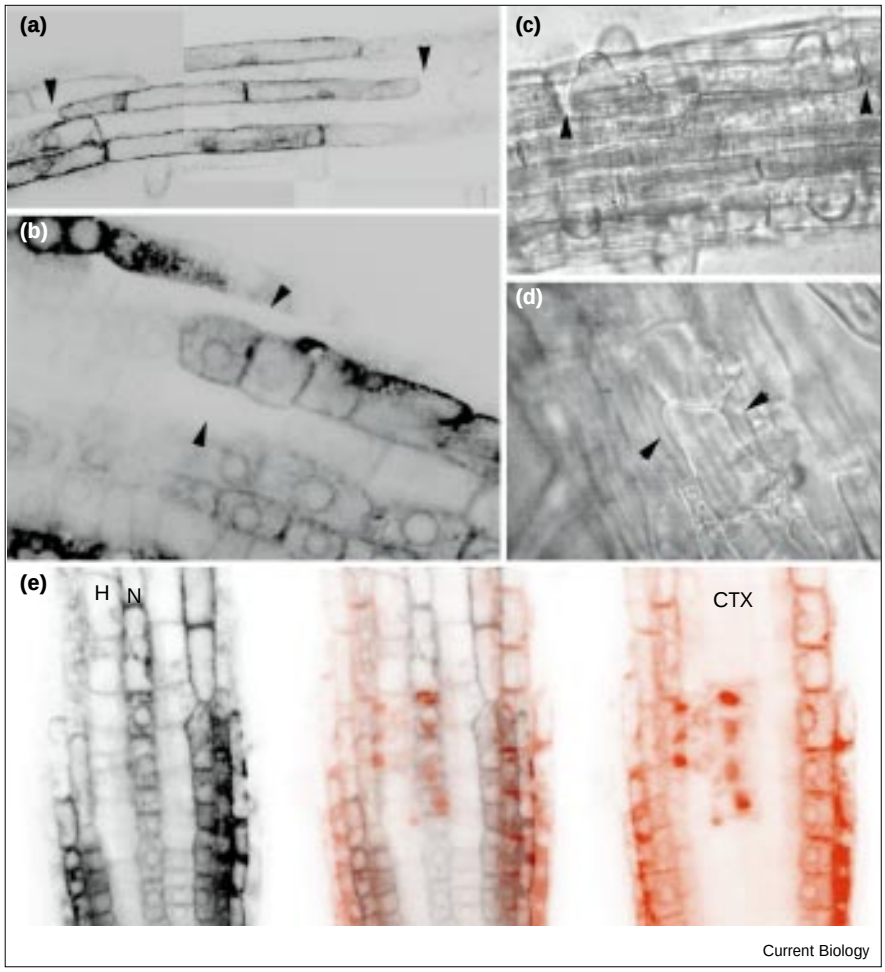
Table 2

Isolation of epidermal cells from their neighbours in epidermis and cortex.

Type of cell isolation	Total number	Total number in which differentiation was scored	Correct differentiation (%)	Total number in which *GL2*-controlled GUS expression was scored	Correct expression of GUS (%)	Total number in which mGFP5 expression was scored	Correct expression of mGFP5 (%)
H cells isolated from H cells in the same file	12	12	67	ND	ND	ND	ND
N cells isolated from N cells in the same file	17	10	100	ND	ND	7	100
H cells isolated from N cells in neighbour files	24	12	100	6	100	6	100
N cells isolated from H cells in neighbour files	27	13	100	7	100	7	100
N cells isolated from underlying cortical cells	40	4	100	ND	ND	40	100

Ablations were carried out in the line J2301 to observe eventual changes in the expression of the GFP marker. A line transformed with the *GUS* gene under the control of the *GL2* promoter, which is preferentially expressed in N-cell files, provided another molecular marker. Differentiation of isolated cells was observed 2 days after isolation. ND indicates not determined.

Figure 6



Maintenance of cell fate is independent of communication between live cells. (a) Epidermal N cells isolated from their N-cell neighbours in the same file do not alter expression of the GFP marker in the line J2301. (b) A similar result is observed when N cells are isolated from H cells in neighbour files. (c) H cells isolated from cells in the same file do differentiate root hairs correctly. (d) The same result is observed when H cells are isolated from N cells in neighbour files. In (a–d) the arrowheads indicate positions at which cells were ablated. (e) Ablation of cortical cells does not alter the pattern of GFP expression in the epidermis as shown by a montage of confocal sections. Left, epidermis with GFP expression; right, confocal section of propidium iodide fluorescence that shows the cell wall of the cortical cells in the middle of the section and dead cortical cells marked by their nuclei brightly stained by the dye; centre, merged image of the two sections. CTX, cortex.

H/*N* was observed according to the hypothesis. Unexpectedly, the correlation between the two parameters was not linear (Figure 7b). When *W*/*L* became higher than 3.0, *H*/*N* values dropped sharply to the order of 1.3, which represents a very small difference in cell-division rate. In other words, when the ACCW was positioned beyond a third of the H-cell width, the differentiation between both cell types in a clone became minimal. This study showed the existence of a spatial discontinuity between two domains of positional information. Thus, in the root epidermis positional information is organised into domains defined by sharp boundaries.

Discussion

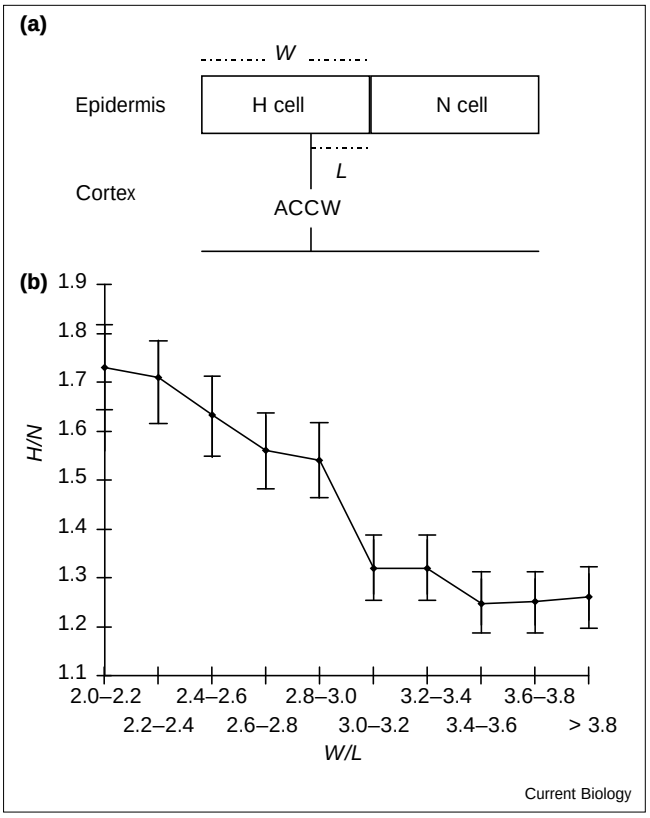
Positional information organises the pattern in root epidermis and is established during embryogenesis

We have observed the expression of molecular, physiological and morphological markers with respect to epidermal cell position over the ACCW in spontaneous clones. This provides clear evidence of the action of positional information as opposed to cell lineage in the specification of cell fate in the root epidermis. Ablation experiments resulting in the displacement of individual cells confirm the importance of positional information. Moreover those results suggest that cell fate is not fixed until late in development; this is further supported by the observation of two-cell clones which are produced at the last meristematic division. Thus restriction in fate occurs during cell elongation, that is, a few hours before the differentiation processes.

We have shown that positional domains involved in the organisation of the root epidermis are apparent by the torpedo stage. This organisation of new domains can be considered as a refinement of the body organisation plan established before heart stage [3,4,22]. The organisation of the root epidermal pattern probably takes place at the transition between heart and torpedo stages.

The mechanism of pattern organisation is unknown. All 16 protodermal cells represent an equivalence group with H-cell fate as a default fate (no expression of mGFP5).

Figure 7



Correlation between the position of the ACCW and cell-fate differentiation in epidermal clones. (a) In epidermal clones originating from H cells, the position of the ACCW relative to the longitudinal wall that bisects the clone (*L*) and the width of the cells in the H file (*W*) was determined, giving a ratio *W*/*L*. (b) The relative degree of differentiation of the epidermal H and N cells of each clone was estimated by the ratio of the number for each cell type, *H*/*N*. Clones were divided into classes that exhibited a ratio *W*/*L* of 2–2.2, 2.2–2.4, and so on up to greater than 3.8. In each class the average ratio *H*/*N* was calculated together with the s.e.m. plotted as standard errors. *H*/*N* decreases sharply when *W*/*L* is greater than 3.0.

We have observed that the GFP marker is expressed initially at random in isolated protodermal cells and this is reminiscent of models developed for the epidermal patterning in animals by lateral inhibition [23]. This remains to be tested either directly by cell ablation or indirectly by the analysis of mutations.

Meristematic function and maintenance of the embryonic pattern

The epidermal pattern is established during embryogenesis before a functional meristem is established. This implies that the pattern must be maintained in concert with the self-perpetuating function of the root meristem. To test the potential involvement of communication between living cells in the maintenance mechanisms, cells were isolated from their neighbours using laser microsurgery. This treatment did not alter the expression of molecular markers, and cells differentiated correctly with the exception of a small number of H cells where root hair differentiation is sensitive to environmental conditions [24]. Those isolated cells were observed during periods equivalent to that during which a change in fate was observed in clones or as a consequence of a change in position following ablations. Therefore, the absence of change in fate was not the result of observations of inadequate duration. Moreover, all ablations were produced in the division zone at a level where fate is still not fixed so the absence of change in fate does not result from earlier determinative events. Hence, communication between an epidermal cell and its live neighbours does not appear to be involved in the maintenance of positional information patterns. However, redundant mechanisms such as positive-feedback loops might be involved in the maintenance of cell fate. Indirectly, positive interactions within a cell file or negative interactions between cells in neighbour files might result in a global positive feedback. In the absence of perturbation, such a mechanism would be sufficient to maintain the cell in its original developmental pathway. Alternatively, fate switching in each cell might be prevented by direct inhibitory action of neighbour cells.

Mechanisms involved in the maintenance of the pattern of the root initials have been investigated in elegant ablation studies. The initials for the various tissue layers are organised as concentric rings of 8 or 16 cells in the young root meristem at the centre of which lies the quiescent centre, a group of four mitotically inactive cells [25]. Ablation studies have demonstrated that quiescent centre cells maintain the undifferentiated state of the surrounding initials but are not involved in patterning [26]. In another study it was shown that a change of position of meristematic initials is accompanied by a change in fate [20]. Hence the root meristem appears to be organised as a structural framework of positional cues. An interesting set of ablations has shown that the maintenance of this framework involves some transfer of information from the daughters of the initials to their mother cell [20]. Our results do not support the involvement of communications between living neighbour cells for maintenance of the pattern of the root epidermis. Hence elements of the pattern must be provided either by other cells in the layers underlying the cortex or by a permanent framework of extracellular matrix.

The nature of positional information in plants

According to our study, positional information is established during embryogenesis and is maintained in a stable way in the root meristem. The nature of this information remains elusive. In plants, the main axes of polarity, both apical–basal and radial, are generally maintained throughout the plant's life. Hormonal gradients have been invoked in this process [27] and in the maintenance of meristematic activity [28]. Recent studies on the development of the

shoot apical meristem have shown that developmental domains have boundaries defined by the expression of genes such as *NO APICAL MERISTEM* [29] and that cross-talk mechanisms regulate the limits of the domains together with the level of cell proliferation [2].

These results and models address the dynamic and flexible state of plant development but offer little to explain the stability of positional information domains such as the one seen in our study. Positional information has been shown to be associated with the extracellular matrix in *Fucus* [30,31]. *Fucus* embryos contain two cell types. Once liberated from their extracellular matrix (cell wall), cells lose their identity [32]. This identity is retained, however, when embryonic cells are isolated from each other inside their wall. Moreover, the cell wall of one cell type induces changes of developmental fate in cells of the other type that come into contact with it.

In the root epidermis there is a striking link between the specification of epidermal cell fate and the position of the underlying cortical cell wall. The position over the ACCW determines trichoblast and H-cell fate whereas the PCCW positions determine the atrichoblast and N-cell fate. Our results suggest the presence of contiguous positional domains as stripes centred on the ACCW and on the PCCW. These domains could result from the establishment of a gradient of an inducer of H-cell fate diffusing from the ACCW. This hypothesis is rather unlikely, as when cortical cells are ablated, the overlying epidermal cells do not change fate as would be expected if a gradient were being destroyed in the space opened in place of the dead cortical cell. An alternative hypothesis is that the ACCW is distinguished from the PCCW by some factors controlling epidermal cell fate. It has been demonstrated that cell walls of the *Arabidopsis* root contain highly localised epitopes [33]. Cell corners are, for example, richer in epitopes for antibodies JIM which recognise arabinogalactan protein epitopes (L.D., unpublished observations). Also, the regular arrangement of protodermal cells with respect to the position of the cell wall in the underlying cell layer is established during heart stage [22,25] presumably by specific growth of the cell wall at the initial T junction in which cues could be localised.

Conclusions

Our study has shown that the root epidermal pattern is determined by positional information which is set up early during embryogenesis. Thus, the organisation of the whole plant during embryogenesis not only involves the definition of the main axes but also of smaller domains. Those patterns are maintained upon activation of the root meristem at germination. However, epidermal cell fate is not fixed until cell morphological differentiation, and cells appear to maintain their fate in response to positional cues. Positional information is organised as discrete domains with strict boundaries and is anchored to a stable structure; this could be the extracellular matrix as shown by the importance of the underlying cell wall in this model.

Materials and methods

Plant material

The following lines were obtained from the Nottingham *Arabidopsis* Stock Centre: wild types, ecotype Columbia, ecotype Landsberg *erecta* and ecotype WS; *GL2* and *TTG* in Landsberg background. The transgenic line containing the construct *pGL2–GUS* was previously described [12]. The J2301 line was screened in a pool of enhancer trap lines (J.H., unpublished observations). Embryos were isolated from ovules dissected with fine tweezers in MS medium containing 10% sucrose. Those were mounted in a small chamber formed between a slide and a coverslip by a silicone joint and were directly observed by confocal microscopy.

Plant growth conditions

Seeds were sterilised in 5% sodium hypochlorite and stratified 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. Plants were grown under light in 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 observation without disturbance.

Confocal microscopy and laser microsurgery

Seedlings were incubated for 60 min in 10 μg/ml propidium iodide (Sigma) in growth medium to stain the cell wall. Optical sections were obtained on live roots using a confocal microscope (Biorad, MRC 1000) with 488 nm excitation line. The propidium iodide fluorescence was monitored with a 580 nm long-pass emission filter, and a 523 nm short-pass filter was used to monitor the expression of mGFP5 [19]. Images were processed using the software Confocal Assistant and Photoshop (Adobe).

The identity of cell files in the meristem was established either by following cell files from the differentiation zone or more frequently by their position relative to the ACCW. Cell files overlying this latter position were termed H-cell files and the other files were termed N-cell files. To perform *W* and *L* measurements, the position of the ACCW at the top of the cortical cells was drawn on the screen of the video monitor and a bottom section of the epidermis was imaged. The measurements *W* and *L* were made at three levels along the clone using the Biorad Software. *W*/*L* was taken as the average of such measurements. To avoid distortion through projection of the sections, only clones with ACCW perpendicular to the section plane were used.

Laser microsurgery was performed using an Argon UV laser coupled to the confocal microscope (420 nm). The laser beam was focused to a 10 μm square area at the centre of the target cell and the cell was scanned for 1–2 sec. Ablation was confirmed by the influx of propidium iodide within minutes or immediately visualised by the degradation of the nuclear envelope visible in the J2301 line. Cells were ablated successively until a suitable configuration was reached. To isolate single cells it was necessary to ablate at least three contiguous neighbour cells on each side to ensure that all contacts were lost during subsequent expansion of the isolated cell. As cortical cell width was less than the width of epidermal cells, ablation of one row of six cortical cells was necessary to isolate one N cell and two contiguous rows of six cells to isolate one H cell. Complete isolation of single epidermal cells thus required the ablation of 12–20 surrounding cells, which caused too much damage and proved not to be possible in our hands.

GUS assay: root epidermal 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 [12] and viewed using DIC optics with a Zeiss Axiophot microscope.

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