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Source: The Arabidopsis Book, 2002(1)

Published By: The American Society of Plant Biologists

URL: https://doi.org/10.1199/tab.0060

First published on April 4, 2002: e0060. doi: 10.1199/tab.0060

Root Hairs

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Introduction

Root hairs are long tubular-shaped outgrowths from root epidermal cells. In Arabidopsis, root hairs are approximately 10 μm in diameter and can grow to be 1 mm or more in length (Figure 1). Because they vastly increase the root surface area and effectively increase the root diameter, root hairs are generally thought to aid plants in nutrient acquisition, anchorage, and microbe interactions (Cutter, 1978; Hofer, 1991).

Root hairs in Arabidopsis have attracted a great deal of attention from plant biologists because they provide numerous advantages for basic studies of development, cell biology, and physiology. The presence of root hairs at the surface of the root and away from the plant body means that they are easily visualized and accessible to a variety of experimental manipulations. Further, the lack of a cuticle layer allows physical and chemical probes to be applied with ease. Root hairs grow rapidly, at a rate of more than 1 μm/min, which facilitates studies of cell expansion. Perhaps most importantly, root hairs are not essential for plant viability, which permits the recovery and analysis of all types of mutants that alter root hair development and function. Also, root hairs become visible on seedling roots shortly after seed germination, which enables genetic screens and physiological tests to be performed rapidly with large numbers of individuals growing on defined media in Petri dishes (Figure 2). Finally, the development of root hairs (and their resident epidermal cells) occurs in a predictable and progressive manner in cells that are organized

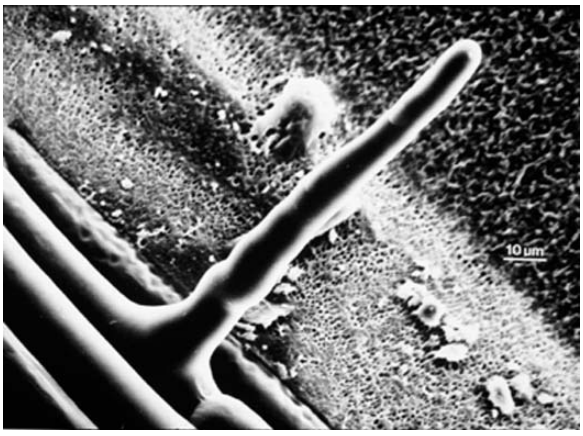

Figure 1. Scanning electron micrograph of a root hair cell. The hair produced by this cell is approximately 1/3 of its final length.

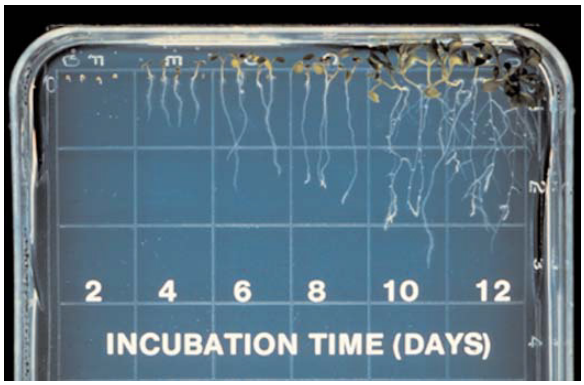

Figure 2. Development of Arabidopsis seedlings growing on agarose-solidified nutrient medium in vertically-oriented Petri plates. The roots grow along the surface of the medium, and root hairs are visualized easily using a low-magnification microscope.

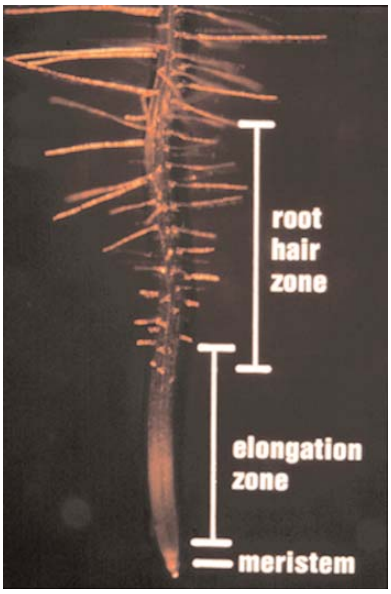

Figure 3. Photograph of a root tip showing the progressive development of root hair cells.

in files emanating from the root tip (Figure 3). This provides the opportunity for detailed analysis of the cellular changes that occur during the entire process of root hair formation.

This chapter provides a summary of the development, structure, and function of root hairs in Arabidopsis. Particular emphasis is placed on recent findings on the molecular genetics of root hair development.

Root Hair Cell Specification

Pattern of Epidermal Cells in the Root

In Arabidopsis, the epidermal cells that produce root hairs (root hair cells) are interspersed with cells that lack root hairs (non-hair cells). Thus, the initial step in the formation of a root hair is the specification of a newly-formed epidermal cell to differentiate as a root hair cell rather than a non-hair cell. This process has been studied intensively during the past several years because it serves as a simple model for understanding the regulation of cell-type patterning in plants.

The Arabidopsis root epidermis is generated from a set of 16 initial cells that are formed during embryogenesis (Dolan et al., 1993; Scheres et al., 1994; Baum and Rost, 1996; see also the chapter on root development in this book). These initials are termed epidermal/lateral root cap initials because they also give rise to the cells of the lateral root cap (Dolan et al., 1993; Scheres et al., 1994). The immediate epidermal daughter cells produced from these initials undergo secondary transverse divisions in the meristematic region of the root, and these divisions (typically 5 or 6 rounds per daughter cell) serve to generate additional cells within the same file (Baum and Rost, 1996; Berger et al., 1998b). Furthermore, anticlinal longitudinal divisions occasionally occur and result in an increase in the number of epidermal cell files; this activity causes the observed number of epidermal cell files to vary from 18 to 22 (Galway et al., 1994; Baum and Rost, 1996; Berger et al., 1998b). The epidermal cells are symplastically connected during much of their development (Duckett et al., 1994).

The root epidermis of Arabidopsis, like other members of the family Brassicaceae, possesses a distinct position-dependent pattern of root hair cells and non-hair cells (Cormack, 1935; Bunning, 1951; Dolan et al., 1994; Galway et al., 1994). Root hair cells are present outside the intercellular space between underlying cortical cells (epidermal cells located outside an anticlinal cortical cell wall; the “H” position), whereas non-hair cells exist over a single cortical cell (epidermal cells located outside a periclinal cortical cell wall; the “N” position) (Figure 4). Because the Arabidopsis primary root always possesses eight files of cortical cells, there are eight root-hair cell files

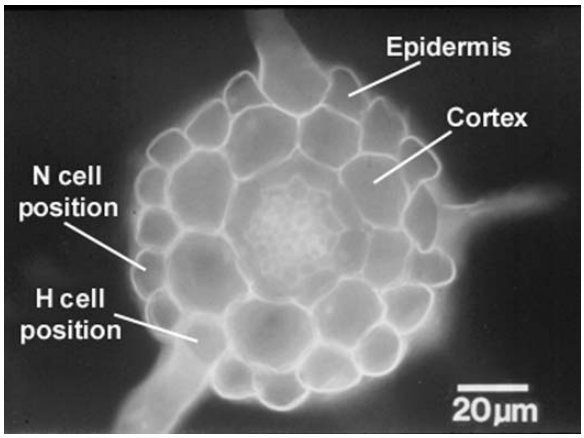

Figure 4. Transverse section of an Arabidopsis root, showing the position-dependent pattern of hair cells and non-hair cells. The hair-bearing cells are located outside the space separating two cortical cells (the H cell position), whereas the non-hair cells are located outside a single cortical cell (the N cell position). Three hairs are visible in this section; the other cells in the H position possess hairs that are located outside the field of view.

and approximately 10 to 14 non-hair cell files (Dolan et al., 1994; Galway et al., 1994). The simple correlation between cell position and cell type differentiation implies that cell-cell communication events are critical for the establishment of cell identity in the root epidermis.

Nature of the Cell Patterning Information

The timing and directionality of the putative positional signal(s) that directs epidermal cell fate in Arabidopsis are presently unclear. It is known that the patterning information must be provided at an early stage in epidermis development, because immature epidermal cells destined to become root-hair cells (trichoblasts) can be distinguished from immature non-hair cells (atrichoblasts) prior to hair outgrowth. Specifically, the differentiating root-hair cells display a greater rate of cell division (Berger et al., 1998b), a reduced cell length (Dolan et al., 1994; Masucci et al., 1996), greater cytoplasmic density (Dolan et al., 1994; Galway et al., 1994), a lower rate of vacuolation (Galway et al., 1994), unique cell surface ornamentation (Dolan et al., 1994), and distinct cell wall epitopes (Freshour et al., 1996).

A more-precise understanding of the timing of the patterning information has been provided by the recent use of two reporter gene fusions, a *GLABRA2* (*GL2*) gene construct (Masucci et al., 1996; Lin and Schiefelbein, 2001) and an enhancer-trap GFP construct (line J2301; Berger et al., 1998c). Each of these reporters are expressed in the N-cell position (epidermal cells located outside a periclinal cortical cell wall) within the meristematic region of the root (Figure 5). Careful examination using these sensitive reporters reveals position-dependent gene expression within, or just one cell beyond, the epidermal/lateral root cap initials, which implies that patterning information may be provided (and cell fates begin to be defined) within these initial cells (Masucci et al., 1996; Berger et al., 1998a).

The presence of differential gene expression within the initial cells of the root meristem led to the possibility that the epidermal cell pattern may be established during embryogenesis, at the time the basic root structure and meristem initials are formed (Scheres et al., 1994). Indeed, the analysis of the J2301 enhancer-trap GFP (Berger et al., 1998a) and the *GL2::GFP* (Lin and Schiefelbein, 2001) reporters show that the epidermal cell specification pattern becomes established during embryonic root development in Arabidopsis (Figure 6). The *GL2::GFP* exhibits the earliest expression, beginning at the early heart stage, which is prior to the formation of the root meristem. For each of these reporters, expression is detected in a position-

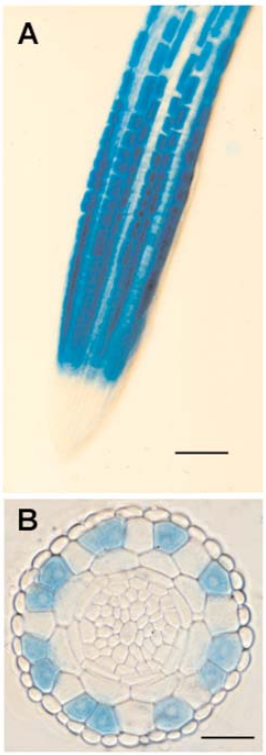

Figure 5. Expression of the GL2::GUS reporter fusion during root development.
(A) Surface view showing preferential expression in the meristematic region. Bar = 50 μm.
(B) Transverse section showing preferential expression in the N-cell position of the epidermis. Bar = 20 μm.

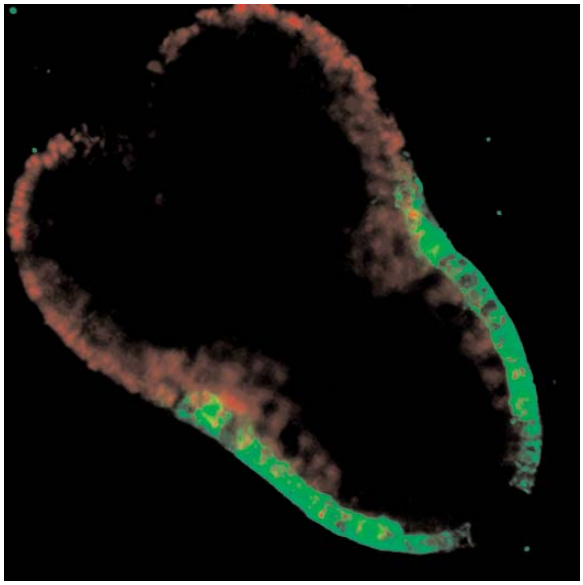

Figure 6. Embryonic expression of the GL2::GFP reporter fusion in the torpedo stage embryo. This median longitudinal view shows GFP accumulation in protodermal cells in the future hypocotyl and root.

dependent pattern that mirrors the post-embryonic pattern (Berger et al., 1998a; Lin and Schiefelbein, 2001). Thus, it appears that positional information is provided during embryonic root development to establish the proper pattern of gene activities that ultimately leads to appropriate post-embryonic cell type differentiation.

To determine whether positional information is also provided to epidermal cells post-embryonically, two sorts of experiments have been conducted. In one, a detailed analysis of peculiar epidermal cell clones was performed (Berger et al., 1998a). The clones examined were ones derived from rare post-embryonic longitudinal divisions of epidermal cells, which causes the two resulting daughter cells to occupy different positions relative to the underlying cortical cells. The cells within these clones expressed marker genes and exhibited cellular characteristics that are appropriate for their new position (Figure 7). In a second set of experiments, specific differentiating epidermal cells were subjected to laser ablation such that neighboring epidermal cells were able to invade the available space (Berger et al., 1998a). Regardless of the original state of the ablated cell or invading cell (trichoblast or atrichoblast), the ultimate characteristics of the invading cell was nearly always determined by its new location rather than its old. Therefore, in each of these sets of experiments, cells had effectively undergone a post-embryonic change in their position and, in response, exhibited a change in their developmental fate. This suggests that positional information is provided post-embryonically, not only embryonically, to ensure appropriate cell specification in the Arabidopsis root epidermis.

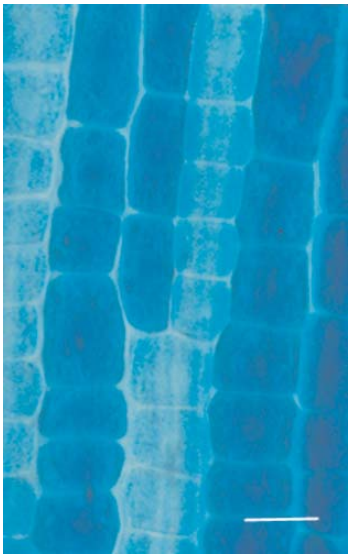

Figure 7. Expression of the GL2::GUS reporter fusion in an epidermal cell clone derived from a rare longitudinal division. Note that only one set of cells in the clone expresses the GL2 marker. Bar = 10 μm.

Laser ablation of specific cells has also provided insight into the directionality of the positional signals that define the epidermal cell types (Berger et al., 1998a). In one set of experiments, plants harboring the J2301 enhancer-trap GFP reporter were subjected to ablations in which immature epidermal cells were isolated from their neighbors within the same file or in adjacent files. In nearly every case, the isolated cells, which had lost contact with their epidermal neighbors, maintained the same reporter gene expression and differentiated according to their original position (Berger et al., 1998a). In a second set of cell ablations in the J2301 line, specific cortical cells were ablated such that the overlying immature epidermal cell(s) were isolated. Regardless of the original state of the isolated epidermal cell (trichoblast or atrichoblast), the ablation of the underlying cortical cell(s) had no effect on their future GFP expression or morphogenesis (Berger et al., 1998a). These results imply that continuous signaling between living cortical and/or epidermal cells is not required to maintain the appropriate cell fate decision. However, it is still unclear whether signaling between cortical and epidermal cells may be required to establish cell fates.

Molecular Genetics of Root Hair Cell Specification

Several mutants have been identified in Arabidopsis that possess a disrupted pattern of root epidermal cell types (Table 1; Figure 8). Three of these patterning mutants, *werewolf* (*wer*), *transparent testa glabra* (*ttg*), and *glabra2* (*gl2*) possess root hairs on essentially every root epidermal cell, which implies that the normal role of the *WER*, *TTG*, and *GL2* genes is either to promote non-hair cell differentiation or to repress root hair cell differentiation (Galway et al., 1994; DiCristina et al., 1996; Masucci et al., 1996; Lee and Schiefelbein, 1999). These mutations differ in their specific effects on non-hair cell differentiation; the *wer* and *ttg* mutations alter all aspects of non-hair differentiation (including the cell division rate, cytoplasmic density, and vacuolation rate) whereas the *gl2* mutations only affect the final cell morphology and do not affect the earlier cellular phenotypes (Galway et al., 1994; Masucci et al., 1996; Berger et al., 1998b; Lee and Schiefelbein, 1999). Thus, WER and TTG may be earlier acting components required for position-dependent non-hair cell differentiation.

The *WER* gene encodes a MYB transcription factor of the R2-R3 class (Lee and Schiefelbein, 1999). It is prefer-

Table 1. Arabidopsis Genes Controlling Root Epidermal Cell Specification

Locus	Gene Product	Chromosomal Location	Mutant phenotype	References / Accession Numbers
CAPRICE (CPC)	MYB protein	II	Reduced number of root hairs	Wada et al., 1997; AB004871
ECTOPIC ROOT HAIR1 (ERH1)	unknown	V	Ectopic root hairs	Schneider et al., 1997
ECTOPIC ROOT HAIR3 (ERH3)	unknown	I	Ectopic root hairs	Schneider et al., 1997
GLABRA (GL2)	Homeodomai n protein	I	Ectopic root hairs	Masucci et al., 1994; DiCristina et al., 1994; L32873
POMPOM (POM1)	unknown	I	Ectopic root hairs	Schneider et al., 1997
ROOTHAIRLESS1 (RHL1)	novel nuclear protein	I	Reduced number of root hairs	Schneider et al., 1998; AF062371
ROOTHAIRLESS2 (RHL2)	unknown	V	Reduced number of root hairs	Schneider et al., 1997
ROOTHAIRLESS3 (RHL3)	unknown	III	Reduced number of root hairs	Schneider et al., 1997
TRANSPARENT TESTA GLABRA (TTG)	WD repeat protein	V	Ectopic root hairs	Galway et al., 1994; AJ133743
WEREWOLF (WER)	MYB protein	V	Ectopic root hairs	Lee and Schiefelbein, 1999; AF126399

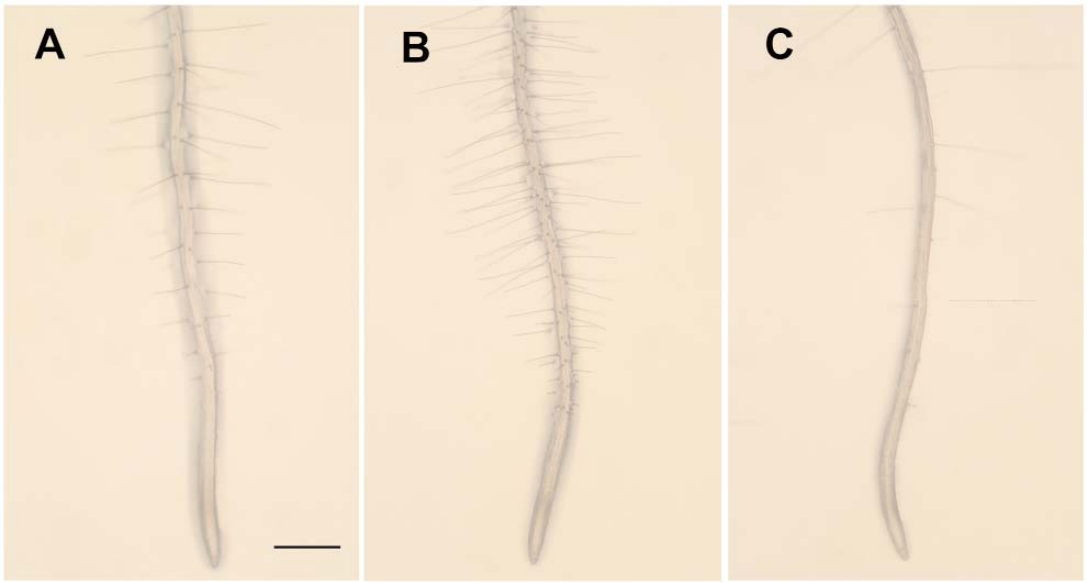

Figure 8. Root hair production in wild-type and cell specification mutants.
(A) Wild-type.
(B) An example of an ectopic hair mutant (wer).
(C) An example of a reduced hair mutant (cpc).
Bar = 500 μm for all images.

entially expressed in developing epidermal cells in the N position, which are the cells whose fate is mis-specified in the *wer* mutant. Unlike *TTG* and *GL2*, the *WER* gene does not influence trichome development, seed coat mucilage, or anthocyanin production.

The *TTG* gene encodes a small protein with WD40 repeats (Walker et al., 1999). Although the protein sequence does not provide a clear mechanistic understanding of TTG's role, it is known that *ttg* mutations can be functionally complemented by expression of the maize R cDNA (under the control of the strong cauliflower mosaic virus 35S promoter) in Arabidopsis (Lloyd et al., 1992; Galway et al., 1994). These data indicate that TTG is likely to activate an Arabidopsis homolog of the maize R (a basic helix-loop-helix transcriptional activator; Ludwig et al., 1989) to specify the non-hair cell fate. The ability of TTG to interact with GL3, an Arabidopsis bHLH protein, in the yeast two-hybrid assay (Payne et al., 2000) suggests that the activation involves protein-protein interactions.

The *GL2* gene encodes a homeodomain transcription factor protein (Rerie et al., 1994; DiCristina et al., 1996), and it is preferentially expressed in the differentiating non-hair epidermal cells within the meristematic and elongation regions of the root (Masucci et al., 1996; Figure 5). As described above, *GL2* expression initiates during the early heart stage of embryogenesis, where it rapidly assumes its N-cell-specific expression pattern (Lin and Schiefelbein, 2001). The embryonic and post-embryonic *GL2* gene expression is influenced by the *WER* and *TTG* genes, with *wer* mutations nearly abolishing *GL2* promoter activity and *ttg* mutations causing a reduction in *GL2* promoter activity (Hung et al., 1998; Lee and Schiefelbein, 1999; Lin and Schiefelbein, 2001). Appropriate cell position-dependent *GL2* pattern is maintained in the *ttg* mutant, but not the *wer* mutant, which implies that WER (but not TTG) is required to specify the positional information for *GL2* expression. Taken together, the emerging picture is that WER, TTG, and an R-like bHLH protein begin to act at an early stage in embryonic development to positively regulate the expression of *GL2* (and perhaps other as yet unidentified genes) in a cell position-dependent manner to specify the non-hair cell type.

A fourth Arabidopsis gene, *CAPRICE* (*CPC*), affects root epidermis cell specification in a different manner. Rather than causing ectopic root hair cells, the cpc mutant produces a reduced number of root hair cells (Wada et al., 1997). This implies that *CPC* is a positive regulator of the root hair cell fate. Interestingly, the *gl2* mutation is epistatic to *cpc*, which suggests that CPC acts in the WER/TTG/GL2 pathway as a negative regulator of *GL2*. A possible explanation for CPC's negative action is provided by the nature of its gene product; *CPC* encodes a small protein with a Myb-like DNA binding domain but without a typical transcriptional activation domain (Wada et al., 1997). Thus, CPC may inhibit GL2 transcription by binding to its promoter and blocking its activation.

These molecular genetic findings led to a simple model for the control of root epidermal cell fate in Arabidopsis (Lee and Schiefelbein, 1999; Figure 9). In this model, the root hair cell fate is proposed to represent the default fate for a root epidermal cell. The pattern of hair and non-hair cell types relies on the relative activity of two competing transcription factors, WER and CPC. Each of these is proposed to have the ability to form a complex with the TTG and an R-like bHLH protein. In immature epidermal cells in the N position, it is proposed that a relatively high level of WER is present and this leads to expression of GL2 (and probably other genes) and non-hair cell differentiation. In immature epidermal cells located in the H position, it is suggested that a relatively high level of CPC exists, which leads to repression of GL2 and permits hair cell differentiation to proceed. At this time, it is unclear whether or how a relative difference in the levels of WER and CPC becomes established in the N and H cell positions.

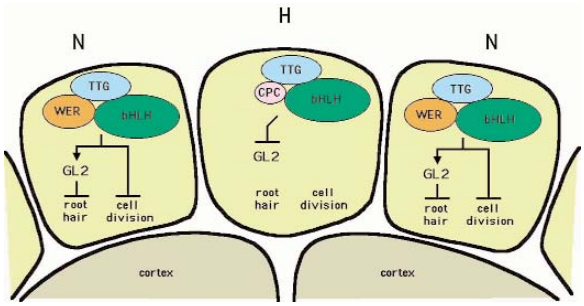

Figure 9. Model for the specification of the root hair and non-hair cell types in the Arabidopsis root epidermis. The proposed accumulation and interaction of cell fate regulators is shown within root epidermal cells destined to be root hair cells (in the H position) or non-hair cells (in the N position). In this model, the default fate for an epidermal cell is a root hair cell. Arrows indicated positive control, and blunted lines indicated negative regulation.

In addition to the genes described above, other loci have been defined by mutation that influence the specification of the root epidermal cells. These include the *roothairless* (*rhl*) mutants *rhl1*, *rhl2*, and *rhl3*, and the *ectopic root hair* (*erh*) mutants *erh1*, *erh2*/*pom1*, and *erh3* (Schneider et al., 1997) as well as the *tornado* (*trn*) mutants *trn1* and *trn2* (Cnops et al., 2000). Each of these alters the early differentiation features of the hair and non-hair cells, which indicates that they affect cell specification rather than a later root hair morphogenesis process. The *RHL1* gene

encodes a small pioneer protein that is nuclear localized, but it does not regulate *GL2*, which suggests that it acts in an independent genetic pathway that specifies the hair cell fate (Schneider et al., 1998).

The cell specification genes described in this section are expected to influence the expression or activity of genes/proteins that control the process of root hair initiation. At present, no direct targets of these cell specification gene products have been identified. There are several candidate genes, such as *RHD6* and *AXR2* described later in this chapter, which are known to control root hair initiation and may therefore be regulated by the products of the specification genes.

Similarities in Epidermal Patterning in the Root and other Tissues

A close relationship exists between cell specification in the root and the above-ground tissues of the Arabidopsis plant. The most striking similarity is found in the epidermis of the hypocotyl. Although hypocotyl epidermal cells do not produce root hairs, there are two distinct files of epidermal cells in the Arabidopsis hypocotyl that arise in a position-dependent manner (Wei et al., 1994; Gendreau et al., 1997; Hung et al., 1998; Berger et al., 1998c). One type of hypocotyl cell file preferentially includes stomatal cells and are present outside an anticlinal cortical cell wall, equivalent to the H position in the root epidermis. The other type of hypocotyl cell file possesses non-stomatal cells and are located outside a periclinal cortical cell wall, equivalent to the N position in the root epidermis (see the chapter on stomata in this book). This means that cells of the hypocotyl epidermis and the root epidermis undergo position-dependent cell differentiation to generate a common pattern of cell types throughout the apical-basal axis of the Arabidopsis seedling.

The similarity in cell specification in the root and hypocotyl epidermis is also apparent in the molecular components employed. The *wer*, *ttg*, and *gl2* mutations significantly alter the patterning of the hypocotyl cell types, causing a greater proportion of ectopic stomata (stomata located outside a periclinal cell wall) (Hung et al., 1998; Berger et al., 1998c; Lee and Schiefelbein, 1999). Furthermore, the *WER*, *GL2*, and J2301 enhancer-trap GFP reporter genes are preferentially expressed in epidermal cells located outside the periclinal cortical cell wall of the root and hypocotyl (Hung et al., 1998; Berger et al., 1998c; Lee and Schiefelbein, 1999) (Figure 10). The similar pattern of specialized and non-specialized epidermal cells in the root and hypocotyl is initiated during embryogenesis, as demonstrated by similar marker gene expression beginning at the heart stage (Berger et al., 1998a; Lin and Schiefelbein, 2001). The parallel pattern of gene activity indicates that the WER/TTG/GL2 pathway is employed in both organs of the seedling beginning during embryogenesis to ensure that cells located outside a periclinal cortical cell wall differentiate into non-hair cells in the root and non-stomatal cells in the hypocotyl epidermis.

In addition to affecting the hypocotyl epidermis, the *TTG* and *GL2* genes are known to also affect trichome formation in the shoot epidermis of Arabidopsis (Koornneef, 1981; Koornneef et al., 1982; Larkin et al., 1997; see also the chapter on trichomes in this book). Furthermore, the shoot and root tissues employ functionally equivalent MYB proteins, WER (in the root) and GL1 (in the shoot) to specify cell fate (Lee and Schiefelbein, 2001). Overlap in cell specification between the root and leaf epidermis was unexpected because the patterning of cell types in these two tissues appears to be quite different; the root epidermis mechanism relies on the positional relationship between epidermal cells and underlying cortical cells whereas the leaf epidermis mechanism relies on sensing trichome density. A further interesting aspect of this relationship is that the WER/GL1, TTG, and GL2 proteins control epidermal hair formation in opposite ways in the root and leaf. They are required for the formation of non-hair cells in the root

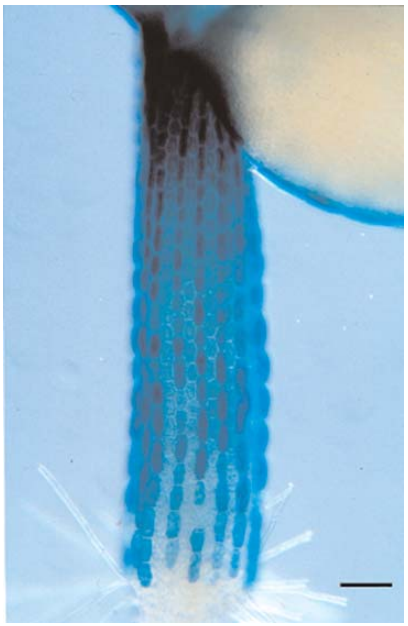

Figure 10. Expression of the GL2::GUS reporter fusion in the hypocotyl epidermis. Cells expressing the GL2::GUS marker are located in the N position. Bar = 100 μm.

and hair-bearing (trichome) cells in the leaf. Although the meaning of this root/leaf overlap is unclear, it may be that TTG and GL2 represent general epidermis transcriptional regulators that have been recruited to participate in very different cell-type specification mechanisms during the evolution of epidermis development in angiosperms.

Hormonal Effects on Root Hair Cell Specification

Results from numerous pharmacological and genetic experiments indicate that ethylene and auxin help promote root hair cell differentiation in Arabidopsis. For example, aminoethoxyvinylglycine (AVG, an ethylene biosynthesis inhibitor) or Ag+ (an inhibitor of ethylene perception) blocks root hair formation (Masucci and Schiefelbein, 1994; Tanimoto et al., 1995) and 1-amino-cyclopropane-1-carboxylic acid (ACC, an ethylene precursor) induces some ectopic root hair cells in Arabidopsis (Tanimoto et al., 1995). Further, mutations affecting the *CONSTITUTIVE TRIPLE RESPONSE* (*CTR1*) locus, which encodes a Raf-like protein kinase proposed to negatively regulate the ethylene signal transduction pathway (Kieber et al., 1993) causes root hairs to form on epidermal cells that are normally hairless (Dolan et al., 1994). Consistent with this, epidermal cells in the H position are more sensitive to the hair-inducing effects of ethylene than cells in the N position (Cao et al., 1999). Also, the hairless root phenotype of the *dwarf* (*dwf*; auxin-resistant) and *auxin resistant2* (*axr2*; auxin, ethylene, and abscisic acid resistant) mutants implicate auxin in root hair formation (Mizra et al., 1984; Wilson et al., 1990). Finally, the hairless *rhd6* mutant phenotype can be suppressed by the inclusion of ACC or indole-3-acetic acid (IAA, an auxin) in the growth media (Masucci and Schiefelbein, 1994).

Although these hormones are involved in root hair development, their role in the specification of epidermal cell fate is less clear. Results from epistasis tests and *GL2* promoter-reporter gene analyses show that the ethylene/auxin pathway does not regulate the TTG/GL2 pathway (Masucci and Schiefelbein, 1996). In addition, studies of the developmental timing of the hormone effects indicate that the ethylene and auxin pathways promote root hair outgrowth after epidermal cell-type characteristics have developed (Masucci and Schiefelbein, 1996; Cao et al., 1999). Nevertheless, mutations in the ethylene and auxin related *AXR2* and *RHD6* genes reduce the cytological differences between the cell types (Masucci and Schiefelbein, 1996), implying that these genes assist in the early establishment of cell identity. Taken together, the results suggest that the WER/TTG/GL2/CPC pathway acts upstream of, or independently from, the ethylene/auxin pathway to define the pattern of cell types in the root epidermis. One of the implications of this proposal is that the newly-formed epidermal cells in the Arabidopsis root may initially be defined as a trichoblast or atrichoblast (due to the action of the WER/TTG/GL2/CPC pathway) although the final pattern of epidermal cell types may be influenced by the hormones (perhaps governed by or intertwined with the effect of environmental factors).

ROOT HAIR FORMATION

Epidermal cells that are committed to hair formation become highly specialized and adopt a characteristic shape. The overall process of hair formation is summarized in Figure 11.

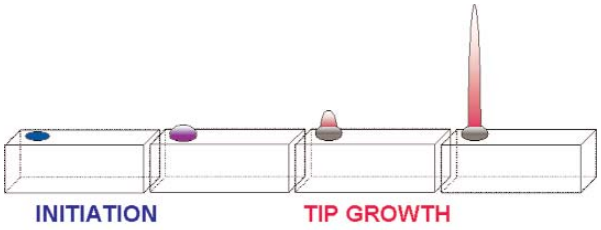

Figure 11. Stages of root hair formation. Root hairs form in two main stages: initiation, when a small, disc-shaped area of the cell wall loosens to form a swelling, and tip growth, when the remainder of the hair grows by targeted secretion.

Root Hair Initiation

Plant cells change shape by modifying their cell walls. After they are generated in the meristem, epidermal cells committed to the root hair fate become wider, longer and deeper by diffuse growth. The hair itself forms when cell expansion becomes localized to a small disc-shaped area of the outer-facing wall about 22 μm across. This process is termed root hair initiation and is summarized in Figure 12.

Before the hair begins to grow, small GTP-binding proteins from the Rop family appear at the growth site (Molendijk et al., 2001, Figure 12B). Rops are unique to plants, but are related to the Rac, Cdc42, and Rho small GTPases that control the morphogenesis of animal and yeast cells (Chant, 1999; Eaton, Wepf and Simons, 1996).

Applying the ARF-GEF (GNOM) secretion inhibitor brefeldin A prevents Rop localisation, suggesting that either Rop itself or a molecule that localizes Rop, is placed at the future site of hair formation by targeted secretion. Rop remains at the tip of the developing hair until growth ends (Molendijk et al. 2001).

Within minutes of Rop localization the root hair cell wall begins to bulge out. At the same time the pH of the wall falls. This pH change may activate expansin proteins that catalyze wall loosening (Figure 12C, Bibikova et al. 1998, Baluska et al. 2000). The mechanism responsible for the pH change is uncertain; it may be due to local changes in wall polymer structure and ion exchange capacity, or to local activation of a proton ATPase or other proton transport activity (Bibikova et al. 1998).

As the bulge enlarges, the endoplasmic reticulum within it condenses (Figure 12D, Ridge et al., 1999) and actin accumulates (Baluska et al., 2000). Under optimal conditions, it takes about 30 minutes for a root hair swelling to form on the surface of the cell (Bibikova et al. 1998).

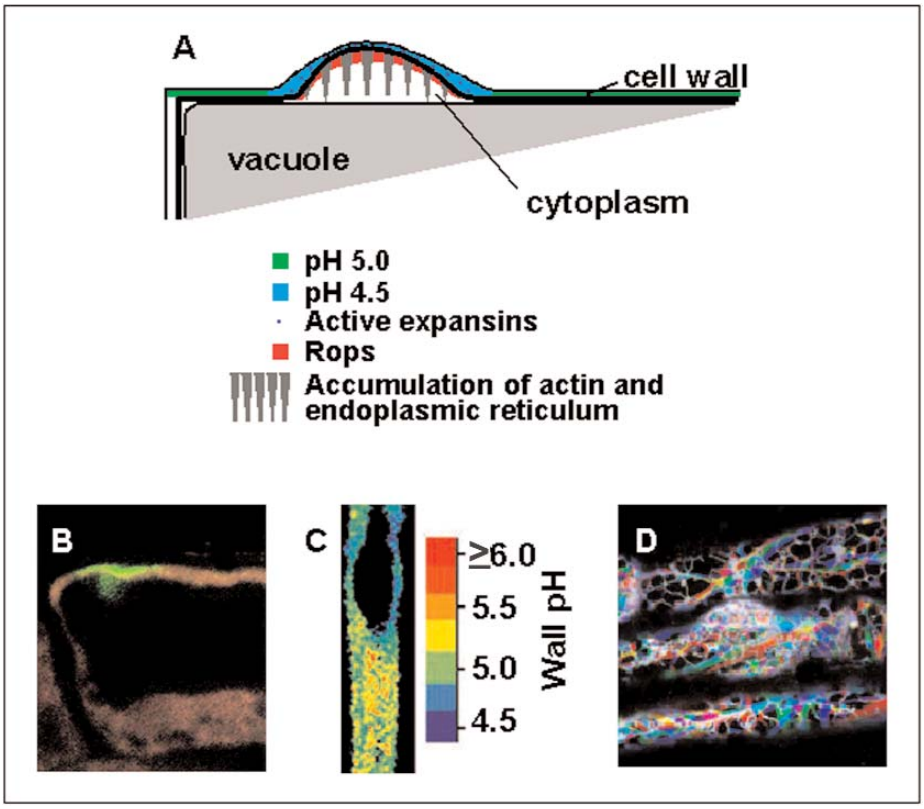

Figure 12. Root hair initiation.
(A) Diagram summarizing the initiation process. Rop protein localizes to the initiation site, and the pH of the cell wall drops to about pH 4 to 4.5. This local pH change is thought to activate expansin proteins that loosen the cell wall. Large amounts of endoplasmic reticulum and filamentous (F) actin accumulate in the developing swelling
(B) Rop at the future site of hair formation. Localization of Rop protein is the first sign that a hair is about to form (see Molendijk et al., 2001).
(C) Acidification of the cell wall at the root hair initiation site. pH was imaged using NERF/Texas Red and pseudo-color coded according to the inset scale (see Bibikova et al., 1998).
(D) Local accumulation of endoplasmic reticulum (ER) in an initiating hair flanked by two non-hair cells. Red, blue and green images were taken 30 seconds apart. White indicates that ER was present in the same location in all three images. (see Ridge et al., 1999).

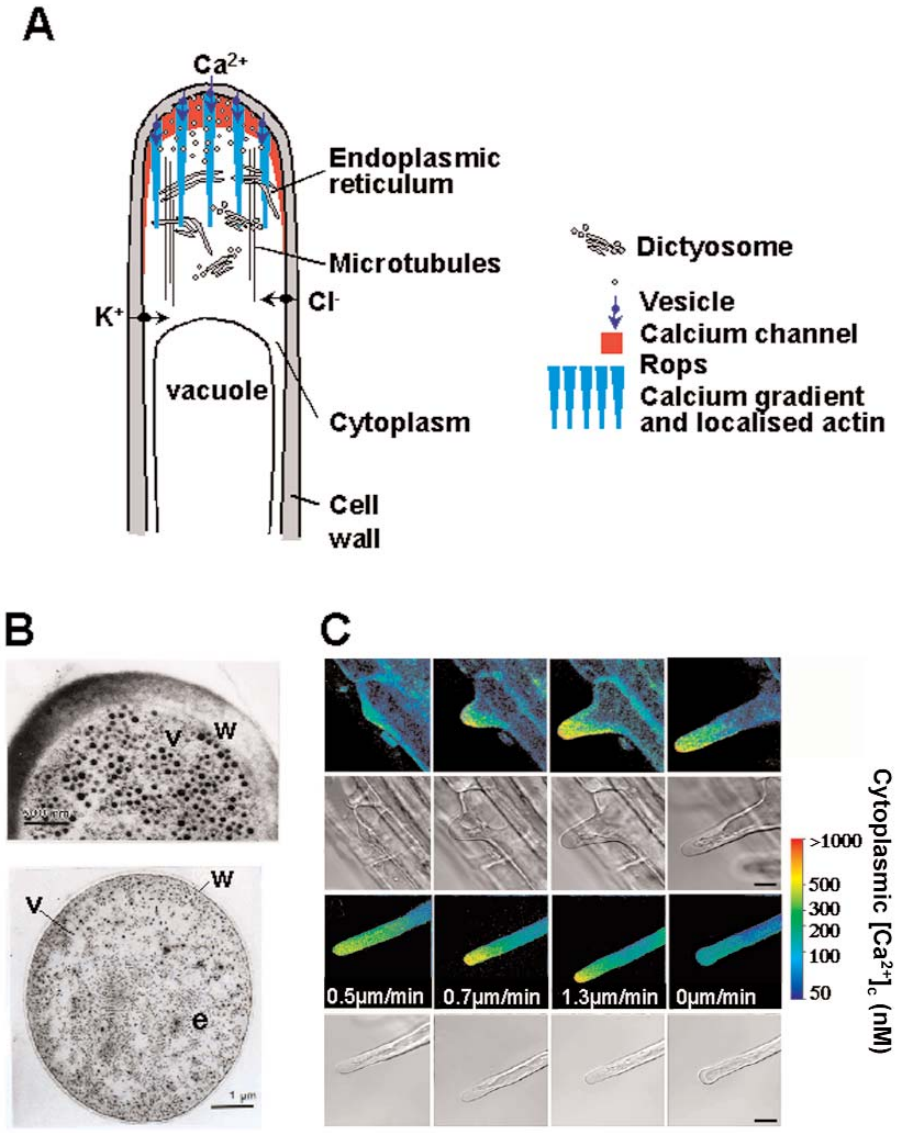

Figure 13. Root Hair Tip Growth.

(A) Diagram summarizing the mechanism of tip growth in Arabidopsis root hairs. The tip is packed with membrane-bound vesicles delivering new cell wall material. These vesicles are made in the endoplasmic reticulum (ER) and dictyosomes which are abundant behind the tip. Rop protein is localized to the tip along with F-actin, and a tip-focused calcium gradient. This calcium gradient is thought to be generated by hyperpolarization-activated calcium channels, which are localized to the plasma membrane at the hair tip. Other channels import osmotically active K+ and Cl- ions, which help to sustain turgor pressure as the hair grows. The direction of growth is controlled by microtubules, which run along the length of the hair.

(B) Cytoarchitecture at the tip of an elongating root hair. Transmission electron micrographs of sections of an elongating hair showing the cell wall (w), vesicles (v), and endoplasmic reticulum (e). Top – The hair apex is packed with vesicles. Bottom – A section from just behind the apex shows dense endoplasmic reticulum surrounded by vesicles.

(C) Tip-growing root hairs have a tip-focused calcium gradient. Time course showing the establishment and maintenance of a calcium gradient in an elongating root hair, and its disappearance when growth ceases. The concentration of cytoplasmic free calcium ([Ca2+]c) was imaged using indo-1 and pseudo-color coded according to the inset scale. [Ca2+]c is shown in the first and third rows with corresponding transmitted light images of the same cell in the second and fourth rows (see Wymer et al. 1997).

Root Hair Tip Growth

Once a swelling has formed, the rest of the root hair is formed by tip growth. This type of growth is used by other tubular, walled cells including pollen tubes. Tip growth is summarized in Figure 13 and a tip-growing Arabidopsis root hair is shown in Figure 14. During tip growth new cell wall material is synthesized and precisely secreted to the cell tip. The cytoplasm inside the tube is highly organized (Figure 13A, B). The tip is packed with vesicles of membrane containing cell wall polysaccharides and cell wall proteins. These vesicles are produced by smooth and rough endoplasmic reticulum and dictyosomes, which are very abundant in the part of the tube just behind the growing apex (Figure 13A, B). The nucleus enters the tube (Figure 14) and moves along behind the tip. Actin is concentrated at the tip throughout tip growth (Baluska et al 2000).

Tip growing cells are very dynamic. Arabidopsis root hairs typically grow at 1 $\mu m.min^{-1}$, rapidly producing and incorporating vesicles. A great deal of the cell contents are moving rapidly around the hair by cytoplasmic streaming (Movie 1).

Root hair tip growth requires calcium (Schiefelbein et al., 1992). When Arabidopsis root hairs are 5-10 µm long the Ca^{2+} concentration at the tip of the swelling increases from about 200 nM to at least 1 µM, and remains very high throughout tip growth (Figure 13C; Wymer et al., 1997; Bibikova et al., 1999). Calcium is imported across the membrane at the tip of the hair by channels that are activated by the negative potential across this membrane (–160 to –200 mV; Lew, 1996; Very and Davies, 2000). This potential is probably generated by a plasma membrane H+ ATPase. The source of calcium for root hair tip growth is uncertain. When hairs are growing through a solution, calcium is apparently imported from liquid surrounding the growing tip. However, root hairs grow well in moist air and are abundant in air pockets in soil (C. Grierson, unpublished observations; Ryan et al., 2001). Under these conditions, calcium for tip growth must be either (1) released from the newly deposited wall, or (2) transported through the apoplast and deposited near the tip of the hair, or (3) released from intracellular stores (Ryan et al., 2001).

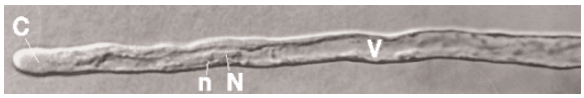

Figure 14. A tip growing hair viewed with differential interference contrast microscopy (DIC). Most of the hair is vacuolated (V), there is an accumulation of cytoplasm at the tip (C), and the nucleus (N) (n denotes the nucleolus) has entered the hair.

The calcium gradient at the tip of the hair is part of the mechanism that controls the direction of growth. If the calcium ion concentration is artificially increased towards one side of the tip, the hair will re-orient to grow in that direction (Bibikova et al., 1997). It is uncertain how calcium controls the direction of growth.

Rop proteins that are involved in root hair initiation (*hyperlink to initiation section above)* also have a strong effect on the direction of tip growth. Rops are active when bound to GTP and inactive when bound to GDP. Plants overexpressing a mutant Rop that is permanently in the active form have balloon-shaped root hairs, suggesting that Rop must be able to cycle from the GTP-bound form to the GDP-bound form for the direction of tip growth to be controlled (Molendijk et al., 2001).

Microtubules also control the direction of growth and ensure that there is only one growth site (Bibikova et al., 1999). When the microtubule cytoskeleton is depolymerized or stabilized using the drugs oryzalin and taxol respectively root hair growth becomes wavy and hairs branch to form two or more tips. The region at the tip where growth can be reoriented by locally increasing the concentration of calcium is larger when microtubules are depolymerized or stabilized (Figure 15; Bibikova et al., 1999). This suggests that microtubules usually limit the area at the tip where growth can take place.

Walled cells require internal pressure, called turgor, to sustain growth. If turgor is too low, the cytoplasm will not fit snugly against the wall, and the intimate contact between the plasma membrane and the wall is jeopardized. In addition, the growth of some plant cells depends on ion channels that are activated when the cell membrane is stretched. For these reasons it is important that plant cells that are growing have mechanisms to keep themselves turgid. At a typical growth rate of 1 µM min^{-1} Arabidopsis root hairs increase their volume by approximately 50 fL min^{-1} (Lew, 2000). To remain turgid whilst increasing in volume the total amount of osmotic ions in the cell must increase. Arabidopsis root hairs actively accumulate several osmotically active ions including K^+ and Cl^- as they grow, but other, unidentified mechanisms are also used to adjust turgor (Lew, 1991; 1998). Experiments using pressure probes and osmotica have been used to show that turgor is regulated by sensing changes in osmolarity, not internal pressure (Lew, 1996).

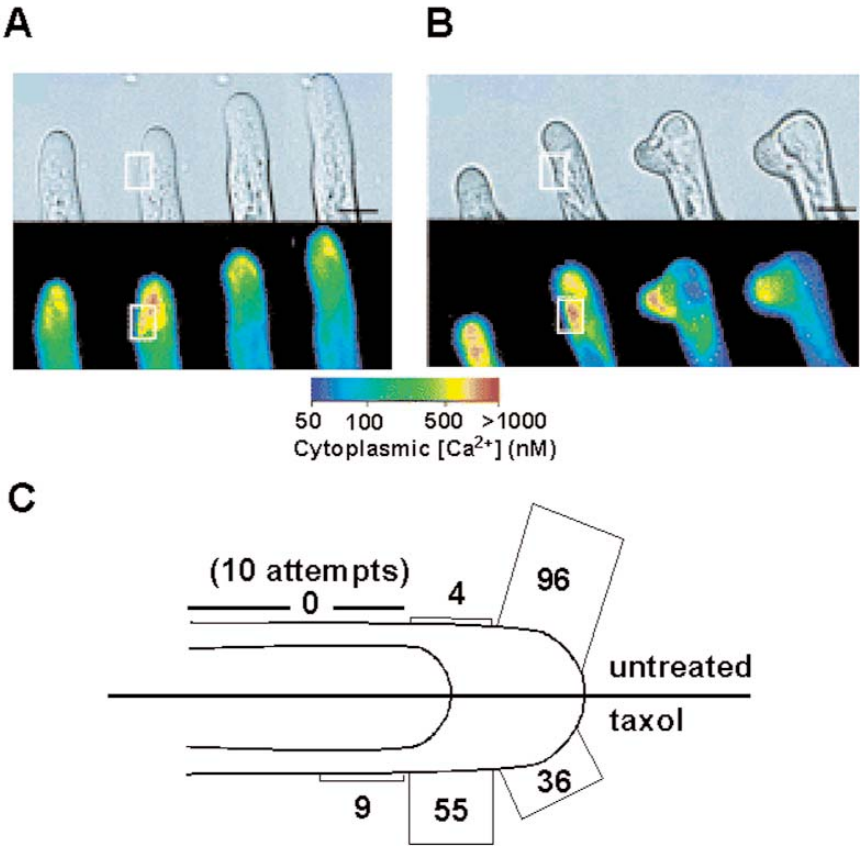

Figure 15. The direction of tip growth is controlled by calcium and microtubules.
(A, B) Cytoplasmic calcium and tip growth in untreated (A) and 10μM taxol treated (B) root hairs after local photoactivation of caged calcium ionophore. The ionophore was activated by illuminating the boxed regions with a UV laser. The concentration of cytoplasmic free calcium ([Ca2+]c) was imaged using calcium green/rhodamine and pseudo-color coded according to the inset scale.
(A) An untreated hair was unaffected by the ionophore.
(B) A taxol-treated hair grew towards the ionophore. Taxol promotes microtubule polymerisation.
(C) Diagram showing the percentage of untreated and taxol-treated root hairs that reoriented their growth in response to locally activated calcium ionophore
(adapted from Bibikova et al. 1999).

Cessation of Tip Growth

Root hair tip growth ceases when hairs reach a predictable length. The end of growth is precisely controlled and coordinated, producing a symmetrical, dome-shaped tip with the same diameter as the hair shaft. When hairs stop growing, the cytoplasm at the tip disperses and the vacuole enlarges into the dome (Figure 13C). Rop protein (Molendijk et al., 2001), the calcium gradient (Figure 13C; Wymer et al., 1997), and calcium channel activity (Schiefelbein et al., 1992; Very and Davies, 2000) are lost from the tip. The route of cytoplasmic streaming adjusts to include the tip of the hair.

Several pharmacological agents including nifedipine and taxol yield hairs with enlarged tips (Schiefelbein et al., 1992; Bibikova et al., 1999). These agents presumably interfere with the coordination of different parts of the tip growth process when growth ceases, resulting in

deformed tips. For example, if vesicle delivery continues after the calcium gradient has been lost, the direction of growth will not be controlled and vesicles will fuse throughout the tip, producing a bulge at the end of the hair (Schiefelbein et al., 1992).

Molecular Genetics of Root Hair Formation

Table 2 lists genes that affect root hair development and Figure 16 shows when each gene is involved. Genes are discussed in the order in which they contribute.

Genes affecting the number of swellings on each hair cell. Wild type hair cells produce a single swelling, which develops into a hair. The *RHD6* gene is required for this process: *rhd6* mutants are nearly hairless, although a small proportion of cells on *rhd6* plants have extra hairs (Masucci and Schiefelbein, 1994). This suggests that *RHD6* is involved in controlling the establishment and the number of swellings. Hair cells on roots mutated in the *TINY ROOT HAIR1* (*TRH1*) gene sometimes have extra swellings (Rigas et al., 2001), implying that *TRH1* prevents multiple swellings from forming on wild type hair cells. *TRH1* encodes a potassium transporter (Rigas et al., 2001). Further research is required to discover how this affects swelling number.

Genes affecting the location of hairs on the cell. Root hairs of many species including Arabidopsis emerge toward the apical end of the cell (nearest the root tip, Leavitt, 1904; Masucci and Schiefelbein, 1994). The Arabidopsis *RHD6* gene affects this positioning**.** Hairs on *rhd6* mutant roots are nearer to the basal end of the hair cell (Figure 17), implicating *RHD6* in mechanisms that encourage hair formation at the apical end of the cell. The fact that *RHD6* determines how many cells form hairs as well as the position of the hair on the hair cell suggests that *RHD6* links mechanisms that control whether a hair will form with mechanisms controlling where on a cell the hair will emerge.

Genes restricting swelling size. As described above, each swelling forms by cell wall loosening. In wild type Arabidopsis the amount of loosening is highly reproducible, and swelling diameter is consistently about 22 µm (Parker et al., 2000, Figure 18). The *tip1* and *root hair defective1* (*rhd1)* mutant plants have large swellings (Figure 18). In *tip1* mutants, swelling diameter is increased by about a third. The *rhd1* mutants have huge swellings that take up most of the outer surface of the hair cell (Figure 18). As *tip1* and *rhd1* are both loss-of-function mutants these results suggest that the *RHD1* and *TIP1* genes restrict swelling size. They presumably do this by restricting the area of wall that is loosened (Parker et al., 2000; Ryan et al., 1998; Schiefelbein et al., 1993; Schiefelbein and Somerville, 1990). The *tip1 rhd1* double mutants have similar swelling dimensions to *rhd1* single mutants, suggesting that *TIP1* cannot effect swelling size unless the RHD1 gene product is present (Parker et al., 2000).

Genes establishing tip growth. Tip growth is established by the time hairs reach 40 µm long (Dolan et al., 1994). Root hairs without functional *ROOTHAIRDEFECTIVE2* (*RHD2*; Figure 19), *SHAVEN1* (*SHV1)*, *SHV2*, *SHV3*, *TRH1*, or *KOJAK* (*KJK)* genes stop growing before this stage (Figure 16, Parker et al., 2000; Schiefelbein and Somerville, 1990; Favery et al, 2001.; Rigas et al., 2001). Mutations affecting the *CENTIPEDE1* (*CEN1)*, *CEN2*, *CEN3*, *SCN1*, *BRISTLED1* (*BST1)*, and *TIP1* genes can also stop hair growth before this stage, but only in certain double mutant combinations (Parker et al., 2000). These results suggest that all of these genes are important for tip growth to be successfully established.

There are at least two ways that growth can fail during the transition from swelling formation to tip growth. In the first case wall loosening may not stop, or the balance between wall deposition and protoplast growth may fail so that the hair bursts. For example, the hairs of *kjk* mutants burst after swelling formation, killing the cells (Favery et al., 2001; Wang et al., 2001). *KJK* encodes an enzyme that synthesizes cell wall components for export to the growing tip. KJK resembles a cellulose synthase, but cellulose is formed at the plasma membrane whereas KJK is found in the endoplasmic reticulum. KJK probably contributes to the synthesis of polysaccharides such as beta-xylans, mannans or xyloglucan (Favery et al., 2001). The *kjk* mutant hairs have weak cell walls that cannot contain the growing protoplast and burst. A second way that growth can fail is if a crucial part of the machinery for tip growth is not functioning. For example, *trh1* mutants do not burst, but are unable to grow (L. Dolan, personal communication). TRH1 is a potassium transporter that is specifically required for tip growth. *trh1* mutant roots have other functional potassium transporters and supplementing *trh1* mutant roots with high levels of potassium does not restore tip growth. These results suggest that tip growth depends on potassium transport that is precisely localized within the cell or coordinated with other events. This specialized potassium transport is carried out by the TRH1 protein and without it growth stops after swelling formation (Rigas et al., 2001).

Genes that prevent branching. Wild type root hairs rarely branch. Plants with mutations in the *SUPERCENTIPEDE1 (SCN1), CANOFWORMS1 (COW1), TIP1, CEN2, CEN1, CEN3, BST1, RHD3,* or *RHD4* genes have more branched hairs than wild type plants. In all cases except *BST1*, the hairs branch after swelling formation so that multiple hairs grow from the same initiation site (Figures 16, 19). *SCN1* has a particularly important role in

Table 2. Arabidopsis Genes Controlling Root Hair Morphogenesis

Locus	Gene Product	Chromosomal Location	Mutant phenotype	References/Accession Numbers
BRISTLED1 (BST1)	unknown	V	Short hairs, sometimes branched	Parker et al., 2000
CANOFWORMS1 (COW1)	unknown	IV	Short, wide, hairs, sometimes branched at base	Grierson et al., 1997; Parker et al., 2000
CENTIPEDE1 (CEN1)	unknown	I	Short, wide hairs, sometimes curled	Parker et al., 2000
CENTIPEDE2 (CEN2)	unknown	V	Short, wide hairs, sometimes branched and/or curled	Parker et al., 2000
CENTIPEDE3 (CEN3)	unknown	III	Short, wide hairs, some with wide bases, some hairs curled and/or branched	Parker et al., 2000
KEULE	Sec1 protein	I	Hairs absent or stunted and swollen	Assaad et al. 2001; AF331066
KOJAK (KJK/AtCSLD3)	Cell wall synthase	*III*	Hairs burst after swellings form	Favery et al., 2001; AF232907
LRX1	Leucine rich repeat/extensin	I	Hairs short, swollen, or branched	Baumberger et al. 2001; AY026364
PFN1	Profilin	-	Long hairs	Ramachandran et al. 2000; U43322
PHYA	Phytochrome A	I	Altered hair length	De Simone et al., 2000; L21154
PHYB	Phytochrome B	II	Altered hair length	Reed et al., 1993; De Simone et al. 2000; X17342
ROOT HAIR DEFECTIVE1 (RHD1)	unknown	I	Wide swellings	Schiefelbein and Somerville, 1990
ROOT HAIR DEFECTIVE2 (RHD2)	unknown	V	Hairs stop growing after swellings form	Schiefelbein and Somerville, 1990
ROOT HAIR DEFECTIVE3 (RHD3)	GTP binding protein	III	Short, wavy hairs, sometimes branched	Schiefelbein and Somerville, 1990; Galway, et al., 1997; Wang et al., 1997; U86081
ROOT HAIR DEFECTIVE4 (RHD4)	unknown	III	Short hairs with bulges and constrictions, sometimes branched	Schiefelbein and Somerville, 1990; Galway, et al., 1999
ROOT HAIR DEFECTIVE6 (RHD6)	unknown	I	Reduced number of root hairs	Masucci and Schiefelbein, 1994
			Site of hair formation closer to basal end of cell	
			Some cells with multiple hairs	
SHAVEN1 (SHV1)	unknown	III	Hairs stop growing after swellings form	Parker et al., 2000
SHAVEN2 (SHV2)	unknown	V	Hairs stop growing after swellings form	Parker et al., 2000
SHAVEN3 (SHV3)	unknown	IV	Hairs stop growing after swellings form	Parker et al., 2000
SUPERCENTIPEDE (SCN1)	unknown	III	Short, wide, hairs, sometimes branched	Parker et al., 2000
TINY ROOT HAIR 1 (TRH1)	Potassium transporter	IV	Hairs stop growing after swellings form	Rigas et al., 2001; AJ296156
			Some cells with multiple swellings	
TIP1	unknown	V	Wide swellings	Schiefelbein et al., 1993; Ryan et al., 1998
			Short, wide hairs, sometimes branched at base	

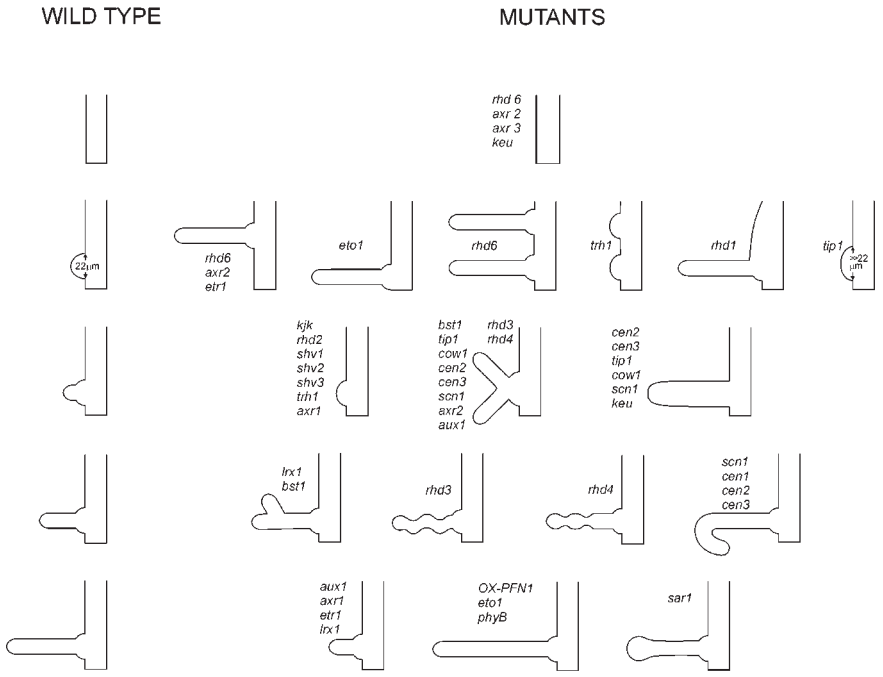

Figure 16. Genetics of root hair morphogenesis.
Diagram summarizing the stages of root hair development that contribute to the shape of the hair cell, and the phenotypes of relevant mutants and transgenic plants. Root hairs are reduced in length to fit into the figure. The developmental stages of wild type hairs are shown on the left. The defects of mutant or transgenic hairs are shown on the right alongside the relevant stage of wild type development. Mutants appear more than once when they affect more than one stage of development. OX-PFN indicates over-expression of the PFN1 gene.

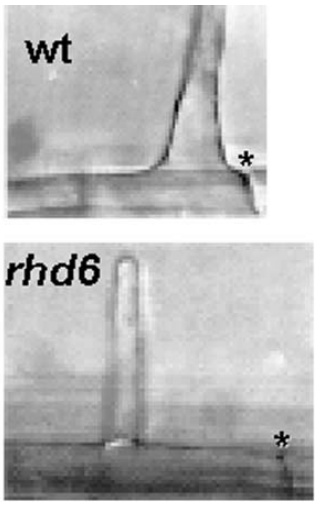

Figure 17. Hairs on the rhd6 mutant emerge at a more basal position on the hair cell than wild type hairs (wt). An asterisk (*) indicates the apical wall of each cell.

preventing branching. Plants homozygous for the loss-of-function *scn1-1* allele have a high percentage of branched hairs, and, in some double mutant combinations that include *scn1-1*, every hair is branched (Parker et al., 2000). The only gene that affects branching whose sequence is known is *RHD3*. *RHD3* encodes a protein with GTP binding domains, but its molecular function remains unclear (Wang et al., 1997).

Genes that sustain and direct tip growth. Several genes that affect tip growth have been identified. The molecular contributions of some of these genes are beginning to be understood.

LRX1 is a cell wall protein with leucine rich repeats and homology to extensins (Baumberger et al., 2001). LRX1 might regulate cell wall expansion. It is expressed in root hairs and localises to the cell wall at the tip of elongating hairs. LRX1 loss-of-function mutants have stunted and branched root hairs (Figure 19) showing that LRX1 affects the amount and location of tip growth.

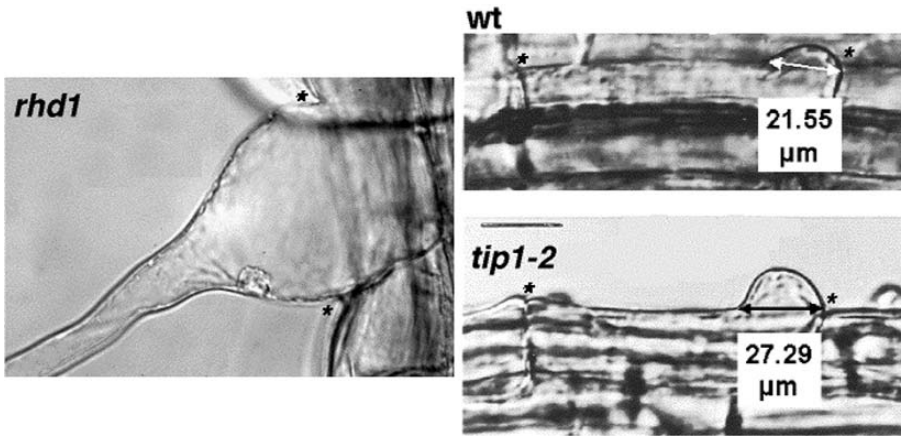

Figure 18. Swellings are wider on tip1 and rhd1 roots. Wild type swellings are about 22 µm across. Swellings on the rhd1 mutant encompass the whole outer cell wall. The tip1-2 mutant has swellings that are about 27 µm across. Asterisks (*) indicate the end walls of each cell (see Schiefelbein and Somerville, 1990; Parker et al., 2000).

PFN-1 encodes one of four Arabidopsis profilin actin-binding proteins, and is expressed in Arabidopsis root hairs. Transgenic plants overexpressing PFN-1 have root hairs that are twice as long as wild type hairs, suggesting that profilin forms part of the mechanism that controls the amount of root hair tip growth that takes place (Ramachandran et al., 2000).

Plants carrying loss-of-function mutations in the *RHD2, SHV1, SHV2, SHV3,* and *KJK* genes occasionally make tip-growing hairs. In all of these cases, the hairs are short and deformed showing that all of these genes are required for normal tip growth (Parker et al., 2000; Favery et al., 2001). The *bst1, cen1, cen2, cen3, cow1, rhd3, scn1, tip1,* and *rhd4,* mutants also have short root hairs so these genes are also required for hairs to achieve their usual length (Parker et al., 2000).

The Sec1 protein KEULE is required for normal root hair development. Loss of function *keule* mutants have absent or stunted, radially swollen root hairs (Figure 19). It is not clear whether KEULE affects root hair initiation, tip growth, or both. In other cell types Sec1 proteins are involved in mechanisms controlling vesicle targeting and vesicle fusion, so it is likely that KEULE contributes to root hair development by facilitating targeted vesicle fusion (Assaad et al., 2001). Several other genes affect the shape of hairs in a way that suggests that they might also control the number or location of vesicles that fuse at the growing tip. The *scn1, cen1, cen2, and cen3* hairs are often curved, showing that these genes are required to keep the elongating tube straight (Parker et al., 2000). The *rhd2-2*, *scn1*, *tip1* and *cow1* all have wide hairs (Parker et al., 2000), suggesting that the *RHD2*, *SCN1*, *TIP1* and *COW1* genes somehow restrict the area at the tip of the hair where vesicles can fuse. The *rhd3* mutant hairs are corkscrew shaped (Figure 19) because vesicle fusion apparently occurs at a point that rotates around the edges of the growing tip rather than being focused in the center (Galway et al., 1997). The *rhd4* mutant hairs have inconsistent diameters (Figure 19) and patches of thick cell wall, suggesting that the amount of material that is deposited varies as the tube grows, producing local constrictions and expansions along the length of the hair (Galway et al., 1999).

Perhaps surprisingly, light signaling can influence root hair length. Mutations in either the *phyA* or *phyB* phytochrome genes affect the length of root hairs grown in the light, showing that phytochrome signaling can influence the amount of tip growth that takes place (Reed et al., 1993; De Simone et al., 2000).

Auxin and ethylene signaling genes affecting root hair growth. Table 3 lists genes involved in auxin or ethylene signaling that play important roles during root hair formation and Figure 16 includes diagrams of their mutant phenotypes. *AUXIN RESISTANT 2* (*AXR2)* is the first to contribute. *AXR2* encodes AXR2/IAA7, a putative transcriptional regulator of auxin-responsive genes (Nagpal et al., 2000). Mutations in the *AXR2* gene that reduce auxin responses reduce hair production. The few hairs that do form on *axr2-1* mutants are closer to the basal end of the cell than wild type hairs, suggesting that auxin signaling encourages hairs to form at the apical end of the cell. The *rhd6* mutant has a similar phenotype to *axr2-1*. Results with *rhd6* confirm that auxin signaling affects the location of hair initation; the site of hair emergence on *rhd6* mutant

roots is restored from its more basal position to normal by treatment with auxin (Masucci and Schiefelbein, 1994).

Treating *rhd6* mutants with an ethylene precursor also restores the position of hair emergence to normal, implicating ethylene in this process. This is supported by the phenotypes of *ethylene response1 (etr1)* and *ethylene overproducer1* (*eto1*) mutants. *etr1* mutants perceive ethylene poorly because they have a damaged ethylene receptor. Like *rhd6* hairs, *etr1* hairs emerge nearer to the basal end of the hair cell. *eto1* plants produce more ethylene than wild type and root hairs form closer to the apical end of the cell (Masucci and Schiefelbein, 1996).

A small proportion of cells on *rhd6* and *axr2* plants have extra hairs (Masucci and Schiefelbein, 1994), suggesting that the growth regulators auxin and ethylene may also be involved in mechanisms that control the number of swellings.

There may be a minor role for auxin signaling in the establishment of tip growth because some hairs on *auxin resistant1* (*axr1*) mutant plants stop growing at this stage (Pitts et al., 1998). *AUXIN RESISTANT1* (*AXR1*) encodes

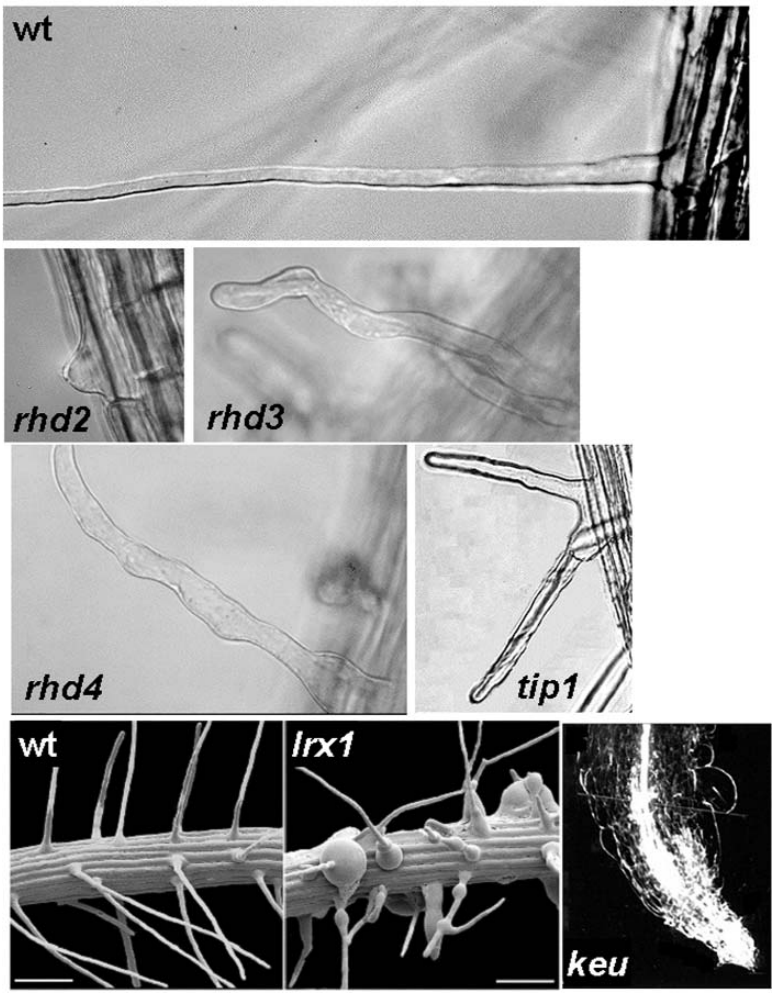

Figure 19. Phenotypes of wild type and mutant root hairs. Light micrographs of single hairs of wild type (wt), rhd2, rhd3, and rhd4, and a branched hair from a tip1 plant. Scanning electron micrographs of wild type (wt) and lrx1 roots. Light micrograph of a keule root tip (keu) with no root hairs. See Table 1 for references.

Table 3. Arabidopsis genes with roles in auxin or ethylene signaling that affect root hair development

Locus	Gene Product	Chromosomal Location	Mutant phenotype	References / Accession Numbers
AUX1	Auxin influx carrier	II	Short hairs, sometimes branched at base	Cernac et al., 1997; Pitts et al., 1998; X98772
AUXIN RESISTANT 1 (AXR1)	Subunit of RUB1-activating enzyme	*I*	Short hairs, sometimes branched at base Some hairs stop growing after swellings form	Cernac et al., 1997; Pitts et al., 1998; L13922
AUXIN- RESISTANT2 (AXR2/ IAA7)	AUX/IAA protein	III	Reduced number of root hairs Site of hair formation closer to basal end of cell	Masucci and Schiefelbein, 1994; Bates and Lynch, 1996; AF332395
AUXIN- RESISTANT3 (AXR3/ IAA17)	AUX/IAA protein	I	Reduced number of root hairs	Leyser et al., 1996; AF040631
ETHYLENE INSENSITIVE2 (EIN2)	unknown	V	Short hairs	Masucci and Schiefelbein, 1994; Pitts et al., 1998
ETHYLENE OVERPRODUCER 1 (ETO1)	unknown	V	Long hairs Site of hair formation closer to apical end of cell	Pitts et al., 1998
ETHYLENE RESPONSE 1 (ETR1/EIN1)	Histidine kinase	*I*	Short hairs Site of hair formation closer to basal end of cell	Masucci and Schiefelbein, 1994; Pitts et al., 1998; L24119
SUPPRESSOR OF AUXIN RESISTANCE1 (SAR1)	Synaptobrevin-related protein	I	Hairs have swollen ends	Cernac et al., 1997; Pitts et al., 1998; AY065357

a subunit of the RUB1-activating enzyme that is necessary for protein degradation required for responses to auxin.

Plants with mutations in the *AXR1, AXR2,* and *AUX1* genes have more branched hairs than wild type plants, suggesting that auxin signaling is involved in mechanisms that prevent branching.

The *axr1, aux1* and *etr1* mutants have short root hairs so all of these genes are required for root hairs to achieve wild-type length (Pitts et al., 1998). As discussed above *ETR1* encodes an ethylene receptor and *AXR1* encodes a component of the auxin signaling machinery. *AUX1* encodes an auxin influx carrier, so the short hairs on *etr1*, *axr1*, and *aux1* mutant plants suggest that ethylene and auxin signaling stimulate elongation (Pitts et al., 1998). In the case of ethylene this is supported by the phenotype of *eto1* mutants, which synthesise more ethylene and have longer root hairs than wild type plants (Pitts et al., 1998). Figure 20 summarizes the effects of auxin and ethylene signaling on root hair growth.

Genes affecting tip growth cessation. One mutant affects coordination of events at the end of tip growth (*hyperlink to section on the end of tip growth above*). When hairs on the *suppressor of auxin resistance 1 (sar1)* mutant stop growing, they have fat tips (Figure 16). SAR1 acts downstream of AXR1 in the auxin response, suggesting that auxin signaling plays a coordinating role at the end of root hair growth (Cernac et al., 1997).

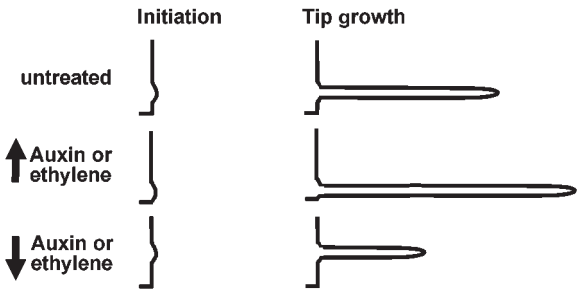

Figure 20. Roles of auxin and ethylene signaling in root hair growth.
Increased auxin or ethylene signaling moves the intiation site to a more apical position and increases the amount of elongation during tip growth. Decreased auxin or ethylene signaling has the opposite effect.

Root Hairs and Nutrient Acquisition

Effect of Root Hairs on Nutrient Uptake

A major function of root hairs is to increase root surface area and hence facilitate the uptake of nutrients from the soil. Plants with more, longer root hairs have an advantage at low nutrient concentrations. For example, the Arabidopsis accessions Co and C24 have high densities of long root hairs and are more efficient at acquiring phosphate (Narang et al., 2000). Similarly, at low phosphorous concentrations wild type is more efficient at taking up phosphorous than the mutants *rhd6* (almost bald) and *rhd2* (hairs stop growing at the swelling stage; Bates and Lynch, 2000a, b). Root hairs contain enzymes and nutrient transporters involved in nutrient uptake. An example is ferric chelate reductase (FCR); wild type plants have twice the FCR activity of a hairless mutant (RM57/*rhd7*), suggesting that root hairs are an important location for this enzyme (Moog et al., 1995).

Effect of nutrients on root hair development

Root hair development is strongly regulated by nutrient concentration. When nutrients are sparse the density and length of root hairs both increase. Hair development is regulated in response to many nutrients including phosphate (Bates and Lynch, 1996), iron (Schmidt et al., 2000), manganese, and zinc (Ma et al., 2001). Phosphate has the strongest and best characterized effect. Root hair density on Columbia roots grown in low phosphorous (1 mmol m^{-3}) is five times greater than on roots grown in high phosphorous (1000 $mmol^{-3}$). The number of hair-forming files is increased in low phosphorous from 8 to 12 files, and more of the cells in these files make hairs than on plants grown on high phosphorous (Ma et al., 2001). Hairs are also three times longer on low phosphorous than on high phosphorous (Bates and Lynch, 1996). Iron deficiency also increases hair density and length; iron-deficient roots produce ectopic hairs and hair length doubles (Schmidt et al., 2000).

Different nutrients control root hair development by different mechanisms. For example, auxin and ethylene signaling are crucial for responses to iron deficiency but have little effect on responses to low phosphorous (Schmidt and Schikora 2001).

CONCLUDING REMARKS

In addition to understanding the development and function of an important cell type, the studies of root hairs in Arabidopsis have provided a useful and simple model to uncover new insights into general principles of plant biology. One of these is the inherent plasticity or flexibility in the development of plant cells. Several examples are now known whereby root epidermal cells that embark on one differentiation program may be caused to switch to the alternate program upon a change in their position or in response to external factors. This includes the effect of laser ablation (Berger et al., 1998a) or ACC treatment (Tanimoto et al., 1995; Masucci and Schiefelbein, 1996) on root epidermal cell differentiation. Increasing evidence supports the idea that plasticity of this sort is important for plants to adequately respond to their environment. Hair density and length are strongly influenced by environmental factors including nutrients and light. Further, patterning can be overridden in response to iron deficiency to produce ectopic hairs. These mechanisms may ensure proper root-hair cell production in the event of extreme environmental conditions or rare abnormal cell divisions.

ACKNOWLEDGMENTS

We thank our colleagues in the root development community, including Farhah Assaad, Fred Berger, Christine Bernhardt, Tanya Bibikova, Liam Dolan, Simon Gilroy, Yan Lin, Alan Lloyd, David Marks, Myeong Min Lee, Roger Lew, Ben Scheres, Takuji Wada, and Amanda Walker, for their helpful discussions. We also thank Nicolas Baumberger, Timothy Colborn, Beat Keller, Moira Galway, Simon Gilroy, and Bob Ridge for figures.

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