

Cellular organisation of the *Arabidopsis thaliana* root

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SUMMARY

The anatomy of the developing root of *Arabidopsis* is described using conventional histological techniques, scanning and transmission electron microscopy. The root meristem is derived from cells of the hypophysis and adjacent cells of the embryo proper. The postembryonic organization of the root is apparent in the mature embryo and is maintained in the growing primary root after germination. Cell number and location is relatively invariant in the primary root, with 8 cortical and endodermal cell files but more variable numbers of pericycle and epidermal cells. The organisation of cells in lateral roots is similar to that of the primary root but with more variability in the numbers of cell files in each layer. [³H]thymidine labeling of actively growing roots indicates that a quiescent centre of four central cells

(derived from the hypophysis) is located between the root cap columella and the stele. This plate of four cells is surrounded by three groups of cells in, proximal, distal and lateral positions. The labeling patterns of these cells suggest that they are the initials for the files of cells that comprise the root. They give rise to four sets of cell files: the stele, the cortex and endodermis, the epidermis and lateral root-cap and the columella. A model of meristem activity is proposed based on these data. This description of *Arabidopsis* root structure underpins future work on the developmental genetics of root morphogenesis.

Key words: *Arabidopsis*, root, meristem, pattern formation, cell fate

INTRODUCTION

Central to our understanding of development is the investigation of the genetic program by which it is directed. The coordinated expression of genes determines cellular characteristics which in turn direct morphogenesis. Genes important for specific developmental processes may be identified by mutation (e.g. Ingham, 1988; Nüsslein-Volhard, 1991; Horvitz and Sternberg, 1991; Mayer et al., 1991). To understand how normal development proceeds and to identify precisely the defects caused by the mutations, a detailed knowledge of the development and structure of the system under analysis is necessary. The value of such analysis is clearly illustrated by the contribution that fate mapping and other techniques of lineage analysis have made in a number of animal systems. For example, the blastula fate maps in *Drosophila* (Wieschaus and Gehring, 1976), and the detailed description of the *Caenorhabditis elegans* cell lineage (Sulston et al., 1983) have laid the foundation for the successful genetic analysis of developmental processes (Sternberg and Horvitz, 1984).

The postembryonic architecture of higher plants is formed from two meristems, which are laid down in the embryo. The cellular organisation of these two meristems is dramatically different. Periclinal chimeras have revealed that the

cells of the shoot meristem are arranged in discrete layers in which the outer layer (L1) undergoes predominantly anticlinal divisions to produce the epidermis of the shoot while the inner two layers (L2 and L3) divide both periclinal and anticlinal producing the major portion of the internal tissue (Satina et al., 1940). Although clonal analysis has identified regions in the meristem with predictable destinies, no strict correlation between meristematic cells and differentiated cell types can be observed (Poethig, 1987; Irish and Sussex, 1992; Furner and Pumfrey, 1992). In contrast to shoots, root meristems are subterminal in location since root cap cells, which slough off as part of a defined terminal differentiation pathway, are produced distally and the cells of the remainder of the root are produced proximally. Cell lineages are easily identified in roots because of the continuous files of cells that arise from repeated transverse divisions in meristematic cells. The cells of the root may arise from tiered sets of initials (closed type) as observed in tobacco and radish or there may be no separation of initials into tiers (open type) such as the organisation seen in *Pisum sativum* or *Fagus sylvatica* (von Guttenberg, 1947; Popham, 1955a,b; Clowes, 1949, 1981). While the organisation of cells in the meristem is often characteristic for that species the structure of the root meristem may change during ontogeny. This is to be seen in the organisation of the meristem of *Helianthus annuus*

and in many other species from a range of families where the initials change from the tiered to the untiered state in older roots (von Guttenberg, 1947, 1964; Armstrong and Heimsch, 1976). Since it has not been possible to obtain stable periclinal chimeras in roots because lateral roots are generally derived from one tissue layer (pericycle) and not all three (L1, L2 and L3) as in shoots, there has been no illustration of the functional tiered organisation of the root meristem by clonal analysis, as there has been in the shoot meristem (Satina et al., 1940; Brumfield, 1943).

To understand the process of morphogenesis in roots, both the developmental history of cell files in the root and the ontogeny of the initial cells must be known. The morphologies, numbers and spatial relationships of the cells must be determined. We present the results of such an analysis for the root of *Arabidopsis thaliana*, a plant ideally suited for subsequent genetic dissection of development (Meyerowitz, 1989). We report here the number, position and morphology of the cells in the *Arabidopsis* root. This study reveals elegant pattern simplicity and little variance in the position and number of cells comprising the primary root. Anatomical data from roots in seedlings and in embryos, together with *in vivo* labeling experiments are combined in a model for the activity of the *Arabidopsis* root meristem initials. This model describes both the postembryonic meristematic activity and the generation of the meristem structure from cells of the suspensor (hypophysis) and the embryo-proper during embryogenesis, and thus provides the foundation for genetic analysis of root meristem formation and activity.

MATERIALS AND METHODS

Plant growth conditions

A. thaliana ecotype 'Columbia' seeds were sterilized in 5% sodium hypochlorite and left to imbibe at 4°C in the dark in sterile water containing 0.1% agarose (Gibco) for 2-5 days. Subsequently, seeds were allowed to germinate on plates containing 1× Murashige and Skoog (MS) salt mixture and 0.5 g/l 2-(N-morpholino) ethanesulfonic acid (MES), pH 5.8, in 1% Duchefa agar. Plates were incubated in a near vertical position in a Heraphyt growth chamber (Heraeus Votsch) at 22°C, 70% humidity, and a 16 hours light/8 hours dark cycle.

For the [³H]thymidine studies, seeds were treated as above until 3 days after germination. Subsequently the seedlings were transferred to agar plates as above, containing in addition 2 µCi/ml [³H]thymidine (TRK.418, 46 Ci/mmol; Amersham).

In order to perform embryo anatomy studies, plants were grown on soil under the same growth conditions as stated above until seed set was apparent.

Light microscopy

Sectioning, staining and autoradiography

Complete seedlings, dissected roots, or imbibed but not germinated seeds were harvested for anatomical studies. For studying embryo development, growing siliques were harvested from soil-grown plants, and subsequently embryos were dissected under a binocular microscope.

All tissues were fixed for 3 hours in 1% glutaraldehyde, 4% formaldehyde in 50 mM sodium phosphate buffer, pH 7.2, rinsed for 30 minutes in the same buffer, and concentrated in 0.6% agarose (Gibco). Agarose blocks were dehydrated in a graded

series of 10%, 30%, 50%, 70%, 90% and 3× 100% ethanol. Technovit 7100 (Kulzer, Heraeus) infiltration was performed according to the manufacturer. 3-4 µm sections were made on a Reichert-Jung 1140 rotary microtome using Adamas disposable steel knives. Sections were stained in fresh 0.05% toluidine blue-O (Merck). For cytoplasmic staining the incubation was performed at room temperature for 1 minute.

In the [³H]thymidine labeling experiments sections were coated with Kodak NBT-2 nuclear emulsion diluted 1:1 with 600 mM ammonium acetate. Upon exposure for 48 hours silver grains were developed in Kodak D19 developer for 3 minutes and fixed in Kodak Fix. A more predominant cell wall staining was achieved by incubating in 0.05% toluidine blue-O at 60°C for 30 seconds.

Sections were mounted in DePeX (BDH) and photographed on a Zeiss Photomikroskop III using Kodak Ektar 25 film.

Immunocytochemistry

2 mm root tip segments were fixed in 2.5% glutaraldehyde in 50 mM cacodylate pH 7.2 for 1-2 hours at 0°C. After two washes in distilled water roots were dehydrated in an alcohol series and embedded in LR White resin (London Resin Company) and polymerized either at 60°C or at room temperature with 0.5% benzoin methyl ether mixed with the resin. 200 nm sections were made on a Reichert Jung Ultracut ultramicrotome and floated onto glass slides. Sections were labeled with JIM 7 rat monoclonal antibody as described previously (Knox et al., 1990), mounted in Citifluor (Agar) and photographed on a Zeiss Universal microscope with TMAX 400 film. Specimens of roots that had undergone secondary thickening were fixed and embedded as described here and 500 nm thick sections cut and labeled with fresh 0.1% Toluidine Blue-O in 0.1% sodium tetraborate for 5-10 seconds followed by a quick wash in water.

Electron microscopy

Plant material was fixed and embedded as described for the antibody labeling above. 100 nm sections were picked up on coated grids and stained with uranyl acetate and lead acetate (Millonig, 1961). Specimens were examined on a JEOL 1200EX transmission electron microscope.

RESULTS

Arrangements and numbers of cell files in the primary root

The external morphology of the *Arabidopsis* root tip is shown in Fig. 1A. There are three distinct but overlapping zones. The meristematic zone constitutes the distal 250 µm of the root. This zone is characterised by small cells overlaid by the root cap. Proximal to this zone the elongation zone is visible, which also spans about 250 µm. The next zone is the differentiation zone, in which elongated cells from the different tissues mature into fully differentiated cells. The distal end of this zone is marked by those epidermal cells that are forming bulges at their distal ends, which will mature into root hairs. In this paper we define the embryonically derived root as the primary root. Roots that arise from the pericycle of this and other roots are lateral roots.

Transverse sections of the primary root reveal that the organisation and numbers of cells is remarkably constant between roots. Fig. 1B shows a colour-coded transverse section taken approximately 1 mm from the primary root tip of an *Arabidopsis* seedling 3 days after germination. This specimen illustrates the characteristic organisation of the

root. The central cylinder is diarch with two clearly differentiated protoxylem elements and at 90 degrees to these are the two protoxylem elements. The outer layer of the central cylinder, the pericycle is composed of an average of 12 cells in circumference (cf Fig. 10). Outside this layer is the endodermis, which is composed of eight cells. In the mature region of the root, protoxylem elements always abut pericycle cells which each face two endodermal cells (all 54 protoxylem elements in 27 roots); protophloem cells almost always flank at least one pericycle cells which faces only one endodermal cell (50 protophloem elements in 27 roots). The cortex, which abuts the endodermis on its outer side, is also a single ring of eight cells. The epidermis is composed of two discrete cell types, the identity of which depends upon their position relative to the underlying cortical cells. Epidermal cells that overlie the anticlinal walls between adjacent cortical cells differentiate into cells that produce root hairs (trichoblasts; Cormack, 1949). The adjacent epidermis cells abut only one cortical cell and generally do not produce a root hair.

Median longitudinal sections display cell files which suggest the meristematic origin of each of the cell types discussed above. Fig. 1C depicts a colour-coded median longitudinal section of the root tip from a 3 day old seedling. Cells of the mature part of the root form contiguous cell files ending at or very close to the characteristic 'central cells'. The longitudinal section depicts lateral and columella root cap cells in addition to the cell types apparent in a transverse section of the mature root.

Serial transverse sections taken at a variety of distances from the root tip of a seedling 3 days after germination are depicted in Fig. 2. Sections closest to the root tip (Fig. 2A) show columella cells (12, in a pattern of four surrounded by eight), surrounded by one complete layer of lateral root-cap cells (between 16 and 32 in circumference; lr-1 in Fig. 2A). In sections made further back from the tip, a complete second layer of lateral root cap cells can be seen (Fig. 2B, lr-2). A 16-cell epidermal layer can now be distinguished immediately within this second lateral root cap layer. Characteristically, each epidermal cell is abutted by either one or two lateral root cap cells on its outer periclinal wall. At this level the centre of the root is occupied by large cells which are either columella cells or 'central' cells (see below). Fig. 2C depicts the next level of sectioning in which epidermal cells are flanked by at least two root cap cell layers. Some of these epidermal cells are split by a further periclinal cell wall (arrow), thereby giving rise to a third root cap cell layer. At this level the future cortex, pericycle and vascular cells can be clearly distinguished by virtue of both their shape and position. Fig. 2D shows that the final number of cell files, as shown in Fig. 1B, arises within 150 μm of the root tip. (Distance from the root tip is measured as distance from the basal wall of central cells). Once the number of cell files has been set down, different cell files mature at different rates. For example, protophloem sieve tube elements become clearly differentiated 300 μm from the central cells but protoxylem elements do not mature until later. Fig. 2D depicts the level at which only one lateral root cap layer remains. The more densely cytoplasmic nature of the future, root hair-forming, trichoblast cells compared to the other epidermal cells can be clearly seen at this early stage. The number of

epidermal cells in circumference has increased to an average of 19 at this level. Pericycle cells appear to remain more densely cytoplasmic than many of the surrounding cells.

In Fig. 2E,F sections through mature root and hypocotyl, respectively, are shown. The central tissues in the hypocotyl are arranged in a similar way to those of the root. The major differences are: larger numbers of cell files in the stele, a second, outer layer of cortex composed of more than eight cell files (Fig. 2F; c-2), and an epidermis with almost twice the number of cells as the root epidermis. In terms of pattern, the most distinctive difference between the root and the hypocotyl is that a single extra layer of cortical cells is present in the latter.

Differentiation of the various cell types is accompanied by dramatic changes in cell morphology which are illustrated in Fig. 3. Electron micrographs of sections approximately 2 μm and 1000 μm above the central cells are depicted in Fig. 3A and 3B, respectively. The cells lying proximal to the central cells are densely cytoplasmic with the nucleus almost filling the cross-sectional area of a number of cells (Fig. 3A). These cells are undergoing transverse divisions forming new cell plates with numerous plasmodesmata; plasmodesmata are also detectable in the older longitudinal walls (data not shown). These cells are extremely small with a diameter of 2-3 μm which makes them some of the smallest plant cells known. Numerous small vacuoles are also evident even at this early stage. Transverse sections of the stele made 1 mm from the central cells show the organisation of cells in the stele (Fig. 3B).

The root is typically diarch. Cells that will later differentiate into metaxylem are located between the two protoxylem elements (mx and px in Fig. 3B). The two protophloem sieve tube elements (st) are located on an axis perpendicular to the xylem axis. Each protophloem sieve tube element is adjacent to an associated companion cell (cc) and two phloem parenchyma cells. Other cells in the stele exhibit varying degrees of vacuolation. Plasmodesmata are also present in the walls of all cells of the stele at this stage of development (not shown).

Origins of cell files in the growing root as predicted by anatomy

Medial longitudinal sections through the primary root show the relationships of the various cell files to each other (Fig. 4A, schematised in Fig. 1C). The cells of the meristem are arranged into three tiers of cells and therefore correspond to the closed meristem organisation of von Guttenberg (1964). The lower tier of cells (protoderm) is composed of the initials of the root cap and epidermis. The middle tier (periderm) is composed of central cells flanked by cells which are contiguous with the files of the cortex and endodermis. The upper tier of cells (plerome) is contiguous with the cells of the stele.

The lower tier forms two types of root cap cells, the central columella and the lateral root-cap, each of which arise from entirely different groups of cells in the tier (Figs 4A, 1C). The columella of the root cap arises from periclinal divisions in the group of cells at the columella base termed 'columella initials', hereafter. The lateral root cap appears to arise from a set of cells forming a collar around the columella initials. Therefore the cells of the lateral root

cap are not clonally related to the cells of the columella. Three layers of lateral root cap can be distinguished in Fig. 4A (lr-1 to lr-3). The epidermis is derived from the same set of initials as the lateral root-cap (Figs 4A, 1C). In addition the lateral root cap and epidermis initials do not appear to give rise to any other cell types. The location of the cell that forms the lateral root cap and epidermis ('epidermal initial') indicates that this cell must undergo periclinal divisions at the same time as the columella initials; otherwise the columella would separate from the lateral root cap. That the structural integrity is preserved by synchronous cell divisions, and not by differential cell expansion, is illustrated by the observation that the number of lateral root cap cell layers is always the same as the number of mature columella cell layers. In addition the position of the periclinal cell walls is contiguous in lateral root cap and columella.

The central tier contains four central cells that are laterally continuous with the cortex and endodermal files (Figs 4A, 1C). It is noteworthy that we have never detected mitotic figures in these cells, in contrast to those occasionally seen

in the surrounding cells. Furthermore, these cells do not need to divide to preserve the junction of the columella and the remainder of the root. Therefore, we regard them as potentially quiescent cells. However, since the relationship of the cell walls in cells of these files are not informative as to the origin of the files, as is the case for the epidermis, anatomical studies cannot provide proof that these cells contribute to the cortical and endodermal cell files.

In the ring of cells encircling the central cells (Figs 4A, 1C), and on which the cortical and endodermal cell columns end, mitotic figures can occasionally be found (data not shown). Usually a new transverse wall is formed in these cells (Fig. 4A, small arrow), but occasionally a new periclinal wall can be formed (Fig. 1C, column to the left). From this we conclude that the two cells may be initials giving rise to sister (in the majority of cases where a transverse wall is first formed), endodermal and cortical cells and are therefore referred to as cortex/endodermal initials hereafter. An anatomical argument supporting the fact that these cells must be initials is that, assuming limited 'slippage' of cell

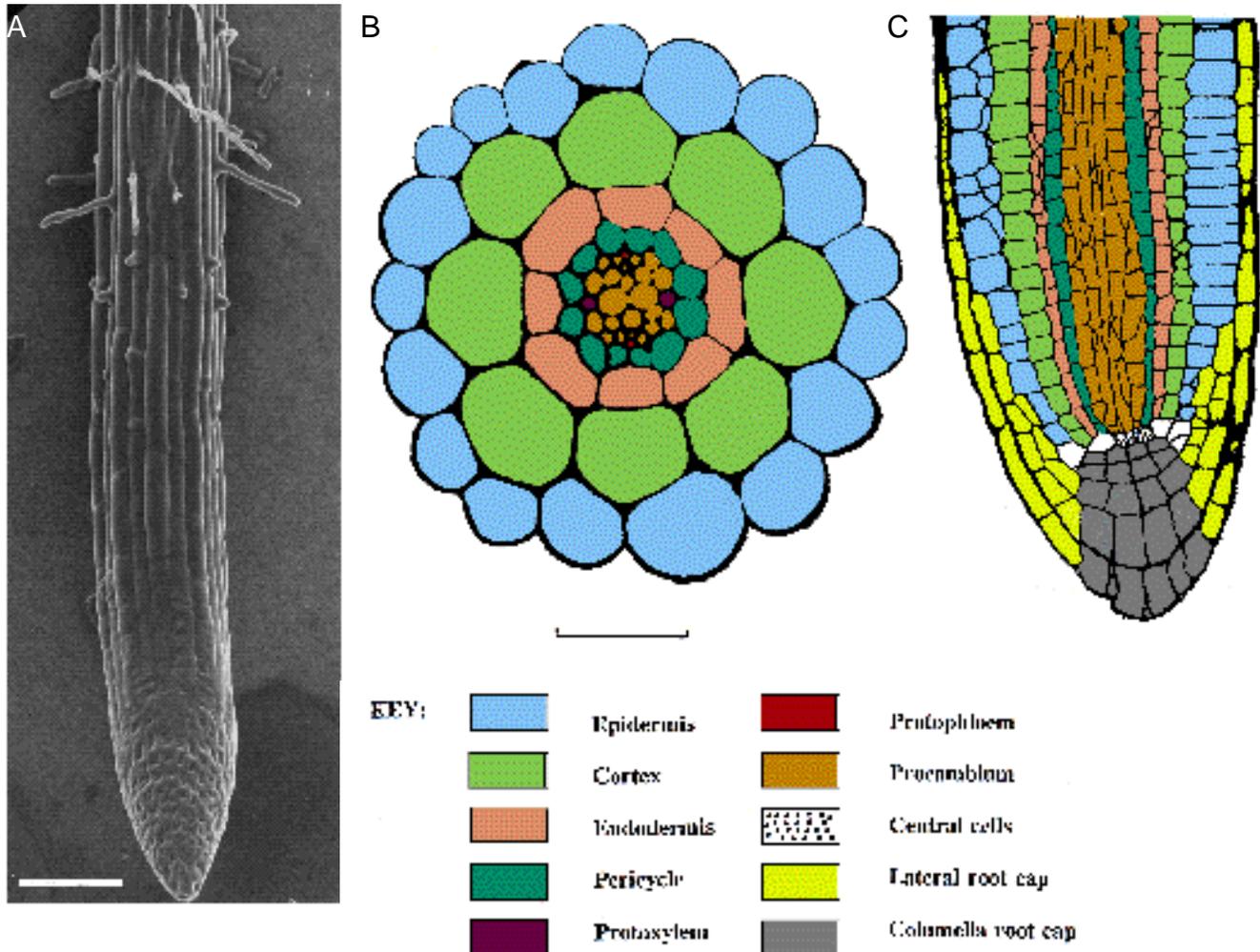


Fig. 1. Organisation of tissues in the *Arabidopsis* root. (A) Exterior view of *Arabidopsis* root. Bar, 100 µm. (B) Transverse section of root approx. 1 mm behind to the root tip, labeled with anti-pectin antibodies, reverse-printed and colour-coded. (C) Median longitudinal section of root tip, labeled with anti-pectin, reverse-printed and colour-coded. The different cell types are indicated in the colour legend. Bar, 25 µm.

walls, they must divide together with the adjacent epidermal initial cells in order to preserve the integrity of the root. The remainder of the central cylinder appears to be derived from the upper tier, located immediately above the central cells (Figs 4A, 1C).

Ultrastructure of cells in the meristematic region

An electron micrograph of an adjacent longitudinal section to the one shown in Fig. 1A is depicted in Fig. 5A. It is clear that the cells of the stele do not undergo vacuolation to the same extent as the the cells outside the stele. The central cells are also less densely cytoplasmic and appear extremely inactive with only a sparse endomembrane system, fewer ribosomes and mitochondria than surrounding cells.

Roots from seedlings grown on MS medium plus sucrose grow faster than roots from seedlings grown on MS medium without sucrose. We analysed whether sucrose addition led to structural alterations in the root meristem. Fig. 5B shows an electron micrograph of a median longitudinal section of a primary root from seedlings grown for 2 days in the presence of sucrose. No obvious differences can be observed in the cellular ultrastructure of the root

meristem grown without or with sucrose, as can be seen in Fig. 5A, and B, respectively. At later stages some alterations in central and columella cell size may be seen, without clear consequences for the meristematic organisation (data not shown). Nevertheless, roots grown in the absence of sucrose appear to vacuolate more quickly than those grown in sucrose.

Embryonic origin of the primary root

Sections of developing embryos reveal that the observed basic pattern of the cellular organisation in the root is set up early. Fig. 4B,C show median longitudinal sections of *Ara - bidopsis* embryos at mature embryo stage and late heart stage (stage 19 and 14, Jürgens and Mayer, 1993). In mature embryos the organisation of putative initials surrounding the central cells can be seen (Fig. 4B). The two lateral root cap layers (Fig. 4B, lr-1 and lr-2) are faced by the two outer columella layers. The most apical columella layer is facing the epidermal initials and a completed division indicative for the formation of a new layer can be seen.

In late heart-stage embryos a similar organisation can be first seen, but now with only one lateral root cap layer (Fig.

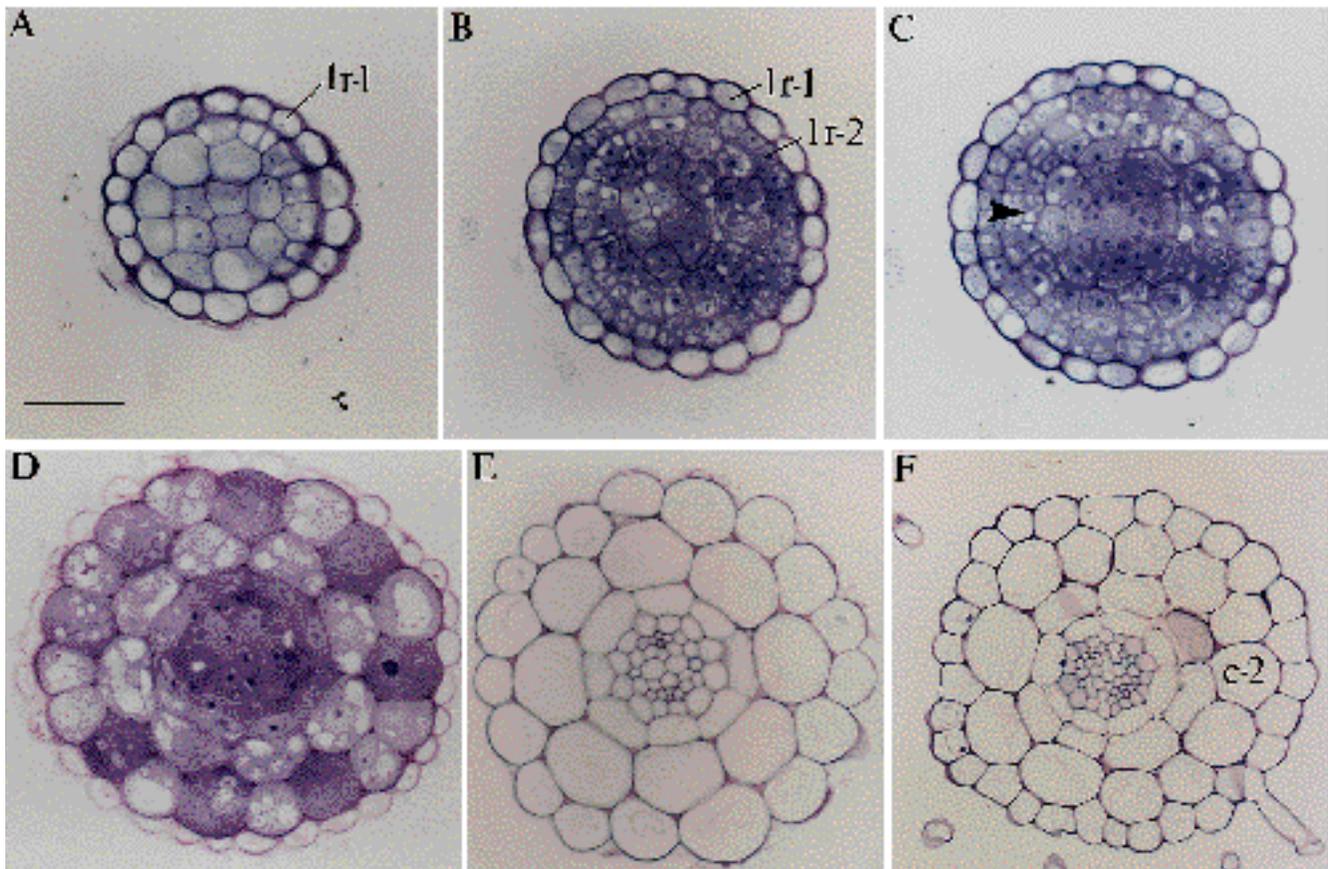


Fig. 2. Radial cellular organisation in root meristem, mature root, and hypocotyl. Series of toluidine blue stained transverse sections from apical root tip to hypocotyl (A to F) of an *Arabidopsis* seedling 3 days after germination. (A) Columella region. (B) Central cell region. Most distal section showing trichoblasts. (C) Central cell region. Most apical section showing vascular cells. Arrowhead: new periclinal wall in epidermal initial. (D) Trichoblast differentiation zone. (E) Mature root zone with strong cell wall staining of xylem and phloem elements. (F) Hypocotyl zone with an additional cortical cell layer. lr-1, first (outer) lateral root cap layer; lr-2, second lateral root cap layer; c-2, second cortical cell layer. Bar, 25 µm.

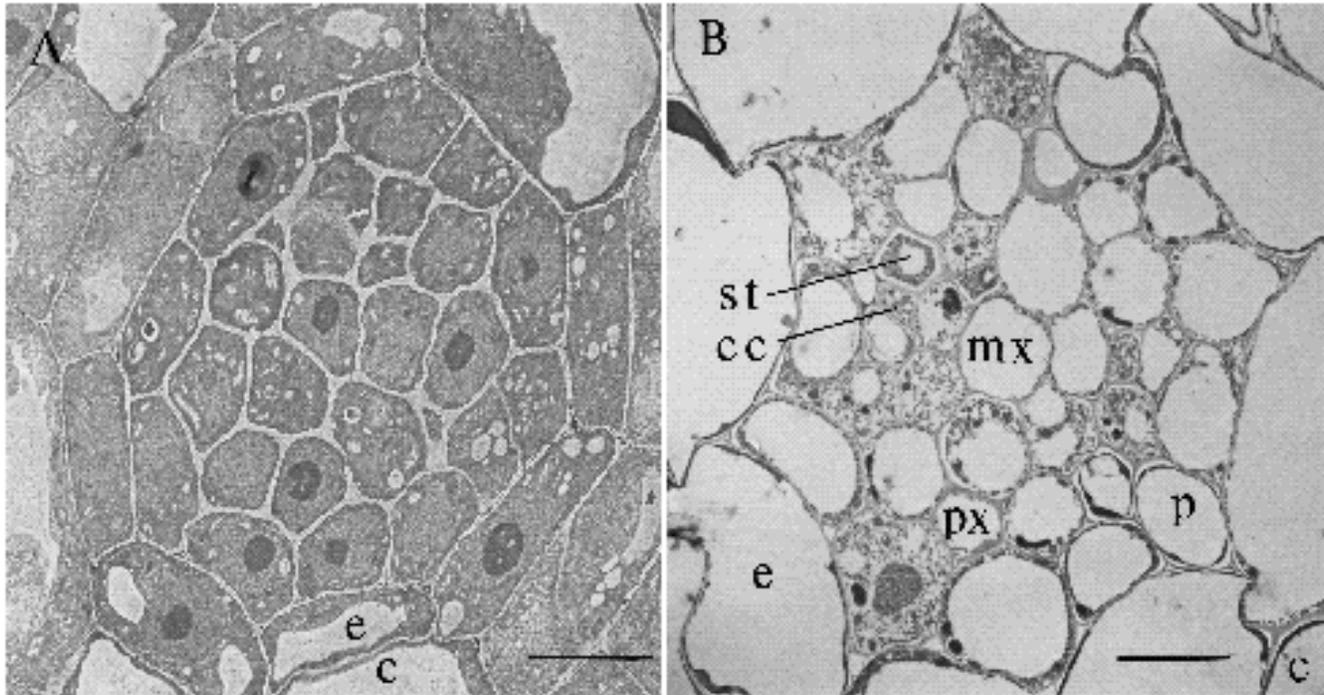


Fig. 3. Ultrastructure of the vascular system at two stages of maturation. Transmission electron micrographs of transverse sections at (A) 2 μm and (B) 1000 μm proximal to the central cells. e, endodermis; c, cortex; p, pericycle; st, sieve tube element; cc, companion cell; px, protoxylem; mx, metaxylem. Bar, 4 μm .

4C, Ir-1), faced by one columella layer. Periclinal divisions increase the number of root cap layers during embryo development.

Columella and central cells form a separate block of cells, which can be identified as staining slightly differently in the heart-stage embryo (Fig. 4C). Both the central cells and the columella initials derive from the hypophysis, which divides producing a lenticular cell that gives rise to the four central cells and below these, a cell divides to form the columella initials. In contrast, the proposed epidermal/lateral root cap, cortical/endodermal, and vascular initials originate from cells derived from the embryo 'proper' (derivatives of the distal cell of the proembryo) (B.S. and K.J. data not shown; for a description of the early stages of *Arabidopsis* embryogenesis, see Mansfield and Briarty, 1991, and Jürgens and Mayer, 1993).

Radial anatomy of the primary root prior to germination

Postembryonic development starts from the mature embryo in the dessicated seed. To establish the original number of the different initials that perpetuate postembryonic root development we sectioned seeds and studied the radial anatomy of the embryonic root. Fig. 6 shows a series of transverse sections through root and hypocotyl prior to germination. Closest to the tip, the spatial arrangement of 12 columella cells surrounded by lateral root cap cells, which was seen in the growing root (Fig. 2) is apparent (Fig. 6A,B). In Fig. 6C the four central cells (c; only two visible in longitudinal section) each abutted by four epidermal initials, are clearly seen. The epidermal initial cells are larger than the central cells. In the next section (Fig. 6D) these central cells,

of which the point of convergence of the cell walls can still be distinguished, appear to flank the first vascular cells and the eight putative cortical/endodermal initials situated outward and over them. Sections higher up in the embryonic root show the cellular pattern present in a mature postembryonic root. However, characteristic features of the postembryonic pattern like dense cytoplasm in the trichoblasts, and vascular cell maturation are absent. It is noteworthy that not all cells in the cortex/endodermal files, undergo the longitudinal division forming these two cell types simultaneously (Fig. 6E, arrowhead). This figure shows how cells in some files have formed endodermis and cortex while the progenitor cell is present in other files. An additional feature to be noticed in Fig. 6E is the presence of only 10 pericycle cells instead of the average 12 seen in the mature root, whereas higher sections (Fig. 6F,G) show an increase to 12 pericycle cells. In summary, the anatomy of the embryonic root is very similar to that of the postembryonic root in terms of cellular patterns. The exact positioning of putative meristematic initials is therefore already established during embryogenesis not only in the longitudinal but also in the radial dimension (cf. Fig. 4C,D).

The hypocotyl region of the embryonic root (Fig. 6H) shows the typical presence of a second layer of cortex cells and an epidermis which now contains on average 26 cell files. One endodermal cell layer in this section occupies the position of both endodermis and innermost cortical cell in the other cell files (Fig. 6H, arrowhead), a situation that is often observed (data not shown). This implies that the endodermis and inner cortex files are formed by a periclinal division in a pre-existing file of cells during embryogenesis.

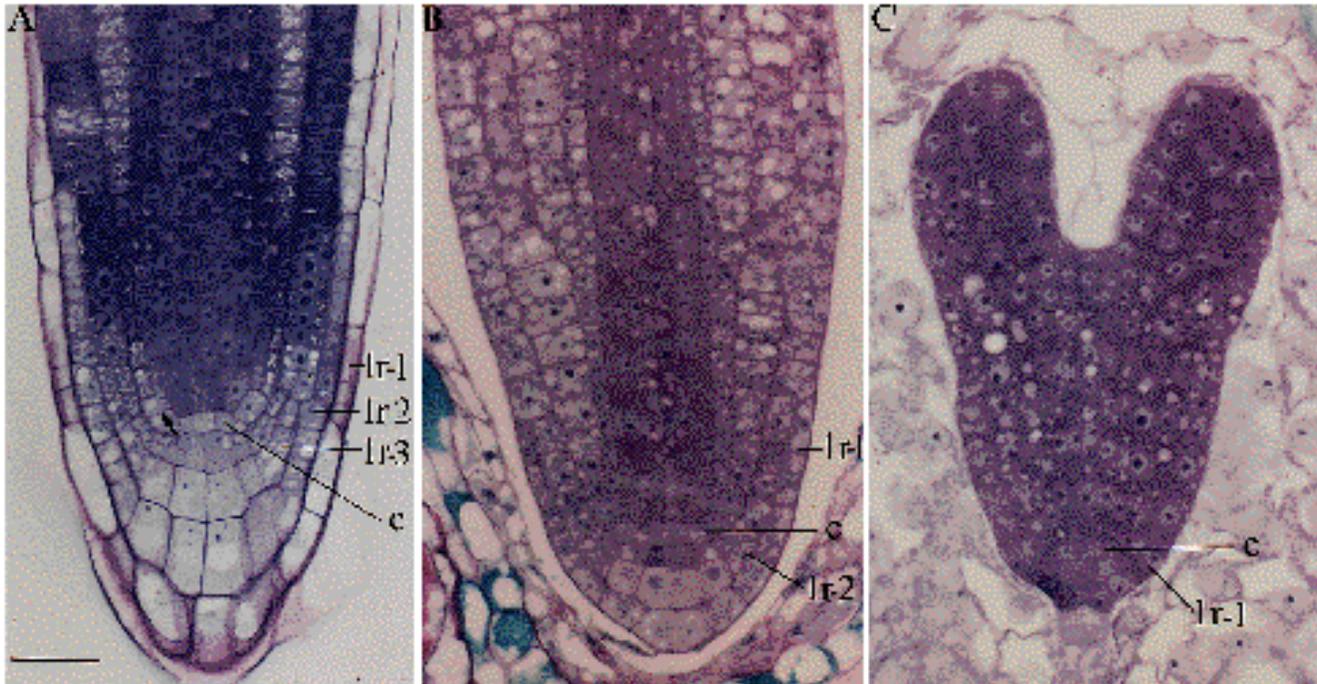


Fig. 4. The origin of cell files in the *Arabidopsis* root. (A) Median longitudinal section of a root tip from a seedling 3 days after germination, stained with toluidine blue, showing typical organisation of cell files in the root meristem converging on central cells. Arrow: new transverse wall typical for cortex/endodermis initial cells. (B) Median longitudinal section of torpedo stage *Arabidopsis* embryo showing arrangement of cells surrounding the central cells. (C) Median longitudinal section of late heart stage embryo with arrangement of cells surrounding central cells, and periclinal division giving rise to first lateral root cap layer. c, central cell; lr-1, first lateral root cap layer; lr-2, second lateral root cap layer; lr-3, third lateral root cap layer. Bar, 25 μ m.

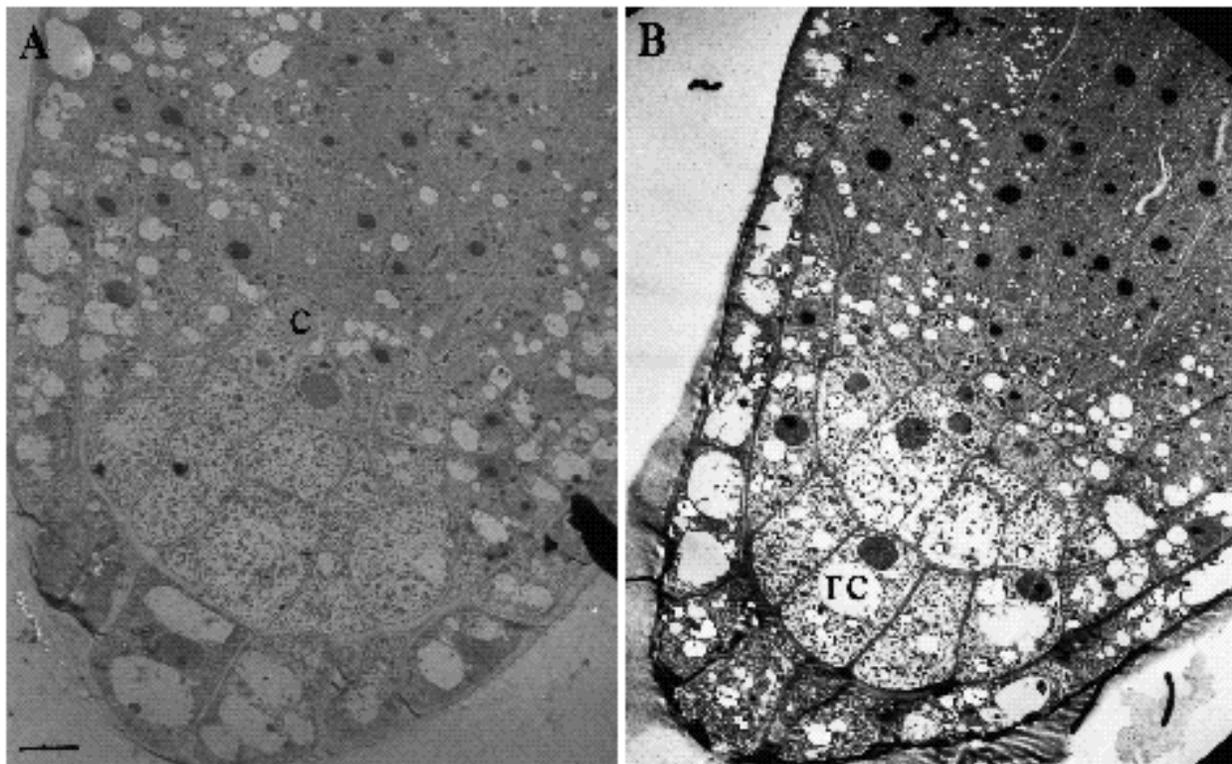


Fig. 5. Ultrastructure of the *Arabidopsis* root meristem initials. (A) Transmission electron micrograph of median longitudinal section of root tip 2 days after germination. (B) Transmission micrograph of median longitudinal section of root tip 2 days after germination on medium containing 1% sucrose. c, central cell; rc, columella root cap. Bar, 5 μ m.

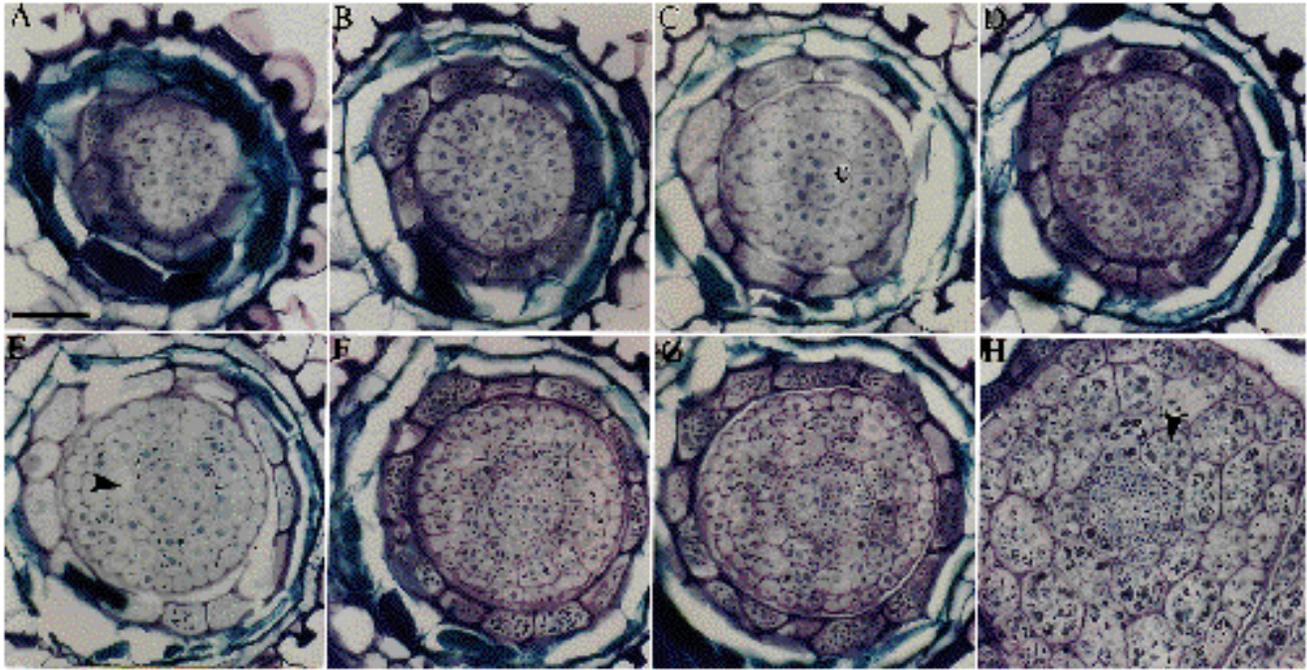


Fig. 6. The radial arrangement of root cells is present in the embryo. Series of toluidine blue-stained transverse sections of imbibed seed from the root tip to the hypocotyl region. (A) Columella region. (B) Columella surrounded by two layers of lateral root cap. (C) Central cell and termination of epidermis and lateral root cap cell files. (D) Termination point of vascular and cortical/endodermal cell files. (E) Region where endodermis forms. Arrowhead: endodermis/cortex progenitor cell lacking periclinal wall. (F) Region where endodermis is completely formed but incomplete pericycle cell number. (G) Cellular root pattern complete. (H) Hypocotyl with additional cortical cell layer. Arrowhead: endodermis/cortex progenitor cell lacking periclinal wall. c, central cell. Bar, 25 μ m.

Cell division in the meristematic zone

To determine if cell cycle times in the root meristematic zone are in agreement with our anatomically derived model of a population of initials surrounding the four quiescent central cells, roots were incubated for various times with [3 H]thymidine, and incorporation of label was examined on sections by autoradiography. This gives an indication of which cells in the root were going through S-phase of the cell cycle.

In Fig. 7 three adjacent longitudinal sections are shown from the root tip of a seedling transferred to [3 H]thymidine-containing medium 3 days after imbibition, and grown on this medium for 4 days. These and similar sections from different roots invariably show that labeled nuclei can be found over any cell when the nucleus is in the sectioning plane, with the exception of the central cells (compare Figs 4A and 1C). The labeling density is highest in the meristematic zone of the root proximal to the central cells. The columella cells label with much lower frequency. This observation suggests that the columella initials divide only once to produce one tier of daughter cells, while other cells in the root are actively dividing for some distance along each file.

In the sections shown, thymidine incorporation can be observed in two epidermal initials (Fig. 7C), two columella initials (Fig. 7A,B), and at least two initials for the stele bundle (Fig. 7A,B,C). In cortical initials, which are larger in size, no labeled nuclei are well depicted in this series, but they have been observed as being labeled in other sections (data not shown).

Frequently nuclei in the third cell layer of the columella

are labeled (Fig. 7B, arrow). We have never observed simultaneous labeling of tier 2 cells, nor new cell plate deposition in this region of the columella, hence we believe that the labeling of this specific layer of columella cells is related to endoreduplication rather than mitosis. In lateral root cap cell files the frequency of labeling is consistent with the frequency of labeling in neighbouring epidermal cell files and no evidence for endoreduplication can be obtained.

In order to get an idea of the increase of the fraction of labeled nuclei with time, we performed similar labeling experiments and analyzed root of seedlings grown for 16, 48, and 96 hours in the presence of [3 H]thymidine. Roughly half of the cells in the meristematic zone, derived from the epidermal, cortical and vascular initials contained labeled nuclei already after 16 hours. In contrast, half of the initial cells surrounding the central cells were labeled only after approx. 48 hours. Labeled nuclei were never observed in the central cells.

These data must be interpreted with caution since it is not possible to discriminate between thymidine incorporation during endoreduplication versus incorporation preceding cell division, and because of the technical difficulty in assigning labeled nuclei to the small-sized cells in the meristem. Nevertheless the data lead us to conclude that four central, quiescent cells are surrounded by columella-, epidermal-, cortical- and vascular initials, which divide at an approximately threefold lower rate than their derivative cells in the meristematic zone. Exceptions are the cells of the columella, which do not divide further but appear to endoreduplicate in tier 3.

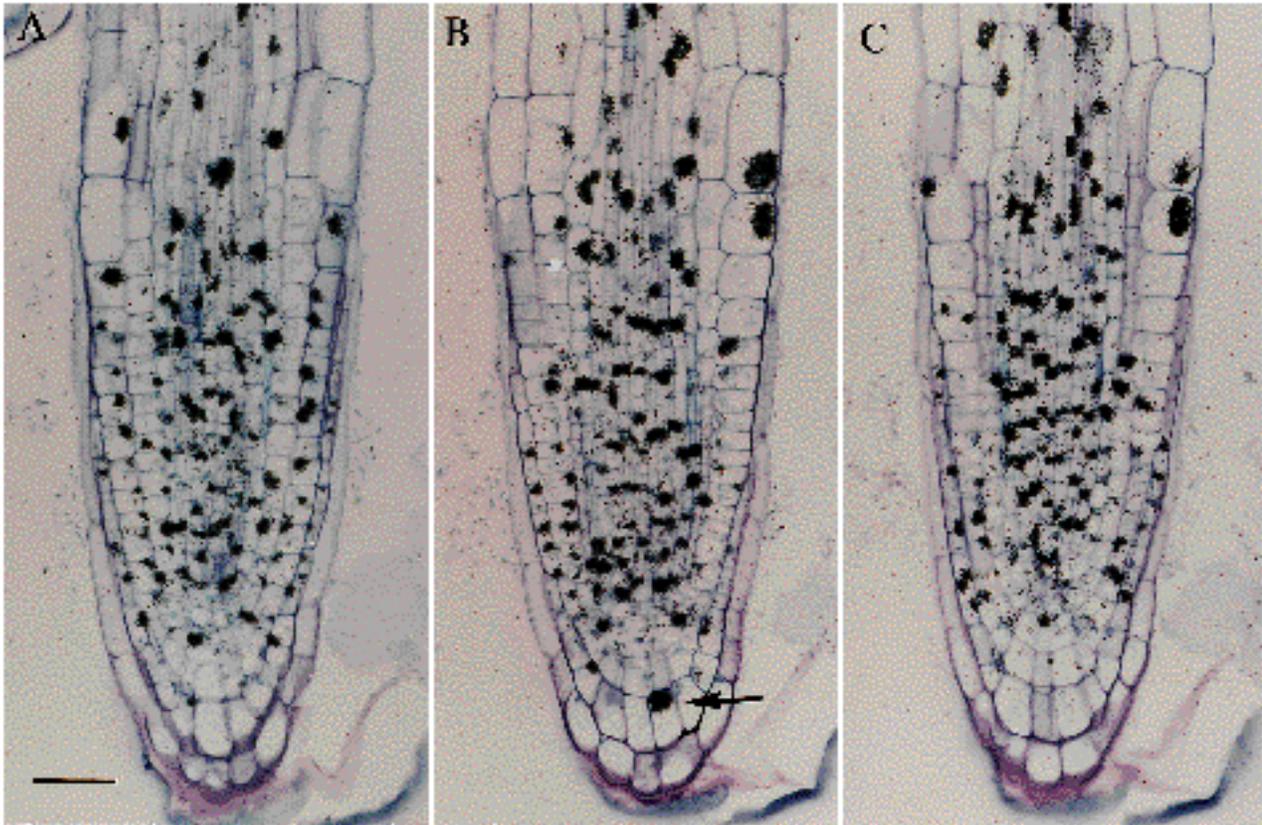


Fig. 7. Cell cycle as measured by [³H]thymidine incorporation is slow in central cells. (A,B,C) Serial longitudinal sections of root tip 7 days after germination and 4 days after the addition of [³H]thymidine. B is the median section; silver grains, shown in black, overlie nuclei in which DNA replication has taken place. Arrow: labeling of tier 3 columella cell nucleus. Bar, 50 μ m.

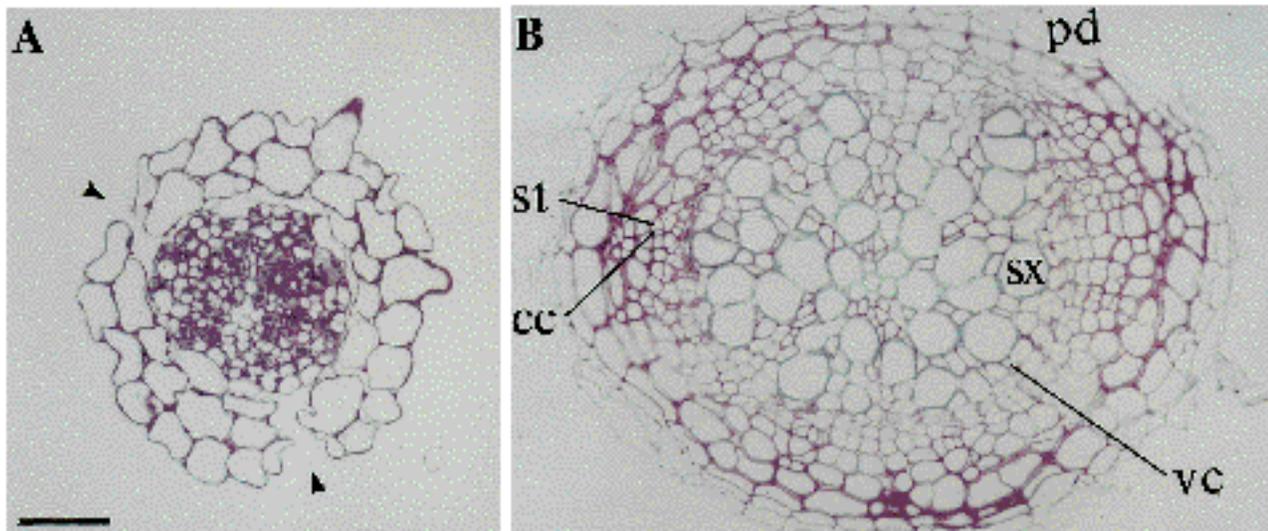


Fig. 8. *Arabidopsis* roots undergo secondary thickening. (A) Transverse section of a 3 week old root which has undergone secondary thickening. Arrowheads: cell separation sites. (B) Transverse section of a root at a later stage of secondary thickening. vc, vascular cambium; sx, secondary xylem; st, sieve tubes; cc, companion cell; pd, periderm. Bar, 28 μ m.

Secondary thickening changes the mature root structure

Transverse sections of roots that have undergone secondary thickening were made to analyse structural alterations asso-

ciated with this process. Fig. 8A is a transverse section of a root in the early stages of secondary thickening. The three outer layers (epidermis, cortex, and endodermis) are still present but in the process of being lost, as illustrated by the

separation of cells in two places (Fig. 8A, arrowheads). The new cells produced during secondary thickening are derived from two populations of cells in the stele of the primary root.

The morphology of an older secondary thickened root as depicted in Fig. 8B is the result of the activity of these two cell populations. One of these groups of cells arises from

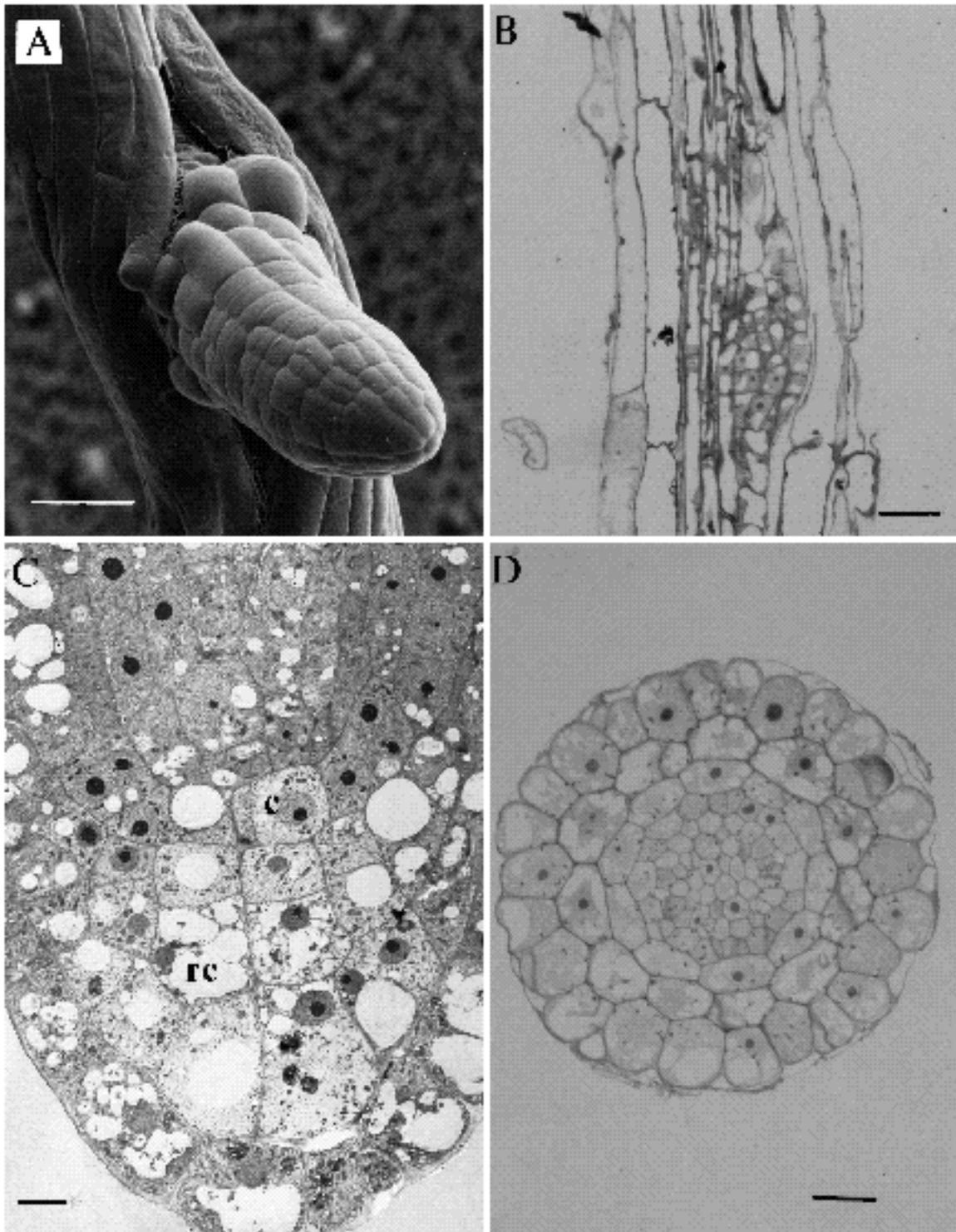


Fig. 9. Lateral roots originate from the pericycle and exhibit a morphology comparable to the primary root. (A) Scanning electron micrograph of an emerging lateral root. Bar, 50 μm . (B) Pericycle cell divisions elaborating a lateral root primordium. Bar, 30 μm . (C) Transmission electron micrograph of a median longitudinal section with characteristic organisation of the meristem. Bar, 5 μm . (D) Transverse section of a lateral root showing organisation of tissues similar to main root. c, central cell; rc, columella root cap. Bar, 20 μm .

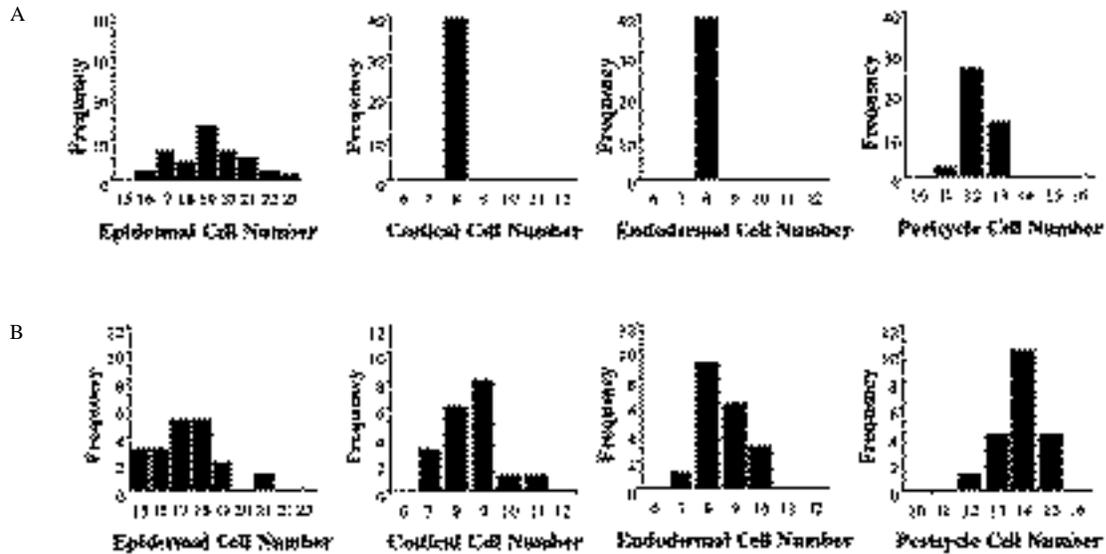


Fig. 10. Conserved numbers in primary and lateral roots. (A) Histograms showing frequency distribution of cell numbers in different cell layers of primary roots. (B) Histograms showing frequency distribution in different cell layers of lateral roots.

parenchymal (thin-walled) cells located between the primary xylem and phloem. These cells form a vascular cambium (vc) composed of cells that are thin in cross sections and as thickening progresses, form an oval ring with the phloem on their outside and the xylem on their inside. Secondary xylem is composed of large lignified xylem vessel elements (sx), staining blue with toluidine blue, inter-

persed among non lignified cells that stain red. The original primary protoxylem and metaxylem are located in the centre of the root with newer elements being formed on the outside. Phloem is differentiated among the population of cells which are cut off from the outside of the vascular cambium. Sieve tube elements (st) and adjacent companion cells (cc) are embedded in a layer of cells with apparently unthick-

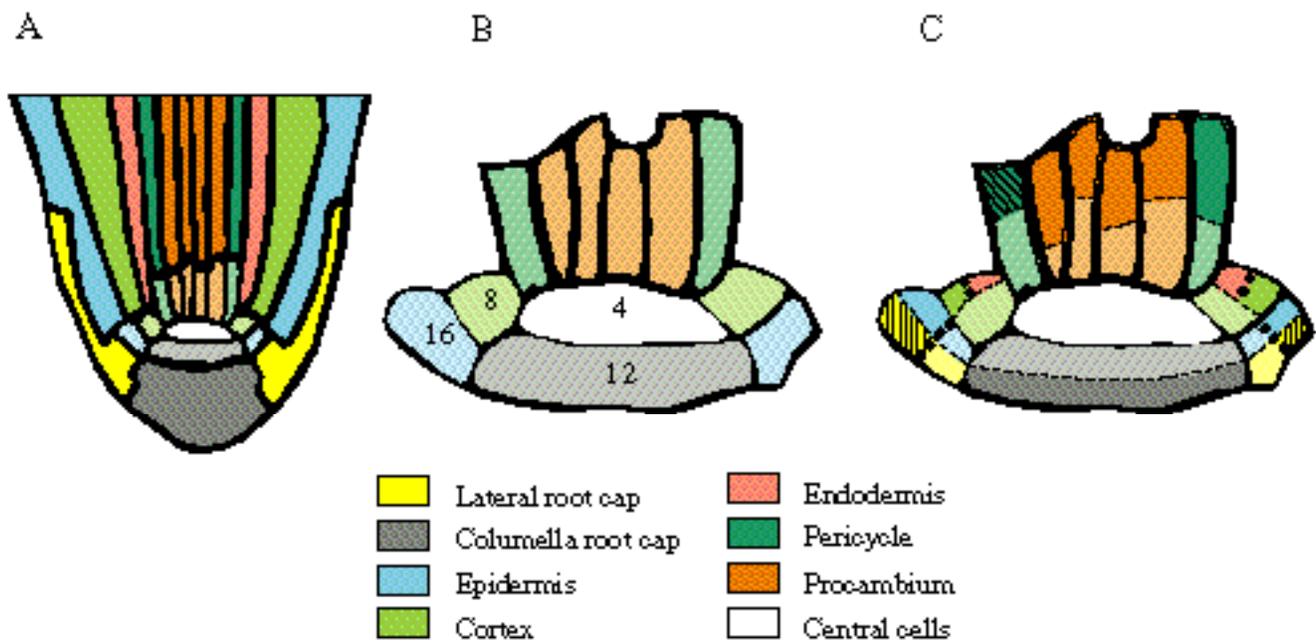


Fig. 11. A model for cell division patterns in the primary root meristem of *Arabidopsis thaliana*. (A) Schematic drawing of position of initials (less intense colouring) for all tissue layers surrounding the central cells in the mature embryo of Fig. 4B. (B) Magnification of the initials in the 'core meristem' outlined in A. Fixed numbers of the different initials as found in mature embryos are depicted. (C) Division patterns of the initials that allow re-establishment of identically grouped initials and derivatives, which can form all different tissue layers. The order of divisions is depicted as follows: dashed lines, first division; dots, second division; striped, third (radial) division. Different cell types are depicted in the colour legend.

ened cell walls. To the outside of the phloem is a layer of thickened fibre cells which are derived from the pericycle of the unthickened root. These fibres are surrounded by the cells of the periderm (pd) which are formed by predominantly periclinal divisions in a layer of cambium known as the phellogen. This layer is composed of approximately three cell layers in this specimen and it appears that the cells on the periphery are lost as growth proceeds.

Lateral roots: a different story

Lateral roots arise endogenously at a distance behind the primary root meristem. Lateral root formation is initiated by periclinal and anticlinal divisions in pericycle cells of the primary root (Fig. 9B). Upon formation of a lateral root primordium by pericycle derivatives, a meristem is formed from a subset of these cells. The anatomy of primary and lateral roots were compared to assess whether this difference in ontogeny is reflected in different arrangements of cell files in the root.

The electron micrograph in Fig. 9C shows that cells at the distal end of cell files in lateral roots do not differ significantly in morphology from those in primary roots. The cytoplasm of the central cells (c) and columella cells (rc) is again less dense than that of the surrounding cells in the root (cf. Fig. 5). The precursor cells in the lateral root primordium from which these central and columella cells are formed, and the central and columella cells in young lateral roots have a cytoplasmic density comparable to the surrounding cells (B.S. and L.D., unpublished data). This may indicate that these cells acquire a more quiescent character in the course of lateral root development.

Fig. 9D shows a transverse section through a lateral root in which there are 11 cortical cell files. The organisation of the lateral roots is similar to that of the primary roots in that it is always diarch, and shows the same organisation of tissue layers. However, there is much variability in the numbers of cell files in each layer, as summarised in Fig. 10. The number of cortical and endodermal cell files is more variable in lateral roots than in primary roots. Cell file numbers in the cortex range from 7 to 11 with an average of 9 while there are between 7 and 10 endodermal cells also with an average of 9.

DISCUSSION

General anatomical features of the *Arabidopsis* root

The anatomy of the *Arabidopsis* root has a similar although simpler tissue organisation to that described for other members of the Brassicaceae (Cormack, 1947; von Guttenberg, 1947; Bunning, 1951; Peterson, 1967; Kuras, 1980; Barlow, 1984). The mature vascular cylinder is diarch with an almost constant number of cells, and a characteristic orientation of pericycle cells facing protoxylem and protophloem elements with regard to the surrounding endodermal cells. Unlike other members of this family with larger roots, the cortex of *Arabidopsis* consists of a single layer of cells. In some other species of the family such as *Brassica napus* and *Sinapis alba*, there are two concentric rings of cortical cells, which are organised such that the cells of each

concentric ring lie opposite each other resulting in the formation of continuous, radial intercellular spaces running from the endodermis to the epidermis (Cormack, 1947). Eight trichoblasts are located in the epidermis overlying the radial walls of these eight cortical cells (Cormack, 1947; Petersen, 1967). Trichoblast cells remain more cytoplasmic at a greater distance from the root apex than the adjacent epidermal cells, a characteristic probably associated with the production of the root hair. The trichoblast cells stain differently to the epidermal cells even before vacuolation has taken place. This suggests that these cells are more metabolically active than the adjacent epidermal cells. The relationship between the position of trichoblasts and the underlying cortical cells which seems to be a characteristic of this family, is conserved in *Arabidopsis* (Cormack, 1947; Bunning, 1951; Peterson, 1967).

Initials

The anatomical studies presented here indicate that the root meristem contains three tiers of initials the organisation of which corresponds to the closed meristem type of von Guttenberg (1964). The closed organisation is to be found in a large number of taxa. Examples are to be found in the Solanaceae, Brassicaceae, Asteraceae and many others. The columella (central part of the root cap) is derived from the lowest tier of initials in the growing root. The lateral root cap and epidermis are derived from a collar of cells located in a ring around the columella initials. Growth of the lateral root cap must keep up with the columella root cap, which is achieved by periclinal divisions in the ring of cells next to the columella, the epidermal and root cap initials. One of these cells divides periclinally producing two cells one outside the other. Each of these daughter cells then divides anticlinally to produce a packet of cells, the outer packet of cells forms the lateral root cap and the inner packet constitutes an epidermal cell file.

The histology alone reveals little structural information that can unambiguously pinpoint the initial cells in the other cell files (cortex, endodermis and stele). Mitotic figures occasionally seen in terminal cells of the cortical and endodermal cell files suggest that these cell files share a common origin. We are of the opinion that, given the positions and activity of columella and epidermal initials, structural integrity of the root meristem can best be explained by assuming a more or less coordinate activity of a set of initials for all tissues, which surround the four central cells as depicted in Fig. 11. However, static anatomical information rarely provides conclusive information about which cells are contributing to the differentiated population of cells. It is for this reason that we tested the proposed model using *in vivo* labeling experiments.

The [³H]thymidine labelings show that all cells surrounding and touching the four central cells have cycling times that suggest active participation in the establishment of the files that originate in these cells. Therefore, these cells meet the definition of 'initial cells', emphasizing the need of these cells to be continuously meristematic (Newman, 1965). In contrast, no labeling of central cell nuclei can be observed in the time span of the experiment, showing that they have a much slower cell cycle. These cells meet the definition for a quiescent centre (Clowes, 1952). Cell

quadrants derived from the hypophysis in the same location as the quiescent centre of *Arabidopsis* have been found in *Brassica napus* (von Guttenberg, 1960; Kuras, 1980) and their quiescence speculated on though never proven.

Our limited data on the [³H]thymidine incorporation rate in the initials suggest that labeling of all rings and plates of initials surrounding the central cells occurs with the same speed. Hence the rate of formation of new lateral root cap cells (and the other files) is the same as the rate of formation of new columella cells. If the rates were different the cells would separate. From the threefold higher incorporation rates in the meristematic zone we conclude that proximal derivatives of the epidermal, cortical, and vascular initials divide at a higher rate than the initials themselves. Derivatives of the columella initials, on the other hand, do not display this increased rate of cell division in non-initial cells.

The model presented in Fig. 11 provides a ground scheme for understanding meristem activity in *Arabidopsis*. There are four central cells derived from the lenticular derivative of the hypophysis. Above this is a layer of stele initials. Surrounding the central cells is a ring of eight initials which form the cortex and endodermis. The lowest file is composed of 12 columella initials, composed of an inner core of four cells surrounded by the remaining eight. These peripheral columella initials are, in turn, surrounded by 16 lateral root-cap/epidermal initials. We call these cells initials because we have obtained evidence for their active participation in pattern formation, and because we lack evidence for engagement of the central cells in pattern formation. Future fate maps will verify this proposed location of the initials in the *Arabidopsis* root.

Clear deviations from the proposed division sequences have been observed. An example is given by the two cells occupying the position of the cortical initial in Fig. 1C. Whereas the majority of our anatomical evidence would predict a transverse division to occur first, in this example a periclinal division has created two cells, which now presumably function as separate cortical and endodermal initials. More examples of plasticity with regard to this scheme have been observed, especially in ageing roots (B.S. and K.J., unpublished data), but we feel they do not undermine the proposed model.

Changes in the organisation of cells in the meristem with age have been observed in many species. The organisation of root meristems in some members of the Asteraceae, such as *Helianthus annuus*, have been shown to change with age. The young primary meristem of *H. annuus* has a closed meristem with tiers of initials while the older root meristem is open and no longer tiered, having what appears to be a single set of initials for all tissues. A number of species, such as *Vicia faba* and *Fagus sylvatica* maintain an open, untiered meristematic organisation throughout the development of their root system (Popham, 1955a,b; Clowes, 1981).

Embryonic origin of the *Arabidopsis* root

The characteristic pattern of dividing initial cells surrounding a static group of central quiescent cells can be traced back to heart-stage embryos, where it is already apparent. The periclinal divisions which give rise to the first lateral root cap cells can be seen as the first divisions characteristic for the proposed epidermal initials. For reasons of struc-

tural integrity this defines all other cells surrounding the central cells as the initials for the other root tissues. Sections of intermediate stages of embryogenesis (after the heart stage) are consistent with this model for the elaboration of the root meristem architecture (K.J. and B.S., unpublished data; see also Jürgens and Mayer, 1993).

The radial pattern of cell files in the mature root can be traced back to rings and plates of initials, organised in tiers. The anatomy of roots from seedlings prior to germination reveals that this radial pattern is already established during embryogenesis and only minor changes in the cellular pattern occur upon germination.

We draw three conclusions from the embryo studies. First, cells derived from the hypophyseal suspensor give rise to both the columella initials and the four central cells. Cells derived from the embryo proper are specified as initials for the other root tissues. The radial organisation of these proposed initials is laid down during embryogenesis and preserved during post-embryonic root development. Second, this specification is evident at the late-heart stage of embryogenesis, when the first layer of lateral root cap is initiated. Third, the division patterns of the cells surrounding the central, quiescent cells (as proposed in Fig. 11C) can explain the maintenance of the structure of the root apex from late heart stage through to the mature root that is formed after germination.

The initials of columella, epidermis and lateral root cap, cortex and endodermis, pericycle, and vascular tissue are clearly separated from each other during embryogenesis. The development of this 'closed' meristem has been described in a number of members of the Brassicaceae including *Arabidopsis* (von Guttenberg, 1947; Tyrkaska, 1979; Mansfield and Briarty, 1991).

Variation in cell number

A striking feature of the organisation in the *Arabidopsis* root is the conservation of cell file numbers in the various tissues. The conservation of file number and organisation is stringent in the primary root. There is very little variation in the number of cell files in the cortex and endodermis, with some variability in the epidermis. Therefore the relative invariance in the resulting pattern is probably due to the fact that the primary root meristem is laid down during embryogenesis when the patterns of cell divisions are rigorously controlled. The small variability in cell numbers observed in primary roots and the relative simplicity of the tissue pattern, facilitates the interpretation of the effects of mutants on the cellular level with an accuracy uncommon in other plant systems.

The more variable numbers of cell files in the lateral roots indicate that the patterns of cell division in root meristems laid down during vegetative development are less tightly regulated. Notably, a larger number of cortical cells are coupled to fewer epidermal cells, implying that cell file numbers in a given tissue are not solely determined by the available space. Variations in anatomy between primary and lateral roots have been described in a number of species, most notably pea (Esau, 1965; Torrey, 1955). While the primary root of pea invariably exhibits a triarch pattern of vascular organisation, the laterals vary from diarch to pentarch, a process which is considered to be dependent on

the size of the developing lateral root primordium and independent of the primary root architecture (Torrey, 1955). This supports the observation that the factors that regulate cellular patterns in lateral meristems can be independent of factors involved in patterning the primary meristem. Therefore the variability exhibited by laterals may result from an autonomous developmental plasticity which is responsive to environmental factors. This inherent potential for plasticity in development could also explain the gradual changes in anatomy of an aging primary root meristem (L.D., K.J. and B.S., unpublished data). It is our view that the genetic or epigenetic control of this developmental plasticity, a highly characteristic feature of plants in general, can best be studied after genetic dissection of the development of a relatively invariant, and rigorously controlled meristem like the primary root meristem of *Arabidopsis*.

While the simple anatomy and pattern of cells in regular files might implicate cell lineage in determining cell fate, this should not downplay the role of cell-cell interactions in the developing root. Such interactions are generally considered to be important in plants where cell lineage is usually variable and not of primary importance in determining cell fate. The characterisation of mutations affecting specific aspects of cell differentiation will prove informative in determining the importance of cell-cell interactions. Similar analyses revealed the importance of these interactions during development in *C. elegans* a creature with simple anatomy and cell lineage (e.g. Horvitz and Sternberg, 1991).

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REFERENCES

- Armstrong, J. E. and Heimsch, C.** (1976). Ontogenetic reorganisation of the root meristem in the Compositae. *Amer. J. Bot.* **63**, 212-219.
- Barlow, P.W.** (1984). Positional controls in root development. In *Positional Controls in Plant Development* (ed. P.W. Barlow and D.J. Carr). Cambridge: Cambridge University Press.
- Brumfield, R. T.** (1943). Cell-lineage studies in root meristems by means of chromosome rearrangements induced by X-rays. *Am. J. Bot.* **30**, 101-110.
- Bunning, E.** (1951). Ueber die differenzierungsvorgänge in der Cruciferwurzel. *Planta* **39**, 126-153.
- Clowes, F. A. L.** (1949). Root apical meristems of *Fagus sylvatica*. *New Phytol.* **49**, 248-268.
- Clowes, F. A. L.** (1952). The cytogenetic centre in roots with broad columellas. *New Phytol.* **XX** 48-57.
- Clowes, F. A. L.** (1981). The difference between open and closed meristems. *Ann. Bot.* **48**, 761-767.
- Esau, K.** (1965). *Plant Anatomy*. New York: John Wiley and Son.
- Furner, I. J. and Pumfrey, J. E.** (1992). Cell fate in the shoot apical meristem of *Arabidopsis thaliana*. *Development* **115**, 755-764.
- Horvitz, H. and Sternberg, P. W.** (1991). Multiple intercellular signalling systems control the development of the *Caenorhabditis elegans* vulva. *Nature* **351**, 535-541.
- Ingham, P. W.** (1988). The molecular genetics of embryonic pattern formation in *Drosophila*. *Nature* **335**, 25-34.
- Irish, V. F. and Sussex, I. M.** (1992). A fate map of the *Arabidopsis* embryonic shoot apical meristem. *Development* **115**, 745-753.
- Jürgens, G., and Mayer, U.** (1993). In *A colour Atlas of Developing Embryos* (ed. J. Bard). London: Wolfe Publishing.
- Knox, J. P., Linstead, P. J., King, J., Cooper, C. and Roberts, K.** (1990). Pectin esterification is spatially regulated both within walls and between developing tissues of root apices. *Planta* **181**, 512-521.
- Kuras, M.** (1980). Activation of embryo during rape (*Brassica napus* L.) seed germination. II. Transversal organisation of radicle apical meristem. *Acta Soc. Bot. Pol.* **49**, 387-395.
- Mansfield, S. G. and Briarty, L.G.** (1991). Early embryogenesis in *Arabidopsis thaliana*. II. The developing embryo. *Can. J. Bot.* **69**, 461-476.
- Mayer, U., Torres Ruiz, R. A., Berleth, T., Misera, S. and Jürgens, G.** (1991). Mutations affecting body organisation in the *Arabidopsis* embryo. *Nature* **353**, 402-407.
- Meyerowitz, E. M.** (1989). *Arabidopsis*, a useful weed. *Cell* **56**, 263-269.
- Millonig, G.** (1961). Modified procedure for staining of thin sections. *J. Biophys. Biochem. Cytol.* **11**, 736-739.
- Newman, I. V.** (1965). Patterns in the meristems of vascular plants III. Pursuing the patterns in the apical meristem where no cell is a permanent cell. *J. Lin. Soc. (Bot.)* **59**, 185-214.
- Nüsslein-Volhard, C.** (1991). Determination of the embryonic axes of *Drosophila*. *Development Supplement* **1**, 1-10.
- Peterson, R. L.** (1967). Differentiation and maturation of primary tissues in white mustard root tips. *Can. J. Bot.* **45**, 319-331.
- Poethig, R. S.** (1987). Clonal analysis of cell lineage patterns in plant development. *Am. J. Bot.* **74**, 581-594.
- Popham, R. A.** (1955a). Zonation of primary and lateral root apices of *Pisum sativum*. *Am. J. Bot.* **42**, 267-273.
- Popham, R. A.** (1955b). Levels of differentiation in primary roots of *Pisum sativum*. *Am. J. Bot.* **42**, 529-540.
- Satina, S., Blakeslee, A. F. and Avery, A. G.** (1940). Demonstration of three germ layers in the shoot apex of *Datura* by means of polyploidy in periclinal chimeras. *Am. J. Bot.* **25**, 895-905.
- Sternberg, P. W. and Horvitz, H. R.** (1984). The genetic control of cell lineage in nematode development. *Ann. Rev. Genet.* **18**, 489-524.
- Sulston, J. E., Schierenberg, E., White, J. G. and Thomson, J. N.** (1983). The embryonic cell lineage of the nematode *Caenorhabditis elegans*. *Dev. Biol.* **100**, 64-119.
- Torrey, J. G.** (1955). On the differentiation of vascular patterns during tissue differentiation in excised pea roots. *Amer. J. Bot.* **42**, 183-198.
- Tykaraska, T.** (1979). Rape embryogenesis. II. Development of the embryo proper. *Acta Soc. Bot. Poloniae* **48**, 391-421.
- von Guttenberg, H.** (1947). Studien über die Entwicklung des Wurzelvegetationspunktes der Dikotyledonen. *Planta* **35**, 360-396.
- vonGuttenberg, H.** (1960). Grundzüge der Histogenese höherer Pflanzen. I. Die Angiospermen. Berlin: Gebrüder Bornträger.
- vonGuttenberg, H.** (1964). Die Entwicklung der Wurzel. *Phytomorph.* **14**, 265-287.
- Wieschaus, E. and Gehring, W.** (1976). Clonal analysis of primordial disc cells in the early embryo of *Drosophila melanogaster*. *Dev Biol.* **50**, 249-263.

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