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Clonal relationships and cell patterning in the root epidermis of *Arabidopsis*

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SUMMARY

The development of the post-embryonic root epidermis of *Arabidopsis thaliana* is described. Clonal analysis has identified three sets of initials that give rise to the columella root cap cells, epidermis and lateral root-cap cells, and the cells of the cortex and endodermis respectively. The mature epidermis is composed of two cell types, root hair cells (derived from trichoblasts) and non-hair cells (derived from atrichoblasts). These cells are arranged in sixteen or more discrete files. Each hair cell file overlies the anticlinal (radial) wall of the underlying cortical cells and is separated from the next by one or two non-hair files. The root hair forms as a tip-growing projection from the basal end of the trichoblast i.e. the end nearest the root meristem. The non-hair epidermal cells are significantly longer than the hair forming cells and are located over the outer periclinal (tangential) wall of the underlying cortical cells. The size difference between the two cell types is apparent in the cell division zone before hairs form. This suggests that the signals required for the differentiation of the root epidermis function in the meristem itself. Ectopic hairs are present in the *ctr1* root epidermis suggesting that a Raf protein kinase may play a role in pattern formation/differentiation in the root epidermis and that ethylene may be a diffusible signal involved in specifying pattern in the root epidermis.

Key words: root development, epidermis, root hair, *Arabidopsis*, clonal analysis, ethylene

INTRODUCTION

The *Arabidopsis* root epidermis is composed of two precisely patterned cell types (hair cells and non-hair cells) and presents a unique opportunity to study the molecular-genetic regulation of pattern formation and cell differentiation in plants. The application of such genetic and molecular approaches to the study of pattern formation and differentiation in animal systems has uncovered many of the biochemical mechanisms regulating these processes (Lawrence, 1992). Of particular interest in these studies has been the discovery of the ways in which cells communicate throughout development (for example, see Dougan and Di Nardo, 1992). In contrast there have been few clear demonstrations of short range cell interactions in developmental processes in plants, although some local cell-cell interactions have been described between cells of different germ layers of the shoot meristem (Hake and Freeling, 1986; reviewed by Becraft and Freeling, 1992). However, the biochemical basis of these interactions is not understood in plants.

Surgical experiments have indicated that (short range) cellular interactions may play a role in the regulation of cell differentiation in the radish root epidermis (Bünning, 1951). Bünning found that separation of the developing epidermis from underlying tissue resulted in the differentiation of additional hair cells in the excised tissue at the expense of non-hair cells. He concluded that hair-cell differentiation is the default state for cells in the epidermis and that during normal development, a regulator of hair formation passed from underlying cells to confer the 'hairless' fate to overlying cells. Spatial regulation of this signal would then result in the precisely patterned epidermis characteristic of this family (Brassicaceae). Although these experiments have not been carried out in *Arabidopsis*, the similarity in cellular organisation of the *Arabidopsis* and radish roots suggests that similar factors may regulate pattern formation in each (Cormack, 1947; Dolan et al., 1993).

The reproducible simplicity, small size and cell number of the *Arabidopsis* root provides a useful system for the study of pattern formation and differentiation in plants (Dolan et al., 1993). Underlying the epidermis is a cortex of 8 cell files. The epidermis is therefore composed of two cell types; those that make contact with two cortical cell files, i.e. they lie over the junction between two cortical cell files, and cells that contact only one cortical cell file, i.e. those that lie over the outer periclinal (tangential) wall of underlying cortical cells.

As a first part of our study to determine the biochemical nature of the interactions regulating differentiation in the *Arabidopsis* epidermis, we have determined the clonal relationships of the endodermis, cortex, epidermis and root cap by clonal analysis. We then show that the root epidermis is highly patterned and that such patterning is tightly regulated. We

describe the structural and developmental features of the two cell types in the epidermis and describe early markers of cell fate for each. Characterisation of the root phenotype of *ctr1* (*constitutive triple response 1*) plants indicates that a *Raf*-like kinase plays a crucial role in the signal transduction process regulating the spatial organisation of this epidermis and we suggest a model for the early patterning events in this simple system.

MATERIALS AND METHODS

Plant material

The clonal analysis was carried out in lines of the Landsberg *erecta* ecotype of *Arabidopsis thaliana* transformed with *Ac*-containing *GUS* constructs (Lawson et al., 1994). The morphological characterisation of epidermal development was carried out in the Columbia ecotype. *ctr1* seeds (Columbia ecotype) were a generous gift from Greg Roman and Joe Ecker (University of Pennsylvania, PA, USA).

Plant growth conditions

Plants were grown on Murashige and Skoog (Flow Laboratories, Irvine, Scotland) medium with 2% sucrose as outlined previously (Dolan et al., 1993). After vernalisation, plates were placed vertically in the light to allow the roots to grow down the surface of the agar.

Light microscopy

Fixation

Roots were fixed in 2.5% glutaraldehyde in either phosphate or cacodylate buffer pH 7 at 4°C for 1 hour. Roots embedded in paraffin were fixed in 2.5% glutaraldehyde, 1% osmium tetroxide in phosphate buffer, pH 6.8, at 4°C for 1 hour.

Paraffin embedding

Roots were embedded in paraffin wax (Paraplast), sectioned on a rotary microtome at 7 μm and stained with safranin and fast green as described by Jensen (1962).

Embedding for sequential sectioning

Roots for sequential sectioning were fixed, washed twice in H_2O for 5 minutes, dehydrated in 70% and 90% ethanol for 10 minutes each and infiltrated in 50% Histo Resin (Reichert Jung) for 10 minutes and then in 100% resin for 30 minutes before being polymerised at room temperature for at least 30 minutes. Sequential serial sections of 6 μm and 14 μm were made on a Reichert Jung Ultracut microtome and collected on glass slides. These sections were inspected on a Nikon Microphot-SA compound microscope, with Nomarski optics.

Embedding of glucuronidase-stained material

Roots were grown for 5-14 days and fixed in 0.05% glutaraldehyde for 15 minutes. The glucuronidase reaction was carried out overnight at room temperature, as previously described, in 0.5 mg/ml X-gluc (5-bromo-4-chloro-3-indolyl-beta-D-glucuronic acid, cyclohexammonium salt), in 50 mM sodium phosphate buffer, pH 7, 1 mM EDTA, 0.1 mM potassium ferricyanide and 0.1 mM potassium ferrouscyanide (Jefferson, 1987). Roots were postfixed in 1% glutaraldehyde for 15 minutes and then dehydrated and embedded as described above. Specimens were polymerised between two polythene lined coverslips separated by a coverslip spacer. Embedding of roots in thin sheets allowed whole mounts to be easily photographed. These specimens were then remounted and thick (5-15 μm) sections made. No diffusion of blue precipitate was observed under these conditions.

Cell length measurements

Measurements of root epidermal cell length on specimens growing in situ in Petri dishes were made using long distance working objectives on a Nikon compound microscope.

Cryo-scanning electron microscopy

3- and 5-day old roots were frozen in nitrogen slush at −190°C. Ice was sublimed at −90°C and the specimen sputter-coated and examined on a Cam Scan scanning electron microscope fitted with a cold stage.

Time lapse measurements of root hair growth

3-day old plants growing in Petri dishes were placed on the stage of the microscope and the hair forming region imaged with Nomarski optics using long distance working objectives. Time lapse sequences of hair growth were recorded using a JVC TK-1280E video camera and a JVC time lapse video recorder (BR-S 920E). Recordings were made 1/320 normal speed. Hair growth measurements were made by tracing hair position at fixed time intervals onto cellophane placed over the video monitor and, to determine rates of growth, recordings were played back using a S-VHS JVC video recorder (HR-S4700EK).

RESULTS

The primary root meristem is derived from cells at the basal end of the *Arabidopsis* embryo as indicated in Fig 1. The primary root of the 3-day old seedling is covered in a profusion of hairs. Hairs are tip growing protuberances that emerge from the basal end of specialised epidermal cells (trichoblasts). Hairs form in a zone of differentiation in which cell elongation has ceased (see below). These specialised cells are interspersed with non-hair forming, epidermal cells. The relative distributions of these cell types will be described later.

Clonal analysis shows that epidermal cells are derived from the same cell lineage as lateral root cap cells

Our previous anatomical description of the *Arabidopsis* root epidermis suggested that cells of the epidermis and lateral root cap are both derived from a common set of about 16 initial cells (Dolan et al., 1993). This prediction was tested in this study by clonal analysis. Since there are no useful genetic markers available for such a study in roots we used plants transformed with the construct described in Fig. 2 (Lawson et

Fig. 1. The root meristem is located at the basal end (b) of the seedling and is covered in hairs. The shoot meristem is located at the apical end (a) of the seedling. Throughout this paper position is defined according to this apical basal axis. Scale bar, 400 μm.

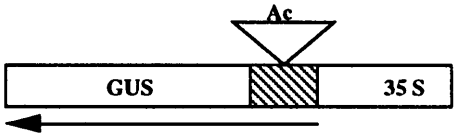

Fig. 2. Schematic diagram of the construct used for clonal analysis. The constitutive promoter (35S) drives expression (arrow) of the *GUS* gene upon excision of the *Ac* transposable element. Expression of the *GUS* gene is visualised histochemically.

al., 1994). The functional portion of the construct contains a constitutively expressed β-glucuronidase gene (*GUS*) into which an active *Ac* transposable element has been inserted. Insertion of this element into the untranslated region of the *GUS* gene prohibits expression. *GUS* gene expression is restored upon *Ac* excision. Such random excision events can be observed because the *GUS* expression can be visualised histochemically through the formation of a blue, precipitate when the chromogenic substrate, X-gluc, is used (Jefferson, 1987). Under the precise conditions described in the Materials and Methods, this labelling is cell autonomous in our hands. *Ac* excisions take place at a variety of times during development resulting in sectors of different sizes depending on how many times the descendent cells divide. We observe sectors that were obviously induced during embryogenesis and a variety of sectors that were induced at a range of times after germination. For the purposes of this study we concentrated on sectors located in the epidermis, root cap, cortex, endodermis and columella of lateral roots. No epidermal sector was found to include any other tissue type except the root cap.

If the cells of the lateral root cap and the epidermis are derived from a common set of initial cells, it would be expected that some, rare, individual sectors would encompass cells of both tissue types. One such sector is shown in Fig. 3. Fig. 3A shows a whole mount of a sector that was subsequently sectioned in Fig. 3B. The sectioned root shows that adjacent cells in the epidermis and lateral root cap are labelled blue.

Since the epidermal and lateral root cap lineages are predicted to diverge early, most sectors in these lineages will be restricted to one or other cell type. Fig. 3C and D show a whole mount and a sectioned root of one such epidermal sector. The sector is seen in only one cell file of the epidermis and is absent from the lateral root cap.

Three photographs taken from a series of sections through sectors induced in an epidermis/lateral root cap initial, in each of two roots are presented in Fig. 4. Fig. 4A is taken through the meristem, B in the region surrounding the central cells of the quiescent centre and C in the root cap. These sections show the extent of a sector, induced in the initial, as it extends basally into the lateral root cap and apically into the epidermis and lateral root cap.

Another prediction of our model (Dolan et al., 1993) is that cells of the central root cap, or columella, are derived from a lineage separate from the lateral root cap (and epidermis). Sectors induced in the central root cap were restricted to this region and never ran into the lateral root cap cells, thereby supporting the prediction that the two root cap lineages are distinct (Fig. 5).

No epidermal sectors were found to include cortical cells,

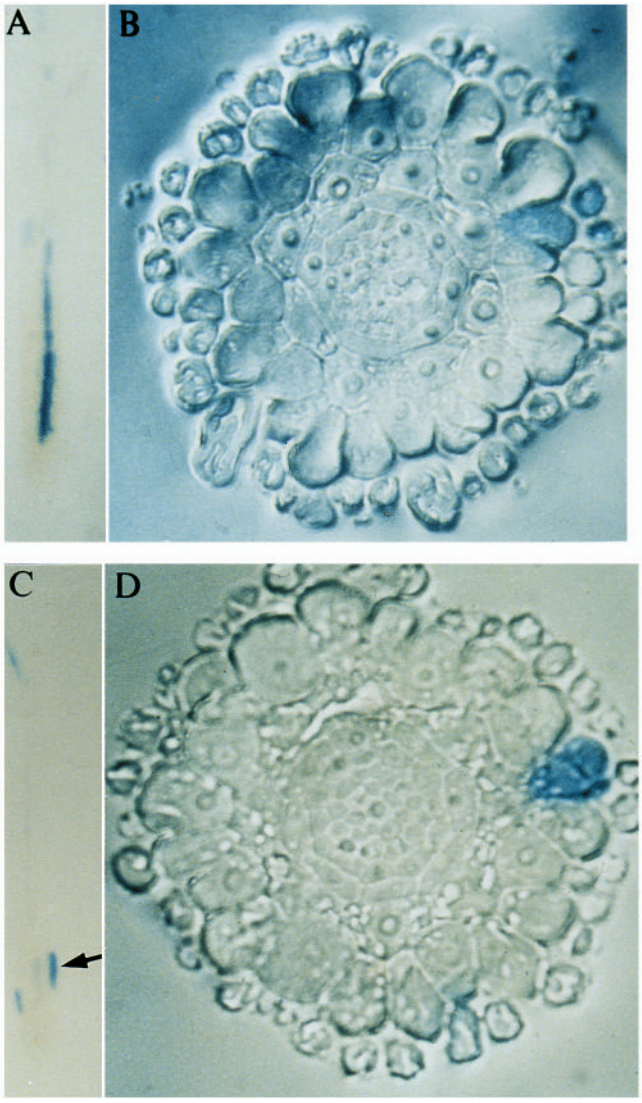

Fig. 3. *Ac* induced sectors indicate that the lateral root cap and epidermis are derived from a common initial. (A) Whole mount of a sector extending from the tip of the meristem into the elongation zone. (B) Cross section of the whole mount showing a single blue epidermal cell file next to a pair of blue lateral root cap cells. (C) An arrow points to a short sector in the meristematic zone (does not run to the root tip). (D) A transverse section through this root showing a single labelled epidermal cell file.

indicating that these are derived from separate initials. Most cortical sectors were restricted to the cortex, although a single sector was found to include a cortical cell file and the adjacent endodermal cell file. This sector ended in a single cell next to the central cells of the quiescent centre (Fig. 4E). This is the predicted location of the cortex/endodermal initial cell and supports the model that a ring of eight initials gives rise to 8 cell-file pairs, each pair comprising a single endodermal cell file and a cortical file (Dolan et al., 1993).

The number of epidermal cell files varies along the length of the root

The number of cell files in the epidermis at different points

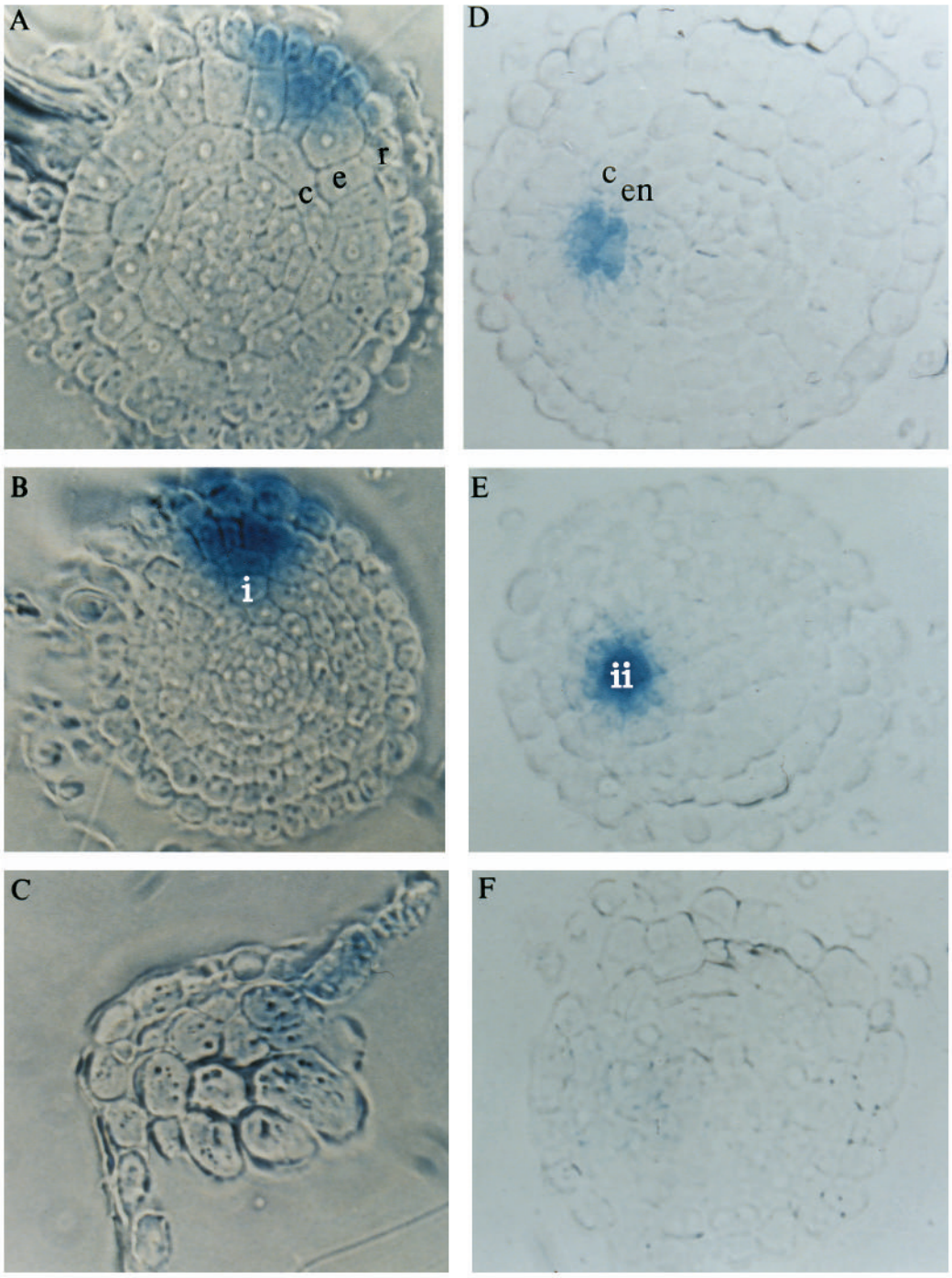

Fig. 4. Sectors indicate the extension of an epidermal sector into the root cap and that the endodermis and cortex are derived from a common initial. (A-C) Serial sections through an epidermal-lateral root cap sector show that it extends into the root cap. (A) Section in the meristematic zone shows the sector located in an epidermal (e) cell and adjacent lateral root (r) cap cells. (B) Section showing the presumed location of an epidermal/lateral root cap initial (i) and root cap derivatives. This section is just below the central cells of the quiescent centre. (C) Section showing very faint staining of cells to the side of the peripheral root cap. The cells at the centre of this section are unlabelled. (D-F) Serial transverse sections through a sector indicate that the endodermis (en) and cortex (c) are derived from a common set of initials. (D) Section through the sector in the meristematic zone shows the labelling of a single cortical cell file and the adjacent endodermal cell file. (E) Section made at the level of the quiescent centre showing that the sector ends in a single cell, the presumptive endodermal/cortical initial (ii). (F) Next section in the series is in the root cap shows that the sector ends at the presumptive initial and does not run into the root cap.

along a root was determined in four serially sectioned roots and the data are presented in Table 1. There was no difference between the number of cell files in the meristematic epidermis (protoderm) and the differentiation/elongation zone, where cell division had ceased, in one root. Cell file number was observed to increase in two cases and cell file number decreased in a single instance. This suggests that the number of epidermal cell files is fluid and may vary, either through longtitudinal anticlinal divisions or by loss of cell files in the meristematic zone in response to environmental or other factors.

The position of hair cell files is determined by the eight, underlying cortical cells

To determine the location of hair and non-hair cells in relation to the underlying cells of the cortex, sequential transverse sections over more than 7 mm of root were made on five wild-type roots. The location (relative to underlying cortical cells) was determined in a total of 248 hair cells. 247 hair cells were located over the anticlinal (radial) wall between two underlying cortical cells (Fig. 6A), while only a single hair cell was found over the periclinal (outer tangential) wall of a cortical cell (Table 2). Very occasionally adjacent files of hair forming

Fig. 5. Columella sectors are restricted to the columella. Arrow points to a blue sector restricted to the columella.

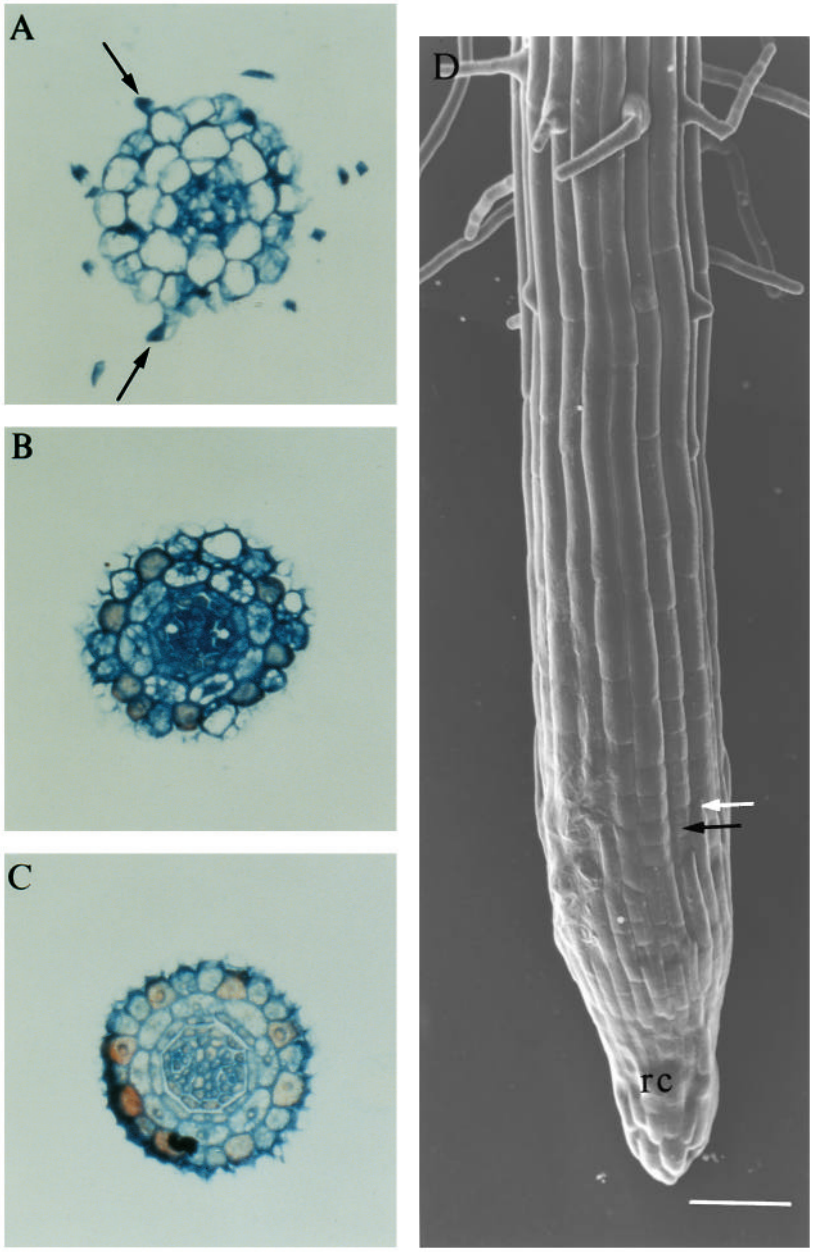

Fig. 6. Zonation in the root. Safranin/fast green stained sections in the root tip shows patterning in the epidermis before root hair differentiation. (A) Cross section in the differentiation zone shows the location of root hairs (arrows) in cells that overlie the anticlinal walls between underlying cortical cells. (B) Section in the zone of elongation shows that trichoblasts are more densely cytoplasmic (stain orange) than neighbouring atrichoblasts. (C) Cross section in the transition between meristem zones and elongation zones shows that trichoblasts are cytologically distinguishable from neighbouring atrichoblasts at this early stage. (D) Cryo-scanning electron micrograph shows the development of the epidermis from its emergence from beneath the root cap (rc) to the differentiation zone where root hairs are growing. Black and white arrows indicate the stage at which the trichoblast and atrichoblast files emerge from beneath the root cap respectively. Scale bar, 50 μm.

cells are observed but the data from the sequential sections indicate that this is a relatively rare event.

Sections made early in the zone of elongation indicate that cytoplasmic differentiation of the cells overlying the cortical walls is apparent before formation of the hairs. Fig. 6A-C shows three transverse sections from a series taken along the root. It is clear that from an early stage (Fig. 6C) the cells overlying the anticlinal cortical walls are more cytoplasmically dense (orange) than neighbouring epidermal cells. Fig 6A shows that it is these relatively cytoplasmically dense cells (located over the anticlinal cortical walls) that eventually form hairs and are therefore designated trichoblasts and their neighbouring cells, atrichoblasts.

The two kinds of epidermal cells can be distinguished at an early stage by at least two cellular features

Examination of the root surface with cryo-scanning electron microscopy (cryo-SEM) indicates that the epidermal cells of each type are arranged in files i.e. there are files of hair forming cells and files of non-hair forming cells (Fig. 6D). Hair cell files are generally separated by either one or two non-hair files. Close examination of the files in the meristematic zone,

Table 1. Change in cell number in the epidermis from meristem to differentiation/elongation zones in serially sectioned roots

Root	Epidermal cell number in the meristem zone	Epidermal cell number in the elongation zone
1	20	19
2	20	20
3	19	20
4	20	17

Table 2. Location of root hair cells in wild-type roots*

Number of hair cells located over anticlinal cortical cell walls	Number of hair cells located over periclinal cortical cell walls
247	1

*Sequential sections made on 5 roots over 7686 μm.

indicates that each cell type can be identified long before the hairs are formed (Fig. 6D). Cellular dimorphism between the files is already apparent soon after the emergence of the epidermis from beneath the root cap. A black arrow points to a 'short cell' and a white arrow points to an adjacent 'longer cell'. Following these cell files through the elongation zone and into the differentiation zone reveals that the 'short' cells belong to the hair forming files and the 'longer cells' to the non-hair forming files.

Cell lengths were measured on 4 roots, two of which were imaged with cryo-SEM and 2 with Nomarski optics. The data are presented graphically in Fig. 7A-D. Each set of curves can be divided into two clear populations, one of longer cells and another of shorter cells. The curves of longer cells (atrichoblasts) correspond to the non-hair forming files and the shorter cells (trichoblast) to the hair forming files.

A second marker of cell fate is seen at the cell surface. Close examination of the surface of the root reveals the presence of deposits on the surface of cells of the non-hair files (Fig. 8A). These structures are observed using the cryo-SEM technique but are occasionally not visible due to the deposition of ice and other substances on the root surface. These structures are not an artefact of the SEM technique since they are also observed on specimens visualised directly with Nomarski optics as they are growing in air along the surface of agar in Petri dishes (Fig. 8B). Whether these are epicuticular waxes or some other crystalline deposit is not known. The chemical composition of these deposits has not been determined.

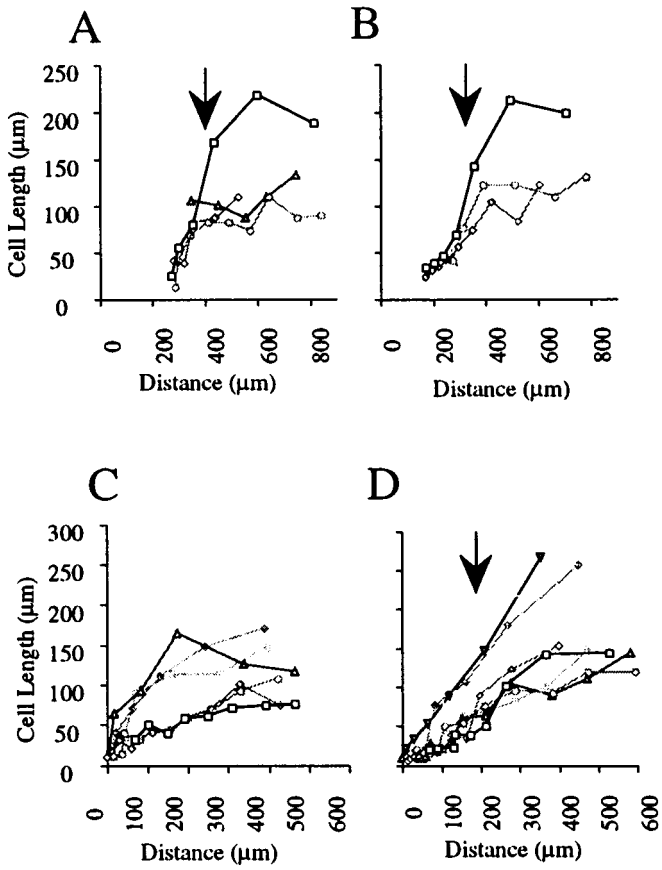

Fig. 7. Epidermal cell length measurements show that shorter epidermal cells form root hairs when root elongation ceases. The arrow marks the time at which the first sign of hair formation (bulge) becomes visible. (A,B) Cell length measurements made on two living roots growing in Petri dishes using long working distance objectives. Distance corresponds to distance from the central cells of the quiescent centre. (A) Cell lengths from a non-hair file are plotted in the upper curve, the lengths of hair forming cells are plotted in the three lower curves. (B) Cell lengths from the non-hair file are presented in the upper curve and cell lengths from two hair forming files are presented in the lower two curves. (C,D) Cell lengths plotted as a function of distance from a chosen point in the root tip in two specimens photographed with cryo-SEM. Distances from the quiescent centre could not be determined with SEM. (C) Three upper curves correspond to non-root hair files and the lengths of the hair forming files are the lower set of curves. (D) Two upper curves are non-hair files and the five lower curves are hair forming files.

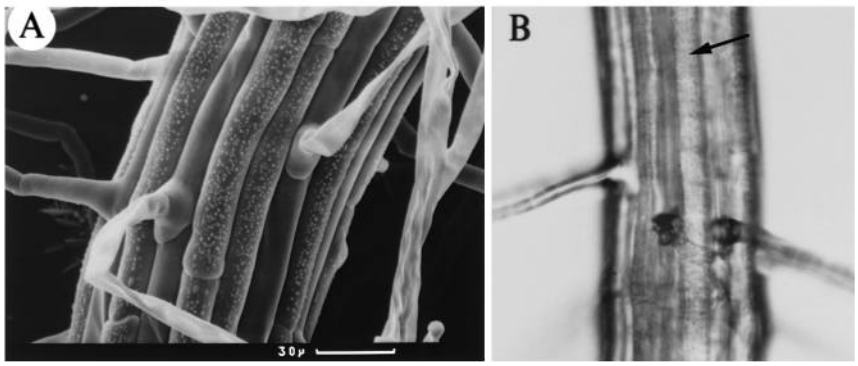

Fig. 8. A speckled deposit accumulates on the surface of non-hair cells. A. A cryo-SEM showing the deposits on the surface of non-hair cells while the hair cells are devoid of such deposits. (B) Deposits visualised on living roots with Nomarski optics. The deposits are absent from the hair files adjacent to the non-hair file (arrow).

Hair growth takes place in three discrete phases

Observations from time-lapse video microscopy, cryo-SEM and light microscopy all support a model of trichoblast differentiation in which three clear structural phases succeed one another. The first is the appearance of a small 'bulge' at the basal end of the trichoblast. The second is characterised by the development of a slow, tip-growing hair (hair proper) from a selected site on this bulge, and there is a third and final phase in which the rate of hair tip-growth increases.

Close examination reveals that the formation of a bulge in the basal region of the outer periclinal wall, takes place shortly before the cessation of elongation in the epidermal portion of the cell (Fig. 7). The stage at which the bulge forms is indicated on the graph with an arrow. The hair proper subsequently grows from this bulge, by a process known as tip-growth, only after the epidermal portion of the root hair cell has ceased elongation. Further confirmation that hairs grow on non-elongating epidermal cells comes from time lapse video recordings. Fig. 9A shows a cell on which a bulge has formed, which then proceeds to grow into a hair as indicated in Fig. 9B-D. Arrows indicate a pair of contaminating spots on the root surface in the vicinity of the growing hair. These spots are separated by a distance of 20 μm throughout the duration of the observations (271 minutes) and confirm that cell elongation has ceased in the region of the root in which hairs are growing.

These time lapse recordings indicated that following bulge formation, hairs exhibit two distinct phases of growth. There is an initial slow phase with a characteristic growth rate of 0.2-0.5 μm/minute followed by a phase with a growth rate of approximately 1-2.5 μm/minute (Table 3). The transition from the early slow phase to the fast phase is variable and takes place when the hair is between 20 and 40 μm in length (Fig. 9A-D).

Table 3. Growth rates of root hairs

Root hair	Early growth rate (μm/min)	Late growth rate (μm/min)
A	0.4	2.5
B	0.3	1.2
C	0.4	1.2
D	0.5	1.1

ctr 1 mutant phenotype indicates that ethylene may be a regulator of differentiation in the epidermis

The surgical studies carried out by Bünning (1951) indicated that a diffusible regulator of root hair formation was important in specifying cell identity in the root epidermis in the related species, radish. It was suggested that this molecule might also play a signalling role in the wound response of plants. One candidate molecule that fits these criteria is the plant growth regulator, ethylene. If ethylene indeed is a signal then the analysis of mutations in the ethylene response pathway may reveal altered patterning of cells in the epidermis. A mutation in the *Constitutive Triple Response* (*CTR1*) gene of *Arabidopsis* causes the plant to behave as if it were grown in the presence of ethylene (Kieber et al., 1993). Our phenotypic analysis shows that mutant *ctr1* plants have ectopic root hairs.

ctr1 plants have stunted, hairy roots (Kieber et al., 1993). To determine if this hairiness could be attributed to the presence of hairs in ectopic locations compared to wild type, i.e. in positionally defined non-hair files, sequential sections were made on two light grown roots over 1170 μm. 144 hair cells were located over the anticlinal cortical cell walls while 40 hair cells were located over the periclinal cortical walls i.e. were ectopic (Table 4 and Fig. 10). It is clear that the strict pattern described in the wild type (Table 2) breaks down in mutant plants.

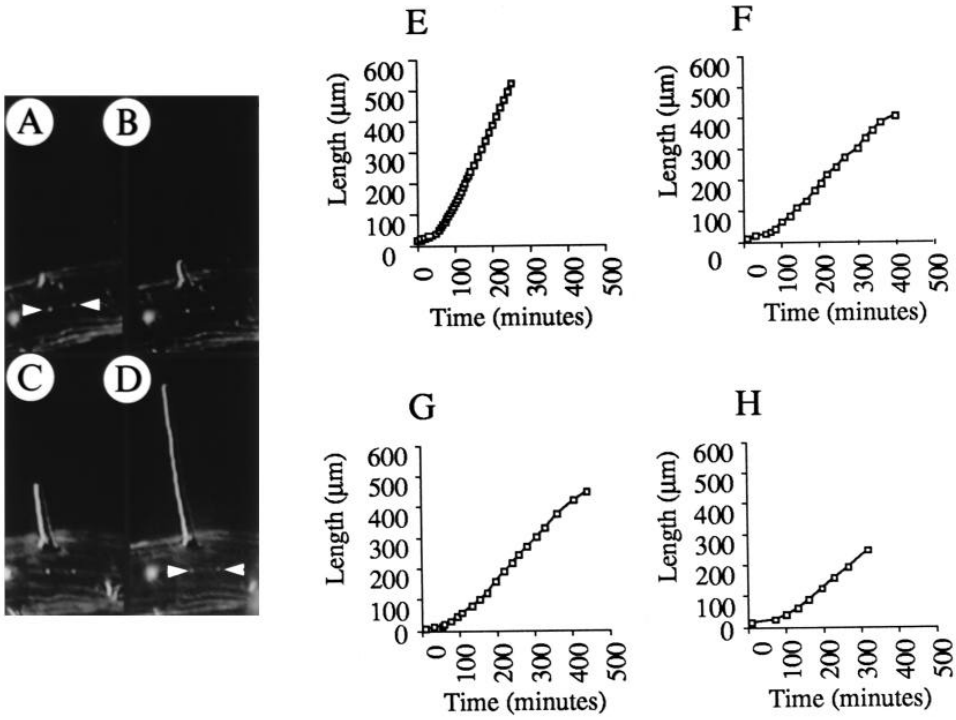

Fig. 9. Time lapse video recordings indicate that hairs grow slowly at first and grow more quickly after they reach a length of 20-40 μm. A-D. Time lapse images of growing hairs visualised with dark-field optics, taken over a period of 271 minutes. Two contaminating spots are highlighted with arrows. They are separated by a distance of 20.6 μm throughout the duration of these observations indicating that the root is not elongating in this region. (A) 0 minutes. (B) 40 minutes. (C) 101 minutes. (D) 271 minutes. (E-H) Plots of root hair length versus time for four growing hairs. Scale bar, 20 μm.

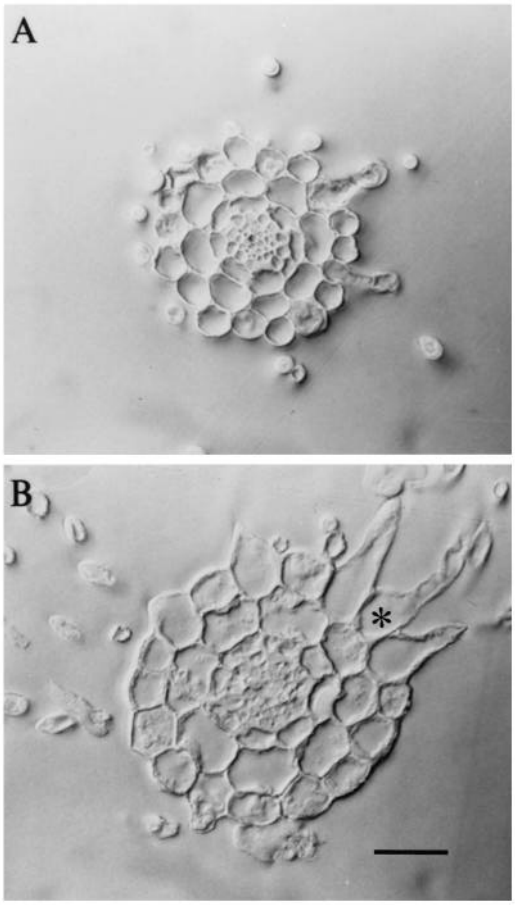

Fig. 10. *ctr1* plants form hairs in ectopic locations. (A) Cross section of a wild-type root showing the location of hair cells over the anticlinal walls of cortical cells. (B) Cross section of *ctr1* root showing the presence of three adjacent hair cell files. The middle hair cell file is in an ectopic location (asterisk). Scale bar, 30 μm.

Table 4. Location of root hairs in *ctr1* roots grown in light*

Number of cells located over anticlinal cortical cell walls	Number of hair cells located over periclinal cortical cell walls
144	40

*Sequential sections made on two roots over 1170 μm.

DISCUSSION

Our description of the development of the *Arabidopsis* root epidermis highlights a number of features that make it a useful system for the study of pattern formation and differentiation in plants. It possesses two cell types that are precisely arranged in space and whose differentiation can be directly visualised because of their superficial location and the small size of the root. The precise spacing of these cells means that mutations in genes that are involved in the regulation or execution of the patterning process can be isolated.

Lineage of the *Arabidopsis* root epidermis

Our earlier anatomical analysis lead to a model of root development that included the prediction that a ring of 16 initials gives rise to both the epidermis and the lateral root cap (Dolan et al., 1993). It was suggested that each of these initials divided periclinally to form an outer cell that gave rise to a double file of lateral root cap cells and an inner cell that underwent transverse divisions and gave rise to a file of epidermal cells and a new initial cell. This initial could then undergo the same pattern of cell divisions. This model was proposed because it was the only pattern of cell divisions that could account for our observations on the orientation of cell walls in the lateral root cap and epidermis of the root.

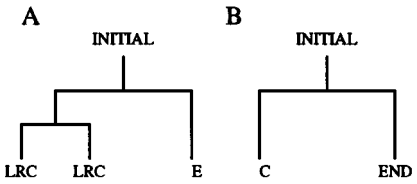

Fig. 11. Summary of the epidermal-lateral root cap (A) and endodermal-cortical (B) cell lineages. C, cortex; E, epidermis; END, endodermis; LRC, lateral root cap.

The sectors described in this paper are consistent with our model in that they now show experimentally that the sequence and pattern of cell divisions predicted do in fact occur. A parallel study using the same marker lines also supports the model (Scheres et al., 1994). The lineage of the epidermis, based on both anatomical and clonal analysis is represented simplistically in Fig. 11A and that of the cortex and endodermis in Fig. 11B. A clonal relationship between the epidermis and lateral root cap has been proposed in a number of species, such as tobacco (Esau, 1941; Williams, 1947) but the absence of clonal analyses in roots has meant that such proposals were of necessity hypothetical (Dolan, 1994). Our sectors also indicate that the columella (central root cap) forms a lineage distinct from that of the lateral root cap/epidermis. That these two cell lineages are separated very early in development, and yet, together form a coherent root cap, suggests that cell communication of some description must be important for the normal coherent development of the cap.

Patterning in the root epidermis

The organisation of cells in the epidermis of *Arabidopsis* is similar to that of other members of the Brassicaceae in that the hair cell files are located over the anticlinal walls of the cortex and the non-hair cell files are located over the outer periclinal walls of the cortex (Cormack, 1947; Peterson, 1967). The hair forming cells therefore abut two cortical cells and the non-hair epidermal cells touch a single cortical cell. Since there are 8 cell files in the *Arabidopsis* root cortex there are only 8 root hair cell files lying over their 8, adjacent, anticlinal walls. Trichoblast differentiation in numerous plants is known to be associated with increased DNA content as a result of endoreduplication (Cutter and Feldman, 1970; Dosier and Riopel, 1978) It is not known if the trichoblasts of *Arabidopsis* exhibit such increase in DNA content but we think it highly likely since trichomes undergo numerous rounds of endoreduplication during cell morphogenesis (Hülskamp et al., 1994). The mechanism responsible for regulation of such position-specific

differentiation is unknown but this cellular organisation led Bünning (1951) to suggest that the hair cells were isolated from the underlying cells (by an air space) and therefore not exposed to a signal acting as a negative regulator of root hair formation in radish (a related species). Surgical experiments in which the undifferentiated epidermis was removed from the underlying tissue resulted in the formation of a greater number of trichoblasts (root-hair forming cells) than in non-surgically removed epidermis. These experiments have since been repeated with the same results (Barlow, 1984). Another model that explains patterning in the epidermis suggests that the location of cells over the anticlinal cortical wall gives them access to larger quantities of nutrients than the other epidermal cells and that the intercellular space underlying the hair cell acts as a reservoir of materials such as sugars (Cormack, 1947; Cormack and Lemay, 1963). This model has not been tested experimentally.

The *ctr1* phenotype and cell signalling in the root epidermis

The genetic dissection of the ethylene response pathway has identified a number of gene products that appear to be involved in a signal transduction phosphorylation cascade. The phenotype of the loss of function, recessive *ctr1* mutation indicates that the wild-type gene product is a negative regulator of this cascade (Kieber et al., 1993; Kieber and Ecker, 1993). *CTR1* encodes a serine threonine kinase Raf homologue (Kieber et al., 1993). Members of the Raf serine threonine kinase family have been shown to play important roles in signal transduction pathways important for cell differentiation in a variety of animal systems. The *pole hole* (*ph*) gene of *Drosophila* encodes the *Drosophila* homologue of the v-*Raf* oncogene and is necessary for the differentiation of the R7 cell of the ommatidium (Ambrosio et al., 1989; Dickson et al., 1992). Similarly the *lin-45* gene product encodes a Raf serine threonine kinase inolved in vulval differentiation in *Caenorhabditis* (Han et al., 1993). The extra root hairs characteristic of the *ctr1* phenotype indicate that the wild type, *CTR1* gene, would then encode a negative regulator of hair formation and that ethylene acts as a positive regulator of hair formation in the root epidermis. Ethylene is known to be a potent inducer of root hair differentiation in a number of species (Crossett and Campbell, 1975; Reid, 1987). The induction of root hair formation by ethylene was first illustrated by Cormack (1935). Light grown roots of *Elodea* fail to form hairs, yet upon exposure to ethylene a profusion of root hairs was observed. Baskin et al. (1992) have shown that ethylene treatment of growing *Arabidopsis* roots induces the formation of extra hairs but their exact location was not determined. We propose that ethylene acts as a diffusible positive regulator and confers the 'hair' character on cells overlying cortical, anticlinal walls. Ethylene may accumulate in the air spaces that form at the junction between trichoblasts and the underlying cortex (Bünning, 1951). The location of trichoblast cells over these spaces may expose these cells to elevated levels of ethylene and thereby induce hairs preferentially in these cells. Changing levels of ethylene in response to environmental conditions could mediate regulation of root hair density on roots, since it is known that physical stimulation of plants induces both ethylene production and root hair formation (Cormack, 1962; Reid, 1987). The activity of *CTR1* in the cells overlying the periclinal cortical walls may serve to inhibit ethylene induced hair formation. This would require that the *CTR1* gene or its gene products may be repressed in the trichoblast files (Fig. 12). This model is presently being tested through the characterisation of the root phenotype of mutants in the ethylene response pathway and by various physiological means.

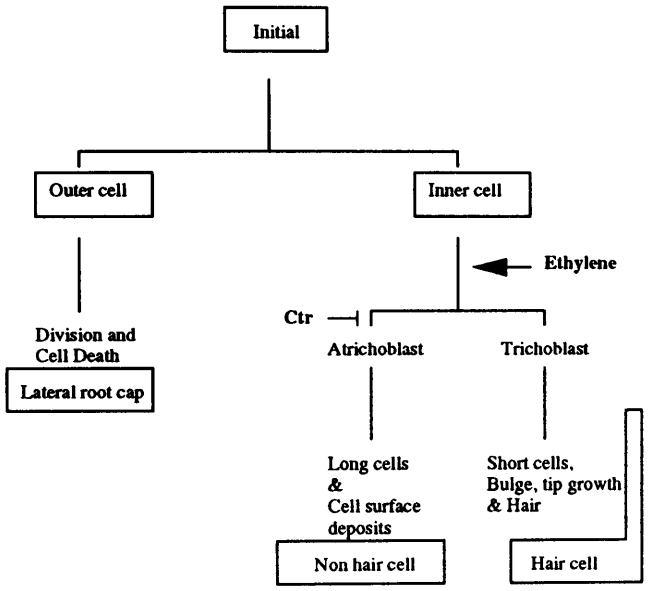

Fig. 12. Schematic representation of events in the root tip involved in the patterning and differentiation of the epidermis and lateral root cap cells. Branch points indicate 'developmental decisions' that are not necessarily related to lineage.

Hair growth and cell morphogenesis

Our analysis of root hair cell morphogenesis indicates that late in the elongation of the epidermal portion of the cell, a bulge forms at its basal end. A root hair then grows from this bulge. The formation of the bulge is accompanied by a local thinning of the cell wall (L. D., unpublished observations). The apical cytoplasmic organisation characteristic of a tip-growing cell then forms and a root hair grows once elongation of the epidermal portion of the cell has ceased.

This temporal uncoupling of the processes of cell elongation (in the epidermal portion of the cell) and hair growth has been noted previously in a number of species (Goodwin and Avers, 1956; Esau, 1965), and gives insights into the way in which this cell develops. It may be that the cell cannot grow conventionally (new wall and membrane material deposited in all regions of the cell) and undergo highly polarised, local tip growth at the same time. Such a polar growth process (the root hair) may require the localised redistribution of elements that are normally employed elsewhere in a non-localised fashion throughout a growing cell. Ongoing genetic studies will identify molecules involved in the formation of pattern and cell differentiation described here.

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