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Root apical organization in *Arabidopsis thaliana* 1. Root cap and protoderm

S. F. Baum* and **T. L. Rost**

Section of Plant Biology, University of California, Davis, California

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Summary. We investigated the development of the root cap and protoderm in *Arabidopsis thaliana* root tips. *A. thaliana* roots have closed apical organization with the peripheral root cap, columella root cap and protoderm developing from the dermatogen/calyptrogen histogen. The columella root cap arises from columella initials. The initials for the peripheral root cap and protoderm are arranged in a collar and the initiation event for these cells occurs in a sequential pattern that is coordinated with the columella initials. The resulting root cap appears as a series of interconnected spiraling cones. The protoderm, in three-dimensions, is a cylinder composed of cell files made up of packets of cells. The number of cell files within the protoderm cylinder increases as the root ages from one to two weeks. The coordinated division sequence of the dermatogen/calyptrogen and the increase in the number of protoderm cell files are both features of post-embryonic development within the primary root meristem.

Keywords: *Arabidopsis;* Root apical meristem development; Dermatogen/calyptrogen histogen; Spiral pattern; Root cap; Protoderm.

Abbreviations: RCP root cap/protoderm; CI columella initial; PI protoderm initial.

Introduction

Our present understanding of the dynamics and organization of the root apical meristem is based on over one hundred years of observations (Hanstein 1870, Janczewski 1874, Clowes 1961a, Barlow 1984, Rost 1994). In describing shoot apical meristem organization, Hanstein (1868) coined the terms dermatogen, periblem, and plerome as designations for histogen layers or tiers that give rise to the epidermis, cortex, and vascular tissues, respectively. In a later study, Hanstein (1870) used these terms to describe the analogous structures in radicles of *Capsella bursa-pastoris*. Contemporary usage designates the dermatogen, periblem, and plerome as giving rise respectively to the three primary meristems; protoderm, ground meristem, and procambium and in turn to the epidermis, cortex, and vascular system (Esau 1953). The dermatogen, periblem, and plerome are considered to be permanent initials, in the sense that they regenerate themselves.

Janczewski (1874) added the term calyptrogen as the histogen that gives rise to the root cap. He characterized roots into five categories based on presumed cell lineage patterns. These patterns can be simplified further to two main groups: *open* and *closed* apical organization (Clowes 1981). In roots exhibiting closed apical organization, like *Arabidopsis thaliana* (Dolan et al. 1993), cell files can be traced down to a single histogen or tier of initial cells. In open organization specific histogen layers are lacking and cell files usually terminate in a zone at the root cap/body junction.

Clowes (1956) challenged the implication of static cell lineages in the histogen concept by his discovery of the quiesent center (QC). Clowes (1961b) and Torrey and Feldman (1977) further clarified the dynamic concept of root apical organization by coining the idea of the distal and proximal meristems as being the actual sources of cells making the root cap and body, respectively. The QC is made up in part by the cells of the histogen tiers, except the histogen that gives rise

* Correspondence and reprints: Section of Plant Biology, University of California, Davis, CA 95616-8537, U.S.A.

to the root cap or root cap plus epidermis (Clowes 1984). The contributing initials are located around the periphery of the quiescent histogen layers.

Another way to understand the organization of root apical meristems is to examine cell patterns and the orientation of cell divisions (Romberger 1963). Schüepp (1916, 1926, as referenced by Romberger 1963, Esau 1953) suggested a classification system based on the appearance of T-junctions. A T-junction is formed by a formative cell division that creates the terminal cell in a cell file. Schüepp used the orientation of the T-junction as the basis for his Körper/Kappe theory of root apical organization. His theory is based on the appearance of inverted Ts (Körper or body) as a component of the root body and non-inverted Ts (Kappe or cap) as a component of the peripheral root cap. The use of the Körper/Kappe theory to describe root apical organization has not gained popular acceptance (Romberger 1963), but patterns of T-junctions are still useful tools to analyze cell lineage patterns.

The signal mechanism which drives cell differentiation in root apices is unknown. Signals, such as cytokinin and auxin, have been suggested to move in the root axis to trigger cell division and differentiation events at the periphery of the tiers of initial cells (Barlow 1984).

In this paper we describe the root apical organization of *A. thaliana* based on the positions of T-junctions that initiate the protoderm and peripheral root cap. We suggest that the triggering event at the periphery of the initial tier occurs in a sequential pattern where one or a few cells at a time are induced to divide to form the tissue-specific initials. The signaling events necessary to make this work would be as simple as inducing the next cell in a sequential series to divide. In this paper we well describe the behavior of the dermatogen/calyptrogen histogen layer and the development of the root cap and protoderm from soil grown plants.

Material and methods

Arabidopsis thaliana ecotype WS (provided by Sandra Russell, E. I. duPont de Nemours and Co., Wilmington, DE) plants were grown in a sandy soil mixture in 4 inch pots. Plants were watered by spraying them every day for one week and then subsequently every other day with 0.3× Hoagland solution (Hoagland and Arnon 1950). The growing conditions were, 16 h of light, 8 h of darkness with 40% relative humidity at 22 °C for one to two weeks.

Pots were submerged in water and roots were gently extracted and severed from the stem. Tissue pieces were fixed overnight in 1.5% glutaraldehyde and 0.3% paraformaldehyde in 0.025 M Pipes buffer. The fixed tissue was rinsed three times for 15 min in buffer, post-fixed in 1% osmium tetroxide for 30 min, dehydrated in a graded ethanol series (10%, 30%, 50%, 60%, 70%, 80%, 95%, and 100%, 15 min per step) and then embedded in Historesin (1 : 1, 100% ETOH : Historesin, 1–2 h; two changes of pure Historesin, for 4 h and overnight, respectively). Plastic blocks were mounted on wooden dowels (with Duco cement) and sectioned on a Reicher-Jung 2050 Supercut microtome. 1.5 or 2 μm thick sections were mounted on gelatin-coated slides. Subbed slides were made by first washing the slides for 15 min in a solution composed of 5% glacial acetic acid in 95% ETOH, rinsed three times in deionized water, and then dipped in a subbing solution composed of 0.5% gelatin in H_2O. Slides were allowed to dry before using. Sections were stained with either 0.05% toluidine blue for 30 s or by a periodic acid-Schiff reaction (periodic acid, 40 °C, 20 min; Schiff reagent, room temperature, 60 min) modified from O'Brien and McCully (1981) and counter-stained with fast green (room temperature, 90 s) and toluidine blue (0.05%, room temperature, 30 s). Sections were viewed and photographed using an Olympus Vanox AHBT photomicroscope and a Galai Scanarray-2 image analysis system. Some images were further processed using Adobe Photoshop.

To determine if the pattern of cell divisions occurring in the peripheral root cap/protoderm (RCP) initials was random or conversely, if there was a pattern associated with the cell divisions, a test was written to evaluate the statistical significance of the closeness of the events. The null hypothesis (H_0) states that cell division events occur randomly within the collar or RCP initials. The alternative hypothesis (H_1) states that cell division events occur closer together than would be expected from randomness. The variables used in the analysis are: N, number of RCP initials in a root; E, number of RCP initials which have recently divided periclinally; n_1, n_2, n_E, positions of recently divided RCP initials within the circle of all RCP initials as seen in cross section.

The test statistic is defined as

$$T = \sum_{\text{pairs}} \text{distance} = \sum_{j=2}^{E} \left(\sum_{i=1}^{j-1} d(n_i, n_j) \right),$$

$$d(n_i, n_j) = \min \{ | n_j - n_i |, N - | n_j - n_i | \}.$$

Here, $d(n_i, n_j)$ represents the shortest distance between cell divisions i and j. This distance is then summed over all possible pairs of cells divisions. T measures the "closeness" of the cell division events. Given N, E, and T_o (observed), a simulation was used to obtain a p-value for testing H_0 vs. H_1. The simulation consisted of repeatedly generating a random sample size E without replacement from {1, 2, ..., N} and calculating the test statistic (T) for the sample. The p-value is the fraction of simulated values of T which are less than or equal to T_o:

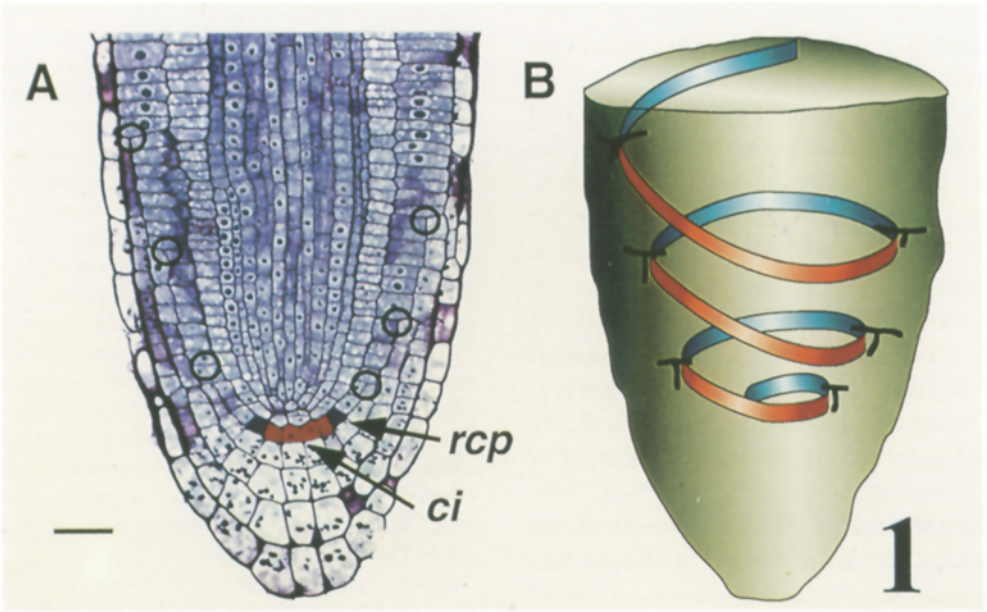
$$p = \frac{\text{number of simulations with } T \leq T_o}{\text{total number of simulations}}.$$

Values less than 0.05 indicate evidence of non-randomness, i.e., cell divisions tend to occur close together. Values less than 0.01 are regarded as strong evidence.

Results

Apical organization and the spiral motif concept

A. thaliana roots have closed apical organization, and the dermatogen/calyptrogen histogen is contiguous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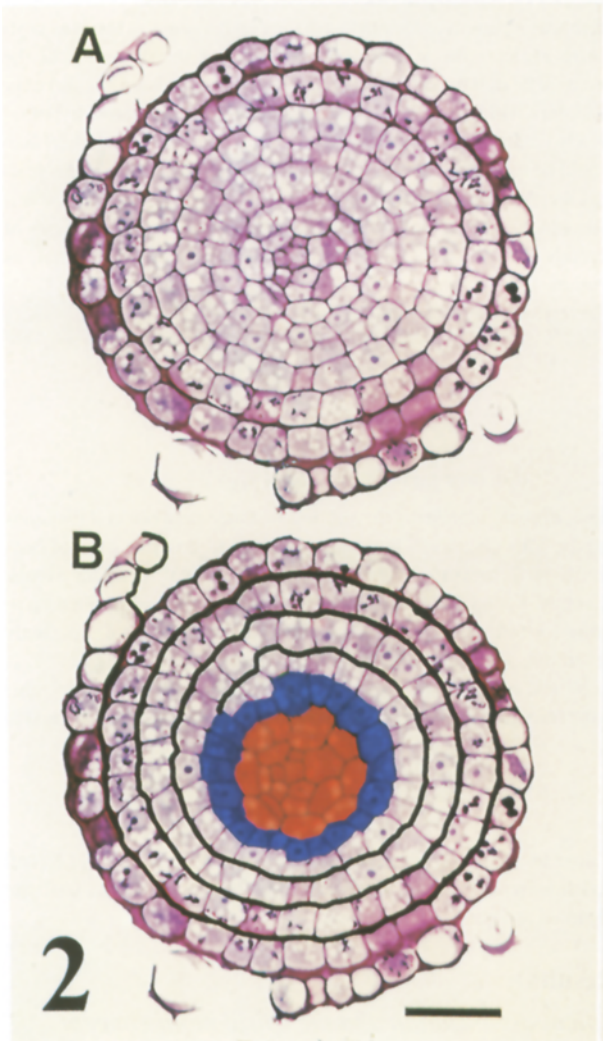
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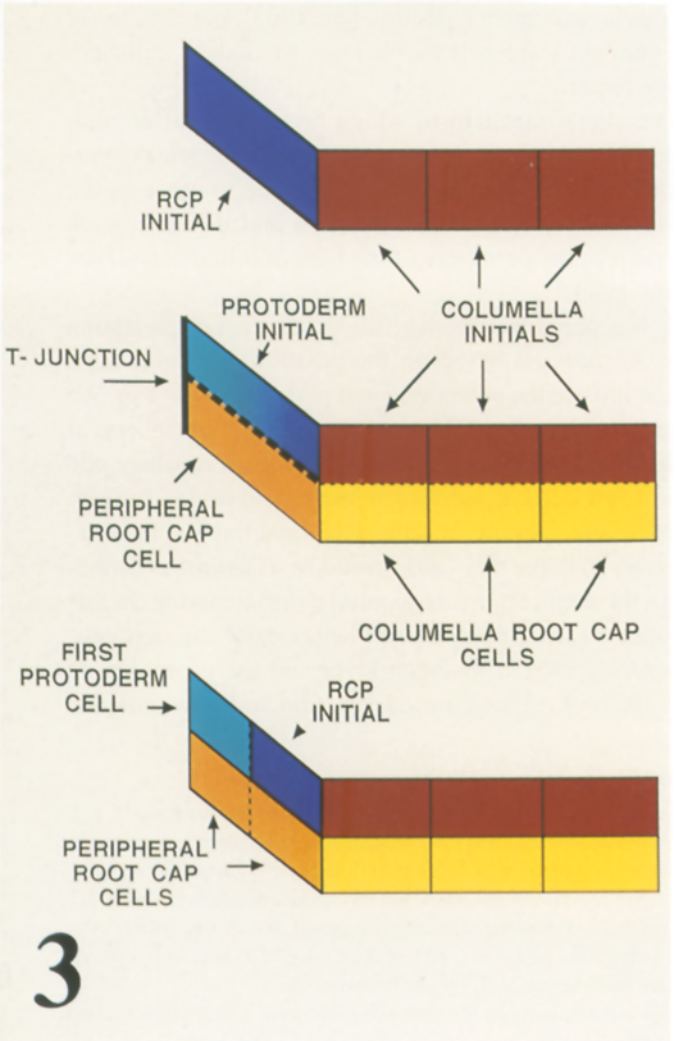

RCP INITIAL
PROTODERM INITIAL
COLUMELLA INITIALS
T- JUNCTION
PERIPHERAL ROOT CAP CELL
COLUMELLA ROOT CAP CELLS
FIRST PROTODERM CELL
RCP INITIAL
PERIPHERAL ROOT CAP CELLS
3

with the protoderm and root cap (Fig. 1 A). This histogen layer has two component parts: (1) the transverse component (as seen in a median longitudinal section) consists of about 20 root cap columella initial (CI) cells which divide to form new root cap columella cells and (2) the second part is composed of peripheral root cap/protoderm (RCP) initial cells arranged in a collar; the cells themselves are oriented at an oblique angle from the CIs, and they give rise to the peripheral root cap and protoderm initial cells. The division patterns in the collar in conjunction with the CIs is such that the resulting root cap resembles a continuous layer of interconnected cones (or spiral cones). In order to clarify the process of initiating the spiral cone root cap, it will be necessary to describe the logic that led us to find it in *A. thaliana* root tips. After that we will describe the development of the root cap and protoderm.

When viewing a median longitudinal section of an *A. thaliana* root tip, the T-junctions made by prior formative periclinal divisions of the RCP initial cells are easy to see on both sides of the root section. If all the RCP initial cells in the collar divided synchronously, then one would expect that the position of the T-junctions would be at the same level on both sides of the root. What we noticed was that these divisions rarely, if ever, occur directly across from each other (Fig. 1 A).

Next, serial transverse sections of roots through the region of the initial tiers (Fig. 2) reveal a spiral composed of peripheral root cap cells. Putting these two observations together we constructed the following hypotheses: (1) if one were to create a three-dimensional reconstruction of the root using serial longitudinal sections and then connect the successive T-junctions, the resulting trace would resemble the pattern of threads on a screw (Fig. 1 B) and (2) the root cap and protoderm are produced from a collar of cells that divide sequentially along with the CIs resulting in a root cap configured as a spiral cone. To verify these hypotheses we analyzed the root cap and protoderm cell lineages. To understand the significance of our data, we will first present our model of cell proliferation within the tier of initial cells which generates the root cap and protoderm.

Root cap and protoderm initiation

Conceptualizing the process of protoderm/root cap initiation requires thinking in four dimensions (length, width, depth, and change over time); but first it is necessary to explain the series of cell divisions in two dimensions as they occur at various points in time. Figure 3 is a schematic diagram of three CIs with one RCP initial. The first event to occur is that the CIs divide transversely in some sequence to form the next increment of columella root cap cells, the proximal row remains as the histogen layer. The RCP initials divide periclinally producing a peripheral root cap cell and a protoderm initial (PI), this is a "T-junction" (Figs. 1 A and 3). The PI divides anticlinally to produce the first protoderm cell proximally and distally it regenerates the RCP initial (Fig. 3). The peripheral root cap cell divides anticlinally producing two peripheral root cap cells (Fig. 3).

The three-dimensional model shown in Fig. 4 allows one to visualize this histogen. Two orientations of the clay model are presented. The red cells are the CIs, the blue cells represent RCP initials or their derivatives positioned in a collar. The yellow lines represent cell walls. The star marks a RCP initial that has recently divided periclinally creating two cells. The cell to the inside is a PI while the cell to the outside is a peripheral root cap cell. This periclinal division creates a T-junction. The next older pair of cells is

Fig. 1. **A** Median longitudinal section of a two-week-old *A. thaliana* root illustrating the staggered pattern of T-junctions. Corresponding T-junctions for the same layer of root cap are at different locations proximal to the tier. Black circles mark the position of T-junctions. *ci* Columella initials; *rcp* root cap/protoderm initials. Bar: 25 μm. **B** A hypothetical three-dimensional reconstruction of the root in **A.** A spiral has been added to illustrate the location of successive T-junctions in all epidermal cell files

Fig. 2. **A** Transverse section of a primary root meristem from a two-week-old *A. thaliana* plant taken at the level of the tier of initial cells. The spiral can be traced to the most recent periclinal division of the RCP initial. **B** The same image as in **A** except it has been color enhanced and the inside tangential wall of peripheral root cap cells has been blackened to accentuate the spiral pattern of peripheral root cap cells. Cells in blue are RCP initials, cells in red are periblem initials. Bar: 25 μm

Fig. 3. A schematic diagram illustrating the series of divisions within dermatogen/calyptrogen layer which includes the columella initials and root cap/protoderm (RCP) initials that give rise to the columella root cap cells, peripheral root cap cells and first protoderm cell

located clockwise to the star-marked cells. The yellow dot marks the youngest RCP initial which was generated by an anticlinal division of a protoderm initial. The yellow line above the yellow dot represents the anticlinal wall. The cell above the yellow dot-marked RCP is a protoderm cell. The anticlinal wall goes through both the PI and peripheral root cap cell creating two peripheral root cap cells opposite the protoderm cell and RCP initial. The sequence of divisions which generate the protoderm and root cap as seen in three-dimensions will now be discussed.

There are two sequences of cell divisions, both in a counter-clockwise direction (Fig. 4). In the first division, the RCP initials divide periclinally. In Fig. 4, starting at the asterisk label, the next RCP initial to divide periclinally is located in a counter-clockwise direction. Once an RCP initial divides, both the PI and the peripheral root cap cell enlarge. This is shown as the blue cells become larger starting with the asterisk marked pair of cells and moving in a clockwise direction. The second division event to occur is for both the PI and peripheral root cap cell to divide anticlinally. The next anticlinal division will occur to the pair of cells located in a counter-clockwise direction from the yellow dot-marked cell, in Fig. 4. The anticlinal divisions will continue in this direction as the pairs of PIs and peripheral root cap cells enlarge. In addition, the newly formed RCP initials will enlarge before dividing periclinally, thus generating a gyre in a spiral-screw pattern. One has to keep in mind that the model is a static representation of a dynamic structure. The RCP initials start out small but each one gets larger and this is represented by progressively larger RCP initials in our clay model.

It was noted in nine two-week-old roots that the direction of the spiral when viewing the root tip from the direction of the shoot was in a counter-clockwise direction; starting from the outside and moving inward. A more thorough investigation of this issue is presently underway.

In two-week-old roots, there is a clear spiral pattern of peripheral root cap cells as seen in transverse section (Fig. 2). However, during our examination of one-week-old roots we found that the spiral pattern was not evident in the outer peripheral root cap layers. This suggests that the mechanism generating the spiral is a post-germination event produced by a pattern of cell divisions that are induced after the root is fully established. Because the sequential pattern of periclinal divisions in one-week-old roots was not as evident as in two-week-old roots, we felt it was necessary to prove statistically that the pattern was present. To test whether the RCP initials were dividing in a sequential pattern, transverse sections were taken from many roots at 2 μm basal to the dermatogen/calyptrogen.

Table 1. Root age, statistic variables, and *p*-values

Root no.	Plant age (week)	*N*	*E*	*p*-Value[a]
1	1	17	9	0.007
2	1	16	9	0.007
3	1	15	8	0.045
4	1	16	5	0.038
5	1	17	15	0.125
6	1	17	14	0.149
7	1	18	14	0.017
8	2	22	17	0.0009
9	2	21	10	0.0018
10	2	21	12	0.0001

N Number of peripheral root cap/protoderm initials
E Number of peripheral root cap/protoderm initials with T-divisions
[a] Cell positions are not shown, but are a key component of the statistic

The RCP initials that had undergone the periclinal division were always grouped together but with an occasional division displaced from the group. To test whether the pattern of periclinal divisions occurred randomly or in a pattern, a statistical test was created (collaboration with Geoff Jones, Dept. of Statistics, U.C. Davis). For the purpose of the test, the null hypothesis (H_0) states that the pattern of periclinal divisions occurs randomly. Table 1 presents the variables used in the test. *p*-Values from the table are in a range from 0.0001 to 0.149 with the majority being less than 0.05. Two-week-old roots exhibited lower *p*-values than one-week-old roots. We reject the H_0 which states that the pattern of periclinal cell divisions occurs randomly, they therefore occur in pattern. This statistical test does not, however, suggest the form of the pattern.

The root cap

The root cap viewed in median longitudinal section includes three to five layers of columella and peripheral root cap cells (Fig. 1 A). One-week-old roots have fewer root cap layers than two-week-old roots. The number of root cap layers decreases incrementally toward the base of the root until finally the epidermis is on the outside.

A spiral pattern of root cap cells can be seen in a cross section of a two-week-old root taken at the level of the periblem histogen. A spiral is traced starting with

the outer layer of the root cap and following it inward (Fig. 2). Two-week-old roots exhibit a very clear spiraling root cap whose origin can be traced to the collar of RCP initials. Roots less than one-week-old show a root cap composed of concentric rings of peripheral root cap cells.

In two-week-old roots, the root cap is a series of interconnected spiraling cones. If you could peel the root cap, it would come off, not in separate layers, but in interconnected cones of decreasing size toward the inside.

The protoderm

For the purpose of this study, the term protoderm is used to denote the region of the epidermis that contains cells which are still dividing. In the longitudinal section in Fig. 5 A, three consecutive T-junctions are shown with the youngest T-junction being designated as 1st T. Distal to the 1st T, is a protoderm cell, two peripheral root cap cells and a RCP initial. After a protoderm cell is formed from a PI, it will divide transversely forming a packet of cells. This packet will expand radially before the cells within the packet divide transversely again. At this level in the root, there are four layers of root cap cells adjacent to the protoderm cell. Between the 1st T and 2nd T are four protoderm cells. All of these cells are derivatives of an original single protoderm cell. The two most distal cells of the packet are the result of a recent division as evidenced by the thin wall between them and their shortened height. All of the protoderm cells in this packet are larger in the radial dimension than the protoderm cell located distal to the 1st T. Below the 2nd T-junction there are three layers of peripheral root cap cells. Between the 2nd and 3rd T-junctions there are six protoderm cells. The two most distal cells in the group are the result of a recent division. The radial width of the six cells is greater than the radial width of the four protoderm cells located distally. At the 3rd T-junction there are two layers of root cap cells. In our illustration, we do not show all the protoderm cells of the oldest packet. What is evident from the illustration is that the radial width of these protoderm cells is greater than any of the protoderm cells located distal to the 3rd T. There is only one layer of peripheral root cap cells opposite the four protoderm cells.

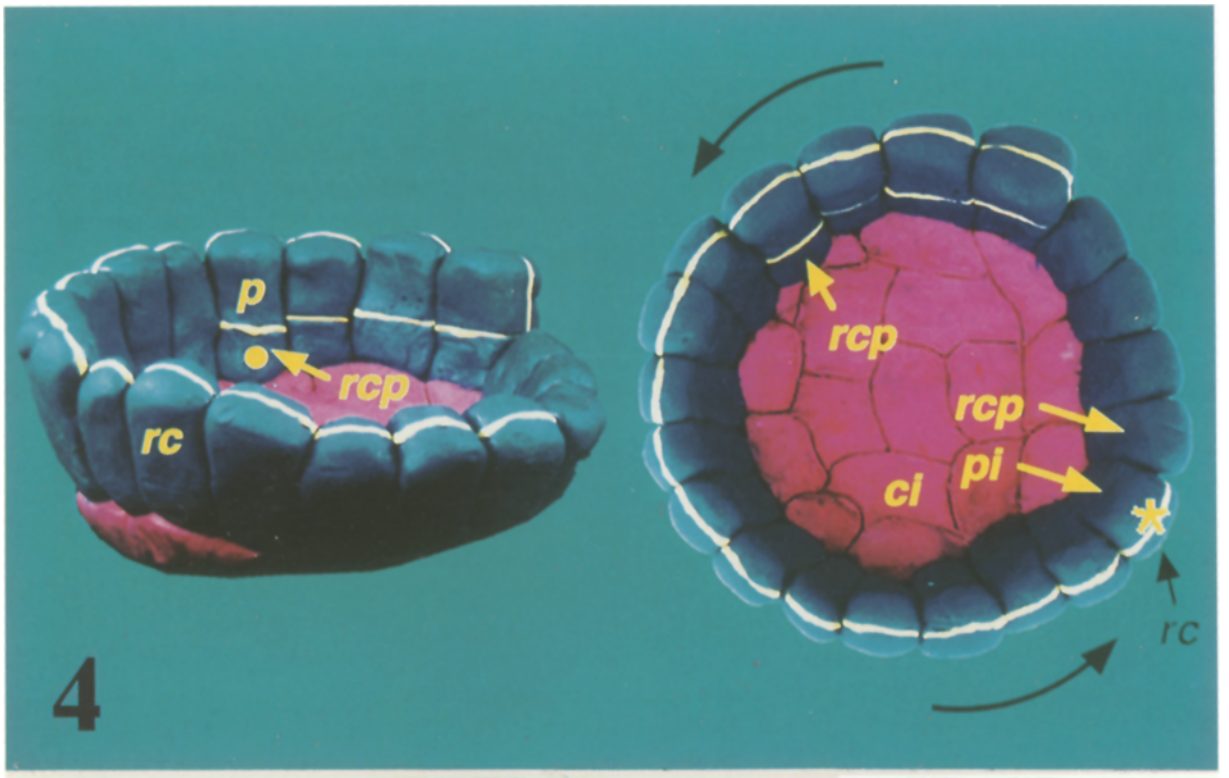
As seen in median longitudinal section, a one-week-old root will have two to three packets of protoderm cells making up a cell file (Fig. 5 A), while a two-week-old root will have three to five packets making up a cell file (data not shown). This implies that the height of the meristem increases as it ages from one to two weeks. Once the protoderm cells are finished dividing, the next step is for the cells to expand in the axial direction, attaining their final mature size.

To document the positions of radial cell divisions and T-junctions within the protoderm, an epidermal cell lineage analysis was performed. This was achieved by analyzing serial transverse sections and schematizing the radial cell division and T-junction positions along the root axis. While analyzing the serial sections, we were able to identify where a T-junction occurred due to both the radial size of protoderm cells and the decrease in root cap cell number adjacent to the protoderm cell. In Fig. 5 B the protoderm cell with an asterisk has three root cap cells outside of it. In the next serial section (Fig. 5 C), the same cell file now has two root cap cells adjacent to it and there is an increase in the radial width of the protoderm cell. These two sections (Fig. 5 B, C) illustrate a T-junction as it would appear if one sectioned transversely through the 2nd T (Fig. 5 A). Figure 5 D and E illustrates how a radial division would appear while viewing consecutive serial sections. In one-week-old roots, there are 15–18 RCP initials giving rise to the protoderm (Table 1), and in a more basal region of the meristem there are 28 (standard deviation is 2.5) epidermal cells as seen in cross section. Radial cell divisions occur in cells located adjacent to two cortical cells. There are 9.5 (2.5) radial divisions in one-week-old meristems.

In two-week-old meristems there are 21–22 RCP initials while in a more basal region of the meristem there are 43 (1.7) protoderm cells as seen in transverse section. There are 32 (12.8) radial cell divisions, most but not all occur in protoderm cells located next to two adjacent cortical cells.

In Fig. 6, the locations of the radial cell divisions that create new files are indicated by dots on the graph. There does not appear to be a pattern associated with the locations of radial divisions along the axes of protoderm cell files (Fig. 6 A, B). Some protoderm cell files only have one radial division while others have two or more. The height of the new cell files is variable. Some are short (ca. 6 μm) while others extend into the mature region of the root (data not shown).

An analysis was made to determine if the sequential pattern established by the RCP initials was maintained in older T-junctions. In order to do this we graphed the positions of older T-junctions along the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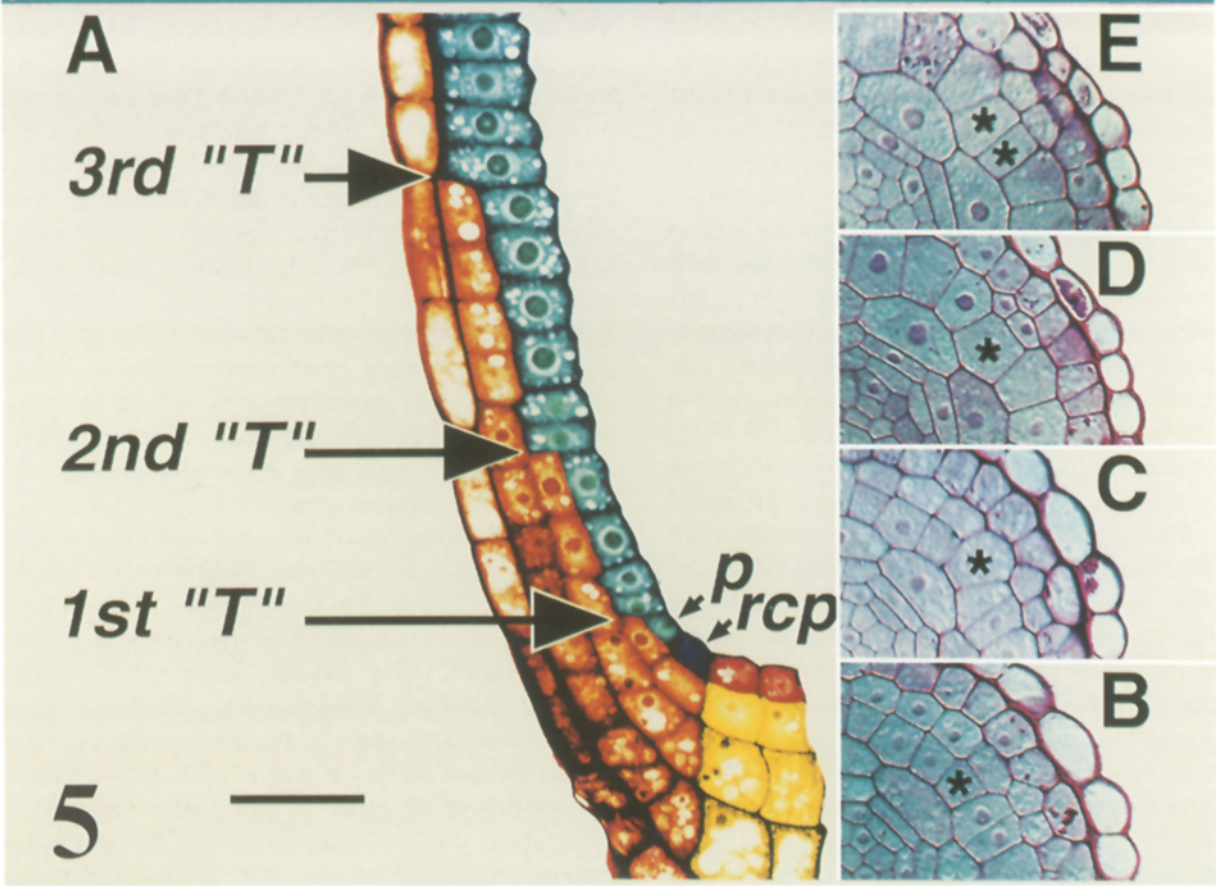
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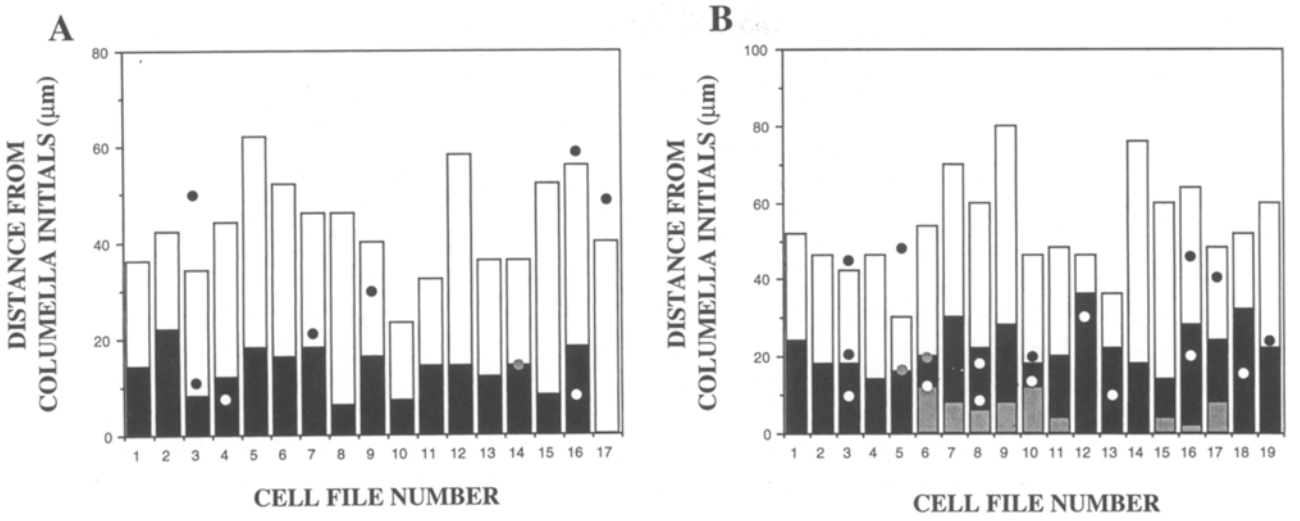

Fig. 6. Position of T-junctions within **A** one- and **B** two-week-old *A. thaliana* roots. Each bar represents a longitudinal epidermal cell file that originates at an initial. **A** Black, 1st T-junction (3 adjacent peripheral root cap layers); white, 2nd T-junction (2 root cap cell layers). **B** Gray, 1st T-junction (4 adjacent peripheral root cap layers); black, 2nd T-junction (3 root cap cell layers); white, 3rd T-junction (2 root cap cell layers). The black, white, or gray dots mark the most distal position within a cell file where radial divisions occur. Refer to Fig. 5 A for clarification

root axis. Figure 6 A and B shows positions of T-junctions in relation to the dermatogen/calyptrogen tier for one- and two-week-old roots. Only those cell files that originated at the tier are presented in the histogram. Files that are formed as a result of a radial division are not presented in the graph. In Fig. 6 A the portion of protoderm cell files that are inside three root cap cell layers and the portion of protoderm cell files that are inside two root cap cell layers are shown. In Fig. 6 B the portion of protoderm cell files that are inside four root cap cell layers and again the portion of protoderm cell files inside two root cap cell layers are shown. The conclusion from this analysis is that as the cells mature the sequential pattern isn't clearly maintained.

Discussion

Basic pattern of root apical organization

An important difference between root tips of various species is the organization of cells in the root apical meristem. Popham (1966), categorized the roots of vascular plants into six types and two subtypes. All of these, however, incorporate one of two common developmental schemes: *open* or *closed* apical organization. In closed organization all longitudinal cell file lineages can be directly followed to a specific tier of initial cells or histogens. These initials (Hanstein 1868, Janczewski 1874) apparently contribute by some developmental mechanism to the initiation of different tissues; for example, in a common monocot

Fig. 4. A clay model of the dermatogen/calyptrogen tier from a two-week-old *A. thaliana* root illustrating how the spiral pattern is initiated and maintained by periclinal and anticlinal divisions of the RCP initials. On the right, a view taken from the direction of the shoot; on the left, side view. The asterisk marks the most recent periclinal division of an RCP initial and the yellow dot marks the youngest RCP initial. The curved black arrows indicate the direction of anticlinal and periclinal divisions in our model. The yellow lines represent cell walls. Activating the division sequences in the direction of the arrows will create the self-generating spiral pattern. *ci* Columella initial; *p* protoderm cell; *pi* protoderm initial; *rc* peripheral root cap cell; *rcp* root cap/protoderm initial

Fig. 5. A A portion of a median longitudinal section taken from a one-week-old root which has been color enhanced to show the positions of T-junctions and packets of epidermal cells. (*p* Most distal protoderm cell within the epidermal cell file.) Two complete packets of epidermal cells are shown, the youngest packet is between 1st T and 2nd T, while the next oldest is between 2nd T and 3rd T. The arrows point to T-junctions. Bar: 25 μm. *rcp* Root cap/protoderm initial; blue cells, protoderm cell file; red cells, columella initials; yellow cells, columella root cap cells; orange cells, peripheral root cap cells. **B–E** Consecutive serial transverse sections through the meristem illustrating the appearance of a T-junction (**B** and **C**) and radial division (**D** and **E**) as viewed in this orientation. The asterisk marks the same epidermal cell file within each pair of cells

type found in grasses there are three initial layers, the plerome (for the vascular cylinder), the periblem/dermatogen (for the cortex and epidermis), and the calyptrogen (for the root cap). The most common type of closed organization in dicot roots also has three layers, the plerome (vascular cylinder), the periblem (cortex), and the dermatogen/calyptrogen (epidermis and root cap); *A. thaliana* has this pattern.

In this study we have characterized the initiation and early development of the protoderm and root cap from one- and two-week-old roots; both of these tissues originate from the dermatogen/calyptrogen. The columella portion of the root cap originates by transverse divisions of the columella initials. The periclinal division of the RCP initial is the first step in the initiation and development of the peripheral root cap and epidermis. It can be hypothesized that the RCP cells might divide in one of three possible patterns: (1) all the cells in the helical collar of RCP initials divide in unison; (2) the RCP initials divide randomly; and (3) they divide in some kind of pattern.

The implication from some published diagrams (Dolan et al. 1993) is that the cells along the periphery of the histogen divide in unison. Our finding taken from median longitudinal sections shows that the periclinal divisions of the RCP initials (T-junctions) are never directly across from each other. The difference in height is small for those corresponding T-junctions near the tip and larger for corresponding T-junctions located basally. This eliminates the first hypothesis.

To test whether the pattern of cell division at the RCP initials was random or not, a statistical test was written to evaluate the closeness of the division events. The low p-values allow us to reject the null hypothesis which states that the RCP initials divide randomly. Therefore, we concluded that there is a pattern associated with the RCP division events.

Anatomical data from two-week-old roots clearly shows that the RCP initials divide in a sequential pattern (producing a continuous layer of interconnected cones) verifying the third hypothesis. Drawings, made by Janczewski (1874: plate 16, no. 2 and plate 15, no. 5) of median longitudinal sections of root apices, also show the presence of T-junctions that are oriented in the same relative position as the T-junctions we observed in *A. thaliana*. Our results taken together with drawings from Janczewski (1874) suggest that other species of plants initiate the peripheral root cap and epidermis in a sequential pattern. We are now verifying this in a survey study of many plants with closed apical organization.

Dynamic nature of apical organization

A. thaliana root meristems exhibit a dynamic change in stature during one to two weeks of growth. Dolan et al. (1993) have stressed the relatively invariant nature of epidermis and cortex cell file numbers in *A. thaliana* ecotype Columbia primary roots grown on agar. Our results using the WS ecotype grown in sand do not support this conclusion. The number of cell files within the cylinders of protoderm and cortex as seen in transverse section increase from one to two weeks of growth. The new protoderm cell files arise from radial divisions of cells mostly located between two adjacent cortical cells. In addition, the number of protoderm cell packets as seen along one side of a MLS also increases as the root ages from one to two weeks. This implies that the position of the most proximal T-junctions in two-week-old roots is farther from the tip than the corrresponding T-junctions in one-week-old roots. Therefore the height of the meristem increases as the root ages from one to two weeks, i.e., the total number of cycling cells presumably increases.

The organization of the primary root meristem in *A. thaliana* is established during embryogenesis (Scheres et al. 1994). The embryonic pattern is maintained for a period of time, after which, based on our data, the primary root exhibits a new post-embryonic developmental pattern. The characteristics of the post-embryonic development include an increase in meristem size and the establishment of the sequential cell division patterns. The idea that there is a number of stages associated with root meristem development is demonstrated by Laskowski et al. (1995) who have shown in *A. thaliana* that as lateral root primordia enlarge, their ability to be excised and grow on artificial media depends on the developmental stage of the primordia before excision. Cheng et al. (1995) have also demonstrated the existence of developmental stages in roots using root meristemless (RML) mutants. The roots of the RML mutants, including both the primary and lateral roots, did not exhibit cell divisions once the root was approximately 2 mm long. This suggested that a component necessary for post-embryonic growth was missing, causing the young root to discontinue further development. This supports the idea from the current study that the root cap is initiated in the embryo as continuous cylinders

and that after one to two weeks a developmental shift occurs resulting in a spiral cone pattern.

Variations in cell division patterns of the protoderm

We have documented the maturation of the protoderm which includes an ordered pattern of transverse divisions followed by radial cell expansions. Variations in the developmental sequence were observed and are a manifestation of the dynamic growth of the meristem. The observed variations include; the irregularity associated with the first transverse division within a packet, distally located protoderm cells have been observed to divide periclinally and tangential longitudinal divisions of some protoderm cells are suspected. Ivanov (1971), in studying transverse division patterns in *Zea mays* epidermal packets, observed a loss of synchrony from one packet to another. Lück et al. (1994) further characterized the development of packets from three different regions of *Z. mays* root meristems. Once a one celled packet is formed the number of cells in a packet increases to 16. Of the 2×10^6 possible division pathways that lead to a 16 celled packet, only a small number of the possible pathways is used. Therefore, the division sequences within a group of packets are variable. We have documented changes in meristem height and have also observed changes in meristem width (data not shown). This is in accordance with published results from *Pisum* (Rost and Baum 1988) that the dimensions of the meristem changes in relation to root length. Increases in the meristem dimensions are presumably attributed to increases in cell number. The added cells would be expected to exert added pressure on cells thereby inducing cells to divide in a plane which counters the force vector, i.e., longitudinal divisions (Lyndon 1990). The variations in cell division patterns negate the linearity of epidermal cell development. Therefore, we expect the positions of consecutively older T-junctions to be juxtaposed in relation to each other, as exhibited by our data, and not be positioned in the spiraling screw pattern in which they were formed.

Our observation that the RCP cells divide in a self-regenerating helical sequential pattern suggests signal transmission around the periphery of the histogen tiers. This type of organization has been observed in fern roots with apical cells (Gunning 1981, Gifford 1993). Barlow (1993) reported a spiral distribution of T-junction divisions in the cortex of tomato roots and suggested that perhaps "a wave of periclinal divisions circulate around the ring of initial cells". The fact that a spiral sequence is suggested in tomato (Barlow 1993) supports this notion. The placement of plasmodesmata in this case would be determined at the time the initial cell divides while its cell plate is made. It needs to be determined in these instances if the numbers or types of plasmodesmata are indicative of selective passage points.

What this means, is that the first step in the development of the protoderm and peripheral root cap cells occurs around a collar of the dermatogen/calyptrogen initial tier. The next cell to become a protoderm/root cap initial in the series will form in the next position in a spiral screw. Spiral screw division behavior has also been seen in *Azolla* (Gunning 1981) and other ferns and lower vascular plants with apical cells (Gifford 1993). Our observation in *A. thaliana* has significance for understanding developmental mechanisms in roots with closed organization because it implies that another critical cell lineage is the continuous helix of epidermal cells. If these findings are confirmed in our observations in other species, the concepts of root development in dicotyledonous plants with closed organization will need to be modified.

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References

Barlow PW (1984) Positional controls in root development. In: Barlow PW, Carr DJ (eds) Positional controls in plant development. Cambridge University Press, Cambridge, pp 281–318

– (1993) The cell division cycle in relation to root organogenesis. In: Ormrod JC, Francis D (eds) Molecular and cell biology of the plant cell cycle. Kluwer, Dordrecht, pp 179–199

Cheng J-C, Seeley KA, Sung ZR (1995) RML1 and RML2, *Arabidopsis* genes required for cell proliferation at the root tip. Plant Physiol 107: 365–376

Clowes FAL (1956) Localization of nucleic acid synthesis in root meristems. J Exp Bot 7: 307–312

– (1961a) Apical meristems. Blackwell, Oxford

– (1961b) Duration of the mitotic cycle in a meristem. J Exp Bot 12: 283–293

Clowes FAL(1981) The difference between open and closed meristems. Ann Bot 48: 761–767

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

Esau K (1953) Plant anatomy. Wiley, New York

Gifford EM (1993) The root apical meristem of *Equisetum diffusum*: structure and development. Am J Bot 80: 468–473

Gunning BES (1981) Microtubules and cytomorphogenesis in a developing organ: the root primordium of *Azolla pinnata*. In: Kiermayer O (ed) Cytomorphogenesis in plants. Springer, Wien New York, pp 301–325 [Alfert M et al (eds) Cell biology monographs, vol 8]

Hanstein J (1868) Die Scheitelzellgruppe im Vegetationspunkt der Phanerogamen. Niederrhein Ges Naturheilk Festschr 50jähriges Jubiläum Universität Bonn 1868: 109–143

– (1870) Die Entwicklung des Keimes der Monokotylen und Dikotylen. A Marcus, Bonn

Hoagland DR, Arnon DI (1950) The water culture method for growing plants without soil. Calif Agric Exp Stat Circ 347: 1–32

Ivanov VB (1971) Critical size of the cell and its transition to division. Ontogenez 2: 524–535

Janczewski E (1874) Recherches sur l'accroissement terminal des racines dans les phanerogames. Ann Sci Nat 5eme Ser Bot 20: 162–201

Laskowski MJ, Williams ME, Nusbaum HC, Sussex IM (1995) Formation of lateral root meristems is a two-stage process. Development 121: 3303–3310

Lück J, Barlow PW, Lück HB (1994) Cell genealogies in a plant meristem deduced with the aid of a "bootstrap" L-system. Cell Prolif 27: 1–21

Lyndon RF (1990) Plant development: the cellular basis. Unwin Hyman, London

O'Brien TP, McCully ME (1981) The study of plant structure: principles and selected methods. Termarcarphi Pty, Melbourne

Popham RA (1966) Laboratory manual for plant anatomy. CV Mosby, St. Louis, MO

Romberger JA (1963) Meristems, growth, and development in woody plants. US Department of Agriculture, Forest Service, Tech Bull 1293

Rost TL (1994) Root tip organization and the spatial relationships of differentiation events. In: Iqbal M (ed) Growth patterns in vascular plants. Dioscorides Press, Portland, pp 59–76

– Baum S (1988) On the correlation of primary root length, meristem size and protoxylem tracheary element position in pea seedlings. Am J Bot 75: 414–424

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