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The Root Cap: Cell Dynamics, Cell Differentiation and Cap Function

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ABSTRACT

The root cap is a universal feature of angiosperm, gymnosperm, and pteridophyte roots. Besides providing protection against abrasive damage to the root tip, the root cap is also involved in the simultaneous perception of a number of signals – pressure, moisture, gravity, and perhaps others – that modulate growth in the main body of the root. These signals, which originate in the external environment, are transduced by the cap and are then transported from the cap to the root. Root gravitropism is one much studied response to an external signal. In the present paper, consideration is given to the structure of the root cap and, in particular, to how the meristematic initial cells of both the central cap columella and the lateral portion of the cap which surrounds the columella are organized in relation to the production of new cells. The subsequent differentiation and development of these cells is associated with their displacement through the cap and their eventual release, as ''border cells'', from the cap periphery. Mutations, particularly in *Arabidopsis*, are increasingly playing a part in defining not only the pattern of genetic activity within different cells of the cap but also in revealing how the corresponding wild-type proteins relate to the range of functions of the cap. Notable in this respect have been analyses of the early events of root gravitropism. The ability to image auxin and auxin permeases within the cap and elsewhere in the root has also extended our understanding of this growth response. Images of auxin distribution may, in addition, help extend ideas concerning the positional controls of cell division and cell differentiation within the cap. However, firm information relating to these controls is scarce, though there are intriguing suggestions of some kind of physiological link between the border cells surrounding the cap and mitotic activity in the cap meristem. Open questions concern the structure and functional interrelationships between the root and the cap which surmounts it, and also the means by which the cap transduces the environmental signals that are of critical importance for the growth of the individual roots, and collectively for the shaping of the root system.

Key words: Cell division; Cell separation; Gravitropism; Lateral root; Meristems; Positional information; Primary root; Regeneration; Root cap; Stem cells

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INTRODUCTION

For an organ as small and seemingly insignificant as the root cap, it is remarkable that it contains so

many biochemical and biophysical processes that are crucial for the survival of the whole plant (Sievers and others 2002). Charles Darwin and his son, Francis, were acutely aware of the importance of the root cap and even imputed to it a cerebral function. The Darwins expressed this opinion in their book 'The Power of Movement of Plants' (Darwin and Darwin 1880) and in the years intervening since this book's publication much critical attention has been devoted to the role of the root cap in gravitropism. However, the cap is also involved in other growth responses, for example, hydrotropism (Takahashi 1997) and thigmotropism (Ishikawa and Evans 1992; Massa and Gilroy 2003), and possibly chemotropism and phototropism also. Fortunately, after many years of only sporadic observation, these other root growth responses are becoming the subject of more systematic investigation (Porterfield 2002). The cap is also the location of phytochrome (Johnson and others 1991), a protein that regulates photomorphogenetic aspects of root development.

The role of the cap in the gravitropic response (gravicurvature) is due not only to its perception of gravity but also to the cap's ability to transduce the gravity signal, so that a second signal, of different form from the first, is transmitted from the cap to the growth zone of the root proper. There it is translated into a growth response. Because this second signal is thought to involve a mobile plant hormone, root gravicurvature has sometimes been employed as a type of bioassay for natural growth regulators suspected of being transported from the cap to the root in the course of the graviresponse (Pilet and Barlow 1987). Tropic movements of roots always involve differential growth in the zone of rapid cell elongation immediately basal to the root meristem, so the question inevitably arises as to whether the cap might not also be involved in the regulation of rectilinear root growth, accelerating or decelerating it in response to a signal received from the cap. Observations of Pilet (1971) on the stimulated elongation of maize (*Zea mays*) primary roots following cap removal bear out this supposition. Furthermore, observations of Clowes (1972) on the stimulation of mitoses in the quiescent center of maize roots, also following removal of the root cap, suggest that the cap could also regulate cell division in the root. However, in each of these cases, the stimulatory effects were short-lived due to the rapid regeneration of the cap and the re-imposition of its putative growth regulatory capacity. Later work (Pilet 1986), which took advantage of the natural variation of cap length, was conclusive in showing that maize primary roots with long caps elongated slower than roots with short caps. Similar variation in the dimensions of root caps of *Ricinus communis*, and of the dimensions of the central, gravity-sensing statenchyma (a term used here to denote a zone of the root cap, mainly within the central portion of the columella, comprised of statocytes (Němec 1964), these being cells that contain large amyloplasts which serve as statoliths for the physical perception of gravity) in particular, were also found to correlate with the degree of root graviresponsiveness (Moore 1985). There is, in addition, some evidence that a correctly structured statenchyma (Figure 1B) *and* a gravity stimulus are both necessary for the regular development of the root tip, including the cap (Moore and others 1986, 1987). More problematic in this area of cap-regulated root growth is the question of whether the cap regulates the so-called ''tonic effect'' whereby a centrifugal force applied axially to the root and its cap affects root elongation (Macdonald and Gordon 1978).

Investigation of the root cap has now reached a point where nearly every cap cell can be assigned some measurable degree of input to the gravitropic response (Blancaflor and others 1998). Moreover, ablation of cap cells by genetic means (for example, see Tsugeki and Federoff 1999) can also be used to probe the quantitative aspects of the interaction between the cap and the growth process of neighboring root tissues without the problems associated with physical damage to the cap inherent to some other types of investigations (laser ablation, decapping). For the remainder of this article, emphasis will be upon (a) the structure of the cap and its relationship to root biology, (b) the dynamics of cap cells and how these might be regulated in response to potential developmental signals impinging on the cap both from within the root and from the external environment, and (c) the spatial specification of cap cell differentiation. The latter topic revisits the concept of ''positional information'' (Wolpert 1996) for which an organ as small as the cap with its spatially well-defined set of cells would, as previously suggested (Barlow 1975, 1981, 1984), be a useful experimental tool for testing hypotheses associated with this concept. Hopefully, the principles that govern developmental processes within the microcosm of the cap will also have points of contact with the principles of development of other plant systems.

Variability of Root Caps

The great majority of plant roots possess a cap. Known exceptions are credited to the roots of a few

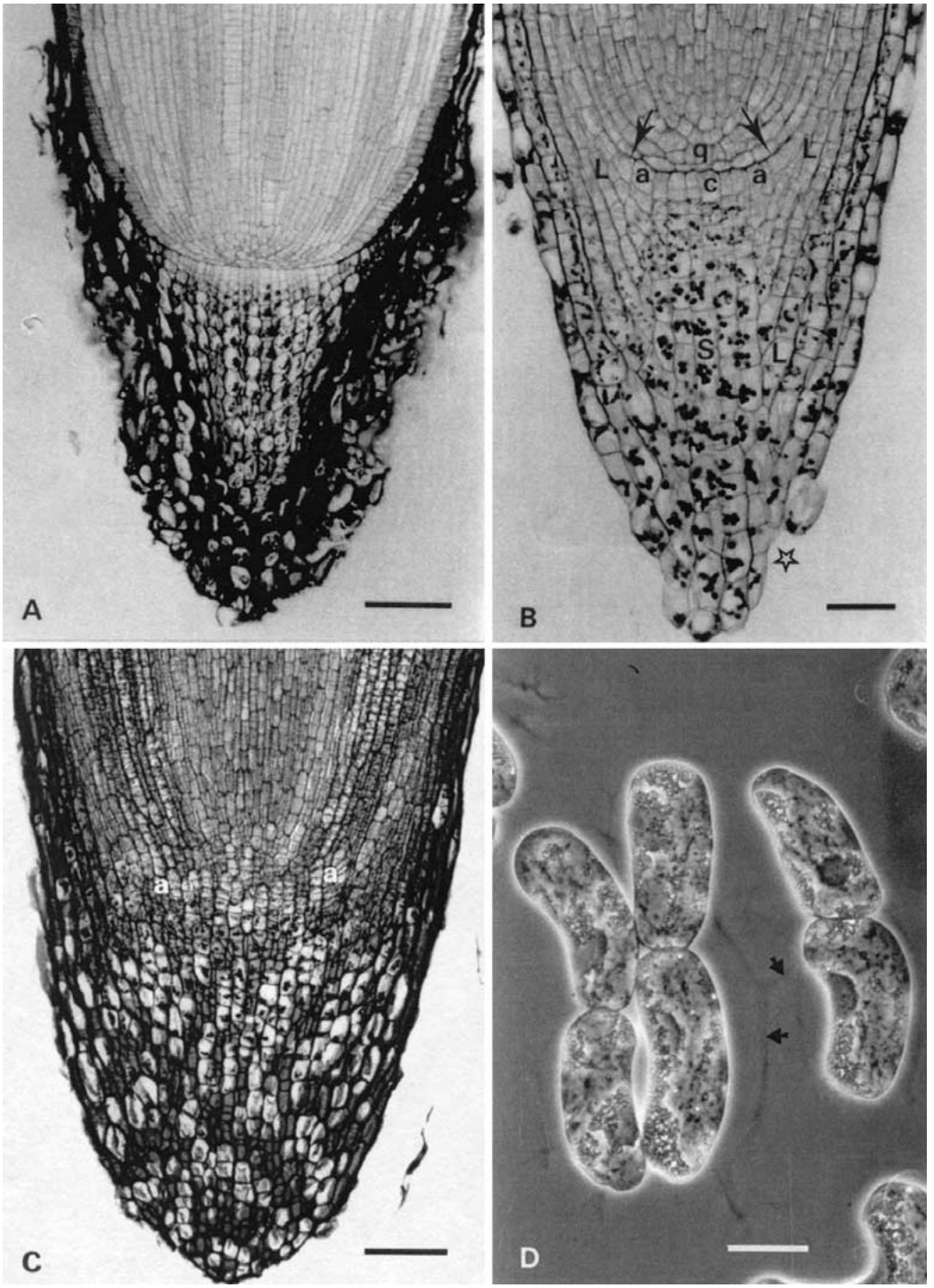

Figure 1. Micrographs of root caps of (**A**) *Zea mays*, (**B**) *Lycopersicon esculentum*, both of whose roots have closed meristems, and of the root cap of (**C**) *Pisum sativum*, whose root has an open meristem. All caps are seen in longitudinal median section. In (**A**), the mucilage and mucilage-secreting outer cap cells of the maize root are heavily stained. In (**B**), the following regions of the root and cap are marked: root-cap boundary (arrow), protoderm initials (a), columella initials (c), quiescent centre (q), statenchyma (S) with prominent starch grains and forming part of the group of axially oriented cells which comprise the columella, lateral root cap (L). Sections stained by PAS reaction. The outer cap cells eventually detach: the star (☆) indicates a possible site from which a cell has detached. In (**C**), the sites of *a*-type initial cells are indicated (a). (D) Phase contrast micrograph of living border cells, released from a maize root cap. Arrows indicate strands of mucilage which are adhering to the cells. Scale bars: A and C – 100 μm; B – 25 μm; D – 10 μm.

aquatic or marshland species, for example, *Pinguicula moranensis* (Brugger and Rutishauser 1989), the roots of parasitic plants whose tips develop as specialized penetrative haustoria (Riopel and Timko 1995), and perhaps the roots of species that normally possess a cap but can lose it when grown in novel environments. Instances of the last-mentioned type of loss were reported for roots of two trees, silver maple, *Acer saccharinum* (Richardson 1955), and horse chestnut, *Aesculus hippocastanum* (Klein and Szabó 1880), when their roots were grown in water culture as opposed to soil. Members of the family Podostemaceae, whose habitat is within cataracts and other white-water environments, possess diverse forms of roots and caps (Rutishauser 1997; Suzuki and others 2002), ranging from asymmetric caps (calyptra) in species like *Podostemon ceratophyllum* and *Indotristrichia ramosissima*, to rudimentary caps, as on the tips of the flattened, ribbon-like roots of *Farmeria metzgeroides*, to the complete absences of a cap as in, for example, *Tristichia trifaria*. It should also be mentioned that some taxa of the Podostemaceae, for example, *Mourera* spp., lack roots altogether.

Wherever a root cap is present, it seems to conform to a common plan of development (Barlow 1975; Sievers and others 2002). Generally, caps of terrestrial plants are conical (Figures 1A–1C), a shape that may be important in facilitating the penetration of the root into its substratum. At least in the early stages of development, all caps possess at their proximal end a meristem from which new cells are derived, a non-meristematic, central columella zone which in many cases acts as a gravity-sensing statenchyma and which is surrounded by a lateral zone, and an outer layer of mucilage-secreting cells (Figure 1A). These latter cells seem inevitably to detach from the cap (Figure 1D), but wherever they remain in the vicinity of the cap they are referred to as ''border cells'' (Hawes and Lin 1990; Hawes and others this issue). Eventually, the root tip grows through the detached border cells and these then aggregate as small clusters at regular intervals along the length of the root (Mosse 1975; Hawes and others this issue). Although the outer, detaching cells of the columella and lateral zone differ in size (Guinel and McCully 1987), cells of both origins secrete mucilage (Juniper and Pask 1973) and, to some extent, share similar cell wall chemistries (Vicré and others 1998). However, a study of *Arabidopsis thaliana* caps by Freshour and others (1996), which made use of antibody markers for wall/membrane epitopes, revealed a significant difference between outer cells of the columella and lateral cap with regard to the presence of an arabinosylated $(1 \rightarrow 6)$-β-galactan epitope; the lateral cap was the only portion of the whole root where walls failed to express this epitope. In maize root caps, the lateral part of the cap (which surrounds the columella) is the site of expression of the gene *zmGRP4* (glycine-rich protein 4), especially in its proximal portion (Matsuyama and others 1999a), the gene product of which accumulates in the mucilage. Two other genes have been found that are specific for lateral cap (Matsuyama and others 1999b). They may be similar to those described by Ponce and others (2000) and used by these authors as markers of root cap regeneration (see p. 26). All three lateral root cap genes appear to be silent in the columella. In pea, the outer cells of the lateral cap, but not those of the columella, express genes coding for a pectin methyl esterase and, under certain circumstances, a putative pectin lyase (Wen and others 1999; MC Hawes personal communication). These particular differential patterns of gene activity between columella and lateral cap may indicate different functions of border cells released from each zone of the cap (see Figures 1A–1C); they may also have something to do with the pattern of cell separation and detachment from the cap.

In relation to this last-mentioned process of cell separation, it is worth recalling that, during the germination of graminaceous species, the root cap separates from the coleorhiza with which, in the embryo, the cap is embedded and forms a continuous tissue (see Figure 2 in Barlow 1975). Thus, in grasses, cap cell separation could commence as part of a pre-germination abscission event that separates cap from the coleorhiza and, hence, border cell production may commence immediately post-germination. In most other taxa, which have no coleorhiza, the timing of the onset of border cell production could be delayed or, indeed, may never occur, as in *Arabidopsis thaliana* (Zhu and Rost 2000).

The mucilage and detached cap border cells play multiple roles in the biology of the root and its interactions with the soil. Different species of plants secrete root cap mucilages with different compositions, and these influence the type of microbial microflora and fauna that are attracted to the root tip, often with beneficial effects to the whole plant (Chaboud and Rougier 1981). The mucilage also provides protective functions, helping the root tip to resist dessication and lubricating the tip's passage through the soil (Bengough and McKenzie 1997). In this latter function, the mucilage is assisted by the border cells (Figure 1D) which function like ball-bearings or rollers in facilitating root elongation (Bengough and McKenzie 1997). Indeed, different

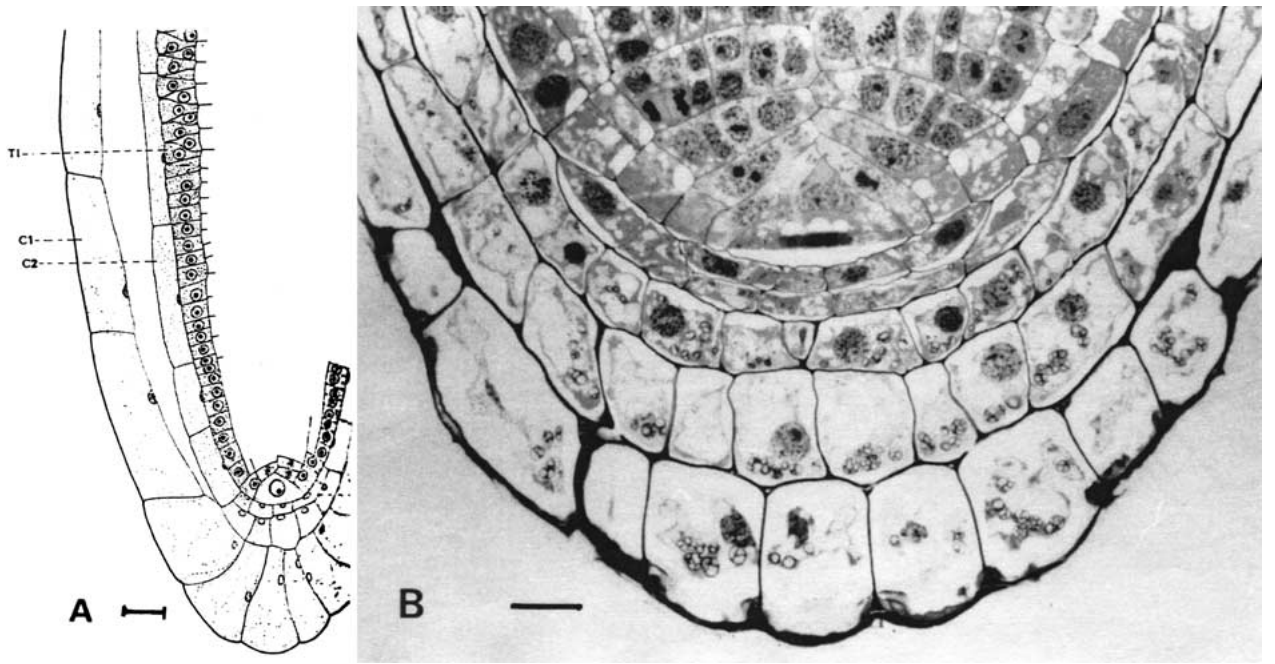

Figure 2. **A.** Partial longitudinal section through a root apex of the leptosporangiate fern, *Azolla filiculoides*, showing two layers, C1 and C2, of root cap cells (here the layers have separated from each other), and the beginning of the development of a third layer around the apical cell. Layer C2 forms a cap over the developing root hairs (trichoblast, TI) which, when they emerge, are forced to grow downwards between the epidermis and the cap. However, elongation growth of the root tip continually pulls the cap forwards, so releasing the root hairs from their confinement. Adapted from a drawing by Leavitt (1902). **B.** Median longitudinal section through the root apex of another leptosporangiate fern, *Ceratopteris thalictroides*. This cap has developed more layers of cells than are present in the cap of *Azolla*. Original micrograph prepared by Dr Alexander Lux, Comenius University, Bratislava, Slovak Republic. Scale bars: A and B – 20 μm.

species of plant have differently shaped outer lateral cap cells, ranging from ovoid (pea) to elongated (rice). Moreover, the sloughed cells may exist either as single cells, small groups (Figure 1D), or even as whole layers of outer cap cells, as occur in rice (Kawata and others 1979). The significance of these different cell shapes and associations for the tribology of root growth has not so far been assessed. A further consequence of the constant sloughing of cells, even when cell production in the cap meristem is reduced, is that the cells or their products may deter pathogens from entering the root (Hawes and others 1998). It should be recalled that after the radicle emerges from the seed, or the lateral root emerges from its covering of endodermal tissue, there is no external boundary to the cap (such as a protective cuticle or some anatomical equivalent of an epidermis), and so the constant flushing of this ''open wound'' at the root apex by the release of border cells may of itself have some protective benefit in deflecting potential pathogens away from the tip.

Another, though less certain, role of the root cap mucilage is to stabilize the seedling upon germination. The polysaccharide fibrils of the mucilage bind to soil particles and thus serve to anchor the tip of the emerging radicle to the soil. Since the peripheral cells of the cap are also embedded in a matrix of mucilage (Figure 1D; see also Guinel and McCully 1987), the additional binding between mucilage and soil would ensure that the mucilage-producing superficial cells were pulled free of the cap as the root tip grows forward. The potential to anchor the root may even hint at the reason for the continued presence of the vestigial cap possessed by the Podostemaceae, the suggestion being that, early in the evolution of these plants, cap mucilage interacted with the biofilms secreted by the cyanobacteria living on the same rocks upon which the podostemonads grow (Jäger-Zürn and Grubert 2000). Such an interaction would seem a likely means of cementing the thallus to the rock and preventing its loss in the torrential environment favored by these plants.

Caps vary in size within a root system, and much of this variation is linked to the branch-order (n) of the root to which the cap belongs. The diameters of roots of branch-order n+1 have been estimated to be

about half that of roots of branch-order *n* (for example, see Barley 1970), and the volume of the cap is likely to follow a similar relationship. There seems to be no compelling grounds for supposing that the cap regulates the physical dimensions of the root of which it is a part, even though the genetic ablation experiments of Tsugeki and Federoff (1999) indicated that the cap could influence the longitudinal extent of the root meristem. Rather, both root and cap development are likely to be subject to a form of higher-order control operating within the embryonic or primordial root apex. An effect of this coordinated development of cap and root within roots of different branch-order is that it defines the gravitational responses of the roots in question (Barley 1970) and, hence, influences the morphology of the root system. For example, compared to the caps of primary or zero-order roots, the smaller caps of first-order lateral roots have fewer gravisensing cells which, in turn, appear unable to support a full positive, orthogravitropic response that is a characteristic of the primary root (Ransom and Moore 1985). This curtailed graviresponse in the lateral root is referred to as plagiogravitropism.

Some bench-mark type of root growth and orientation has to be assumed against which the graviresponse, due to an alteration of statenchyma volume, is interpreted. Generally, the interpretation is that the interactions between roots caps and gravity result, at one extreme, in positive orthogravitopism when the statenchyma is maximally developed, and, at the other, in agravitropism when the statenchyma is absent. But, then, a problem arises in relation to the maintenance, by roots, of gravitropic liminal (set-point) angles which lie between horizontal (diagravitropism) and vertically upwards (negative gravitropism). Here, one would have to concur with the remark of Sir Nigel Ball that ''little seems to be known of the tropistic response of these roots'' (Ball 1969). In these cases, which are by no means unusual within a root system, it is likely that the inherent positive graviresponse of a root can be modified by local physiological conditions to result in a plagiogravitropic response. One such modifying condition was suggested to be the internal supply of sugars reaching the root tip (Morita and others 1983; compare Montaldi 1969). Interestingly, the interphloem pole distances studied by Morita and others (1983) in relation to the growth angle of rice nodal roots might also be related to the rate of delivery of auxin from the phloem for, in *Arabidopsis*, the poles of the protophloem are associated with the auxin permease, AUX1 (Swarup and others 2001), mutations in whose gene (*AUX1*) affect root gravitropism.

It is also worth mentioning that root caps of perennial species undergo seasonal cycles of growth and dormancy. In a study of conifer root apices, Wilcox (1954) found that, during autumn, the outer layers of the cap became suberized. The suberized layers then enclosed a proximal cap zone from which cap growth was reestablished in spring. This seasonal cycle may explain why the caps of conifers maintain such a long meristematic zone (see below): it could be to preserve a reservoir of cells for rapid regrowth of the cap following periods of dormancy.

Root Cap Ontogeny and Self-Maintenance

Root caps of contrasting size are found in the gymnosperm, *Ephedra*, where the caps are up to 1200 μm long (Pillai 1966; Peterson 1983), and in *Brassica* and *Arabidopsis* which have much smaller caps, about 75 μm long (Kuraś 1978; Baum and Rost 1996). Both types of cap arise during embryogeny and develop in intimate association with a suspensor. In *Ephedra*, and in gymnosperms generally, the suspensor is extremely long and contains many files of cells, with the proembryo a seemingly insignificant feature at its tip. By contrast, the suspensors of *Brassica* and *Arabidopsis* have few cells and the embryos are relatively much larger and more prominent. The role of the suspensor is like that of a mammalian umbilical cord: to transport trophic factors from maternal tissue to the heterotrophic embryo. Among these factors are hormones such as gibberellins, which are probably synthesized within the suspensor itself (Ceccarelli and others 1981).

At some point in embryogenesis, the suspensor begins to die, an event which may be coincident with, or even be responsible for, the curtailment of further growth of the cap (Barlow 1982). However, at this stage, the embryonic radicle now contains not only an autonomous hormonal system but also a complete complement of initial, or stem, cells (Barlow 1997), both of which support the growth of the autotrophic seedling and its radicle. The size of the cap, whether in *Ephedra* or in *Arabidopsis,* could, at this pre-germination stage of development, be related to the longevity of the suspensor. The maintenance of cap size in the subsequent post-germination phase of growth depends upon (a) whether all the stem cells of the embryonic cap are maintained, and (b) the manner of coordination between the rates of cell differentiation within the cap, the production of border cells, and the production of new cells by the cap meristem.

Cap size should not be taken for granted, for it is a feature that has almost certainly been determined by natural selection. The cap must be large enough both to assist the passage of the root through the soil and to provide a sufficient signal for root growth orientation (tropisms), but not so large that detached border cells can smother the tip and deplete it of oxygen. The rate of cell detachment is probably also related, or adjusted, to the rate at which the root grows forward, ensuring that the tip can continually advance through the accumulating numbers of released cells.

It is difficult to culture whole isolated root caps so that they remain in a steady state of cell production and differentiation. The maintenance of the correct pattern of meristematic activity, besides requiring the correct trophic or inductive factors normally supplied by the remainder of the root (see van den Berg and others 1997), may also require physical cues provided by the cell wall boundary that separates the root from the cap (Figures 1A, 1B). In theory, both conditions could be artificially supplied to isolated caps in much the same way that physical and trophic factors can maintain, and even define, a particular pattern of development and functional activity in cultured animal cells (Singhvi and others 1994; Pittenger and others 1999). In the natural, *in vivo* situation, the correct trophic and physical conditions may be fulfilled only at the root-cap boundary, thereby enabling the cap to be self-renewing by continually producing cells from a population of autoreproductive initial cells. It has, however, been possible to culture detached border cells (Caporali 1983), and even to regenerate (in alfalfa) new roots from them via an intermediate callus stage (Hawes and others 1991). That only roots formed in response to these culture conditions suggests that border cells have lost totipotency.

Border cells, as well as the protoplasts that can be prepared from cells taken from various regions of the cap (Pilet and others 1985), may be useful tools to examine the important topic of gene repression and chromatin modification during plant cell differentiation (Li and others 2002). Root cap nuclei of maize, for example, show clear changes in chromatin structure during their passage from the cap meristem to the cap periphery (Barlow 1976, 1985). Despite increasing chromatin condensation in the outer cap cells, which is often an indicator of impending cell death, there was no evident sign of nuclear DNA breakage, as evidenced by the TUNEL reaction (which uses terminal deoxynucleotidyl transferase as the probe) (Barlow 1976). [A few dead cells at the tip of maize root caps were recorded by this reaction in the hands of Matsuyama and others (1999b), though the appearance of the cap shown (their Figure 5C) looks atypical and environmentally induced death of cells in the embryo is not unknown in this species.] This result is compatible with the above-mentioned findings on the viability of border cells and with their being sites of expression of a unique suite of proteins and mRNAs (Brigham and others 1995). By contrast, in the case of 2-4 week-old *Arabidopsis* roots, application of the TUNEL reaction did reveal DNA breaks in the outer layer of cap cells (Zhu and Rost 2000). This observation correlated with the death of these cells. It may also account for the non-production of border cells by *A. thaliana* and other Brassicaceae (Niemira and others 1996). Members of the Chenopodiaceae also lack border cells and, interestingly, the absence of border cells in these two families correlates with the much reduced association of their roots with arbuscular mycorrhizae (Niemira and others 1996).

Initial Cells

Cap meristems of angiosperms and gymnosperms consist of two types of initial cell (Baum and Rost 1996; Barlow and others 2001) irrespective of whether the root meristem as a whole is of the ''closed'' or the ''open'' type (Guttenberg 1960), these being morphological terms indicating, respectively, whether or not the boundary between root and cap is discrete (see Figures 1A–1C). Cap columella initials, or *c*-type initials, are located at the proximal end of the cell files comprising the columella (Figures 1A–1C). These cells divide mainly transversely with respect to the root cap axis, and thereby feed new cells into the columella. Their shape is generally rectangular when viewed in longitudinal section. The other type of initials, the protoderm initials, or *a*-type initials, surround the *c*-type initials (Figure 1B). The *a*-type initials divide in multiple directions, thereby feeding cells into the lateral cap which ensheathes not only the columella but also, in some cases, extends up the distal portion of the root proper (see Figures 1A–1C). These *a*-type initial cells can have characteristic triangular or irregular polygonal shapes, as were described by Hayat (1963), working with root caps of *Cassia* spp, but these shapes are likely to be a result of the cells' variable division planes.

Root apical organization in *Azolla* and other leptosporangiate ferns (see Barlow 1997, Figure 6) follows another pattern (Figure 2). The cap is derived by an early periclinal division of the root apical cell. The cap meristem may be active for a short while, all of its dividing cells being *a*-type initials

that divide periclinally and anticlinally; there is no columella. A few tiers of cells result from a correspondingly limited number of periclinal divisions, and the files so created extend back along the root, tightly adhering to and ensheathing the meristem (Leavitt 1902). The root cap of *Azolla* displays a simple pattern of construction, shown in Figure 2A, whereas in Figure 2B is shown the slightly more complex cap of *Ceratopteris thalictroides*. In some ways, the cellular construction of roots of *Azolla* is a prototype for other, more complex root constructions. Its root cap is no exception, the caps of other species being an elaboration of its simple pattern (Figures 1A–1C), which mainly consists of the acquisition of a *c*-type pattern of cellular behavior in the central cells of the cap meristem.

c-Type Initials. The *c*-type initials of the columella are autoreproductive and divide asymmetrically. These features mean that one daughter cell, c_p, continues to be autoreproductive, whereas the other daughter, c_q, has a determinate division probability. In the columella of *Zea mays*, the distal c_q daughter cell undergoes only one further transverse division. To provide a conceptual basis that accounts for this latter point, the production of a *q* cell can be thought of as establishing a transverse division counter, λ_t, within that daughter cell (Lück and others 1997); in the instance of maize, $\lambda_t = 1$ indicates that the c_q cell can accomplish only one transverse division. By contrast, in the columella of the large root cap of *Ephedra nevadensis*, mitoses extend longitudinally and with undiminished frequency for more than 60 cell tiers (Peterson 1983); this would indicate that Λ_t is approximately equal to 6 for the c_q cells of these caps.

The concept of a division counter in the c_q cell assists in understanding the onset of cell differentiation. Usually, differentiation is regarded as beginning when some mysterious cytodifferentiation factor consumes cells at the end of the meristem. In a steady state system, the rate of cell differentiation keeps pace with cell production. But in a situation where λ is held to define the number of future cell divisions, cytodifferentiation commences only when the allotted λ divisions have been accomplished. According to this concept, the meristematic state is dominant over the differentiating state. When the course of division, defined by λ, is completed, the differentiating state is then enabled. To elevate the concept of a division counter to the level of a hypothesis would require the linking of λ to some cytophysiological variable. This link might be made, as suggested later, through the hormone, auxin. The value of λ that applies in the columella meristem may thus be directly related to the auxin content of either the c_q cells themselves, or of the c_p cells from which the c_q cells are descended.

Table 1. Frequency of Mitoses (Transverse plus Longitudinal) in each of the Proximal Tiers of the Cap Columella in Primary Roots of *Zea mays*

Tier of cap columella	Percent mitoses in tiers 1–5				
1	84	71	92	71	52
2	1	11	2	11	4
3	13	18	6	16	39
4	1	0	0	2	6
5	0	0	0	0	0
Reference	1	2	3	4	5

Roots were of different age, or were grown under different conditions, n, total number of mitoses scored, was ≥54.
References: 1, 2, 3-respectively, 5 mm, 45 mm and 105 mm roots of Z. mays cv. LG11 (aLivingstone 1987, and P.W. Barlow unpublished); 4, 5–20 mm roots of Z. mays cv. Mephisto grown in either loose sand (4) or compact sand (5) (aIijima and others 2003). Statistical comparison of the values in columns 1–3 reveals that they are significantly different from each other, as are the values in columns 4 and 5.

In the meristem of the root proper in *Z. mays*, it was found that cells sharing a common ancestry divided according to spatially defined sequences, or pathways, *P* (Lück and others 1995). The same should also be true for cells in the root cap columella meristem, even when $\lambda_t = 1$ for the c_q cells. A pathway, Pc with variable timesteps, *i*, between successive mitoses in the c_q cell, seems to be the simplest way of accounting for the pattern of mitotic indices (MIs) observed in the meristematic tiers of the columella. A study of the MIs in the columella of roots of various ages (Livingstone 1987; PW Barlow unpublished) revealed that the MI in tier 3 was often higher than the MI in tier 2, whereas the MI in tier 1 was higher than in either, and the MI in tier 4 was the lowest (Table 1). In view of the rapid cell division cycle in tier 1 (Clowes 1980), it seems reasonable to assume that a minimal interdivisional time (with, say, $i = 1$ timestep) is an attribute of the c_p daughter cell in tier 1, whereas its sister cell, c_q, in tier 2 has either a similar or a longer interdivisional period (say, $i = 1$, 2, or 3 timesteps for each variant of *P*c).

The filiations of four variant pathways, Pc_1–Pc_4, are shown in Figure 3. Mitoses always occur in tier 1, but then, according to which variant pathway is followed, they occur with varying frequencies in tiers 2–4. Unfortunately, mitotic indices derived from meristems are always estimated from observations on fixed material. Hence, it cannot be known for sure whether columella files in different root caps, or different files within a single cap, fol-

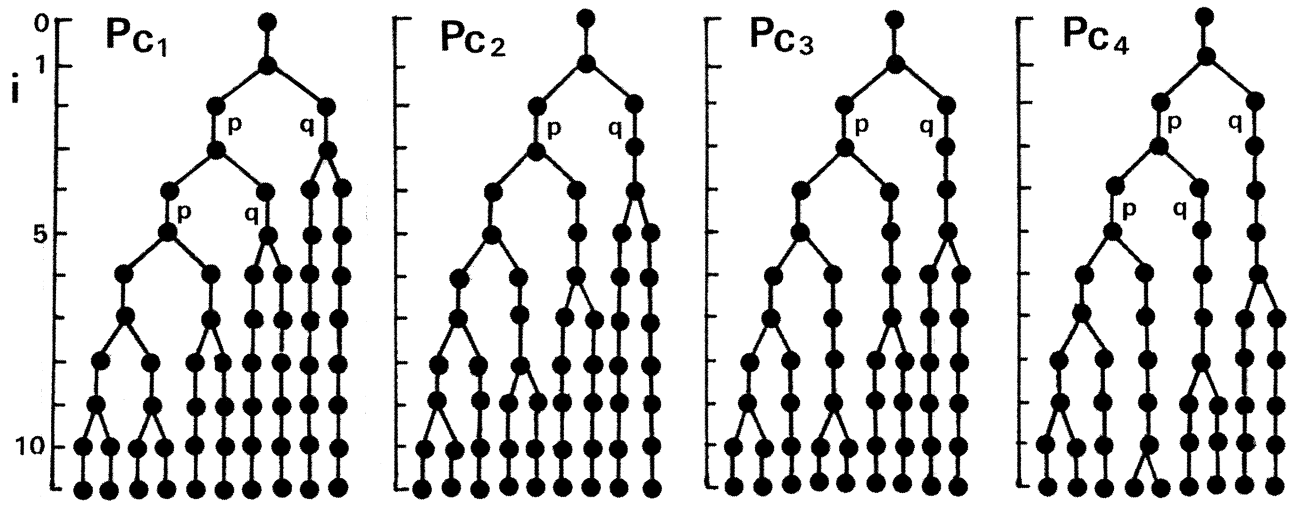

Figure 3. Filiation of cell divisions within the root cap columella of *Zea mays*. One pathway, *P*c, is chosen. There may be a variable number of timesteps (*i*) before division is initiated in the daughter cell, *q*, of the columella initial, *c*. The other daughter of the initial, *p*, remains meristematic. Four variants of *P*c are shown, Pc_1 … Pc_4, with incrementally increasing numbers of timesteps before the daughter, *q*, divides. In the diagram, the nodes (●) represent cell states, the links (|) represent the passage from one timestep to the next. Cell division is indicated by a bifurcation of the links at a node. Reading each filiation from left to right at any timestep gives an indication of the relative proximal-distal position of the cells in the columella files. Thus, tier 1 is always on the left, and higher numbered, more distal tiers are to the right.

low similar or different variants of *P*c. Nevertheless, suppose that equal numbers of columella files are engaged in Pc_1, ..., Pc_4, as depicted in Figure 3. Then, summing the number of mitoses that occur between timesteps $i = 2$ and $i = 10$ results in tiers 1, 2, 3, and 4 being the sites for 16, 4, 7, and 3 mitoses, respectively. This evaluation is rather similar to the distribution of MIs shown in the second column from the right in Table 1 (denoted therein by reference 4). However, in the next column (denoted by reference 5) significantly more divisions ($P < 0.05$) are present in tier 3 than in the former set of values (reference 4). The difference between the two distributions of MI relates to two sets of roots: one set was grown in loose sand, whereas the other set was grown in compact sand. Evidently, environmental conditions have here made a subtle, but nevertheless significant, impact on the cell division sequence.

Plant cells of a given species and cell type divide at an approximately constant size (Francis 1998). The timesteps (*i*) between divisions for each variant pathway *P*c shown in Figure 3 represent steps towards the achievement of the divisional cell size. Thus, if the rates of cell elongation in tiers 1 and 2 are similar, then the c_p and c_q cells will require a similar number of timesteps to reach mitosis, as in variant Pc_1. But if cell growth rate decreases with distance from the c_p cell in tier 1, the effect would be to increase the number of timesteps required for the c_q cell to reach mitosis. The form of the cell growth distribution along the meristematic portion of the columella would therefore define which variant of *P*c is adopted. One such experimentally determined growth distribution for the root cap columella of *Z. mays* is shown in Figure 4 (line a).

a-Type Initials. Compared to the *c*-type initials, the *a*-type initials that surround them and that give rise to the lateral cap, divide in a more complex manner. In cultured tomato (*Lycopersicon esculentum*) roots, the autoreproductive (a_p) daughter cell of an *a*-type mother cell produces derivative cells in defined directions (Barlow and others 2001). Derivatives produced in either the S and E directions (by transverse and periclinal cell divisions) are determinate cells (a_q) which establish the cell lineages of the lateral cap and the epidermis, respectively. Those cells produced (by radial longitudinal divisions) in either the B direction, or its opposite, the F direction, remain as *a*-type autoreproductive cells. In tomato, a value of $\lambda_t = 5$ applies to the a_q cell initiating the epidermal lineage (a_{qE}). Each a_{qE} cell therefore produces 32 cells along the length of each epidermal file. Similarly, a value of $\lambda_t = 5.5$ applies to each a_{qS} cell, resulting in approximately 50 cells in each packet descended from a_{qS} within the lateral cap (Barlow and others 2001). The cell packets extend apically towards the columella, and basally up alongside the epidermis (Figure 5). In *Arabidopsis*,

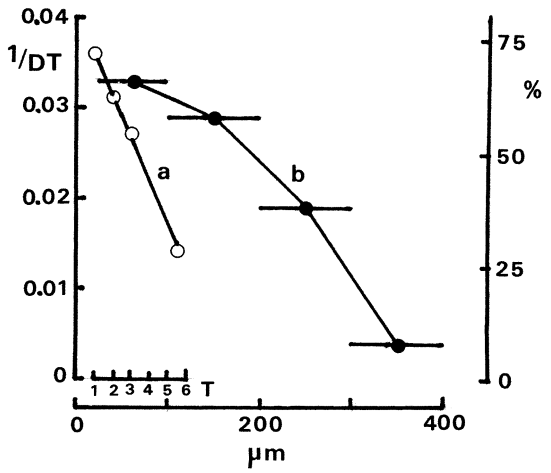

Figure 4. Relative rates of cell growth along the length of the cap columella of *Zea mays*. Two measures of cell growth, indicated by lines **a** and **b**, are shown. Line **a** applies along tiers 1–6 (minor abscissa labelled T) and plots the reciprocal of the cell volume doubling time (h) (left-hand ordinate labelled 1/DT) against these tier positions within the columella. Line **b** plots the % increase of cell volume per day (right-hand ordinate labelled %) along the length of the columella measured from the root-cap boundary (major abscissa labelled μm). Horizontal bars indicate the length of the columella over which the indicated value was estimated. Since there is little, if any, vacuole development in the columella, cell growth is related to the synthesis of new cytoplasm and the increase of nuclear volume. Data from Barlow (1977a, 1977b).

the number of epidermal cells descended from a_{qE} is 16 (Baum and Rost 1996), and so here a value of $\lambda_t = 4$ applies to its productions. In some other species (for example, *Z. mays*), however, there are no centrifugal E-directed productions, and the epidermis is derived from an initial cell in the root proper. Whether or not the epidermis and lateral cap derive from a common initial represents a fundamental difference in root apical organization.

Another type of production from the *a*-type initial cell is found in the tomato root cap, but only during its phase of enlargement (Barlow and others 2001). Here, a derivative, a_{qW}, is produced in the centripetal W direction thereby establishing a new *c*-type columella initial (see Figure 5). Thus, the tomato cap widens with the addition to the columella of *c*-type initials derived from the protoderm *a*-type initial. The W-directed cell productions are a flexible feature of probably all root caps composed of columella and lateral portions. Lateral root caps with a construction similar to that of tomato have been described for *Arabidopsis* and clover, *Trifolium repens*, by Wenzel and Rost (2001) and Wenzel and others (2001), respectively. In *Arabidopsis* only one additional columella file is added by each *a*-type initial during the early post-germination growth of the cap. Modern analyses of root cap formation recapitulate similarly detailed studies made by workers of an earlier era. Wagner (1939), for example, examined cap development in *Sinapis alba* and *Vicia faba*. The patterns of developments in these two species have features clearly in common with those found in caps of *Arabidopsis* and tomato, and of *Trifolium*, respectively.

Longitudinal Divisions in the Columella

There is one aspect of cell production in the cap columella that is somewhat puzzling, and that is the presence of longitudinal divisions (Figure 6). Such divisions are infrequent compared to the numbers of transverse divisions. In the columella of *Z. mays*, longitudinal divisions account for 10% or less of all divisions in tier 1 (Clowes 1980). Some longitudinal divisions also occur in tier 2 (Figure 6B). Similar frequencies were observed by Harkes (1976) in the cap columella of oat, *Avena sativa*, although here all divisions were confined to tier 1. Since median longitudinal sections of cap were scored by both workers, it is likely that the observed longitudinal divisions were perpendicular to the plane of section, whereas those divisions that were parallel to the plane of the tissue section were probably unrecorded. Although they might seem superfluous (given a source of *c*-type initials from *a*-type initials), the longitudinal divisions in the columella nevertheless establish additional files of cells. The importance of these divisions is that they could replenish the population of plasmodesmata on the longitudinal walls of the columella initials which, on average, become less frequent as the roots become older (Zhu and others 1998a). Plasmodesmata are involved in the movement of morphogenetic factors, and those on the longitudinal walls may participate in the movement of auxin out of the tier 1 columella cells during the root graviresponse (see later). Such longitudinal divisions may therefore help maintain a positive graviresponse in ageing roots.

Using 1-2 μm-thick resin sections cut transversely through tier 1 of the cap columella, and also through the layer of quiescent center cells just above cap tier 1, a survey was made of the orientation of the new longitudinal division walls (PW Barlow unpublished). Divisions were classified with respect to the radius of the circular cross section of cap as either being periclinal (if perpendicular to the cap radius), radial (if parallel to the radius), or in-

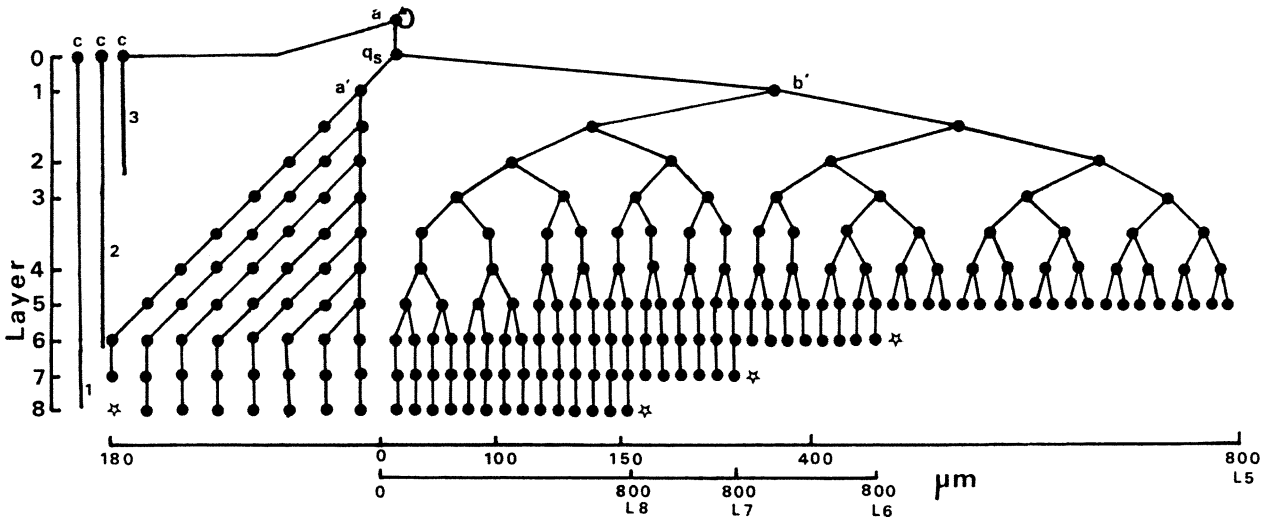

Figure 5. Descendance of cells comprising the lateral portion of the root cap of 7-day old, *in vitro*-grown roots of tomato (*Lycopersicon esculentum*). Only the transverse cell divisions are considered in this diagram, the conventions (nodes and links) of which are explained in the legend for Figure 3. The cell pattern corresponding to this division scheme is illustrated in Figure 1B (region marked L). The descendance is initiated by an S-directed production from an *a*-type initial. One daughter, q_s, is produced; the other daughter retains its initial status as an *a*-type initial (**a**). The q_s cell divides repeatedly. Its apically directed daughter (**a′**) produces all the cells that comprise the lateral cap. The basally directed daughter (**b′**) undergoes a more extensive set of divisions, the cellular products of which extend proximally, as a cap-derived 'skin', and cover the meristem of the root proper. Repeated productions of cell q_s and its descendents result in layers of cells, as shown in Figure 1B. The number of descendents of b′ in each layer (the layer number is indicated at the left-hand abscissa, 0 and 1 being innermost, 8 being outermost) can be found from summing the number of cells at the corresponding horizontal level. These numbers are based on the mean number of cells found within cellular packets (groups of cells of common descent) within the layers. Eventually, cells in the outer layers begin to be released from the cap flank. Release of cells starts when they become caught up in the zone of rapid cell elongation, and then proceeds towards the apex. The sites where cells have been lost in each layer are marked with ☆, and include also one lateral cap cell (as shown in Figure 1B). The most distal cells in the lateral cap make contact with the columella. In the early stages of cap development, the columella is itself widening by the addition of new *c*-type initials. These *c*-type initials are W-directed productions of the *a*-type initial, as shown here. Counting outwards from the columella axis, there may be 1 (extending to the tip of the cap), 2 or 3 (a short file at the base of the cap) axial files of columella. The numbered columella files (1–3) descended from the indicated *c*-type initial are shown at the left, and are represented by a vertical line. It can also be deduced which columella file connects with which layer of peripheral cap. The two ordinates (labelled μm) indicate the axial distance, in either the distal or proximal direction, from the tip of the root proper (0 μm). These distances apply, as indicated on the ordinates, to the cell layers L5, L6, L7, and L8 of the dermatogen. In layers L6–L8, cell divisions have ceased, but the cells continue to elongate and to be displaced basipetally before being shed from the surface at either 800 μm proximally from the root tip (that is, at the junction of meristem with rapid elongation zone), or 180 μm distally (that is, from the tip of the cap). Accompanying the transverse divisions shown here are radial division. As the proximal cells of the lateral cap move upwards along the epidermis towards the elongation zone, they undergo two or three radial divisions. At the position 0 μm, three radial divisions occur in the first five layers as they are displaced centrifugally. These radial divisions enable the number of cells in the circumferential plane to keep pace with the increasing diameter of the root.

termediate. Observations were made on caps of *Z. mays* roots of three different ages (up to 140 mm length) (Figure 6C), as well as on caps of recently germinated roots of wheat (*Triticum aestivum*), rice (*Oryza sativa*), and pea (*Pisum sativum*). No consistent pattern of orientation of new longitudinal division walls in tier 1 was found among the four species. Division walls in the central zone of the columella (a circular area, 40 μm in diameter) were predominantly periclinal in rice, but in wheat there were equal frequencies of periclinally and radially oriented division walls. In both maize and pea, the division walls in the columella were predominantly radial, but in a similar circular area within the quiescent center just above the columella they were predominantly periclinal. One tentative conclusion from these observations is that the predominant plane of these longitudinal divisions does not conform to what would be expected on the basis of the division plane being perpendicular to the principal direction of growth for a flat disc, a form characteristic of the root-cap boundary wall upon which

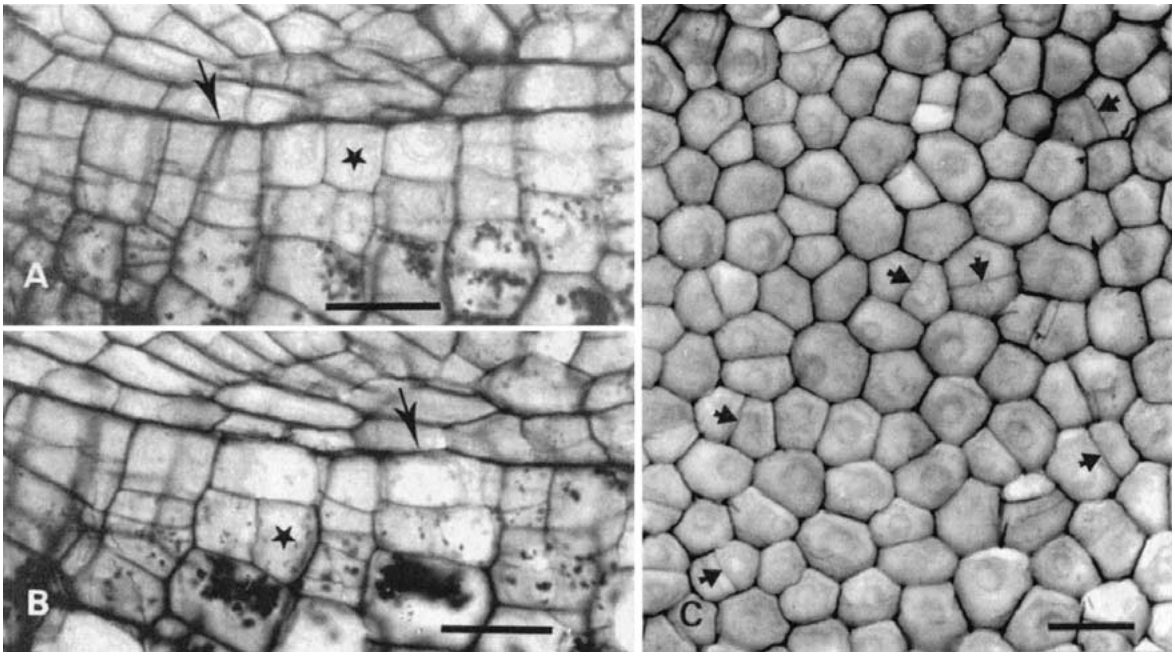

Figure 6. Median longitudinal sections (**A, B**) through the root apex of *Zea mays* to show cap meristem cells (stars) which have recently undergone longitudinal divisions in tier 1 (**A**) and in tier 2 (**B**). These divided cells may be lying at the point of isotropic growth of the root-cap boundary (arrowed). (**C**) Cross-section of tier 1 of a maize root cap. Recently inserted division walls are arrowed. The geometric center of the tier is in the center of the micrograph. Scale bars: 20 μm.

tier 1 columella cells and quiescent center cells are both situated. If this principle applied in these two zones, a predominance of radial divisions would be expected because of the greater amount of circumferential growth needed to maintain this discoid portion of the boundary wall. Moreover, the centrifugal growth of the contiguous layers (tiers) of cap and quiescent center in the maize and pea roots would be expected to yield similar frequencies of division wall orientation, yet they actually yielded completely different results.

One way out of this problematic area is to suggest that longitudinal divisions in the cap columella are regulated in accordance with information inherent to the state of the cells. For instance, it may be that each *c*-type cell newly produced from an *a*-type cell retains a potential to participate in at least one non-transverse division. The longitudinal divisions in cells of tier 1 of the columella may, therefore, be the result of the age-structure of the cell walls that has been handed on by production from a parental *a*-type initial cell. Assigning a value of $\lambda_{pr} = 1$ to the *c*-type daughter cells of *a*-type initials might, in formal terms, satisfy the observation of such otherwise unexpected divisions. (The term λ_{pr} is used here to distinguish this division counter for periclinal and radial division from the λ_t value mentioned earlier, which applies to transverse divisions.)

Conditions for Establishing Initial Cells

How could the different divisional patterns of *c*-type and *a*-type initial cells be regulated in the root cap? The following proposal is derived from observations on the roots of grass species, all of which have a closed type of meristematic construction, but it may also apply to species with an open type of meristem. The clue to the behavior of the initials – that is, the direction that their cell productions will take – lies in the shape of the distal surface of the root proper to which these initials are attached. This surface is like that of a disc or a flattened dome. In a newly germinated maize root, the root-cap boundary at the center of the dome is relatively thin, but becomes thicker just at the point where the files of epidermal cells curve steeply to extend up the flank of the root (Figure 1A). Using the end walls of epidermal cell packets as markers of packet position, it was possible to determine a strain rate along this boundary, from the center of the dome outwards (PW Barlow unpublished). Strain rate is minimal at the center of the discoid portion of the root-cap boundary, but increases further away (Figure 7). The relationship between this pattern of extension and the form of this boundary is as follows. A flat disc can enlarge centrifugally and remain flat only as long as its circumference grows at a rate that

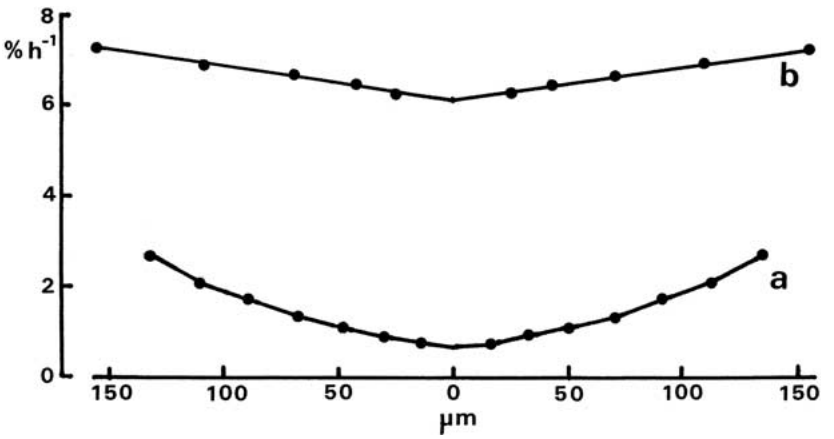

Figure 7. Radial growth of the root-cap boundary of a maize root (see Figure 1A and Figure 6C) in terms of estimated mean relative elemental radial extension rates ($\mu m \cdot \mu m^{-1} \cdot h^{-1} \times 10^{-2}$, or $\% \ h^{-1}$) at different distances (μm) from the center (0 μm) of the apical dome at the root tip. Estimates were averaged from data collected from median longitudinal sections on two successive days following germination, between 8 and 30 h (line a), and 30 and 41 h (line b). The increase in rates at these two times is compatible with an observed increase in the rate of root elongation and also with an increased mean curvature (K) of the apical dome, the values of which increases from $K = 1.06 \times 10^{-3}$ at 8 h to $K = 2.64 \times 10^{-3}$ at 41 h. The trend in values indicates that the dome becomes slightly more pointed with time. The formula by which K was calculated is given in Barlow and Rathfelder (1984). In the disc-like center of the dome (0–40 μm, approx.), the relative circumferential extension rate would be approximately × 2 Π the value of the radial extension rate, and would then decline rapidly as the radial rate increases, thus establishing the cylindrical form of the root. (Data from PW Barlow unpublished).

accommodates the increase of its radius. That is, if the radius of the disc increases by one unit, the circumference must increase by 2π units. If, however, circumferential growth slows to less than 2Π units, but radial growth does not diminish, the disc would change shape and become like a dish, that is, like the apical dome. This is what happens to the root-cap boundary wall. Growth of its disc-like central portion is at first equal along all radii and the circumference grows as described. Then, after a certain radius has been achieved, radial growth accelerates while circumferential growth diminishes. As the consequent curvature of the boundary (that is, the domed apex of the root proper) is established, so the radial growth component is transformed into the longitudinal growth component of the root flank. The magnitude of the circumferential growth component on the root-cap boundary wall regulates the shape of the root apex. The alteration of growth polarities at the apical dome may be regulated largely by changes in microtubule orientation in the epidermal cells which lie on the proximal side of the root-cap boundary. These orientations change from random, at the dome where there is disc growth, to hooped, as the radial/longitudinal growth component picks up (Baluška and others 1992).

The continual generation of the domed surface of the root proper, as outlined above, together with the relative growth rates that contribute to this shape, may have an impact on the direction and rate of cell production from the cap initial cells. The near absence of radial growth in the central portion of the dome favors the persistence of S-directed productions from the *c*-type initials. The acceleration of radial growth away from the center eventually creates a point, the isotropic point, on the root-cap boundary where the circumferential and radial rates are equal. This condition would favor not only the establishment of *a*-type initials with their multiple-choice E-, W-, B-, or F-directed cell productions but may also activate the *TORNADO* genes which, in *Arabidopsis* roots, are concerned with the onset of lateral cap and epidermis development (Cnops and others 2000). The longitudinally dividing cells of the maize root cap that are marked in Figures 6A and 6B are located at an isotropic point, just before the root-cap boundary becomes thicker and begins to curve upwards along the epidermal surface. At this point, also, the frequency of longitudinal divisions in tier 1 is at a maximum (Table 2). The same principle is evident in the open meristem of *Pisum* (Figure 1C; Table 2). This system of control of initial cell type and behavior via the growth properties of root-cap boundary can therefore account for variations in root and cap size during root growth. Increase or decrease in the number of columella cell files (*c*-type initials) in the radial plane is the result of shifts in the isotropic point on the root-cap boundary. But how this shift occurs is so far unknown. Whatever the nature of the switch between *c*-type and *a*-type initials, it has to be reliably perceived to set in motion a cascade of gene activity directed towards the development of either columella or lateral zones of the cap.

Lateral Cap and Detaching Superficial Cells

The detachment of cells, such as border cells, from one another and their release (Figure 1D) from the

Table 2. Frequency (%) of Longitudinal Divisions in Tier 1 of the Root Cap, or in the Zone of the Root Meristem which includes the Quiescent Center (QC) just above Tier 1, in Relation to Distance from the Center of the Cap in Roots of *Zea mays* and *Pisum sativum*

Species and zone	Distance from center (μm) and longitudinal division frequency (%)						
Z. mays	0–25	25–50	**50–75**	**75–100**	100–125	125–150	
Cap Tier 1	4	14	**39**	**35**	7	0	
P. sativum	0–40	40–80	80–120	**120–160**	160–200	200–240	240–280
Cap Tier 1	2	4	20	**47**	21	6	0
QC	0	3	12	**40**	34	10	0

n is > 150 in all cases. Newly inserted division walls were scored within concentric rings at the distances indicated from the geometric center (0 μm) of either tier 1 or the root zone above.
The emboldened values are those that apply at the position of radial and circumferential isotropic growth on the root-cap boundary.

surface of the cap seem to be regular features of all root caps of terrestrial plants. Evidence from transformed hairy roots of both *Pisum sativum* and *Artemisia annua* suggests that cap cell release is under genetic control (Wen and others 1999; Weathers and Kim 2001). Although the level of activity of pectin methylesterase (PME) in whole caps of pea roots was found to be positively correlated with the separation of border cells (Stephenson and Hawes 1994; Wen and others 1999), the border cells themselves do not seem to contain detectable amounts of this enzyme. Moreover, conditions that initiate border cell separation also initiate the synthesis of PME mRNA. Peripheral cap cells of *Ephedra* spp. also detach (see Pillai 1966, Figure 5), and so the great length of these caps is evidently not due to a failure of the cell release process. It may also be surmized that the enzymatic activity of outer cap cells of lateral root primordia brings about the disruption of surrounding mature cortical cells as the primordia make their way through the parental root to the exterior (Bonnett 1969); a similar process was described earlier for the separation between cap and coleorhiza in germinating grass embryos. Cell detachment obviously defines the periphery and, hence, the dimensions of the cap; it also makes the cap an interesting object of study from the point of view of the relationship between cell reproduction and cell differentiation because if cell production from the meristem were halted, but detachment from the cap proceeded unabated, the entire cap would eventually be lost.

The root cap has no boundary cuticle, as does the root epidermis, and so the peripheral cells are directly exposed to the environment in which the root is growing – soil, air, water, and so on. It seems that simply being at or near the surface of the cap is a sufficient condition to initiate this final stage in the cap-cell differentiation pathway – that is, enzyme induction, cell separation and mucilage production, and finally cell detachment from the cap surface (Barlow 1984). Superficial cells in many other plant systems behave similarly. Cells on the surface of callus tissue, for example, also detach and, like the outer cap cells, synthesize mucilage (Wright and Northcote 1974). Proliferating cells on the surface of the stele of cultured carrot hypocotyl swell up and detach in a cap-like manner (Guzzo and others 1995). The remarkable cultured cellular ''aggregates'' of *Solanum lycopersicoides* even form detachable root primordia, complete with starch-filled root caps (Tylicki and others 2000)! In each case, the tissues that release these cells are not covered by any impermeable or rigid cuticle. Naturally, the very act of detaching from the root cap means that the released cells uncover other cap cells that were formerly internal. These in their turn are induced to detach, thus exposing another cohort of cells, and so on. Clearly, such a process of detachment is self-perpetuating and is an extreme form of homogenetic induction. The surface of the cap is thus like a perpetual open wound.

A simple model for the induction of this final stage of differentiation in the outermost layers of cells is that it is a stretch-activated response, the driving force being the growth of the underlying cells. Indeed, a hypothesis of stretch-induced mucilage secretion as a trigger for cell separation in the outer cap cells would also account for the mucilage produced in quiescent center cells of *Z. mays* immediately following their exposure by the removal of the root cap (Barlow and Sargent 1978). Decapping leads to the rapid expansion (stretching) of the exposed root-cap boundary (Barlow and Hines 1982). Stretching, or deformation by pressure, of cell membranes in the outer cap cells in response to the mechanical impedance offered by soil conditions, may also account for the intensified devel-

opment of their dictyosomes (Iijima and Kono 1992). Compared to the outer cap cells of lightly compacted control roots of *Z. mays*, those of strongly compacted roots showed 30% more dictyosomes and a more than 2-fold increase in the total cross-sectional area of their secretory vesicles.

Turnover of Cap Cells

The rate of cap cell release defines the rate at which border cells accrue in the root environment, or rhizosphere. By the same token, the rate of release defines the rate at which formerly internal columella or lateral cells are recruited to the superficial population; and, similarly, the rate of cell production by the meristem determines the rate of entry of cells into either the young statenchyma of the columella as well as into the lateral cap. Thus, the cap is a dynamic system where the cells are always in flux, its cells being born in the cap meristem and being released from the cap periphery (Figure 5). In maize, the flow of cells from meristem to flank or tip takes from 3 days (through the lateral cap) to 7 days (through the columella) (Barlow 1978), a result verified by computer simulation using known values for cell cycle time within the cap meristem (Livingstone 1987). Interestingly, the value of 7 days is similar to the time it takes for cells to make the transit from the initial zone in the root proper to the zone of maturation about 1500 μm away.

A transit rate of seven days was also recorded for root cap columella of adventitious roots of *Cissus sicyoides* (Sambin and others 1978). In both *Cissus* and *Zea*, the cell transit time was estimated from the rate of displacement of caffeine-induced binucleate cells along the cap. Cell displacement, from cap meristem to cap flank, has also been followed by marking cell nuclei with tritium (^{3}H) and visualizing, by autoradiography, their positions within the cap at different times following their marking. By this means, Harkes (1973) found that derivatives of the cap meristem took 5–6 days (at 25°C) to reach the edge of the cap, while Philips and Torrey (1971) estimated that cap renewal in *Convolvulus arvensis* took 6-9 days (at 23°C). Innocenti and Stefani (1977) estimated a renewal time for caps of *Allium cepa* of only 3 days (at an unstated temperature). For maize caps, the ^{3}H-labelling technique produced results similar to the binucleate cell transit times (PW Barlow unpublished). It should be noted that much more rapid cap renewal times of only one day have been estimated for the caps of maize (Clowes 1976) and rice (Chaboud and others 1982). The timing of the cap replacement process is of interest because not only does it relate directly to the process of border cell production but it also places a time-scale on the processes of cell differentiation within the cap (Barlow 1977a).

In a steady state situation, the rate of passage through the various locations in the cap, each of which is associated with a different stage of cell differentiation (Figure 1B), would be constant, and the cap would remain a constant size as long as such conditions applied. A crucial question, therefore, is whether the passage through the various compartments can be modified. It seems that it can, and that the cell flux is subject to environmental conditions (Clowes and Woolston 1978; Clowes and Wadekar 1988). One intriguing question is whether cell release from the cap surface affects cell production in the meristem; if so, the two processes could be co-ordinated by both internal and environmental signals. In the experiments of Clowes and Woolston (1978), different steady state conditions were maintained by altering the density of the roots in the growth medium. The rate of cell production was found to correspond to the approximate rate of cell release. However, it is known that if division of meristematic cells is inhibited, cell release from the periphery can continue (Barlow 1977b).

Other experiments indicate that the steady-state situation can be broken. Increasing the temperature of root culture, for instance, resulted in rates of cap cell release exceeding those of cap cell production (Clowes and Wadekar 1988). Moreover, when cell release from the cap periphery was stimulated by mechanical impedance during a 24-h period (Iijima and others 2000), no evidence was found of any short-term acceleration of cell production in the meristem (Iijima and others 2003), although, as indicated in Table 1, the pattern of columella cell division was altered as a result of the impedance.

In the experiments of Clowes and Woolston (1978), it was found that more cells were released from the cap when the roots were grown at low density (fewer cells per volume of growth solution) than at a higher density. It might be assumed that the number of released cells would positively correlate with the quantity of mucilage secreted from the superficial cap cells. A study of this very point by Chaboud and Rougier (1991), also working with maize roots, confirmed that at low root densities more carbohydrate and protein was released into the medium than when roots were grown at high densities. The significance of these findings remains to be explained and, indeed, are even somewhat puzzling in the light of observations that modest increases in CO_2 (which might be expected to be associated with higher density root cultures) stimulate border cell release (Zhao and others 2000). It is

interesting to speculate whether the cap mucilage or some property of the border cells could act as a signalling system within or between roots, as suggested by Bennet and Breen (1991).

An emerging view is that border cell release and cell division in the cap meristem are tightly correlated (Brigham and others 1998). For example, when 1000–4000 border cells have been produced by pea roots grown on germination paper in Petri dishes, cell divisions in the cap meristem ceases – and so does the further production of border cells. Removal of the border cells by washing (which must involve a considerable alteration to the external environment in which the roots had previously been growing) reactivates division in the cap meristem. Conversely, reduction in meristematic activity by means of an anti-sense RNA for a gene whose expression is correlated with the cell cycle was associated with an inhibition of border cell production (Woo and others 1999). Unfortunately, correlations do not necessarily indicate causes, and further critical experiments are required to highlight the link between border cell release and cell birth in the meristem.

Regulation of Cap Size and Pattern of Cap Cell Differentiation

Cap Size

The question of how the overall size of the root cap is established is one that relates to the larger and more general question of how the size of any plant organ is regulated (Mizukami and Fischer 2000; see also West and others 2001). The radial dimension of the root cap is regulated by the growth characteristics of the root-cap boundary, modulation of circumferential growth being critical in this respect. The longitudinal extent of the cap and its total volume are regulated by the output of cells from the meristem which, in turn, is partially governed by the value of λ_t. The other critical component of cap size regulation is the timing, or positioning, of cell separation and release. Thus, regulation of cap size is a rather unique problem in plant biology because its basis lies in the coordination between the release of old cells from the cap and the production of new cells in the cap meristem. In addition, cell separation and cell release might depend on the size to which the cells are capable of growing. Thus, cell separation may be due to the combined effect of growth cessation at the cap surface and the continued expansion of the underlying cells. Since polyploid cells are larger than diploid cells, the longevity of cap cells, and hence their contribution to cap size, may be enhanced by an ability to double their nuclear DNA content. In tomato, for example, all lateral cap cells become polyploid (their nuclei have 4C-8C DNA content) whereas all columella cells remain diploid (2C DNA content) (PW Barlow unpublished) – a finding, incidentally, of general interest with respect to the regulation of DNA content in somatic cells.

Another possible control of cell separation, which complements the already mentioned stretch hypothesis for mucilage production, may result from some alteration in the pattern of sugar metabolism. Sugars are converted into starch within the amyloplasts of the central columella cells. The avidity of the amyloplasts for sugar might have the effect of starving any cell that lies downstream of this sugar supply. The production of new cells by the meristem would bring about the displacement of older cells into even more downstream positions. As a result, the anabolic processes could be put into reverse – the starch being degraded and the released sugars secreted as mucilage by the sugar-activated dictyosomes. In fact, the mutant, *ageotropic*, of maize is blocked in sugar metabolism at the inner/outer cap cell interface (Moore and Miller 1993). Compared to its wild-type parent, cv. Kys, the mutant has enlarged amyloplasts in the statenchyma but fails to secrete mucilage from the superficial lateral cap cells. Nor are cells released from the cap of *ageotropic* maize (Miller and Moore 1990). This supports the supposition that the secretion of mucilage (which in the mutant accumulates within the outer cells) plays some part in peripheral cap cell separation. It was also suggested (Miller and Moore 1990) that the mucilage plays some role in the gravitropic reaction and, hence, its absence from the exterior of the cap results in the agravitropic phenotype. Later, Baluška and others (1996) presented direct evidence that cap mucilage could regulate cell growth in the elongation zone of the root. In the case of *Arabidopsis*, where the outer cap cells die, the plasmodesmata and endoplasmic reticulum degenerate (Zhu and Rost 2000). Cap cell death may therefore be associated with the physiological isolation of these cells.

Gradients of Morphogens

The problem of organ size was sometimes discussed in forums devoted to the development of a ''theoretical biology'' (Wolpert 1970). One solution arrived at was that organ size is achieved by means of a concentration gradient of some morphogen relevant for organ development (Crick 1970). The

Table 3. Proteins Associated with the Differentiation and Function of Distinct Groups of Cells in the *Arabidopsis* Root Cap

Protein and function	Location of gene or protein expression	Effect of mutation, as expressed mostly in the root cap	Reference
AUX1 Auxin permease	Mostly in Tier 3 of the Columella (2nd tier of statenchyma). Lateral cap	*aux1*. Agravitropic root. Failure to accumulate IAA in root apex	1
AXR1 Involved in protein degradation, but affects auxin response		*axr1*. Reduced number of columella files	2
AXR3 Mediator of auxin response		*axr3*. Starch grains absent from columella	2
PIN1 Regulator of auxin efflux	Xylem parenchyma	*pin1*. Altered auxin distribution in cap columella	3
PIN3 Regulator of auxin efflux	Tiers 1–3 of Columella. Lateral cap	*pin3*. Defective gravitropism. Failure to re-route auxin from cap columella to root cortex. Absence of PIN3 from cap columella cells	4
PIN4 Regulator of auxin transport	Tier 1 of Columella. Lateral cap initials	*pin4*. Disordered cap columella divisions. Extra tiers of cap cells. Failure to maintain auxin gradient in cap columella	5

References: 1-aSwarup and others (2001); 2-aSabatini and others (1999); 3-aGälweiler and others (1998); 4-aFriml and others (2002b); 5-aFriml and others (2002a).

steepness of this gradient (which is a measure of the distance between high and low concentrations) is the determinant of tissue dimensions (Slack 1987; Wolpert 1996) as well as the pattern of cell differentiation along the hypothetical gradient. It is not hard to suppose that, in the case of the root cap, the source of the morphogen, and hence the high-point of the gradient, is at the proximal, meristem end, and that the sink for the morphogen, and hence the low-point of the gradient, is at the cap periphery where the morphogen may be catabolized or simply leak from the surface.

It was the work of Crick (1970) which established that gradients of the correct dimensions for developing tissues were feasible and, hence, put the morphogen concept on a firmer footing than hitherto. However, his estimate of the distance over which gradients could be set up was based on the assumption that diffusion was the means by which the morphogen was dispersed within the tissue. Such a basis now seems somewhat obsolete for plant development, at least, where so many carriers and permeases for small molecules, including the potential morphogen, auxin, have now been discovered (Palme and Gälweiler 1999; Friml and Palme 2002) and shown to be crucial for patterned development (Table 3).

While auxin may be carried from cell to cell via permeases and then eventually be inactivated in association with proteolysis and the cessation of cell growth (Gray and Estelle 2000), sucrose, another candidate morphogen, is exuded from the surface of the cap (Chaboud 1983). In the cap, as in other root tissues, sucrose may move via plasmodesmata. Because these structures are particularly abundant in transverse walls of the cap columella, they would favor acropetal movement of sucrose. By contrast, plasmodesmata are not so abundant on the longitudinal walls of the central statenchyma cells, thus making them less reliable for morphogen movement from the columella to lateral cap. However, in the early stages of root growth, the frequency of plasmodesmata across longitudinal and transverse walls of columella initial cells is about equal in both maize (Juniper and Barlow 1969) and *Arabidopsis* (Zhu and others 1998a, 1998b).

Observations that provide evidence supporting the general concept of positional information have

come from studies of organ regeneration (Wolpert 1970). When part of an organ is amputated and this missing piece is regenerated from a source of proliferating pluripotential cells, the new cells acquire properties appropriate to their position in the regenerating whole. Moreover, the regenerated portion usually corresponds in size to that which was lost. The root cap is no exception. A new cap, apparently of a size and function similar to the original cap, will regenerate from a decapped portion of a root. In the process of cap regeneration many anatomical features of cell differentiation which are normally spatially separated overlie each other, as though compressed together within the remaining tissue of the root tip. As the tip continues to grow, so the spacing of these features is reestablished, as though they were being separated out within the tissue, and a new cap is eventually restored.

Features of maize root cap cell differentiation which have been studied in connection with cap regeneration include mitotic activity, nuclear endoreduplication, amyloplast development, mucilage production, cell separation (Barlow 1974, 1984; Barlow and Sargent 1978; Barlow and Hines 1982) and, more recently, cytoskeletal microtubule orientations (Barlow and Parker 1996). Ponce and other (2000), using three different maize mRNA probes for gene activity concerned with mucilage and cell wall syntheses, have now confirmed, in molecular terms, what the older, anatomical studies of regeneration had revealed. Importantly, during the regeneration process, a molecular marker for the distal cap columella (gene expression for a GDP L-fucose) separated from other markers strictly related to outer cap tissue. The control of cap size might, therefore, depend on the regeneration of a feedback system involving markers related to specifically outer cell features and a more internal feature (quiescent center development, for example). Only when the correct spatial pattern of cells involved in this feedback, underpinned by the correct pattern of gene activity, has been established can the correct cellular dynamics of the cap – from cell production to cell release – then be established also (compare Hawes and Lin 1990; Brigham and others 1998). A dynamic system of this sort may, as already discussed, help maintain cap dimensions.

The Self-Maintaining Cap

From the foregoing discussion, it is possible that plasmodesmatal frequencies within the cap during its steady-state, self-maintaining phase of development, may determine morphogenetic gradients and, hence, regulate the patterns of gene activity and cell differentiation. In other words, mitotic activity in the meristem, rate of cell growth, competence of plastids to become amyloplasts, and dictyosomes to differentiate, may all be gradient-related features.

In the absence of other candidates, sugars and auxins have to be considered for roles as possible cap morphogens. As determined by the visualization, in *Arabidopsis*, of the activity of the DR5:GUS construct consisting of an auxin-responsive promoter and a glucuronidase reporter, an auxin gradient was proposed to be present along the cap columella, the highest concentration being in the columella initials (Sabatini and others 1999). No significant amounts of auxin were detected in the lateral cap. It is also important to note that there is no evidence that auxin itself is synthesized in the root cap but, rather, is synthesized in the meristem of the root proper (Müller and others 1998) from whence it is transported into the cap by the auxin permeases, PIN1 and PIN4, and by the AUX1 protein (Friml and Palme 2002). Auxin appears to be concerned with the development of the statenchyma and for promoting the cell divisions in the columella initials, evidence for this coming from the *axr1* and *axr3-1* mutants of *Arabidopsis*, both of which show defects in these two characters (Sabatini and others 1999) (Figures 8A–8C, Table 3). But whether auxin concentration *per se* conveys the information for specific cell fates is unknown, although, according to Wolpert (1996), there is evidence that in at least one animal system different cell types are developed at different positions along a morphogen concentration gradient. Surface cells of clover (*Trifolium pratense*) and oat root caps have a much increased rate of synthesis of xyloglucans and polygalacturonic acid/rhamnogalacturonan I compared with columella cells (Lynch and Staehelin 1992, 1995). Could these differences in polysaccharide synthesis be related to the changing auxin levels along the length of the columella, particularly since it is known that auxin can regulate the types of polysaccharides synthesized by cap tissues (Harris and Northcote 1970)?

The Enlarging Cap

In the embryo of *Arabidopsis* the first sign of auxin accumulation is in the prospective cap columella (Sabatini and others 1999). Moreover, auxin transporter molecules (for example, PIN1) seem to be arranged within the embryo in such a way that auxin is directed towards the future radicle end of the embryo (Hamann 2001), with PIN4 finally directing it into the cap (Friml and others 2002a) (Table 3).

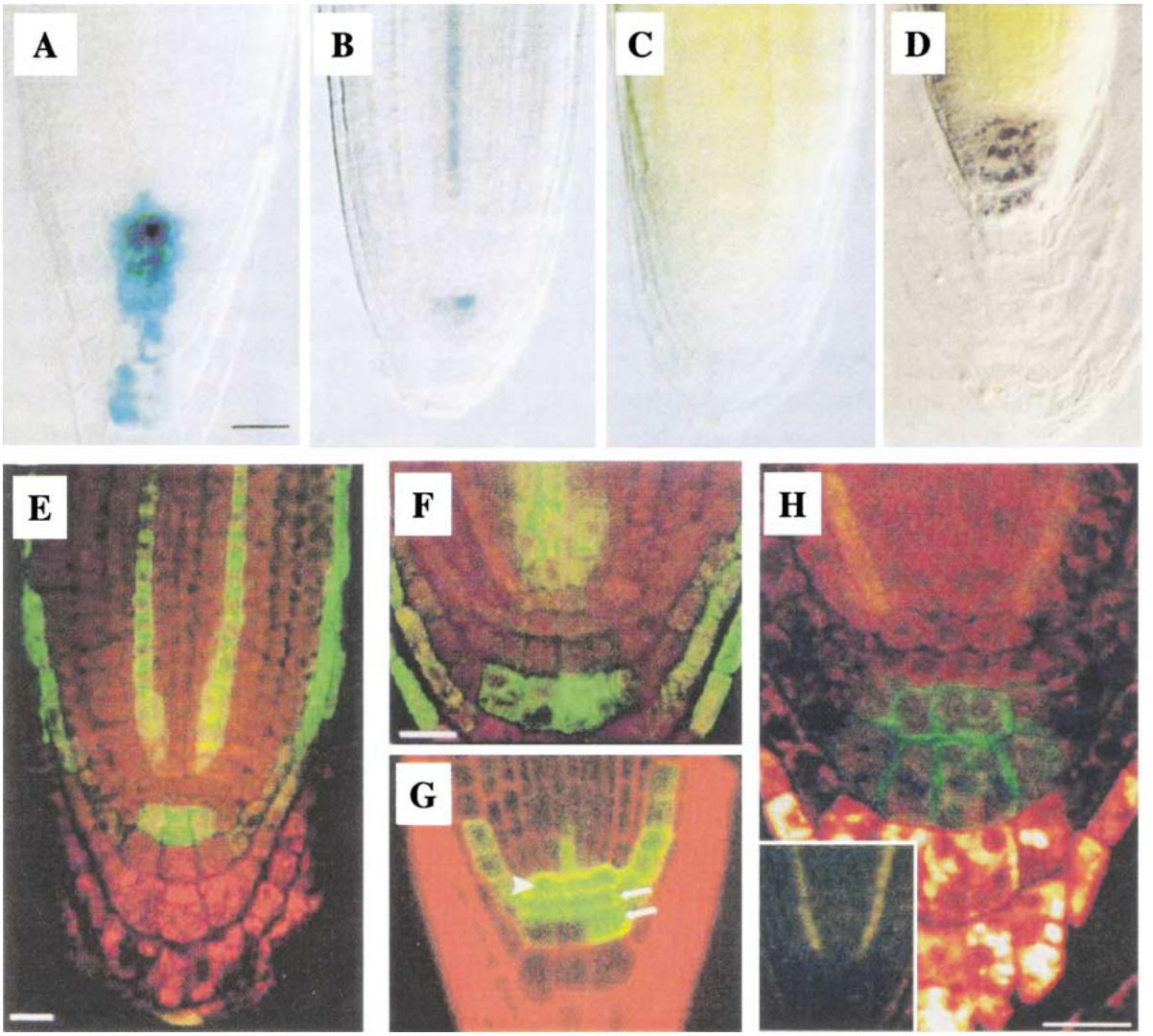

Figure 8. *Arabidopsis* root apices and root caps showing features of mutations in auxin permease genes. (**A**) Expression of the DR5::GUS auxin reporter in the root cap of wild-type seedling. The columella shows a strong reaction indicating the presence of auxin. (**B**) Reduced expression of DR5::GUS in the cap columella of *axr3* mutant indicating a lower auxin level. (**C**) Absence of starch in the cap columella of *ax3r* mutant (DIC optics of cleared root tip). (**D**) Additional tiers of cap in the *pin4* mutant. (A, B, C from Sabatini and others 1999, D from Friml and others 2002a, all reproduced with permission of Cell Press). (**E, F**) Immunolocalization of AUX1 protein to the columella and to the lateral root cap, and also to vascular tissue within the root proper. (From Swarup and others 2001, reproduced with the permission of Cold Spring Harbor Laboratory Press). (**G**) Expression of J2341, a GFP-revealed marker of *Arabidopsis* root columella initial cells. In the wild-type root apex, the marker appears only in cells of tier 1 of columella, but here, in the *pin4* mutant, the marker is present in tier 2 cells as well as in those of tier 1(arrows). The quiescent center is also labelled (arrowhead) in the mutant, but not in wild-type (modified from Friml and others 2002a, reproduced with permission of Cell Press). (**H**) Localization of PIN3 protein to the columella cells of wild-type seedings, especially tiers 2 and 3. Inset shows absence of protein signal from the cap of a *pin3* mutant seedling. (From Friml and others 2002b, reproduced with permission of Macmillan Magazines Ltd). Scale bars: **A**-25 μm (and the same magnification applies to **B**, **C** and **D**); **E**, **F**, **G** and **H**-20 μm.

Auxin accumulation may induce cap-building cell divisions. The level of IAA, detected by DR5:GUS, is highest in tier 1 of the cap columella of *Arabidopsis*, which is normally the only tier where cells divide (Figure 8A). Disturbance of this auxin pattern in *Atpin4* mutants, where IAA is now additionally found in both quiescent center and vascular cylinder initials, is accompanied by supernumerary mitoses in the quiescent center and columella. Moreover, in *Atpin4* mutant roots the expression of J2341, a GFP-based marker of columella tier 1 cells, was found also in cells of the quiescent center and

columella tier 2 (Figure 8G) (Friml and others 2002a). In the light of these recent findings, the earlier results of laser ablation experiments by van den Berg and others (1997) can be further interpreted. In this work, a quiescent center cell was ablated. The neighboring columella initial cell (tier 1) then failed to divide and, instead, differentiated as a statenchyma cell, as indicated by the presence of starch grains. The observations indicate not only that the export of IAA to the columella initial was probably halted by the ablation but also that cell division is dominant to cell differentiation. The basis for this last-mentioned feature may lie in the acropetal auxin gradient in the cap: a high auxin level in the columella initials maintains their division status, whereas a lower auxin level permits differentiation. Interestingly, up to eight additional tiers of columella cells were found in caps of the *Atpin4* mutant (Figure 8D). This suggests that the impaired transport of auxin into, and then through, the columella in the mutant might also impair the usual cell separation process at the cap periphery.

Auxin Permeases and Root Cap-Regulated Gravity Response

The discovery and exploitation of the *PIN* mutants in *Arabidopsis* has revolutionized understanding of root gravitropism. The coupling of immunofluorescent localization of auxin permease proteins (Figures 8E, 8F, 8H) with a visible marker of IAA through the use of the DR5:GUS gene construct (Figures 8A, 8B) indicates a prominent role for this hormone in root gravitropism (Chen and others 1999). Following maximal gravistimulation, the asymmetric rearrangement of AtPIN3 on the plasma membrane of the columella initials (Table 3) seems quite significant since it could lead to an efflux of auxin into the lateral cap region (Friml and others 2002b). How these permease rearrangements are effected is unknown. They may be brought about by corresponding rearrangements of the actin cytoskeleton (Muday 2000) acting in concert with the all-important, gravity-perceiving statoliths (amyloplasts) (Sievers and others 2002). Similarly, the visualization of IAA moving from the lateral cap into the outer cortex and epidermis of gravistimulated *Arabidopsis* roots (Rashotte and others 2001) permits a conclusion to be reached which an earlier generation of researchers could only dimly perceive through the use of radioactive markers (Ohwaki and Tsurumi 1976) and sophisticated quantitative assays (Pilet 2002). In principle, these earlier workers were correct in what they so laboriously surmized: namely, that IAA is transported acropetally in the stele of the root and passes into the cap from whence it is re-exported, upon gravistimulation, to the underside of the root and then transported basipetally within the outer cells of the cortex.

An interesting feature of the recent work on this basipetal transport during gravitropism, as revealed by DR5:GUS, is that the auxin flow appeared to lag behind the actual stimulation of differential growth. That is, the time-courses of the auxin flux and of the tropism seem not to match. This might be because the GUS staining reaction is not sensitive enough to reveal all the IAA, and that its advancing front is not, in fact, detected. Alternatively, it could be that there is some other gravity-induced signal that travels in advance of the IAA. What this signal could be is speculative, but it might be an electrical signal (originating in the cap?) which outpaces the slower basipetal auxin transport system. A discussion of electrical and other signals emanating from the cap in relation to the gravitropic response of roots and the relationship of these to auxin movement can be found in Monshausen and Sievers (2002).

Exchange of Cells Between Root and Root Cap in Relation to Open and Closed organizations of Root Meristems

Although all roots possess a cap (a few exceptions were mentioned earlier, however), in some species the cell files of the cap are in continuity with the main body of the root, whereas in others the cell files of root and cap have no continuity and the cap appears to develop independently of the root proper. Root meristems displaying the former type of cellular organization have been termed ''open'', whereas those of the latter type are termed ''closed'' (Guttenberg 1960). These terms adequately convey the idea of whether the meristems of the cap and the root are continuous or discrete. Anatomically, both types of meristematic organization are usually well defined, although the primary apical meristems of some species (for example, the Asteraceae) may regularly switch from open to closed during postgermination growth (Armstrong and Heimsch 1976). The converse transition, from closed to open, has also been recorded (see Chapman, this issue). From the phylogenetic point of view, morphologists regard the open type of meristem, as expressed in actively growing radicles and roots, as being ancestral to the closed type.

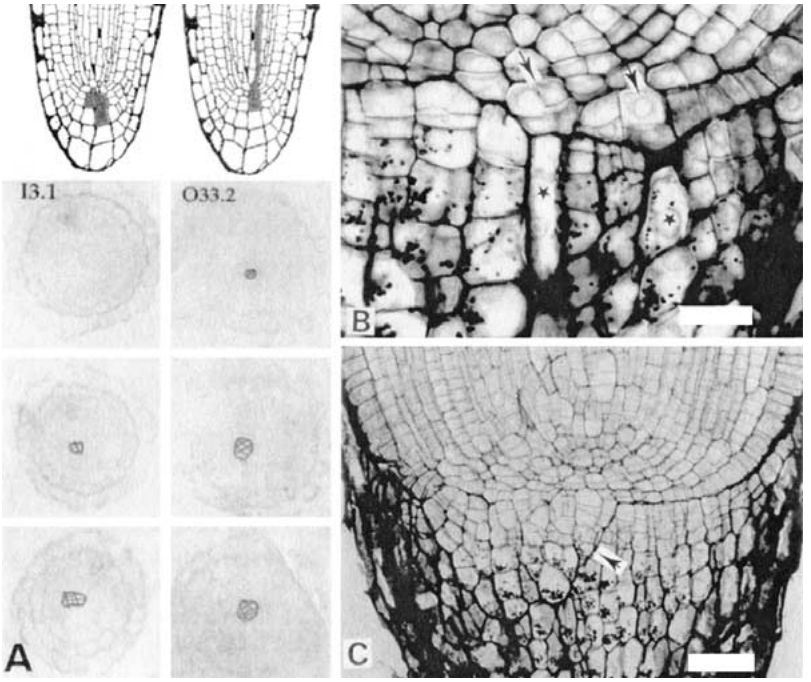

Figure 9. The passage of cells from the quiescent center into the root cap indicating that the root-cap boundary can be breached in a normally closed meristem. (**A**) Genetically marked tracts of related cells (shaded in the upper scheme) rendered visible in the light microscope by the GUS reaction applied to longitudinal sections from two different roots of *Arabidopsis thaliana*. The three lower panels indicate the marked cells (grey) in cross-sections through the cap initials (lower), quiescent center (middle), and proximal portion of the meristem (upper). The sections are 5 μm apart. (Adapted from Kidner and others 2000.) (**B**) Longitudinal section through an apex of *Zea mays* grown at 5°C for four days and then allowed to recover for three days at 20°C. A few cells in tier 1 of the cap have failed to divide (asterisks) and this has provoked adjacent cells in the quiescent center to divide (arrows), distort the root-cap boundary, and begin to replace the damaged cap cells. (**C**) Another recovering *Z. mays* apex (four days at 20°C) with extensive intrusion into the former cap of descendents of the quiescent center. The meristem appears to be closed on the right-hand side, where no damage has occurred, but open on the left-hand side, where cells have passed from quiescent center into the cap. The former root-cap boundary is marked (arrows). The intruded cells have differentiated normally, as cap cells, due to the specifications of positional information. Scale bars: B-25μm, C-50 μm.

The cellular behavior associated with the open and closed types of apical meristems has been analyzed, notably by Guttenberg (1960) and Clowes (1981, 1982). Clowes paid special attention to the rates and distributions of cell divisions in the stelar and cortical portions of the quiescent center at the tip of the root proper as well as in the root cap initials, for it is these features that define whether or not the cell files of root and cap are in continuity. When there is no continuity of files, and hence no passage of cells from root to cap, a definite root-cap boundary becomes established. Numerous studies of closed meristems have, nevertheless, revealed circumstances when this boundary can be breached and the meristem in question temporarily become open. One such recent study (Kidner and others 2000), using genetically marked cells within closed root meristems of *A. thaliana*, has shown that the descendents of quiescent center cells can occasionally breach this usually discrete boundary (Figure 9A). However, in this work, a heat shock was used to activate the genetic marker and this shock could have had a disruptive effect on the usual regulation of cell division rate and orientation in the quiescent center. Moreover, as roots of *Arabidopsis* age, the organization of their primary meristems naturally changes from closed to open (Baum and others 2002), which again could also account for some of Kidner and others' (2000) findings.

None of the above studies have indicated why the activation of cell division in the quiescent center which leads to the switch from closed to open meristematic organization, brings about a rupture of the root-cap boundary. It cannot simply be because of the occurrence of cell divisions in the cortical or epidermal portions of the quiescent center since additional cells can build up here without disrupting this boundary. Clowes (1982) suggested that in *Helianthus annuus* and *Cucurbita pepo* root apices (both with open meristems) there may be periods when the cap columella initials are quiescent and this condition may be sufficient to provoke a corresponding proliferation of cells in the quiescent center. Presumably, the quiescence of the cap initials would also have to be coupled with a weakening of the root-cap boundary to enable a surge of forward-directed axial growth by the cells of the quiescent center to penetrate this boundary. Similarly, when division activity in tier 1 cap initials in *Z. mays* is terminated by a period of low temperature, cellular descendents of the quiescent center repopulate the damaged portion of cap (Figures 9B, 9C). In this case, there may be a turgor pressure differential across the root-cap boundary resulting in the

sub-vital tier 1 cells being crushed by the more vigorously growing cells of the quiescent center. At the same time as cells ''flow'' forward from the quiescent center they fall under the influence of root apex-specific positional information which determines their pattern of differentiation as that pertaining to root cap. As a consequence, there is a re-differentiation of a new root-cap boundary and a re-establishment of the cytological properties usual for the zone in question. These events occur in much the same way as they do during the embryogeny of the root and its cap (Barlow 1975), but exactly how the root-cap boundary forms in undisturbed or in regenerating root tips is an open question. It is also interesting to note that when the cap is being regenerated by a decapped root tip, its development is not dependent upon cell division. In the presence of inhibitors (5-aminouracil, hydoxyurea) which slow, or even abolish, cell division, regeneration of a statenchyma and of an assemblage of walls resembling a root-cap boundary can still be discerned. Regeneration can, therefore, be a morphallactic process, with cell differentiation occurring in the correct relative position within the apex, even though the usual accompaniment of cell division has been largely blocked (Barlow 1981).

A recent study by Nakajima and others (2001), using roots of *A. thaliana*, of the action of the gene *SHORT-ROOT* (*SHR*), which codes for a putative transcription factor, seems to have relevance to the problem of the open/closed meristem. In mutant *shr* root apices, initial cells for the endodermis do not form and, consequently, the root contains only a single cortical cell layer. The *SHR* gene is necessary for the periclinal longitudinal divisions that create the endodermal initial cells. The *SHR* protein is synthesized in the stele, but can move from there into the adjacent cortical layer. When SHR was over-expressed in a transgenic line (*SCRpro*::*SHR*) not only were additional endodermal layers found but so, too, were additional tiers of cortical cells lying within the quiescent center. This latter result is similar to what occurs in cultured root apices of tomato bearing the *gib-1* mutation (Barlow 1992) which impairs gibberellin biosynthesis. In *gib-1* roots there are successive cycles of cap formation, one partial cap forming from descendants of the quiescent center before yet another cap forms in the same way. Guttenberg and others (1955) also described similar rounds of division and differentiation that periodically breach the root-cap boundary in the developing root apices of dicot species. These results may be interpreted as follows. In a closed meristem, such as *Arabidopsis* or tomato, a division-regulating protein (such as SHR) periodically migrates from the stelar to the cortical zone of the quiescent center and therein initiates, by periclinal cell division, a new layer of cells. At the same time, there is impairment of division activity in the cap initials and this is accompanied by a weakening of the root-cap boundary. The distal daughter cells of the periclinal division then escape the effects of whatever component of positional information normally imposes quiescence, and these cells are then enabled to divide more actively, just as do cap initials. In fact, these cells become the actual cap initials at the same time that positional information reinstates, in the affected meristematic area, the cellular properties appropriate to a closed type of meristem. In open meristems, by contrast, there may be a greater movement of a factor like SHR from the stele to the cortex, bringing about more frequent periclinal divisions in the distal cortical zone of the quiescent center – so frequent, in fact, that the cells' response to positional information is inadequate at defining a root-cap boundary, and hence, an open type of meristematic organization develops.

Concluding Remarks

The above discussion concerning the root cap and its relationship with the overall organization of the root apex indicates how this organ is integrated into the general biology of the root. It also indicates the dynamic interplay of cell division and cell differentiation. As in many growing systems in plants, new cells produced by division are constantly in demand for cell differentiation, the differentiation process continually encroaching upon the meristem. This is clearly shown in the experiments of van den Berg and others (1997) where indirect laser-generated damage to a cap initial cell prevented this cell from dividing and producing new cells in an apical (acropetal) direction whereupon it was encroached upon by an opposing, basipetally moving differentiation process. At the same time, the events of division and differentiation throughout the meristem and cap are under the higher control of positional information which specifies the fate of cells at any particular position relative to certain critical reference points in the organ. Border cells, one might suppose, are free from such controls and, hence, are autonomous, only requiring access to vital solutes leaking from the root to maintain their function and survival.

The root cap also upholds Charles Darwin's prescient view that a mobile growth-promoting influence could be responsible for tropisms. This

hypothesis has been substantiated by the recent visualization of auxin movement in the root and root cap. However, there is still much to learn with respect to root gravitropism, especially in the area of how the gravity signal is transduced and converted into auxin movement. Clearly, there is also more to the cap than simply housing a control system for gravitropism and other sensory functions, and it is now becoming more widely appreciated that its border cells may also have a special role in maintaining the growth and well-being of the roots and of the whole plant. The fact that cap cells can proceed from their birth in the cap meristem to their release into the soil in a period of a few days, *and* carry out sensory functions on the way, surely makes the cap one of the most remarkable parts of the plant. The small size of most caps and the increasing ease with which the fine details of cellular function can be probed certainly commend the root cap as a useful object of research. Not only could there be practical benefits from cap research for plant management, but the cap also represents a system for the study of the deeper processes of cell turnover and organogenesis in plants.

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