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Root Caps and Rhizosphere

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Abstract

In this paper we discuss recent work on the physiological, molecular, and mechanical mechanisms that underlie the capacity of root caps to modulate the properties of the rhizosphere and thereby foster plant growth and development. The root cap initially defines the rhizosphere by its direction of growth, which in turn occurs in response to gradients in soil conditions and gravity. The ability of the root cap to modulate its environment is largely a result of the release of exudates and border cells, and so provides a potential method to engineer the rhizosphere. Factors affecting the release of border cells from the outer surface of the root cap, and function of these cells and their exudates in the rhizosphere, are considered in detail. Release of border cells into the rhizosphere depends on soil matric potential and mechanical impedance, in addition to a host of other environmental conditions. There is good evidence of unidentified feedback signals between border cells and the root cap meristem, and some potential mechanisms are discussed. Root border cells play a significant mechanical role in decreasing frictional resistance to root penetration, and a conceptual model for this function is discussed. Root and border cell exudates influence specific interactions between plant hosts and soil organisms, including pathogenic fungi. The area of exudates and border cell function in soil is an exciting and developing one that awaits the production of appropriate mutant and transgenic lines for further study in the soil environment.

Key words: Root caps; Rhizosphere; Plant growth

Introduction

Haberlandt (1914) defined the cap as a mucilage-covered, bullet-shaped barrier functioning primarily to protect the meristem physically and to lubricate its passage through the soil. This much-cited classical description of the cap as a slimy battering ram may have led inadvertently to an impression of this organ as an important yet relatively passive part of the root system, like cuticle or bark. Recent studies outlined below suggest a more complex, dynamic, and specialized system that does provide mechanical protection of the root, but also serves to 'engineer' the properties of the invaded environment (reviewed in Hawes and others 1998; Hawes 2000). Thus, the root cap responds to a symphony of signals from the soil environment to (1) control direction of movement; (2) facilitate penetration into soil; and (3) define microbial ecology by the regulated delivery of biologically active root exudates into the rhizosphere.

An often-cited distinction between plants and animals is that of mobility: whereas animals can propel themselves away from danger and toward nutrients and safety, plants are less obviously mobile. Survival of plants nevertheless depends on the

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ability of root systems to establish themselves in locations where water and nutrients are available to be delivered to the rest of the plant, without destruction of root meristems by predators and/or environmental stress. This complex feat is accomplished by the production of root systems with myriad root tips, each elongating by up to a millimeter or more per hour, and covering territory at a rate of meters per day (Lynch 1995). As with animals, plant movement is conditioned by the need to avoid danger and to seek water and other nutrients (Darwin 1880).

Root 'motility' is generated through the action of the root apical meristem, and a region called ''the region of elongation'' (Baluška and others 1996a). However, it is the root cap that senses, processes and transmits signals to the meristem and region of elongation to control the direction of movement. In large part, the capacity of the root cap to direct movement of the root and modulate properties of its rhizosphere defines the architecture and functioning of the entire plant (Aiken and Smucker 1996). Despite its importance, studies of root cap gene expression have been surprisingly limited (Hawes and others 2000; Ponce and others 2000; Tsugeki and Federoff 1999). In the following sections, we describe structural components of the root cap and outline what is known about how the root cap directs root system architecture in response to environmental signals. Data consistent with the hypothesis that border cells (formerly called 'sloughed root cap cells') delivered by the cap facilitate penetration of the soil and modulate microbial ecology at the root-soil interface are presented in the context of newly emerging information about root cap dynamics in higher plants.

Root Cap Structure

The root cap has been a popular model system to study cellular functions like cytokinesis, differentiation, and secretion for more than 100 years (reviewed in Battey and others 1999; Driouich and others 1993; Firn and Digby 1997; Rougier 1981; Sievers and Braun 1996; Van den Berg and others 1997). This small (less than 1 mm^2 even in plants with large roots) cluster of specialized cells is organized in structured tiers (Figure 1). After generation in the cap meristem in pea and maize, the best characterized systems, new cells differentiate progressively through a series of distinctive morphological changes that are correlated with specialized functions (Feldman 1984; Moore and McClelen 1983; Moore and others 1986). These include the synthesis of starch grains ('statocytes') which participate in gravity sensing, within the columella of the cap (Chen and others 1999, 2002; MacCleery and Kiss 1999; Moore and Evans 1986; Rosen and others 1999; Wolverton and others 2002). As differentiation progresses, the starch is degraded and a high molecular mass polysaccharide mucilage is synthesized and exported to form a water-soluble capsule surrounding the cap periphery (McCully and Boyer 1997; Rougier 1981). This polysaccharide layer is similar in composition to middle lamellae—like a primary cell wall, without the cellulose (Moody and others 1988).

As cells reach the cap periphery they are programmed to separate from each other and from the cap by the activity of cell-wall-degrading enzymes localized in peripheral cells of the cap (Hawes and Lin 1990; Hawes and Stephenson 1994; Wen and others 1999). This enzyme activity results in precise solubilization of interconnections to release single cells or small groups of cells. Remarkably, in most species the integrity of the cell wall is maintained during this process of polysaccharide solubilization, such that viability is retained after the cells detach from the cap (Table 1). In cereals and legumes, border cell populations can remain more than 95% viable over a period of weeks in culture (Hawes and Wheeler 1982; Hawes and Pueppke 1986; Knudson 1919). Border cells from maize reportedly survive for a week or more after detachment from roots grown in nonsterile soil (Vermeer and McCully 1982). *A. thaliana* is a distinct outlier: cap turnover occurs only sporadically and when it does occur, there are no border cells *per se*; instead there is abscission of an entire root cap whose cells are dead (Hawes and others 1997; and unpublished observations). This apparently is associated with programmed cell death (Zhu and Rost 2000). In tomato, whose border cell viability is low, aspects of programmed cell death also reportedly occur during cap turnover (Wang and others 1996). A similar process may occur in representatives of the Asteracae family (sunflower and zinnia), whose border cells are dead by the time cell separation is complete (Table 1). The genetic basis for such diversity is not known.

Though most tested species produce border cells, marked diversity occurs in magnitude of border cell production among plants (Hawes and Pueppke 1986). The number of cells produced by a given root in a 24-h period is roughly conserved at the family level and varies from a hundred or so for the nightshade family, to several thousand for cereals and legumes, to 10,000 for pine and cotton

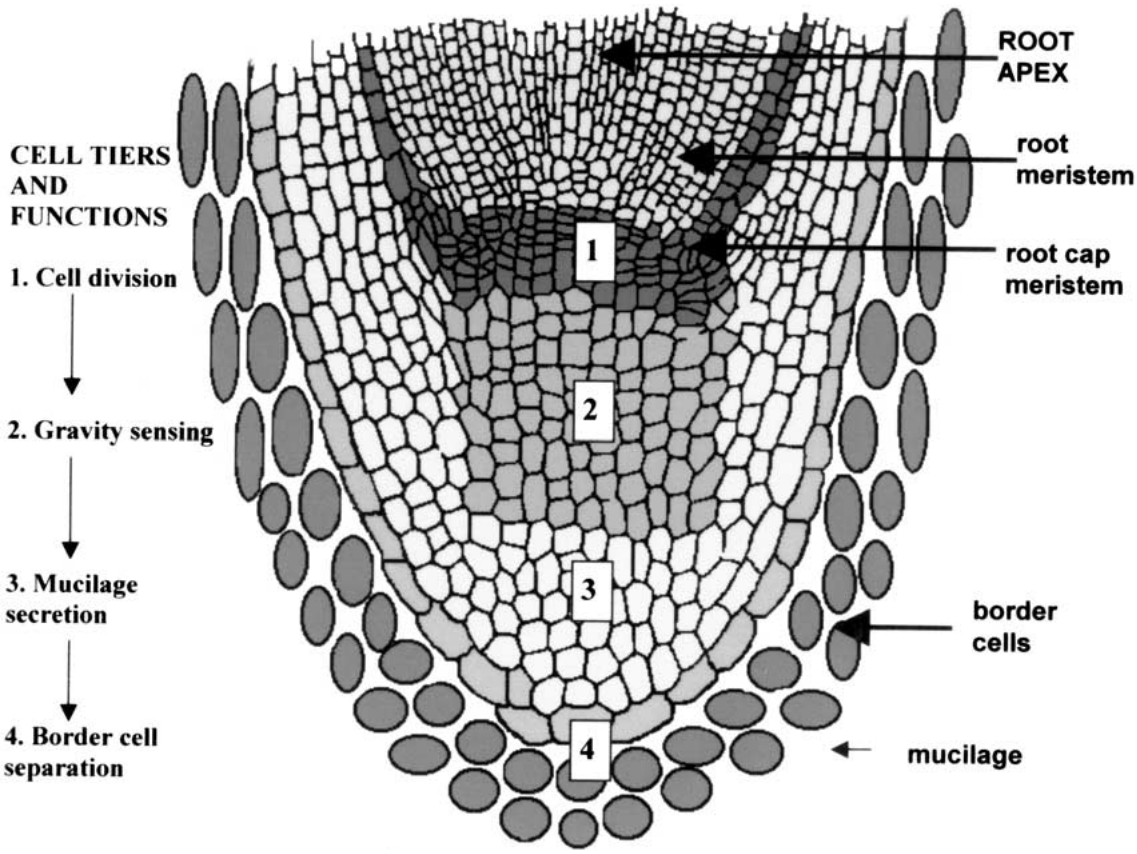

Figure 1. Root cap structure and development (from Barlow 1975). As cell division occurs in the root cap meristem, cell tiers are displaced toward the periphery of the cap. In the columella region, cell tiers exhibit distinct morphologies reflecting their specialized functions. As each tier is displaced, previous functions cease and new functions are initiated within the progressively differentiating cells. The time required for the entire cap to be displaced by a new set of cells ranges from 24 h to 7 d, depending on growth conditions (Barlow 1978; Clowes 1976, 1980).

(Table 1). The underlying mechanisms controlling diversity of the cap turnover process among divergent families remain unexplored, and the physiological ramifications are not known. There does not appear to be a strong correlation between root size or habitat and cell number (Hawes and Brigham 1992).

Most of what is known about border cell biology is based on *in vitro* studies, in which roots are grown in conditions such that existing cells are not lost due to abrasion and/or immersion in free water. Even under such conditions the cells can easily be overlooked. When roots are grown on 1.0% agar overlaid with filter paper to prevent penetration into the agar, it is difficult to discern the presence of border cells even with a dissecting microscope (Figure 2A). Only when examined with a scanning microscope are the contours of the border cells, layered over the cap periphery, apparent (Figure 2B). This is because the cells are embedded within the extruded mucilage layer; this mucilage is 99.9% water at matric potentials wetter than −50 kPa (Read and others 1999). In dry conditions, the cells remain tightly appressed to the root cap periphery (Guinel and McCully 1987) (Figure 2A,B). However, upon immersion of the tip, the mucilage rapidly absorbs 1000 times its weight in water, causing an apparent expulsion away from the tip as the enfolded border cells swell away from the periphery (Figure 2C) and begin to disperse into suspension (Figure 2D). With slight agitation of the surrounding water, the root periphery is rendered free of mucilage and cells (Figure 2E). All border cells are released into suspension (Figure 2F) where they can be induced to divide and grow when supplied by appropriate nutrients (Figure 2F, inset).

Upon differentiation into border cells, which by definition means that interconnecting links between cells have been solubilized (Hawes and Lin 1990), border cells in cereals and legumes undergo a dramatic switch in gene expression (Brigham and others 1995; Hawes and others 1998; Matsuyama

Table 1. Variation in Border Cell Numbers and Viability among Plant Species[a]

Species	Number produced in 24 h	Viability
Apiaceae		
Daucus carota	2300–2500	>95%
Asteracae		
Helianthus annus	1200–2000	0
Zinnia elegans	1000–1500	0
Tithonia spp.	1800–3600	>95%
Brassicacae		
Arabidopsis thaliana	0	NA
Brassica alba	0	NA
Brassica oleracea	0	NA
Brassica rapa	0	NA
Cucurbitacae		
Citrussus lanatum	1800–2400	>95%
Cucumis melo	1800–2500	>95%
Cucumis sativa	2400–3100	>95%
Luffa cylindrica	1100–1500	>90%
Euphorbiaceae		
Ricinis communis	1600–2700	>95%
Fabaceae		
Glycine max	2900–3700	>90%
Phaseolus vulgaris	2700–3500	>90%
Pisum sativum	3500–4500	>90%
Sesbania exaltata	3100–3900	>90%
Sesbaniajavonica	3100–4600	>95%
Vigna unguiculata	3800–6000	>90%
Gramineae		
Avena sativum	1800–2300	>95%
Oryza sativa	1500–2100	>95%
Panicum miliaceum	900–1200	>95%
Secale cereale	1200–1980	>95%
Triticum aestivum	1100–1500	>95%
Zea mays	2500–4000	>95%
Liliaceae		
Yucca spp.	1000–1500	>95%
Malvaceae		
Gossypium hirsutum	8000–10,000	>95%
Solanaceae		
Lycopersicon esculentum	50–200	50–60%
Nicotiana tabacum	<100	50–60%
Petunia hybrida	<100	50–60%
Solanum melongena	<50	60–70%
Capsicum annuum	80–100	50–60%
Pinaceae		
Abies spp.	8000–11,000	>90%
Picea spp.	8000–11,000	>90%
Pinus spp.	8000–11,000	>90%

[a]*From aHawes and Pueppke 1986; aHawes and others 1997; and unpublished observations.*

and others 1999; Zhang and others 1995). The profile of proteins expressed by border cells is completely distinct from that of the root cap, from which the population of border cells was derived only a few hours previously. Most of the border cell specific proteins are exported into the external environment as soon as they are synthesized; thus, of border cell-specific proteins synthesized during a one-hour period, most can be collected from the supernatant (Brigham and others 1995). Active

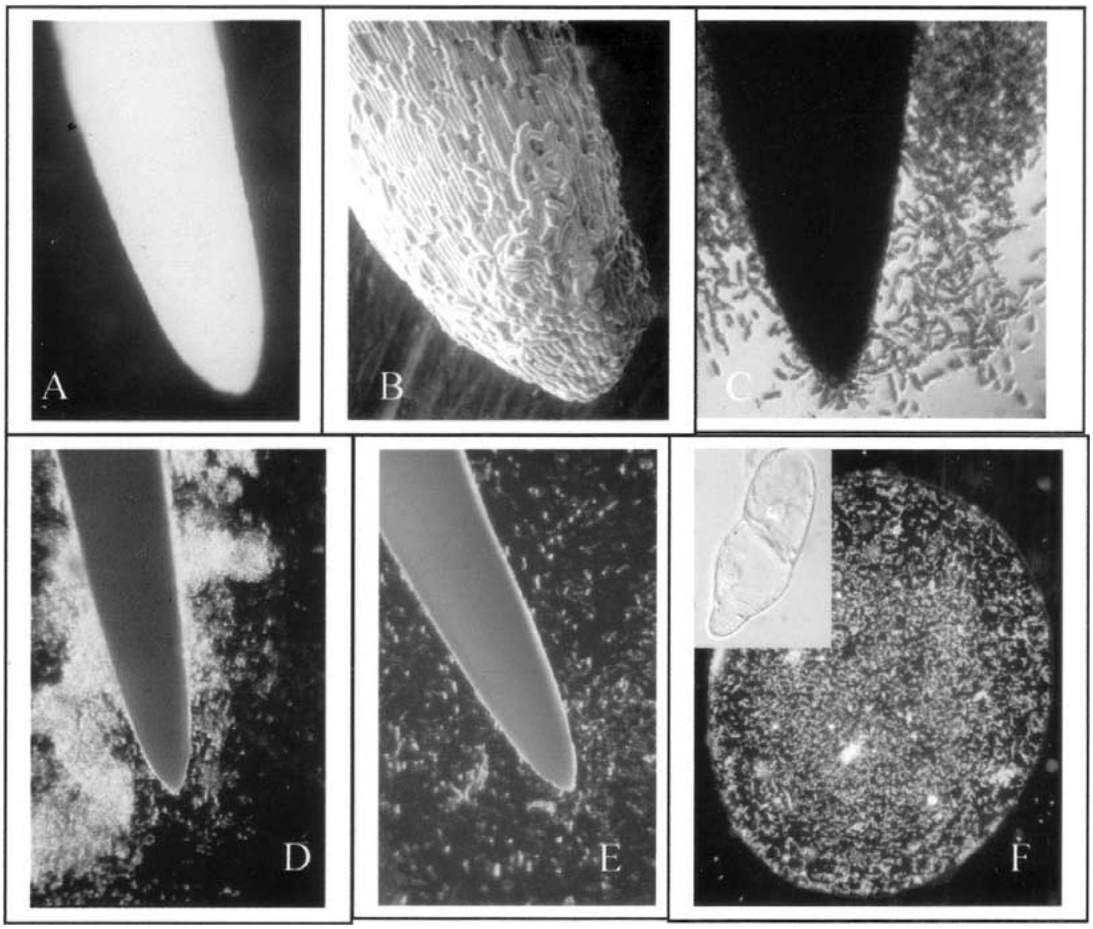

Figure 2. Root border cells of legumes. (**A**) When germinated on 1% water agar overlaid with filter paper and examined with the naked eye, the root cap periphery of a 25-mm long root is smooth. (**B**) Upon examination with scanning electron microscopy, outlines of border cells are apparent. (**C**) Upon immersion into water, the mucilage rapidly expands and border cells swell away from the periphery. (**D**) The population of cells disperses into suspension spontaneously within minutes. (**E**) Gentle agitation of the water disperses all cells. (**F**) Thousands of border cells are released into suspension, where they can be induced to divide (inset) and grow into organized tissue when supplied with appropriate nutrients (Hawes and Pueppke 1986). Magnification: A,C = 10×; B = 20×; D,E,F = 6×; inset = 500×.

secretion of proteins into the external environment might be predicted for a population whose function is to modify the properties of that external environment. The detached cells continue to undergo changes in morphology and structure even after separation in legumes and cereals, elongating by up to 100% in length and producing lignified secondary cell walls (Hawes and Wheeler 1982; Hawes and Pueppke 1986; Guinel and McCully 1987).

The discovery that plasmodesmata differentiate progressively and remain functional in detached border cells is consistent with the hypothesis that the cells retain a capacity for intracellular communication after separation from the cap periphery (Zhang and others 1995). Recent studies suggest that components of the border cell/mucilage layer can convey external signals controlling root development and function. Thus, changes in elongation of cells in the root meristem occur in response to removal of the border cell/mucilage capsule, and reciprocal changes occur when excess capsule is added to root tips (Baluška and others 1996b). Furthermore, a product secreted by border cells into the mucilage controls mitosis in the cap meristem (Brigham and others 1998). Thus, when border cells are removed, mitosis is induced within minutes and conversely, when extracellular products from border cells are added back to caps, mitosis is repressed. These data suggest that there may be efficient communication between border cells and root cap initials. By contrast, in *A. thaliana*, which does not release living border cells, plasmodesmata deteriorate as cap turnover proceeds 2 weeks after germination, and the dying cap cell layers are

symplastically isolated from the functional cap layers (Zhu and Rost 2000).

Overall, the results are consistent with the possibility that in most plants, the border cell/mucilage capsule constitutes a continuous, barrier-free apoplastic pathway which wraps around the root apex to link the external environment with cells in the cap and cells in the root meristem (Baluška and others 1996b). However, the process of cap development and function appears to be fundamentally different in *A. thaliana* (and perhaps other members of the *Brassicacae* family) than in other species examined, at least under controlled laboratory conditions, and therefore extrapolation about root cap function in general, based on studies in this interesting model plant, should be made with caution. The following summary applies to species other than *A. thaliana*.

A Model for Root Cap-Rhizosphere Interactions

Directional Movement

When plants are supplied with basic needs, new cells are synthesized within the root meristem more or less continuously, and these are the cells that give rise to root growth (reviewed in Barlow 2003, this issue). New cells from the root meristem proceed through a transition phase before entering into a period of rapid elongation. This transition is correlated with dramatic changes in gene expression and cell wall structure, with resultant changes in cell shape, size, and organization (Baluška and others 1996a). Rapid uptake of solutes into an enlarged vacuole appears to be the driving force for cell expansion, resulting in a tenfold increase in cell volume over a 13-h period following cell division. The overall effect is that a given root tip can be found several centimeters past its point of origin from one day to the next. These two linked but independent activities, cell division and cell elongation, enable the root to 'move' through the soil. When the root meristem is removed, or damaged irreversibly by pathogens or toxins, growth of that root branch stops.

Roots seldom just grow in a straight line, but instead respond to external stimuli by rapidly changing the direction of growth (Darwin 1880). Bending occurs within the region of elongation because the cells on one side of the root elongate more rapidly than cells on the other side. However, the primary site of signal perception leading to directional movement is the root cap, not the two tissues (the root meristem and region of elongation) which actually generate growth (Baluška and others 1996a; Darwin 1880; Feldman 1984; Pilet 1998; Sievers and Braun 1996). Removal of the root cap eliminates tropic responses to gravity and other environmental stimuli, even when the root meristem and region of elongation remain intact. The root can still grow, but it can no longer avoid danger and seek nutrients to support the rest of the plant. Not surprisingly, the root meristem is programmed to synthesize a new cap when the existing one is exised. This regeneration of a new cap occurs from a population of mitotically inactive cells designated the quiescent center (QC). In experiments in which both the root cap and QC are excised, a new root cap re-forms, but not until after a new quiescent center redevelops (Feldman 1976; Jiang and Feldman 2003 this issue).

By virtue of their capacity to sense and transmit signals controlling the direction of root growth, root caps are primary determinants of root architecture (Aiken and Smucker 1996). Although there is an undefined genetic component determining root architecture and depth, environmental factors also affect root distribution and shape. Gravity is unique among environmental signals in that it is present continuously, it is unidirectional, and it is essentially of constant intensity (reviewed in Correll and Kiss 2002). Its dramatic influence on root morphology has made it a favored subject for many years, and root sensing of gravity therefore is better understood than its responses to other stimuli (Tsutsumi 2003 this volume). Roots can change their growth patterns in response to numerous other environmental stimuli including distribution of nutrients and water in the soil, in addition to temperature, heavy metals, light, soil composition and texture, carbon dioxide and oxygen, electrical gradients, fungi and bacteria, and touch (for example, see Curl and Truelove 1986; Darwin 1880; Kochian 1995). Moreover, the plant has the capacity to respond in a hierarchical fashion to multiple stimuli. Thus, as one example, a root growing on agar which offers its only source of water will defy gravity and propel itself straight into the air if the agar is loaded with a toxic level of aluminum (Hawes and others 2000; Miyasaka and Hawes 2000).

Our understanding of signal perception and transmission leading to root growth, much less directional growth, is in its infancy (Barlow 2003 this issue; Correll and Kiss 2002). Little is known about mechanisms underlying the architecture of root systems, or what determines how roots are distributed in the soil. Of particular importance is root cap-mediated responses to water, because survival for

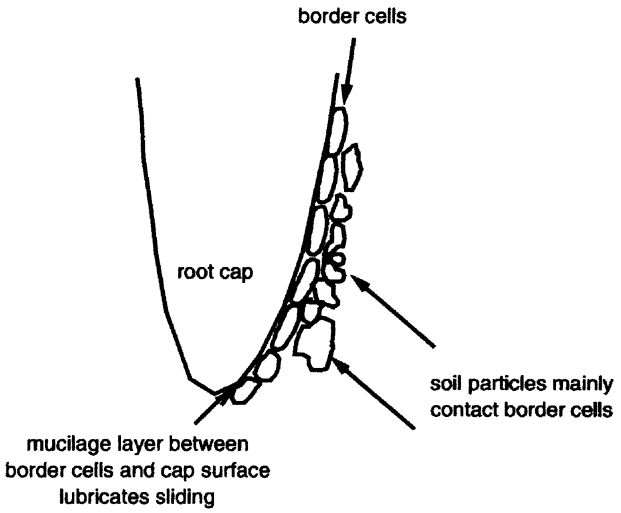

Figure 3. A model describing the role of border cells in penetration of compacted soil by the root cap. Border cells form a sacrificial layer that slides over the cap surface, lubricated by intercellular mucilage.

most plants depends upon the capabilities of the root cap to sense and move toward water. The phenomenon of root growth responsiveness towards moisture gradients is known as hydrotropism (Takahashi 1997). Historically, plant scientists have paid little attention to hydrotropism even though lack of sufficient water in the soil remains the single most important factor affecting world agriculture and thus food security. Recent studies have established that hydrotropism is amenable to genetic analysis and that an abscisic acid (ABA) signaling pathway participates in sensing water potential gradients through the root cap (Eapen and others 2002; Takahashi 1997). A negative root hydrotropic mutant (*nhr1*) of *Arabidopsis thaliana* was selected by using a screening system with a water potential gradient (Eapen and others 2002). This mutant continues to grow downwardly into the medium with the lowest water potential contrary to the positive hydrotropic and negative gravitropic response seen in wild type-roots. Conversely, root mutants lacking the capacity to sense gravity retain the ability to sense water (Takahashi 1997). Such behavior may be explained by the existence of multiple receptors which feed into a network of separate but interrelated pathways that ultimately induce expression of genes needed to alter behavior (for example, see Jenkins 1998).

Root Penetration of Soil

In the field, roots grow through a complex three-dimensional maze of soil pores. Physical protection of the root meristem during this penetration process has been long presumed to be a role of the root cap and its associated mucilage and border cells but the hypothesis remained unexamined (Haberlandt 1914). In recent years, a systematic approach to testing the hypothesis has been undertaken by examining root penetration behavior in a range of environments (Bengough and others 1997; Bengough and Kirby 1999; Bengough and McKenzie 1997; Iijima and others 2000; Kirby and Bengough 2002). The results to date, summarized below, suggest that the physical properties of the border cell-mucilage layer are consistent with the hypothesis that they can faciliate penetration, and direct tests of the model indicate that this does occur.

Roots must deform the soil to make a hole if a continuous pore, bigger than the nominal root diameter, does not already exist. Much of the resulting soil deformation results from stresses applied by the surface of the root cap pressing against the soil. For maize roots grown in loose sand, passage of the root cap produces a hole about half the diameter of the elongation zone (M. Iijima and others unpublished). In compacted sand, the figure is smaller, about one third of the diameter of the elongation zone, mainly because the root diameter increases by 70%, whereas the cap diameter decreases only slightly. The magnitude of the mechanical stresses in the soil around the root tip has been shown theoretically to be greatest at the apex of the root cap (Kirby and Bengough 2002), decreasing with distance behind the apex and distance from the root surface. The pressures on the cap surface can average up to 1 MPa in compacted or dry soil. This limit is a little smaller (because of the yield threshold of the cell walls) than the maximum turgor pressure generated by the expanding cells of the elongation zone. Locally, these stresses may be exceeded due to soil heterogeneity if, for example, a large particle of sand is encountered that indents the root surface.

Friction can account for 80% of the penetration resistance of soil. Root caps appear to be designed as low-friction bodies, at least in relatively wet soil. Coefficients of friction are approximately 0.04 for maize and pea root caps, when measured against a moist ground glass surface (Bengough and Kirby 1999). Comparisons between the penetration resistances of soil to metal probes and to plant roots also suggest that the surface of the root tip is relatively low friction (Bengough and Mullins 1991), with roots only experiencing between one-half and one-eighth of the resistance to a conical metal probe (Bengough and Mullins 1990). The combined presence on the cap surface of a mucilage layer and border cells is likely to be more effective at de-

creasing friction than either component acting independently. A conceptual model for friction decrease is illustrated in Figure 3. Border cells act as cellular cushions for soil particles, sliding over the root cap and epidermis, and preventing local peaks in stresses between soil particles and the root surface. The mucilage layer between the border cell and the root surface is more likely to remain intact under this more uniform stress regime and, therefore, be more effective at decreasing friction.

For such a mechanism to work, there must be a sufficient area of border cells to cover a substantial portion of the root cap surface. Each border cell released from the root cap will only shield a small area of the root cap surface, and we cannot merely assume that there will always be sufficient border cells to completely cover the root cap. The faster the rate of root elongation, the greater the area of new soil-interface that the root cap pushes past, per unit time. Are there enough cells produced by a maize root to accommodate the model in Figure 3? The answer depends on soil physical conditions. Numbers of maize border cells released into sand increased from 1930 d^{-1} to 3220 d^{-1}, as a result of increasing compaction (Iijima and others 2000). This is an order of magnitude increase in the number of border cells per unit root elongation, from 60 mm^{-1} in loose sand to more than 700 mm^{-1} in compact, because of the slower root elongation rate in compacted sand. This release rate of border cells is sufficient to cover the whole of the root cap in compacted sand, but only about 7% of the cap surface area in loose sand (assuming, for simplicity, average border cell release rates stated above (Iijima and others 2000). Hence, border cell release occurs in sufficient numbers to decrease friction for maize roots in compacted sand, but we do not know the situation for other species.

Rhizosphere Ecology

The rhizosphere is defined as a narrow zone surrounding roots in which microbial populations are higher than in bulk soil as a result of nutrients supplied by the root (Curl and Truelove 1986). When exudates are collected from undamaged roots, then hydrolyzed and subjected to composition analysis, they can yield just about any biological product known to occur in plants. The fact that the magnitude of such exudation can be substantial and can have a very significant impact on microbial growth, gene expression, and behavior is widely recognized (for example, see Griffin and others 1976; Mosse 1975; Rogers and others 1942; Rougier 1981). However, often overlooked is the fact that root exudates as they occur in the rhizosphere are not a generic hydrolyzate of soluble amino acids and sugars available to be consumed by any organism in the vicinity. Instead, the composition and biological activity of the material delivered by plants is *dictated by the genotype of the plant* (Atkinson and others 1975; Neal and others 1970, 1973). Conversely, whether or not a given component of root exudate is available to be used by a given microorganism is *dictated by the genotype of the microorganism in question*.

In young, healthy, uninjured root systems the bulk of root exudates is delivered by the root cap as it moves into new territory as living plant cells with all the normal cellular specificities regarding plant-microbe recognition and relationships (reviewed in Hawes 1990; Hawes and Brigham 1992; Hawes and others 1994, 1996, 1997, 1998, 2000). In legumes, direct measurements of root exudation in hydroponics have shown that sloughed organic materials (including border cells and associated mucilage) account for 95 to 98% of the total dry weight of material released into the rhizosphere (Griffin and others 1976). Iijima and others (2000) estimated that about 10% of the total carbon released to the rhizosphere by young maize radicles growing in unsaturated compacted sand was contained within the border cells. This figure does not include the substantial quantity of mucilage closely associated with border cells, and will also depend substantially on conditions in the rhizosphere. When marked growth of a pathogenic fungus is seen to occur in the rhizosphere, the hyphae invariably are found to be growing on a cluster of detached cells, and little or no growth is evident elsewhere on the root surface (Figure 4A). A similar relationship occurs between bacteria and border cells: when substantial growth of bacteria is seen in the rhizosphere of a plant, separate from the root, it invariably is found to occur in association with one or more border cells (Figure 4B).

Root border cells not only deliver much of the bulk weight of root exudates but they also produce and secrete an array of specific extracellular signals that can attract, repel, and control growth and gene expression in soilborne organisms (Table 2). Of fundamental importance in understanding rhizosphere dynamics is the recognition that *each of these activities is host- and genotype-specific*. In one dramatic example, zoospores of *Pythium dissotocum* are instantaneously and specificially attracted to cotton border cells (Goldberg and others 1989). The zoospores encyst and penetrate the cells within minutes, and can fully digest a population of thousands of border cells within an hour (Figure 4C). Similar recognition and digestion occurs between border

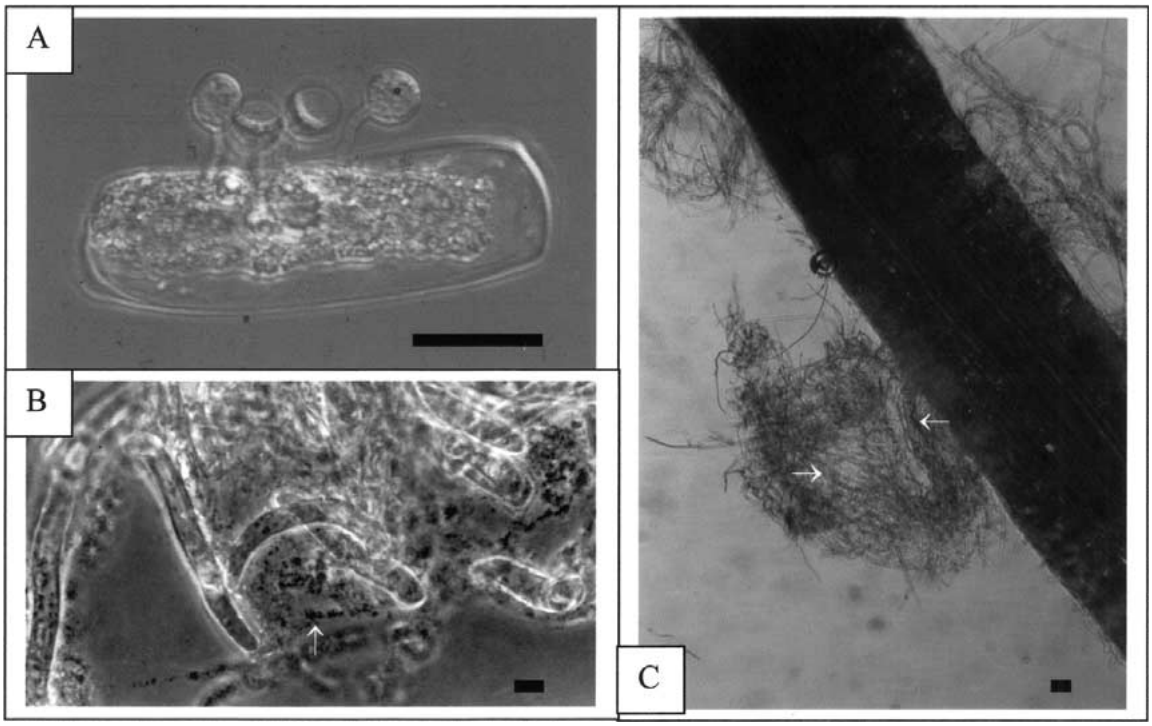

Figure 4. Border cells and soilborne microorganisms. (**A**) Species-dependent invasion of host border cell by zoospores of a pathogenic fungus. Zoospores encyst, germinate, and penetrate host cells in less than one minute; cell death and digestion of cellular contents ensues. No invasion occurs in border cells of a nonhost plant species, and no growth of the fungus occurs even with lengthy co-cultivation. (**B**) Growth of *Agrobacterium tumefaciens* bacteria occurring in close association with pea border cells (Hawes and Smith 1989). (**C**) Growth of *Nectria haematocca* in the rhizosphere of pea occurring in associationwith groups of border cells (arrow) (Gunawardena and Hawes 2002). Scale bar = 10 μm.

Table 2. Documented Biological Effects of Border Cells*

	Source
Bacteria	
Stimulation of sporulation	Gochnauer and others 1990
Genotype-specific binding	Hawes and Pueppke 1987
Genotype-specific stimulation of growth	Hawes and Smith 1989
Species-dependent chemoattraction and repulsion	Hawes and others 2000
Induction of nodulation genes in *Rhizobium*	Zhu and others 1997
Nematodes	
Species-dependent chemoattraction and repulsion	Zhao and others 2000
Stimulation of secretion	Zhao and others 2000
Fungi	
Host-specific infection and mantle formation	Gunawardena and Hawes 2002
Host-specific induction of border cell production	Gunawardena and Hawes 2002
Host-specific induction of defense responses	Sherwood 1987
Exported signals and enzymes	
Signals for repression of mitosis	Brigham and others 1998
Extracellular enzymes	Rogers and others 1942; Price 2002
Mucilage secretion	Hawes and others 1998
Phytoalexins and other antibiotics	Brigham and others 1999
Aluminum-binding products	Miyasaka and Hawes 2000

**Reviewed in Hawes and Brigham 1992; Hawes and others 1998, 2000.*

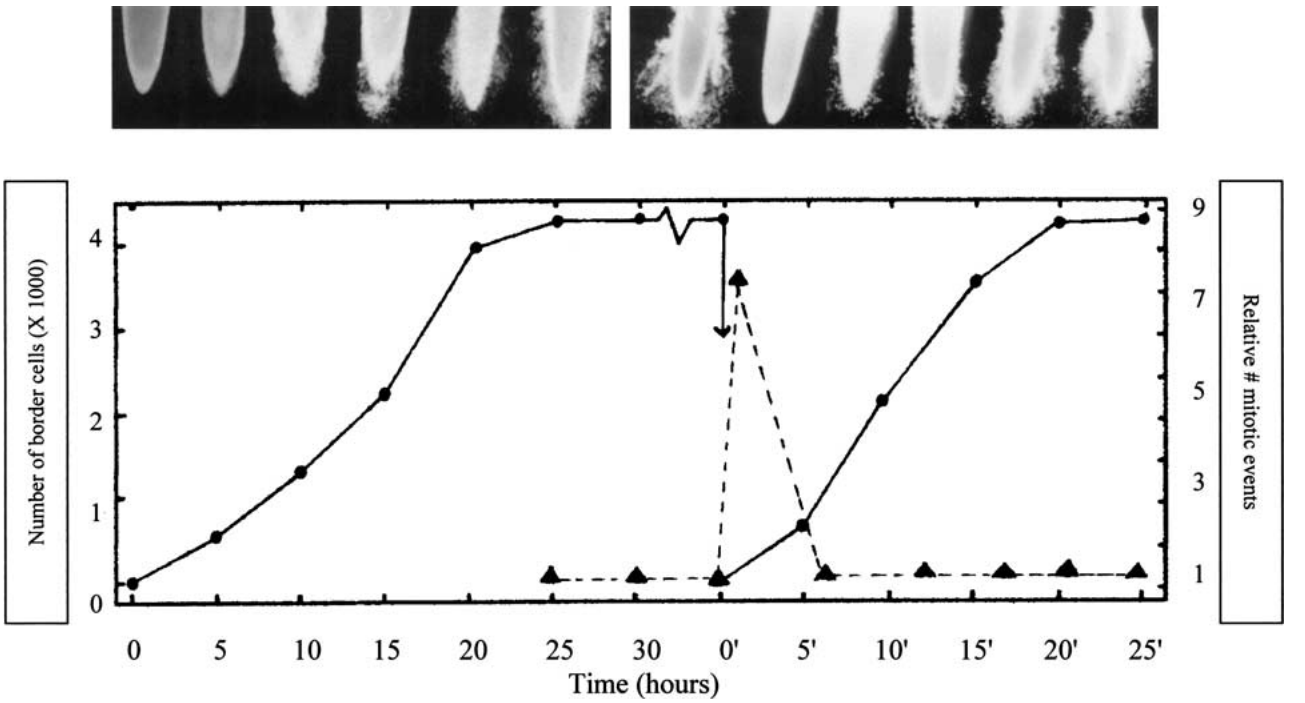

Figure 5. Developmental regulation (left) and experimental induction (right) of border cell production in correlation with mitosis in the root cap meristem. After emergence of the radicle (time 0), several hundred border cells can be harvested within 5 h; cell number per root increases to a species-specific set maximum (3500 ± 500 cells in legumes), and then cell production ceases after 20–24 h. If existing cells are removed (arrow) by gentle agitation of the tip in water (time 0′), mitosis is induced 7- to 9-fold within 15 min in correlation with a global switch in gene expression throughout the cap. Experimental induction of cap turnover by removal of existing cells synchronizes cap development from plant to plant; cell number increases over time until 20–24 h when a new set of border cells is present on the cap periphery and cap turnover again ceases.

cells of cucumber and zoospores of *P. catenulatum*. However, in the inverse combinations—*P. dissotocum* on cucumber and *P. catenulatum* on cotton—the interactions are completely inert because genes required for recognition and response are absent. Thus, there is no attraction, no encystment, no penetration and killing of border cells, and no germination and growth of the fungus. The thousands of zoospores will continue to swim in the same vessel as the thousands of border cells for days because at the molecular level, in the absence of the appropriate matching genotypes, the plant and pathogen are functionally 'invisible' to each other. Eventually the zoospore starves to death despite the abundance of nutrients, just as it would if it landed on the leaf surface of a resistant plant: as in an intact plant, the nutrients delivered to the rhizosphere are packaged in living cells, and living cells of most species have the capacity to resist invasion and digestion by most microorganisms.

Similarly, the abundance of nutrients potentially available in the high molecular mass polysaccharide mucilage secreted by the root cap will only be made available to microorganisms with appropriate enzymes to solubilize the matrix into digestible component sugars. In some cases, extracellular plant enzymes may degrade the mucilage into component sugars which will be available as signals and nutrients, but only to microorganisms with appropriate enzymes to utilize them. As an example, a border cell specific extracellular galactosidase releases galactose into the rhizosphere (Price 2002). However, not all bacteria can recognize and respond to galactose: some bacteria are attracted, some are repelled, and some do not recognize the sugar at all (Hawes and Smith 1989). The same is true for digestibility of galactose. Every molecule delivered by the root cap as root exudates is subject to the same plant-microbe genotype specificity as galactose. As such, the host and genotype specificity contained in the border cell-mucilage system is a powerful mechanism for defining community structure as the cap invades virgin territory (Hawes and others 1998, 2000). This new community then is left behind in the established rhizosphere of the root system. A capacity to regulate timing of the delivery of root

exudates in response to environmental signals provides the root cap with an additional mechanism for modulating rhizosphere structure, as described below.

Controlled Delivery of Products

All tiers of the root cap, including border cells, are derived from the root cap meristem (Figure 1). Root cap turnover has long been presumed to be a continuous process which, like root growth, is responsive to temperature, water, and other factors but in the absence of severe stress is predictably constant within a given set of conditions (Brigham and others 1998; Hawes and Lin 1990; Hawes and others 2000). A given cell generated in the cap meristem of maize can traverse the entire cap and separate as a detached border cell within one day, or it can take a week or more, depending on environmental conditions (Barlow 1975; Clowes 1976, 1978, 1980; Feldman 1984; reviewed in Hawes and Brigham 1992).

During early development, the process of cap turnover and border cell separation is reasonably predictable. In cereals and legumes, no border cells are present at germination until the root is 5 mm in length (the process is somewhat faster in emerging lateral roots), and then cell number increases to a species-specific set maximum number of cells within 24 h (Figure 5). Once a full set of border cells is present on the cap periphery and is not removed (as when seedlings are germinated in air or on water agar overlaid with filter paper), cap turnover ceases. Even after a week or more, the same original set of border cells remains present at the cap periphery even though root growth proceeds unabated (Hawes and Lin 1990). Cells within the cap, then, such as gravity-sensing statocytes, remain static in a differentiated state. This inhibition of cap turnover occurs partly because border cells produce an extracellular signal which, when it accumulates in the cap mucilage to a critical threshold level, represses mitosis in the cap meristem (Brigham and others 1998). While in this state, with an ever-aging border cell/mucilage capsule on its periphery, the root cap continues to function as a sensory organ to facilitate root growth responses to gravity and other signals but stops undergoing progressive differentiation and turnover.

When border cells are removed by immersion of the root tip in water, mitosis in the root cap meristem is induced within 5 min, together with a global switch in gene expression as cells throughout the cap proceed to differentiate (Brigham and others 1998). Within 1 h, several hundred new border cells together with a proportional amount of cap-secreted mucilage are released at the cap periphery. Within 24 h, an entire new border cell/mucilage capsule is present, and differentiation within the cap again ceases. Mitosis in the cap meristem is tightly coordinated with cap development. Thus, inhibition of cell-wall-degrading enzyme activity at the cap periphery inhibits border cell production and also inhibits cap turnover (Wen and others 1999); when border cell production is restored, cap turnover proceeds as well. Conversely, slowing the cell cycle by antisense inhibition of a meristem-localized UDP glucuronosyltransferase results in a corresponding delay in production of border cells (Woo and others 1999).

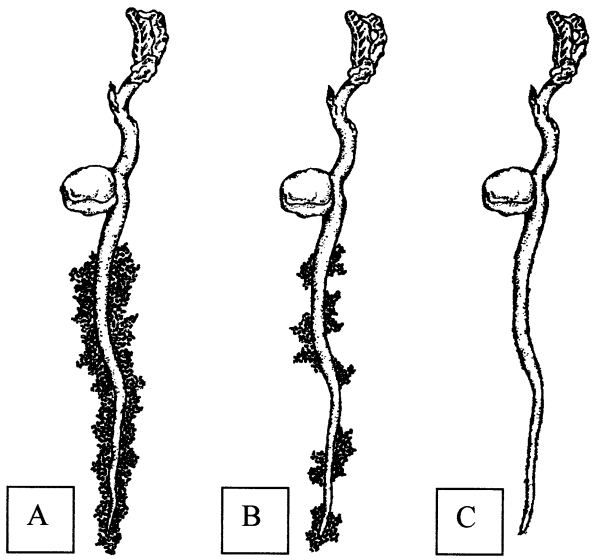

Figure 6. Variation in border cell production under conditions where cells are removed as they are delivered to the cap periphery. Pea seeds (*Pisum sativum* L. cv. Little Marvel) were germinated until the radicle emerged to a length of 1–2 mm. Plates were poured with water agar (0.8%) to a depth of 2 cm, and wells (ca. 1 × 1 cm) were cut into the agar. Seedlings were placed into the wells, and the plates were oriented vertically with roots penetrating the agar and growing downward. Border cell distribution was monitored with a Zeiss dissecting microscope, and drawings were made from photographs over the course of one week: (**A**) uniform distribution of border cells along root, presumably due to continuous release of cells from the growing tip; (**B**) non-uniform distribution of border cells surrounding the root as a result of discontinuous release from the tip; (**C**) absence of border cells, presumably due to absence of production during the period of observation. All examples were maintained under identical conditions, and diversity of distribution in some cases occurred on roots grown in the same Petri plate.

Table 3. Endogenous and Environmental Factors Influencing Production of Border Cells

Factors that induce border cell production	Source
Removal of cells by agitation in water	Hawes and Lin 1990
Removal of cells by manual wiping from periphery	Brigham and others 1998
Dipping of tip in water for 1 sec	Brigham and others 1998
Exposure to aluminum	Miyasaka and Hawes 2000
Growth in compacted soil	Iijima and others 2000
Increased carbon dioxide (in pea)	Zhao and others 2000
Infection of root caps by pathogenic fungi	Gunawardena and others 2002
Factors that inhibit border cell production	
Inhibition of cell cycle	Woo and others 1999
Inhibition of cell wall degrading enzymes	Wen and others 1999
Other unknown signals	Hawes and Brigham 1992
'Factor B' extracellular signal released by border cells	Brigham and others 1998
Colonization by endophytic bacteria (in wheat)	Milus and Hawes unpublished
Crowding of roots in solution culture	Clowes 1976, 1980
Factors that have no apparent influence on border cell production	
Infection of border cells by pathogenic fungi	Gunawardena and Hawes 2002
Colonization by nonpathogenic fungi	Gunawardena and Hawes 2002
Touch	Brigham and others 1998
External pH (range from 5–7)	Unpublished
Colonization by bacteria (most tested species)	Unpublished
Heat shock	Unpublished
Increased carbon dioxide (in alfalfa)	Zhao and others 2000
Reduced oxygen (from 21% to 15%)	Zhao and others 2000
Changes in diurnal light/dark cycles	Unpublished

These experiments illustrate that root cap turnover is not necessarily continuous, but can respond to an apparent feedback effect in which accumulation of border cells results in suppression of mitosis. These data are consistent with the possibility that cap turnover, at least in pea, involves a steady-state system which is active only when there are fewer than 4000 cells present on the cap periphery. Nevertheless, it would be logical to predict that under natural conditions in the soil, abrasion or contact with water in the soil would never result in such an accumulation of border cells on the cap periphery, such that turnover would be continuous. Yet results to date indicate that the situation is not so simple. Thus, even under conditions in which border cells are removed as soon as they are produced, cap turnover may or may not be constant, and can vary from one seedling to the next in a single culture plate (Mosse and others 1975) (Figure 6). Conversely, even when a full set of border cells is present cap turnover is not necessarily suppressed: in pea, increased carbon dioxide acts as a signal which overrides the normal controls on border cell production, such that twice the normal number of cells is produced on a single root (Zhao and others 2000) (Table 3). To confound the situation further, alfalfa border cell production is impervious to increased carbon dioxide, suggesting that cap responses to the environment can be species-dependent. These new results highlight the need for caution in making any assumptions about how the root cap operates its rhizosphere delivery system on a moment-by-moment basis in the ever-changing soil environment.

Future Perspectives

To date, research on root cap biology and its relationship with the rhizosphere has raised more questions than it has answered. For example, if border cells are so important in penetration and modulation of rhizosphere ecology, how does *A. thaliana* get along without them? Given the discovery that roots can turn cap turnover on and off, the simplest hypothesis is that turnover may occur under natural conditions even if it does not occur in culture (Brigham and others 1998; Hawes and others 1990, 1998). The fact that the cells in *A. thaliana* and a few other species are dead on release may not preclude a capacity to define rhizosphere properties and may even facilitate the process by the programmed dumping of cell contents. With regard to penetration of soil, a phalanx of dead cells or even a whole dead cap rolling along the surface of

the cap could be envisioned to serve the purpose of reducing mechanical friction as easily as living cells. The impact of border cells, living and dead, is being examined using transgenic plants whose border cells are altered by inhibition of specific genes controlling their production and properties (for example, see Hawes and others 2000; Wen and others 1999; Woo and others 1999). For example, susceptibility of root tips to infection by pathogenic fungi was found to be drastically increased in transgenic alfalfa with reduced border cell production (Hawes and others 2002). If the presence of border cells is important for penetration, then plants with altered border cell production will be predicted to have altered ability to become established in soil environments.

The discovery that root caps can exert tight control on the delivery of root exudates may shed light on one long-standing mystery: How can plants afford to commit such a large amount of energy to the release of root exudates into the soil? Studies tracking carbon at specific points in time have revealed numbers as high as 90% of the total fixed carbon being 'lost' to the rhizosphere (Curl and Truelove 1986). One possible answer to how this could happen is that it doesn't. The only time wholesale loss of all the border cells from the cap periphery occurs is when there is a flush of free water delivered into the site where the root tip is present. Notwithstanding exceptions (that is, monsoons, overflowing rivers, or agricultural systems like hydroponics and rice paddies), the presence of standing water around root systems is a relatively rare occurrence for most plants in most soils. Therefore, it is reasonable to propose that cap turnover may be considerably faster in solution culture than in loose soil, such that much of an existing border cell-mucilage capsule can exist for some time on a given root cap, as it does in aeroponics or on filter paper. If so, a recycling of the components of the mucilage may provide an additional mechanism to regulate carbon loss. Thus, extracellular cell wall-degrading enzymes released by border cells may render the material available for uptake back into the cap for synthesis of new cell walls when mitosis is induced. Transgenic plants with altered expression of polysaccharide synthesizing and solubilizing enzymes can be used to test predictions of this hypothesis, and these studies are underway (Price 2002; Wen and others 1999). If, for example, recycled sugars from root cap mucilage are needed for the synthesis of new cell walls in the cap, then inhibiting the expression of border cell-exported saccharidases would be predicted to result in root cap cells with altered structure.

A more complex problem involves the relationship among border cells, the root cap, and the root apical meristem, and how this may differ among diverse plant species. How can a border cell product which is sufficiently soluble to suppress mitosis in the cap meristem (Brigham and others 1998) have no influence on mitosis in the apical meristem? One possibility is that the quiescent center (QC) functions in some way as a molecular buffer between them. Recent work has suggested that one function of the QC cells is to regulate developmental activities of the root cap, implying that there is a long-range cross-talk between the quiescent center and root cap cells (Ponce and others 2000). Mitotic quiescence in the QC has been linked directly to high levels of auxin that accumulates in these cells via polar transport (Kerk and Feldman 1995). Interestingly, inhibition of polar auxin transport in maize roots for 24 h activates mitosis in the QC cells and inhibits the specific expression of root cap genes (G. Ponce and others unpublished observations). These results are consistent with the hypothesis that communication between the QC and root cap cells in maize roots can be regulated by polar auxin transport, and that this cross-talk seems to regulate several activities in the root cap. Furthermore, preliminary experiments have shown that induction of mitosis in the root cap meristem by removal of existing border cells is correlated with increased activity in the QC (G. Ponce and others unpublished observations). Given that the QC is known to be programmed to replace lost root caps, it would not be surprising to find that communication between the QC and root cap cells plays a role in the regulation of root cap development leading to the delivery of border cells.

Conclusions

The root cap is a multifunctional molecular relay station that not only detects, integrates and transmits information about the environment to appropriate plant organs, but also functions to specifically modulate properties of the soil habitat in advance of the growing root (Hawes and others 2000). The cap maintains its own independent developmental patterns in response to the environment while simultaneously directing movement generated by the root meristem and region of elongation (Ponce and others 2000). As it proceeds in its self-appointed direction, the root cap has the capacity to release mucilage-encased border cells to facilitate mechanical penetration into an unpredictable matrix of soil, sand, and organic matter (Bengough and McKenzie

1997). The detaching cells exhibit unique profiles of proteins which are rapidly exported into the external environment (Brigham and others 1995). These products may foster the ability of border cells to function in warding off toxins and pathogens, soliciting relationships with beneficial microorganisms, and creating a chemical milieu favorable to the uptake of the molecular harvest thereby generated (Hawes and others 1998, 2000).

Detailed analyses of the root cap over the last century have defined a conceptual framework which offers opportunities not only to understand plant development but to use the information for crop improvement. As it moves, the cap processes incoming signals whose rapid perception and processing may be fostered by the unique dynamics of the border cell-mucilage interface (Ponce and others 2000). These signals drive molecular responses that control the size, shape, and function of a plant throughout its lifespan. Genetic variation in cap structure and function among different species makes the stimulus-response network extremely wide and provides opportunities to exploit natural diversity in cap function to improve crop productivity. As an example, how the root cap senses moisture gradients in the soil and translates it into movement toward water is still unknown. The potential benefit of this knowledge alone could be substantial. Directing roots to grow deeply, as opposed to remaining near the soil surface, might allow plants to take advantage of substantial ground water supplies, thereby reducing the need for irrigation. Similarly, for crops that traditionally are irrigated, maintaining roots within a certain soil horizon (depth) could conserve both water and fertilizer; since the bulk of the root mass would be in the upper regions of the soil, needs for deep fertilization or irrigation would be lessened. Given the capacity of the root cap to amplify subtle incoming signals into major behavioral responses, small changes in root cap function could be predicted to generate large changes in plant productivity.

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