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Root Hairs

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Roots hairs are cylindrical extensions of root epidermal cells that are important for acquisition of nutrients, microbe interac- tions, and plant anchorage. The molecular mechanisms involved in the specification, differentiation, and physiology of root hairs in Arabidopsis are reviewed here. Root hair specification in Arabidopsis is determined by position-dependent signaling and molecular feedback loops causing differential accumulation of a WD-bHLH-Myb transcriptional complex. The initiation of root hairs is dependent on the *RHD6* bHLH gene family and auxin to define the site of outgrowth. Root hair elongation relies on polarized cell expansion at the growing tip, which involves multiple integrated processes including cell secretion, endo- membrane trafficking, cytoskeletal organization, and cell wall modifications. The study of root hair biology in Arabidopsis has provided a model cell type for insights into many aspects of plant development and cell biology.

INTRODUCTION

Root hairs are long tubular-shaped outgrowths from root epider-mal 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 effec-tively increase the root diameter, root hairs are generally thought to aid plants in nutrient acquisition, anchorage, and microbe inter- actions (Hofer, 1991).

Root hairs in Arabidopsis have attracted a great deal of at-tention from plant biologists because they provide numerous advantages for basic studies of development, cell biology, and physiology (Schiefelbein and Somerville, 1990). 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 cu- ticle 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 impor- tantly, 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 grown on defined media in Petri dishes (Figure 2). Finally, the development of root hairs (and their

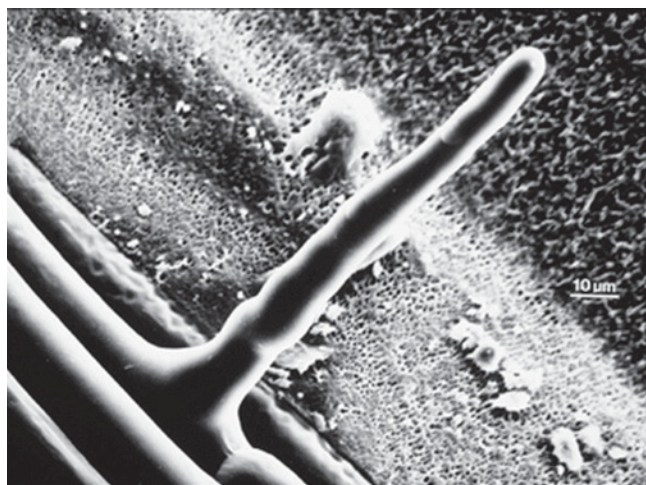Figure 1. Scanning electron micrograph of a root hair cell. The hair pro-duced by this cell is approximately 1/3 of its final length.

resident epidermal cells) occurs in a predictable and progressive manner in cells organized in files emanating from the root tip (Fig-ure 3). This provides the opportunity for detailed analysis of the cellular changes that occur during the entire process of root hair formation.
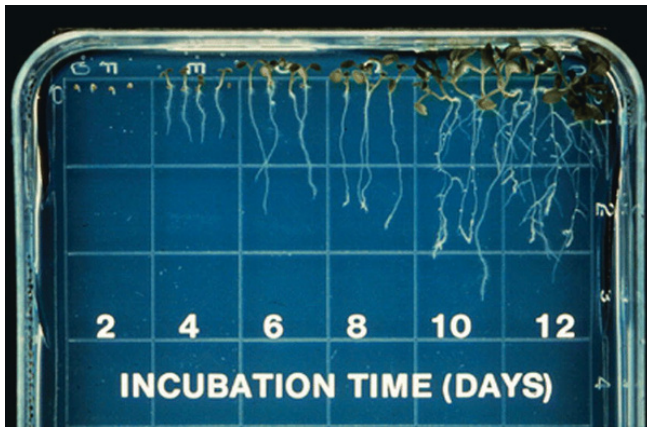
Figure 2. Development of Arabidopsis seedlings growing on agarose-solidified nutrient medium in vertically-oriented Petri plates. The roots grow along the surface of the medium, and root hairs are visualized easily using a low-magnification microscope.

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

This chapter provides a summary of the development, structure, and function of root hairs in Arabidopsis. Particular emphasis is placed on recent findings using molecular genetics to explore root hair development. Recent reviews emphasizing varied aspects of Arabidopsis root hairs have been published (Ishida et al., 2008; Schiefelbein et al., 2009; Tominaga-Wada et al., 2011; Benitez et al., 2011; Ryu et al., 2013).

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 first step in root hair development is the specifi- cation 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 pattern- ing in plants.

The Arabidopsis root epidermis is generated from a set of 16 initial (stem) 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). Further- more, 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 in the seedling root to vary from 18 to 22 (Galway et al., 1994; Baum and Rost,
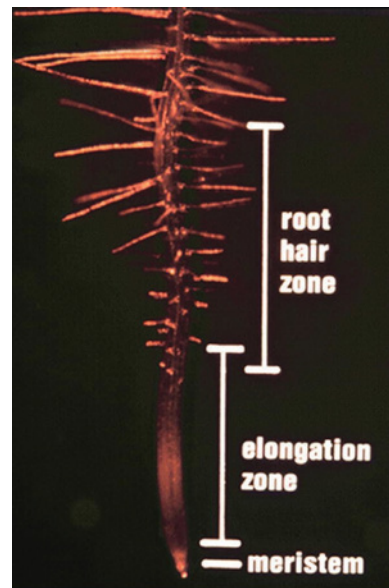
1996; Berger et al., 1998b). The epidermal cells are symplastically connected during much of their development (Duckett et al., 1994).

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; 1949; Bunning, 1951; Dolan et al., 1994; Galway et al., 1994). Root hair cells are present outside the intercellular space between two underlying cortical cells (i.e., located outside an anticlinal corti- cal cell wall, called the "H" position), whereas non-hair cells are present over a single cortical cell (i.e., located outside a periclinal cortical cell wall, called the "N" position) (Figure 4). Because the Arabidopsis primary root consistently possesses eight files of cor- tical cells, there are eight root-hair cell files 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 differ- entiation implies that cell-cell communication events are critical for the establishment of cell identity in the root epidermis.

An exception to this pattern exists near the root-hypocotyl junc- tion, in a region containing 3-7 tiers of cells called the collet (Par- sons, 2009). Here, every epidermal cell forms a root-hair-like exten- sion during early seedling growth (Scheres et al., 1994; Lin and Schiefelbein, 2007; Sliwinska et al., 2012). Consistent with this ex- ceptional pattern, genes that specify the non-hair fate are not active in this region (Lin and Schiefelbein, 2007). Interestingly, this region differs from the rest of the root by possessing a second (incomplete) layer of cortical cells (Lin and Schiefelbein, 2007), due to transition from the cellular anatomy of the hypocotyl (two cortical layers) to the root (one cortical layer). Furthermore, the root hairs in the collet arise synchronously, rather than the progressive formation of root hairs within cell files at the root apex (Sliwinska et al., 2012).
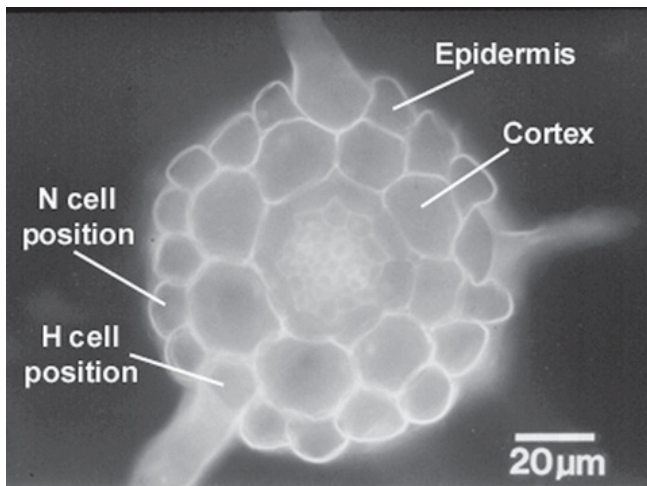
Figure 4. Transverse section of an Arabidopsis root, showing the position-dependent pattern of hair cells and non-hair cells. The hair-bearing cells are located outside the space separating two cortical cells (the H cell position), whereas the non-hair cells are located outside a single cortical cell (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.

Nature of the Cell Patterning Information

The information that directs the position-dependent epidermal cell pattern is 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 dif- ferentiating 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 use of two reporter gene fu- sions, a *GLABRA2* (*GL2: At1g79840*) 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 report- ers are expressed in the N-cell position (epidermal cells located outside a periclinal cortical cell wall) within the meristematic re- gion of the root (Figure 5). Careful examination using these sensi- tive 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 and/or their immediate daughters (Masucci et al., 1996; Berger et al., 1998a). The presence of differential gene expression in the early meri- stem led to the possibility that the epidermal cell pattern may be initiated during embryogenesis, when 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
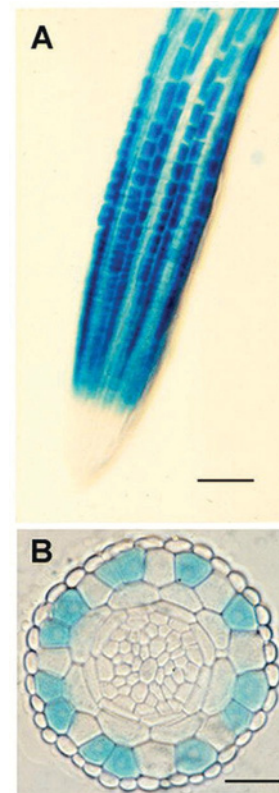

Figure 5. Expression of the *GL2::GUS* reporter fusion during root devel- opment.

(A) Surface view showing preferential expression in the meristematic re- gion. Bar = 50 μm.

(B) Transverse section showing preferential expression in the N-cell posi- tion of the epidermis. Bar = 20 μm.

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-dependent epidermal pattern that mirrors the post-embryonic pat- tern (Berger et al., 1998a; Lin and Schiefelbein, 2001). Thus, it appears that positional information is provided during embryonic root development and acts 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 re- sulting 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 ap- propriate for their new position (Figure 7). In a second set of ex- periments, specific differentiating epidermal cells were subjected to laser ablation such that neighboring epidermal cells were able
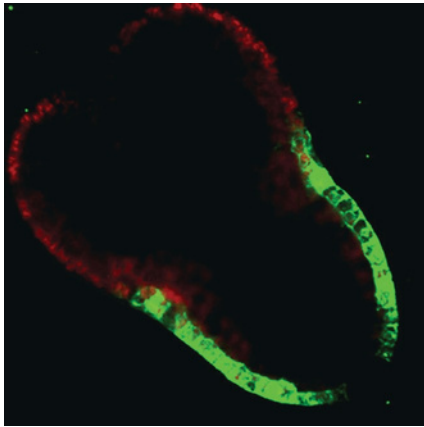
Figure 6. Embryonic expression of the *GL2::GFP* reporter fusion in the torpedo stage embryo. This median longitudinal view shows GFP accumu- lation in protodermal cells in the future hypocotyl and root.

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 were 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-embry- onically, not only embryonically, to ensure appropriate cell speci- fication in the Arabidopsis root epidermis.

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 epi- dermal 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, specific cortical cells of the J2301 line 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 abla- tion 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 pos- sess a disrupted pattern of root epidermal cell types (Sup- plemental Table 1; Figure 8). Mutations in the *WEREWOLF (WER: At5g14750), TRANSPARENT TESTA GLABRA (TTG: At5g24520), GLABRA3 (GL3: At5g41315)/ENHANCER OF GLA-*
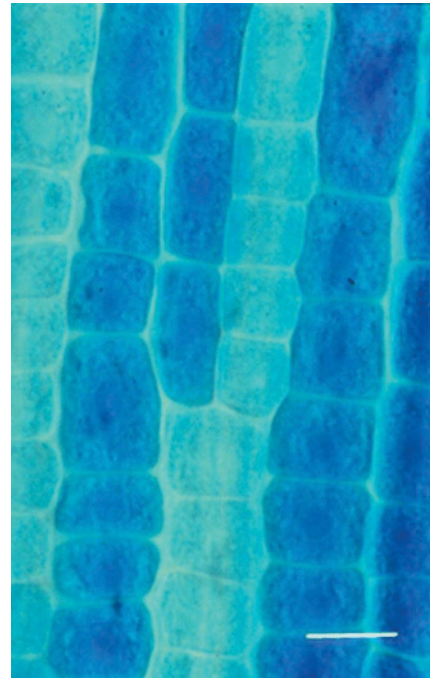
Figure 7. Expression of the *GL2::GUS* reporter fusion in an epidermal cell clone derived from a rare longitudinal division. Note that only one set of cells in the clone expresses the *GL2* marker. Bar = 10 μm.
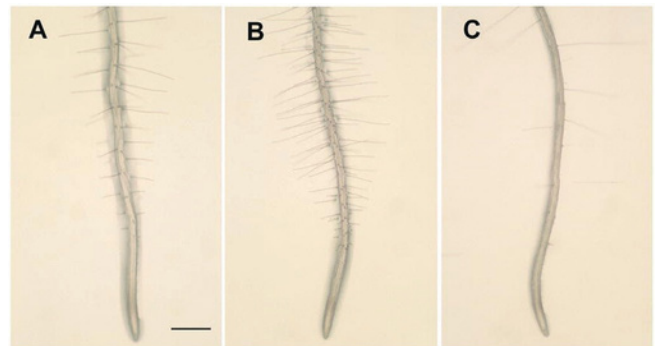
Figure 8. Root hair production in wild-type and cell specification mutants.

(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.

BRA3 (EGL3: At1g63650), and *GL2* genes cause root hairs to form on essentially every root epidermal cell, which implies that the normal role of *WER, TTG, GL3/EGL3,* and *GL2* is to promote non-hair cell differentiation and/or repress root hair cell differen- tiation (Galway et al., 1994; DiCristina et al., 1996; Masucci et al., 1996; Lee and Schiefelbein, 1999; Bernhardt et al., 2003). These mutations differ in their specific effects on non-hair cell differen- tiation; for example, 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 cellu- lar phenotypes (Galway et al., 1994; Masucci et al., 1996; Berger et al., 1998b; Lee and Schiefelbein, 1999). Thus, WER and TTG may be earlier (and more broadly) 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 preferentially expressed in developing epidermal cells in the N position, which are the cells whose fate is mis-specified in the *wer* mutant. In addition to the MYB DNA-binding domains, the WER protein possesses a phosphatidic acid (PA)-interacting region involved in nuclear localization (Yao et al., 2013).

Unlike *TTG* and *GL2*, the *WER* gene does not influence trichome development, seed coat muci- lage, or anthocyanin production. A closely related gene, *MYB23* (*At5g40330*), encodes a protein with a similar biochemical func- tion as WER, and it exhibits N-cell-specific expression that is dependent on WER, GL3/EGL3, and TTG (Kang et al., 2009).

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 its role, TTG is able to physically interact with the basic helix-loop-helix (bHLH) tran- scriptional activators GL3 and EGL3, which act in a partially func- tionally redundant manner (Bernhardt et al 2003). GL3 and EGL3 also physically interact with WER (Bernhardt et al., 2003; Song et al., 2011), which implies that a tripartite transcription factor complex is responsible for directing the non-hair cell fate.

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* ex- pression 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, GL3/EGL3,* and *TTG* genes; *wer* mutations nearly abolish *GL2* promoter activity, while *ttg* and *gl3 egl3* mutations reduce *GL2* promoter activity (Hung et al., 1998; Lee and Schiefelbein, 1999; Lin and Schief- elbein, 2001; Bernhardt et al., 2003). Because appropriate cell position-dependent *GL2* expression is present in the *ttg* and *gl3 egl3* mutants, but not the *wer* mutant, it implies that WER is most critical for conveying positional information for *GL2* expression. Taken together, the current view is that WER, TTG, and the GL3/ EGL3 proteins act in a transcriptional complex 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.

Another Arabidopsis gene, *CAPRICE* (*CPC: At2g46410*), af- fects root epidermis cell specification in a different manner. Rath- er 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. Inter- estingly, the *gl2* mutation is epistatic to *cpc*, which suggests that CPC acts in the WER/TTG/GL3/EGL3/GL2 pathway as a nega- tive 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 single R3 Myb repeat with the bHLH- and

DNA-binding domains but without a typical transcriptional activa- tion domain (Wada et al., 1997). Consistent with this structure, CPC appears to inhibit the function of the WER-GL3/EGL3-TTG complex by interfering with WER binding to GL3/EGL3 in a com- petitive manner (Lee and Schiefelbein, 2002; Tominaga et al., 2007; Song et al., 2011; Kang et al., 2013). Interestingly, CPC is capable of moving from cell-to-cell in the developing root, en- abling it to act as a signaling molecule to repress non-hair cell fate specification in neighboring H cells (Kurata et al., 2005; Kang et al., 2013). Its preferential accumulation in the H cells is pro- posed to be due to trapping of CPC protein by EGL3 (Kang et al., 2013). Because CPC transcription is positively regulated by the WER-GL3/EGL3-TTG complex, its negative effect on the action of this complex represents an intercellular negative feedback loop (Ryu et al., 2005). In addition to CPC, several related R3 Myb proteins have been shown to act in a partially redundant manner, including ones encoded by *TRIPTYCHON* (*TRY: At5g53200*) and *ENHANCER OF TRY AND CPC1* (*At1g01380*) (Schellman et al., 2002; Kirik et al., 2004; Simon et al., 2007; Serna, 2008; Wang et al., 2010).

Another transcriptional feedback loop affects expression of the *GL3* and *EGL3* genes. Although protein fusions of GL3 ac- cumulate in the N-cell position, the *GL3* and *EGL3* genes are preferentially transcribed in the H-cell position due to negative regulation by the WER-GL3/EGL3-TTG complex (Bernhardt et al., 2005). This implies that, like the CPC family proteins, the GL3 and EGL3 bHLH proteins may also move through plasmodes- mata; although in this case, from the H to the N cells. Based on mathematical modeling, these interlocking intercellular feedback loops have been suggested to provide stability and robustness to the establishment of the cell-type pattern (Savage et al. 2008).

The *SCRAMBLED* gene (*SCM: At1g11130*) differs from the preceding genes because its mutant phenotype does not elimi- nate one of the epidermal cell types but merely alters the dis- tribution of root hair and non-hair cells (Kwak et al, 2005). *SCM* encodes a leucine-rich-repeat receptor-like kinase (LRR-RLK) that appears to enable epidermal cells to perceive their relative cell position (Kwak et al, 2005), and as a result, they achieve distinct gene expression patterns and adopt appropriate fates. In- terestingly, the *SCM* gene is itself under the transcriptional feed- back regulation of the WER-GL3/EGL3-TTG complex, because N-position cells exhibit a complex-dependent reduction in SCM accumulation, relative to H-position cells (Kwak and Schiefelbein, 2008). This negative regulatory loop may serve to amplify SCM signaling in the H-position cells.

These molecular genetic findings led to a simple model for the control of root epidermal cell fate in Arabidopsis (Lee and Schief- elbein, 2002; Figure 9). In this model, the root hair cell fate is pro- posed 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 sets of transcription factors, the R2R3 WER and MYB23 proteins vs. the one-repeat Mybs CPC, TRY, and ETC1. These are able to form an active or inactive complex, respective- ly, with the TTG and GL3/EGL3 proteins. In immature epidermal cells in the N position, it is proposed that a relatively high level of WER is present and this leads predominantly to formation of the active complex, expression of *GL2* (and probably other genes), and non-hair cell differentiation. On the other hand, immature epi- dermal cells located in the H position are proposed to accumulate
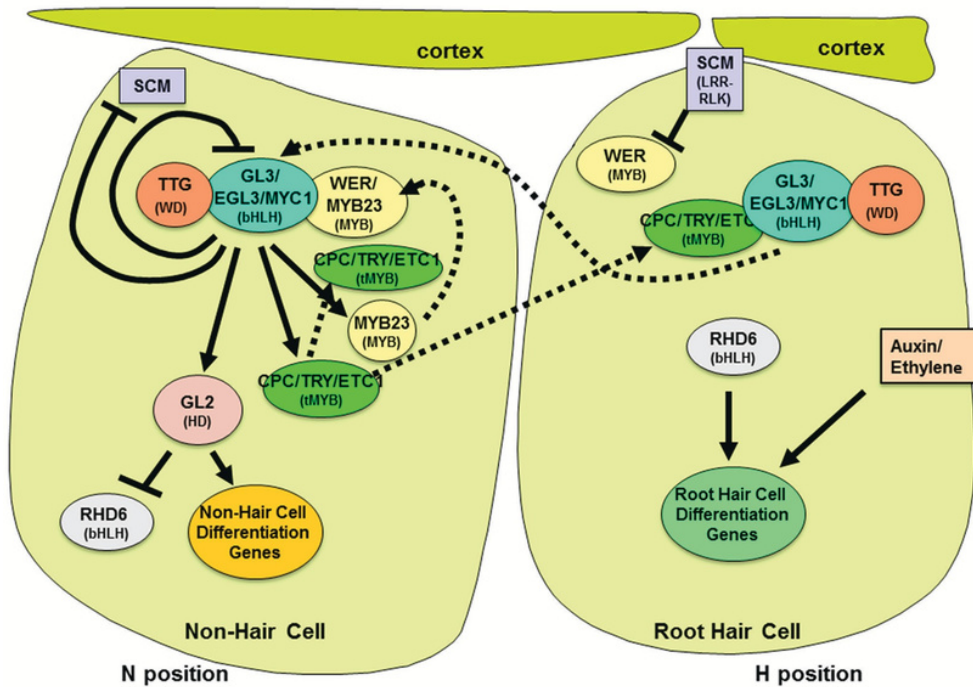
Figure 9. Model for the specification of the root hair and non-hair cell types in the Arabidopsis root epidermis. The proposed accumulation and interaction of cell fate regulators is shown within root epidermal cells destined to be root hair cells (in the H position) or non-hair cells (in the N position). In this model, the default fate for an epidermal cell is a root hair cell. Arrows indicate positive control, blunted lines indicate negative regulation, and broken lines indicate intercellular or intracellular protein movement. The effects on the downstream genes is modified from Bruex et al., 2012.

a relatively high level of CPC, which leads to inactive complexes, repression of *GL2,* and hair cell differentiation. The SCM receptor is proposed to mediate the effects of a position cue and initiate differential accumulation of the WER and CPC regulators. The downstream feedback loops, including the negative effect of CPC and positive regulation by MYB23, are thought to stabilize and amplify the initial asymmetry in gene expression patterns.

In addition to the genes described above, other loci have been defined by mutations that influence the specification of the root epidermal cells. These include the *ROOTHAIRLESS* (*RHL*) genes *RHL1 (At1g48380), RHL2 (At5g02820),* and *RHL3 (At3g20780),* and the *ECTOPIC ROOT HAIR* (*ERH*) genes *ERH1* (*At2g37940*), *ERH2/POM1* (*At1g05850*) and *ERH3* (*At1g80350*)
(Schneider et al., 1997), as well as the *TORNADO* (*TRN*) genes *TRN1/LOP1* (*At5g55540*) and *TRN2/TET1* (*At5g46700*) (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 three *RHL* genes encode components of DNA topoisomerase VI complex and are involved in endoreduplication (Guimil and Dunand, 2006). The RHL1 is nuclear localized, but it does not regulate *GL2,* which suggests that it acts in an independent genetic pathway that is required for the hair cell fate (Schneider et al., 1998, Sugimoto-Shirasu et al. 2005).

Only a few genes/proteins have been identified that likely act upstream of the transcription factors that determine epidermal cell fate. These include a gene affecting histone acetylation, *HISTONE DEACETYLASE18* (*HDA18*: *At5g61070*). Mutations in *HDA18* or treatment with trichostatin A (a histone deacetylase inhibitor) cause N-position cells to differentiate as root hair cells (Xu et al., 2005), implying that epigenetic factors also influence epidermal cell fate (Guimil and Dunand, 2006). Mutations in the *JACKDAW* (*JKD*: *At5g03150*) gene, encoding a putative zinc-finger transcription factor, cause multiple patterning defects in the Arabidopsis root, including abnormal arrangement of the hair and non-hair cell types (Hassan et al., 2010). JKD is likely to act indirectly to influence differential expression of the transcriptional regulators of epidermal patterning.

The WER-GL3/EGL3-TTG transcriptional regulatory complex described in this section influences the expression or activity of many downstream genes/proteins that control root-hair or non-hair cell differentiation. Large-scale identification of downstream genes has been conducted using several microarray-based transcript profiling strategies, including a morphogenesis mutant approach which identified genes differentially expressed in the *rhd2* mutant vs. wild-type (Jones et al., 2006), a cis-element-based approach centered on a putative root-hair specific element (Won et al., 2009) and cell-sorting approaches to identify genes preferentially expressed in root hair cells and non-hair cells (Brady et al., 2007) or genes that exhibit differential transcript accumulation in the hairy *ttg, wer myb23,* and *gl3 egl3* mutants relative to the
hairless *cpc try* double mutant (Bruex et al., 2012).

Computational Modeling of Root Epidermal Patterning

Recent advances in computational power and software have attracted investigators to apply computational modeling to the Arabi- dopsis epidermal patterning system. The efforts thus far have cen- tered on modeling the accumulation of the central transcriptional complex, largely based on the classic "reaction-diffusion" mecha- nisms described by Turing and others (Turing, 1952). These stud- ies have shown that simple rules, including local self-enhancement and long-range inhibition, can generate distinct patterns of gene expression in a field of cells that mimic the observed root epidermal pattern (Benitez et al., 2007; Savage et al., 2008).

Similarities in Epidermal Patterning in the Root and other Tissues

A close relationship exists between cell specification in the root and in the above-ground organs of the Arabidopsis plant. The most striking similarity is found in the epidermis of the hypocotyl. Although the hypocotyl does 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 is present outside an anticlinal cortical cell wall, equivalent to the H-cell position in the root epidermis. The other type of hypocotyl cell file possesses non-stomatal cells and is 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 un- dergo analogous position-dependent cell differentiation to gener- ate 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 epi- dermis is also apparent in the molecular components employed. The *wer*, *ttg*, and *gl2* mutants exhibit alterations in the pattern- ing 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), whereas *cpc* and *try* mutants have reduced stomata for- mation (Serna, 2008). 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/CPC/TRY/ GL2 pathway is employed in both organs of the seedling begin- ning 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, GL3/EGL3, CPC/TRY* and *GL2* genes are known to also affect trichome formation in the shoot epidermis of Arabidopsis (Koorn-
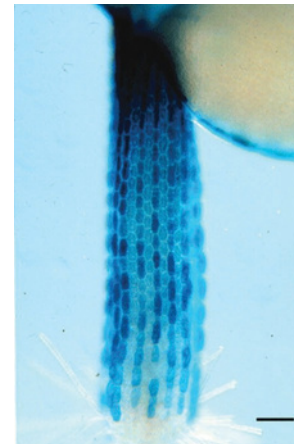

Figure 10. Expression of the *GL2::GUS* reporter fusion in the hypocotyl epidermis. Cells expressing the *GL2::GUS* marker are located in the N position. Bar = 100 µm.

neef, 1981; Koornneef et al., 1982; Larkin et al., 1997; Kirik et al., 2004; see also the chapter on trichomes in this book). Also, gene expression patterns suggest that the WRKY protein encoded by *TRANSPARENT TESTA GLABRA2* (*TTG2*; *At2g37260*) acts in both trichome and non-root hair cell specification (Johnson et al., 2002; Ishida et al., 2007; Simon et al., 2013). Interestingly, the shoot and root epidermis employ functionally equivalent MYB proteins, WER (in the root) and GLABROUS1 (*GL1*: *At3g27920*) (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 mecha- nism relies on the positional relationship between epidermal cells and underlying cortical cells whereas the leaf epidermis mecha- nism relies on sensing trichome density. A further interesting as- pect of this relationship is that the TTG, CPC/TRY, GL3/EGL3, 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 and hair-bearing (trichome) cells in the leaf. It may be that these proteins are part of an epidermal transcriptional cassette that has been recruited to participate in both 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 experi- ments indicate that auxin and ethylene help promote root hair cell differentiation in Arabidopsis. For example, treatment of Ara- bidopsis seedling roots with 1-amino-cyclopropane-1-carboxylic acid (ACC, an ethylene precursor) induces ectopic root hair cells (Tanimoto et al., 1995). Further, mutations affecting the *CON- STITUTIVE TRIPLE RESPONSE* (*CTR1: At5g03730*) locus, which encodes a Raf-like protein kinase proposed to negatively regulate the ethylene signal transduction pathway (Kieber et al.,

1993) also leads to some ectopic root hair formation (Dolan et al., 1994; Ikeda et al., 2009). 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 exhibited by mutations in the *AUXIN RESISTANT2* (*AXR2: At3g23050*; auxin, ethylene, and abscisic

acid resistant) and *AUXIN RESISTANT3* (*AXR3: At1g04250*; auxin and ethylene resistant) genes implicate auxin in root hair formation (Mizra et al., 1984; Wilson et al., 1990; Leyser et al. 1996). Furthermore, mutations in the *ROOT HAIR DEFECTIVE 6* (*RHD6: At1g66470*) bHLH gene (Menand et al., 2007), which cause a hairless root phenotype, can be suppressed by the inclu- sion of ACC or indole-3-acetic acid (IAA, an auxin) in the growth media (Masucci and Schiefelbein, 1994).

Although these hormones influence root hair cell differentiation, their role in the specification of epidermal cell fate is less clear. Re- sults from epistasis tests and *GL2* promoter-reporter gene analyses indicate that the ethylene/auxin pathway does not significantly affect the WER/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). Mutations in the *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 togeth- er, 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.

In addition to auxin and ethylene, other plant hormones influ- ence root hair development. The addition of brassinolide and *bri1* mutations cause opposite changes in the expression of cell fate regulators and in the root hair pattern, indicating that brassino- steroids act at an early stage to promote the hair cell fate (Kup- pusamy et al., 2009). Furthermore, the synthetic strigolactone GR24 increases root hair length, perhaps by interfering with aux- in-regulated cell expansion, suggesting a role for strigolactone in later stages of root hair formation (Kapulnik et al., 2011). Finally, application of jasmonic acid promoted root hair growth in a dose- dependent manner, which may require crosstalk with ethylene and auxin pathways (Zhu et al., 2006).

One of the general implications of the hormone research is that newly-formed epidermal cells in the Arabidopsis root may initially be defined as a trichoblast or atrichoblast (due to the cell-

position-dependent action of the intrinsic WER/TTG/GL2/CPC pathway) but the final differentiation/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.

Root Hair Initiation

Plant cells change shape by modifying their cell walls. After they are generated in the meristem, epidermal cells adopting 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 pro- cess is termed root hair initiation and is summarized in Figure 12.
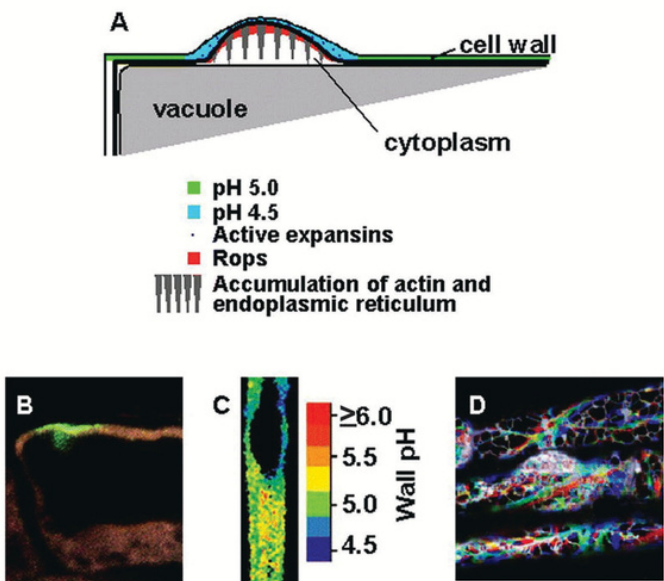
Figure 12. Root hair initiation.

(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 im- aged 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).
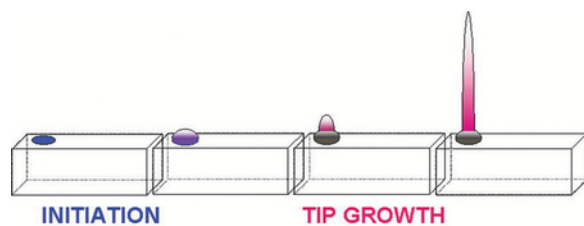

Figure 11. Stages of root hair formation. Root hairs form in two main stages: initiation, when a small, disc-shaped area of the cell wall loosens to form a swelling, and tip growth, when the remainder of the hair grows by targeted secretion.

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 small GTPases (e.g. Rac, Cdc42, and Rho) that control the morphogenesis of animal and yeast cells (Vernoud et al., 2003; Chant, 1999). Recent identification that the cytoplasmic domain of the receptor-like kinase FERONIA (*FER*: *At3g51550*) recruits the Rop proteins, and that loss of FER results in defective root hair growth, raises the possibility that this receptor assists in Rop accumulation at the apical plasma membrane domains in the tips of root hairs (Duan et al., 2010). Rops remain at the tip of the developing hair until growth ends (Molendijk et al. 2001). It is proposed that guanine nucleotide exchange factors for Rops (RopGEFs) are also required for FER-dependent action in root hair development (Huang et al., 2013).

Within minutes of Rop localization, the root hair cell wall begins to bulge out and 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; Monshausen et al., 2007). The mechanism responsible for the pH change is unclear; 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 F-actin accumulates (Baluska et al., 2000). Under optimal conditions, it requires about 30 minutes for a root hair swelling to form on the surface of the epidermal cell (Bibikova et al. 1998).
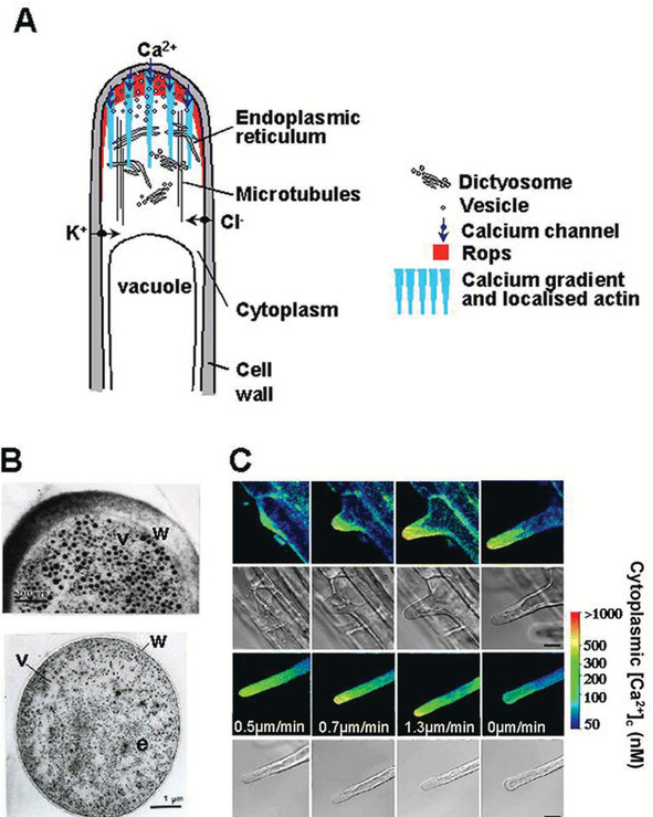
Root Hair Tip Growth

Features of Tip Growth. Once a swelling has formed, tip growth begins. Tip growth is an extremely polarized type of cell expansion that results in the formation of a tubular cell (Figure 13, 14). Tip growth is often rapid; Arabidopsis root hairs typically grow at a rate of 1 μm min−1 or more. Besides root hairs, other tubular- shaped cells including pollen tubes are formed by tip-growth. Tip growth is sustained by exocytosis of vesicles in the root hair apex. These vesicles contain cargo molecules such as cell wall polysaccharides and cell wall (glyco)proteins, which will be in- corporated into the newly-forming cell walls. In addition, integral membrane proteins, such as cell wall synthases and membrane transporter proteins are carried to the plasma membrane where they function in tip-growth expansion. It has been estimated that over 9000 exocytosis events per minute are required to sustain root hair tip growth (Ketelaar et al., 2008). These vesicles are produced by smooth and rough endoplasmic reticulum and Golgi complexes.

During tip growth, the cytoplasm is highly polarized. In the tube of growing root hairs, only a thin layer of cortical cytoplasm surrounds the large central vacuole while cytoplasm accumulates in the sub-apical and apical parts of the cell (Figure 13A, B). The nucleus is typically located at the base of the cytoplasmic dense area and tracks the tip at a fixed distance of approximately 75 μm until root hair growth stops (Figure 14) (Ketelaar et al., 2002). In the sub-apical cytoplasm all cellular constituents that sup- port tip growth accumulate. Together, they are referred to as the

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).

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.

tip-growth unit (Emons and Ketelaar, 2009). The extreme apex contains a large number of vesicles while other membrane struc- tures are absent from this area (Galway et al., 1997; Ketelaar et al., 2007). Continuous delivery of new vesicles is assisted by cytoplasmic streaming, the mixing cellular contents (Figure 13, Supplementary Movie 1. Ten second loop showing streaming at the tip of a growing root hair at x100 magnification.).

Calcium in Tip Growth. Root hair tip growth requires cal- cium (Schiefelbein et al., 1992). When Arabidopsis root hairs are 5- 10 µm long the Ca^{2+} concentration at the tip of the swell- ing 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). The calcium gradient at the tip of the hair appears to be part of the mechanism that controls the direction of growth, by facilitating fusion of exocytotic vesicles with the api- cal plasma membrane and subsequent delivery of cell wall cargo to the expanding cell wall (Figure 13) (Pei et al., 2012). If the calcium ion concentration is artificially increased toward one side of the tip, the hair will re-orient to grow in that direction (Bibikova et al., 1997). Further, treatment with a Ca^{2+} channel blocker (e.g. nifedipine) yields hairs with enlarged tips, presumably due to a breakdown in the calcium gradient and decentralized vesicle fu- sion at the tip (Schiefelbein et al., 1992).

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 mem- brane 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) trans- ported through the apoplast and deposited near the tip of the hair, or (3) released from intracellular stores (Ryan et al., 2001).

Rop GTPase proteins that are involved in root hair initiation (*hyperlink to initiation section above)* continue to function during tip growth. Plants overexpressing a mutant Rop GTPase that is permanently in the active (GTP-bound) form have balloon-shaped root hairs, suggesting that Rop GTPases must cycle from the GTP- bound form to the GDP-bound form for the direction of tip growth to be controlled (Jones et al, 2002). *RHO-RELATED PROTEIN FROM PLANTS2* (*ROP2:At1g20090*) initiates the generation of re- active oxygen species (ROS) by the NADPH oxidase, *ROOT HAIR DEFECTIVE2* (*RHD2*: *At5g51060*) (Foreman et al., 2003; Jones et al., 2007). ROP GTPase-stimulated ROS accumulation activates calcium channels in these cells, which in a positive feedback loop, further activates RHD2 oxidase activity via increased Ca^{2+} levels. While the identity of this Ca^{2+}-channel remains uncharacterized, members of the cyclic-nucleotide-gated channel family of proteins have been implicated in this process in pollen tubes, and may play similar roles during the formation of this gradient in root hair cells as well (Frietch et al., 2007). This tip-focused Ca^{2+} gradient is main- tained as long as tip growth occurs in these cells, and it disappears when these cells stop growing (Wymer et al., 1997).

Actin in Tip Growth. In addition to tip-focused Ca^{2+} gradients, the actin cytoskeleton plays a prominent role in root hair growth, firstly by acting as a backbone over which cytoplasmic streaming occurs and secondly by organizing the cytoplasm of the subapical region during tip growth (Figure 1). When the actin cytoskeleton is depolymerized by treatment with actin inhibitors, tip growth in both pollen tubes and root hairs is inhibited (Gibbon et al., 1999; Baluska et al., 2000; Ketelaar et al., 2002; 2003). However, al- though F-actin accumulates in the tips of newly initiated root hair bulges, treatment with Latrunculin B, which interferes with F- actin polymerization, did not block bulge formation. This indicates that selection of sites for future root hair tip-growth and bulge forma- tion are both independent of actin (Baluska et al., 2000).

Once tip-restricted elongation of the root hair is initiated, the actin organization is highly polarized. In the root hair tube, thick bundles of F-actin support cytoplasmic streaming in the cortical cytoplasm (refer to Root hair tip growth section). In the (sub)api- cal part of elongating root hairs, the accumulation of cytoplasm is supported by an F-actin configuration that correlates with root hair tip growth: fine F-actin. Fine F-actin is a dense and highly dynamic actin configuration that is much more sensitive to ac- tin inhibitors than the thick actin bundles located in more distal regions of the root hair tube. When root hairs are treated with low concentrations of actin inhibitors, the fine F-actin is specifi- cally depolymerized; cytoplasmic streaming continues and thick F-actin bundles loop through the tip. This results in the disappear- ance of the (sub)apical accumulation of cytoplasm and growth inhibition (Miller et al., 1999; Ketelaer et al., 2002, 2003; Ketelaar, 2013). When treated with a lower concentration of actin inhibitors, the fine F- actin is partially depolymerized, growth continues, but tip diameter increases (Ketelaar et al., 2003). This suggest that fine F-actin plays a role in determining root hair width. Similar ob- servations has been made in pollen tubes (Gibbon et al., 1999).

Acting Binding Proteins (ABPs). ABPs play an important role in determining the higher-order organization of the actin cytoskeleton. ABPs can be divided into several groups: actin nucleating proteins, proteins that increase actin turnover, actin- crosslinking proteins, and motor proteins (Ketelaar, 2013). The activity of some ABPs is regulated by the local concentration of calcium ions. It has been hypothesized that the modulation of ABP activity by the tip-focused Ca^{2+} gradient is responsible for the formation and maintainance of fine F-actin in the tip of grow- ing root hairs (Ketelaar, 2013; Pei et al., 2012). ABPs that have a known function in actin organization during root hair growth are discussed below. It is likely that many more ABPs function in actin organization during root hair growth and remain to be identified due to redundancy with proteins from the same or dif- ferent families.

Actin nucleating proteins include the Arp2/3 complex (Higgs and Pollard, 2001) and formins (Cvrcková et al., 2004). The Arp2/3 complex nucleates a new actin filament from the side of an existing filament with a fixed angle of 70 degrees (Higgs and Pollard, 2001). In root hairs of some Arabidopsis mutants with disrupted Arp2/3 complex activity, wavy, swollen and branching root hairs with reduced length have been observed (Mathur et al., 2003a; 2003b), while defects in tip growth are absent in others (Brembu et al., 2004; El-Assal Sel et al., 2004; El-Din El-Assal et al., 2004).

Formins employ the large pool of actin monomers that is bound to the actin sequestering protein profilin for actin nucleation and polymerization (Michelot et al., 2005; Ye et al., 2009; Vidali et al., 2009; Zhang et al., 2011a; Van Gisbergen and Bezanilla, 2013).

Plant formins can be divided into two classes: class 1 formins that generally posess a trans-membrane domain and class 2 formins that display a variety of interactions (Van Gisbergen and Bezanilla, 2013). Formins have been identified as key regulators in tip growing pollen tube and moss protonemal cells (Staiger et al., 2010; Van Gisbergen and Bezanilla, 2013) by specifically nu- cleating actin filaments in the subapical cytoplasmic domains of these tip-growing cells (Cheung et al., 2010). The role of formins in root hair growth has been less well studied. Overexpression of the Arabidopsis class 1 formin gene *FORMIN8* (*FH8*: *At1g70140*) cause an accumulation of F-actin in growing root hair tips and defects in actin organization and growth defects such as growth reduction, tip swelling, wavyness and branching (Yi et al., 2005). Using an approach in which FH8 that lacked the actin-nucleating FH2 domain is overexpressed, Deeks et al. (2005) showed that the amount and length of root hairs was reduced.

Profilins are actin monomer sequestering proteins. While profilin-bound actin monomers cannot spontaneously nucleate or polymerize (Valenta et al., 1993; Gibbon et al., 1998), they can be used for formins mediated actin filament nucleation and elongation (see F-actin nucleating proteins). Overexpression of the Arabidopsis profilin gene *PROFILIN1* (*PFN1*: *At2g19760*) causes the formation of root hairs that are twice as long as root hairs in wild type plants (Ramachandran et al., 2000).

Actin Depolymerizing Factor (ADF) binds both G-actin and F-actin. It increases actin turnover by severing actin filaments and increasing the dissociation rate from their minus ends (Maciver and Hussey, 2002). The actin severing activity from the maize ADF, ZmADF3, is enhanced by high Ca2+ concentrations through phosphorylation by a Calcium Dependent Protein Kinase (CDPK; Smertenko et al., 1998; Allwood et al., 2001). This suggests that, in tips of growing root hairs, enhancement of ADF activity plays a role in fine F-actin formation. Root hairs of Arabidopsis plants that overexpress the *ACTIN DEPOLYMERIZING FACTOR1* (*ADF1*: *At3g46010*) gene have irregular actin organization and are shorter (Dong et al., 2001). Root hairs with reduced *ADF1* expression are longer and possess more longitudinally oriented actin cables (Dong et al., 2001).

ACTIN INTERACTING PROTEIN (*AIP1*: *At2g01330*), an evolutionarily conserved WD repeat protein, enhances ADF activity (Allwood et al., 2002; Staiger et al., 2010). In root hairs of Arabidopsis lines in which *AIP1* expression is reduced by RNAi, bundled F-actin cables reach into the apical area of growing root hairs that normally contains fine F-actin. The growth velocities of these root hairs are up to 5-fold reduced as compared to wild-type hairs (Ketelaar et al., 2004). When *AIP1* is overexpressed, root hair tip swelling and inhibition of root hair growth occur. In these root hairs, the actin cytoskeleton formed very prominent bundles that looped through their tips (Ketelaar et al., 2007).

Besides a role in cAMP production (Moutinho et al., 2001), the cyclase-associated protein family member CYCLASE-ASSOCIATED PROTEIN 1 (*CAP1: At4g34490*) serves as a nucleotide exchange factor for actin monomers that increases ADP to ATP substitution rates over 50-fold (Chaudhry et al., 2007). In root hairs of Arabidopsis *cap1* mutants both actin and cytoplasmic organization are severely disrupted and root hair growth velocities are decreased. This results in the formation of shorter root hairs (Deeks et al., 2007). When Arp2/3 complex and CAP1 are disrupted simultenously, root hair growth is aborted after bulge formation, suggesting that CAP1 and the Arp2/3 complex function synergistically in actin organization during root hair growth (Deeks et al., 2007).

Villins are proteins that bundle actin filaments. Most plant villins have, besides an actin bundling activity, Ca2+/calmodulin dependent actin severing, F-actin capping and monomeric actin binding activities (Tominaga et al., 2000; Yokota et al., 2005; Huang et al., 2005; Khurana et al., 2010; Zhang et al., 2010; Zhang et al., 2011b). Thus, while villins are actin bundling proteins at low Ca2+ concentrations, they increase actin turnover at higher Ca2+ concentrations. Mutations in the *VILLIN4* (*VLN4: At4g30160*) gene form root hairs with a finer actin organization, lower elongation rates, and decreased cytoplasmic streaming rates (Zhang et al., 2011b). Disruption of the lily villin 135-ABP by microinjection of a specific antibody against this protein causes defects in actin bundling In fully grown root hairs from *Hydrocharis dubia* (Tominaga et al., 2000). These observations suggest a role for villins in actin bundling during root hair growth. Ca2+ dependent actin severing and capping activities of villin are involved in the regulation of actin dynamics in the apical area of pollen tubes (Qu et al., 2013). Since pollen tubes possess a similar tip-focused Ca2+ gradient, it is likely that the Ca2+-dependent functions of villins also contribute to the regulation of actin dynamics in the tips of growing root hairs.

Myosin XI motor proteins function in actin-dependent organelle trafficking and actin-mediated cytoplasmic restructuring in plants (Lee and Liu, 2004; Reisen and Hanson, 2007; Hoffmann and Nebenführ, 2004; Van der Honing et al. 2010). The Arabidopsis genome contains 13 myosin XI genes (Reddy and Day, 2001). Reduced root hair growth and altered actin organization is observed in the *MYOSIN XI K* (*ATXIK: At5g20490*) mutant (Park and Nebenfuhr, 2013), as well as lines in which multiple myo- sin XI genes are disrupted (Ojangu et al., 2007; Prokhnevsky et al., 2008; Peremyslov et al., 2008; Peremyslov et al. 2010). Also, myosin XI motor activity is decreased under high Ca2+ conditions (Tominaga et al., 2012), resulting in reduced cytoplasmic streaming velocities (Doree and Picard, 1980; Woods et al., 1984; Takagi and Nagai, 1986; Kohno and Shimmen, 1988). This Ca2+ dependent inhibition of myosin XI may explain the reduced cytoplasmic streaming velocity that is observed in growing root hair tips (Sieberer and Emons, 2000).

The Microtubule Cytoskeleton. As in other plant cells, root hairs possess a prominent array of cortical micortubules (CMTs; Sieberer et al., 2005). In Arabidopsis, CMTs in the root hair tube are oriented net-axially, i.e. with their average orientation parallel with the long axis of the tube (Ketelaar et al., 2002; Van Bruaene et al., 2004). When cytoplasm starts to accumulate during the initiation of root hair tip growth, a dynamic array of endoplasmic microtubules (EMTs) accumulates in the subapical cytoplasmically dense area (Van Bruaene et al., 2004). The EMTs originate from the perinuclear cytoplasm and run axially towards the hair tip (Van Bruaene et al., 2004). Although a bundle of EMTs occasionally reaches into the extreme tip, generally both CMTs and EMTs are absent from the extreme apex of growing root hairs (Marc et al., 1998; van Bruaene et al., 2004). Since microtubules are sensitive to increased Ca2+ concentrations (Cyr, 1991, 1994), the high Ca2+ concentration in the tip of growing root hairs may prevent them from polymerizing into the tip (Sieberer et al., 2005).

Microtubule organization in root hairs is disrupted by alterations in a gene encoding an armadillo repeat-containing kinesin-related protein, *MORPHOGENESIS OF ROOT HAIR 2* (*MRH2/ARK1 At3g54870*). Null mutants of *MRH2/ARK1* show an increased amount of EMTs and root hairs are wavy and occasionally branch, suggesting that MRH2/ARK1 regulates tip growth by limiting the assembly and distribution of endoplasmic microtubules (Jones et al., 2006; Sakai et al., 2008). Furthermore, the *mrh2/ark1* mutations act as genetic enhancers of the *CA-rop2* phenotype. Interestingly, an armadillo repeat domain-containing MRH2/ARK1 fragment could bind to polymerized actin in vitro and the *mrh2/ark1* mutant showed increased sensitivity to actin depolymerization. Thus, MRH2/ARK1 not only controls MT organization in root hairs, it may also be involved in the coordination of actin and microtubule organization during root hair growth (Yang et al., 2007).

When Arabidopsis root hairs are treated with the microtubule inhibitor oryzalin or microtubule stabilizer paclitaxel (taxol), their growth velocity does not change, but their growth direction becomes wavy and occasionally root hairs branch (Bibikova et al., 1999; Ketelaar et al., 2002; Preuss et al., 2004). This suggests that microtubules help maintain a straight growth axis, but are not directly involved in maintenance of tip-restricted expansion rates. When root hair tip growth is transiently disrupted, the function of microtubules in root hair growth becomes even more clear: in the presence of microtubules, root hair growth recovers in the original orientation, whereas if microtubules are non-functional, the direction of hair growth recovery is random (Bibikova et al., 1999; Ketelaar et al., 2002; Figure 15). Taken together, these observations suggest that the microtubule cytoskeletal network functions only indirectly in controlling root-hair tip growth in Arabidopsis. However, it likely plays a more prominent role when changes in root hair growth direction are necessary, such as when root hairs curl around nitrogen-fixing bacteria during the formation of root nodules in legumes (Sieberer et al., 2005).
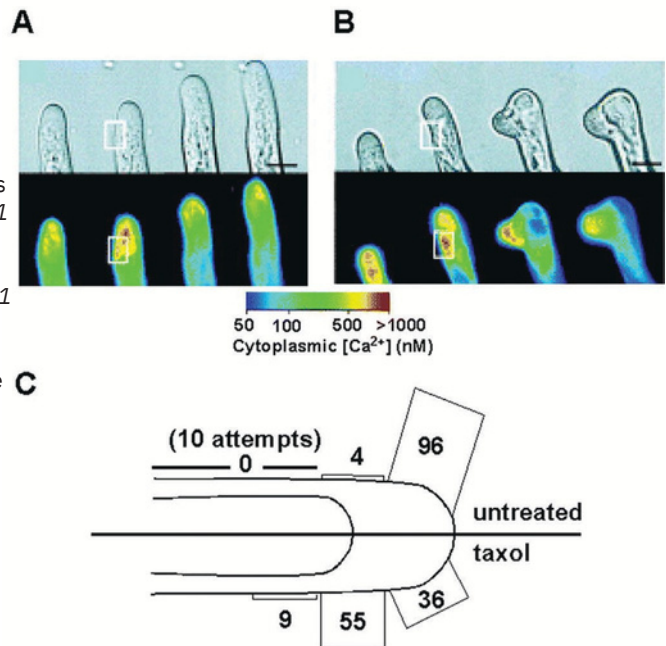

Figure 15. The direction of tip growth is controlled by calcium and microtubules.

(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).

Polarized Membrane Trafficking in Root Hairs

Polarized expansion of root hairs is dependent upon the continuous formation and delivery of newly-synthesized cell wall components to the growing tips of these cells; as a result, the control of mem- brane trafficking plays a critical role in root hair formation. Members of the Rab GTPase family are important regulators of eukaryotic membrane trafficking, and they carry out these regulatory functions through their specific localization to distinct subcellular organelles within these cells (Zerial and McBride, 2001; Vernoud et al., 2003; Nielsen et al. 2008). During root hair tip growth, newly-synthesized cell wall components are packaged into secretory vesicles that emerge from plant trans-Golgi network (TGN) membranes, and are subsequently targeted and delivered to the apical plasma mem- brane region of growing root hairs. In Arabidopsis, formation and delivery of at least one subpopulation of these secretory vesicles is regulated by the specific recruitment of members of the RabA family of plant Rab GTPases to these membrane compartments (Preuss et al., 2004; Ovecka et al., 2010; Kang et al., 2011). In their active, GTP-bound conformation, Rab GTPases function through the re- cruitment of cytosolic "effector proteins" to their target membranes. During tip-growth in Arabidopsis root hairs, RAB GTPASE HOMO-

LOG A4b (*RAB-A4b: At4g39990*) recruits the lipid kinases, PHO-SPATIDYLINOSITOL 4-OH KINASE BETA1 (*PI4Kβ1: At5g64070*) and PHOSPHATIDYLINOSITOL 4-OH KINASE BETA2 (*PI4Kβ2: At5g09350*), which phosphorylate the inositol head group of phos- phatidylinositol (PI) to generate PI-4P (Mueller-Roeber and Pical, 2002). Regulation of PI-4P levels through the coordinated action of these lipid kinases, as well as a PI-4P phosphatase, ROOT HAIR DEFECTIVE4 (*RHD4: At3g51460*), play important roles in regula- tion and delivery of secretory cargo to the tips of growing root hairs (Preuss et al., 2004, 2006; Thole et al., 2008). Additionally, phos-

phorylation of PI-4P to PI-4,5P2 by 1-PHOSPHATIDYLINOSITOL-4-PHOSPHATE 5-KINASE3 (*PIP5K3: At2g26420*) is required for appropriate control of tip-growth in root hairs (Kusano et al., 2008; Stenzel et al., 2008). Enrichment of PI-4P and PI-4,5P2 likely par- ticipate in organizing polarized membrane trafficking in root hair cells by acting as targeting determinants that stimulate recruitment of other proteins playing important roles in root hair tip-growth that contain PI-4P or PI-4,5P2 binding domains (Bubb et al., 1998; Yoo et al., 2012).

Root Hair Cell Walls

Root hair cell walls are organized in two distinct layers that appear to reflect when and where they are deposited during root hair development. Initial cell wall deposition occurs at the extreme apical 30-50 μm of the growing root hair (Newcomb and Bonnett, 1965; Galway et al., 1997). Fibrillar elements that may be cellulose microfibrils are observed in these apical root hair cell walls, but unlike in cells undergoing diffuse expansion, these elements appear to be shorter and randomly oriented (Newcomb and Bonnett, 1965). Additional inner cell wall lay- ers containing parallel arrays of cellulose microfibrils appear to be deposited later, and these are often organized in a helical orientation along the length of the root hair. Cellulose synthesis is required for root hair tip growth (Park et al., 2011; Galway et al., 2011), and while known components of the cellulose synthase complexes CESA3 and CESA6 are not observed in apical plasma membranes in actively growing root hair cells, a functional, fluorescently-tagged CELLULOSE SYNTHASE- LIKE PROTEIN D3 (*CSLD3/KJK/RHD7: At3g03050*) was en- riched at the tips of growing root hairs (Park et al., 2011). Both *CSLD2* (At5g16910) and *CSLD3/KJK/RHD7*, members of the Cellulose-Synthase-Like (CSL) super family, are required for root hair growth (Wang et al., 2001; Bernal et al., 2008; Galway et al., 2011; Yin et al., 2011; Yoo et al., 2012). While mannan synthase activity has been detected for some CSLD proteins (Yin et al., 2011), a CSLD3 chimera containing a CESA6 cata- lytic domain restored root hair growth in a *csld3* null mutant (Park et al., 2011), raising the intriguing possibility that CSLD proteins may synthesize cellulose-like polysaccharides in tip- growing root hair cells.

Xyloglucan and pectin cell wall polysaccharides are also re- quired for tip-growth in root hair cells. Xyloglucan is a β-1,4 linked glucan polymer with extensive side chain modifications contain- ing xylose, galactose, and fucose sugars. Members of the *CSLC* family synthesize the glucan backbone of xyloglucan (Cocuron et al., 2007), while xylosyl residues are attached to the glucan backbone by a series of xylosyltranferases, encoded by *XY- LOGLUCAN XYLOSYL TRANSFERASE*1 (*XXT1: At3g62720*), *XXT2* (At4g02500), and others. *Arabidopsis xxt1/xxt2* double mu- tants grew normally, but root hairs in these mutants are shorter and possess bulged bases (Cavalier et al., 2008). Recently, XY- LOGLUCAN-SPECIFIC GALACURONOSYL TRANSFERASE1 (*XUT1: At1g63450)* was shown to be a galacturonosyltransferase that incorporates galacturonic acid residues into root-hair-specific xyloglucan polysaccharides (Won et al., 2009; Peña et al., 2012). Homogalacturonan (HG), rhamnogalacturonan I (RG-1) and rhamnogalacturonan II (RG-II) are three major forms of pec- tin and together these polysaccharides comprise ~35%-40% of Arabidopsis primary cell walls. Disruption of genes encoding UDP-4-KETO-6-DEOXY-D-GLUCOSE-3,5-EPIMERASE-4-RE- DUCTASE1 (*UER1: At1g63000)* or UDP-D-GLUCURONATE 4- EPIMERASE 6 (*GAE6: At3g23820*), two enzymes required for synthesis of pectin precursors, affected root hair length (Pang et al., 2010). Furthermore, FucA1, a sugar analog fucose alkyne, is selectively incorporated into cell walls in newly initiated root hair bulges, suggesting a prominent role for these polysaccharides during root hair initiation (Anderson et al., 2012).

In addition to the major polysaccharide classes, numerous classes of structural proteins are secreted and incorporated into growing plant cell walls. In Arabidopsis, two members of the expansin family of cell wall proteins, EXPANSIN A7 (*EXPA7: At1g12560*) and EXPANSIN A18 (*EXPA18: At1g62980*), exhibit root-hair-specific expression (Cho and Cosgrove, 2002; Jones et al., 2006; Won et al., 2009; Bruex et al., 2012), and altering their levels by treatment with exogenous protein preparations or by RNAi-based suppression, resulted in altered root hair tip growth (Cosgrove et al., 2002; Lin et al., 2011). Many hydroxyproline-rich glycoproteins (HRGPs) are found in cell walls (Velasquez et al., 2011; Mohnen and Tierney, 2011). Proline residues in these pro- teins are post-translationally modified to form hydroxyproline, in a process catalyzed by a family of membrane-bound prolyl 4-hy- droxylases (P4Hs) (Velasquez et al., 2011; Mohnen and Tierney, 2011). Elimination of the root-expressed *PROLYL 4-HYDROXY- LASE* (*P4H*) genes, *P4H2* (*At3g06300*), *P4H5* (*At2g17720*), or *P4H13* (*At2g23096*) resulted in shortened root hairs (Velasquez et al., 2011), which indicate important roles for HRGPs during deposition of plant cell walls in tip-growing root hair cells.

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 ac- tively accumulate several osmotically active ions including K+ and Cl- as they grow (Figure 13), but other, unidentified mechanisms are also used to adjust turgor (Lew, 1991; 1998). Experiments us- ing pressure probes and osmotica have been used to show that turgor is regulated by sensing changes in osmolarity, not internal pressure (Lew, 1996).

Cessation of Tip Growth

Root hair tip growth ceases when hairs reach a fairly predictable length. Growth can be prolonged by increasing the expression of the small GTPase ROP2 (Jones et al., 2002) or the transcription factor ROOT HAIR SIX-LIKE4 (*RSL4/bHLH54: At1g27740*; Yi et al., 2010). Growth is sustained by auxin (Lee and Cho 2006), which is supplied by neighboring non-hair cells that transport auxin through the epidermis from the root tip toward the shoot (Jones et al., 2009). As the root tip grows away from young root hair cells, they receive less and less auxin, until the auxin supply is lost and growth ceases (Jones et al., 2009). 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). Simultaneously, Rop protein (Molendijk et al., 2001), the calcium gradient (Figure 13C; Wymer et al., 1997), and calcium channel activity (Schief- elbein et al., 1992; Very and Davies, 2000) are lost from the tip.

Molecular Genetics of Root Hair Formation

Supplemental Table 1 lists genes that affect root hair development and Figure 16 shows the stage each gene is involved. Genes are discussed here in the order in which they contribute.

Genes affecting the number of swellings on each hair cell. Wild-type root hair cells produce a single swelling on their outer wall, which develops into a hair. The *RHD6* gene is required to initiate this process: *rhd6* mutants are nearly hairless, and among the few cells that produce hairs, some of them initiate more than one hair (Masucci and Schiefelbein, 1994). This suggests that *RHD6* is involved in controlling the establishment and the num- ber of swellings. Hair cells on roots mutated in the *TINY ROOT HAIR1* (*TRH1: At4g23640*) gene sometimes have extra swellings (Rigas et al., 2001), implying that *TRH1* prevents multiple swell- ings from forming on wild type hair cells. *TRH1* encodes a potas- sium transporter (Rigas et al., 2001). Further research is required to discover how this affects swelling number. Extra swellings are also produced if there is excess ROP activity, for example on plants with mutations in the RhoGDI1 negative regulator SUPER- CENTIPEDE (*SCN1: At3g07880*) or plants overexpressing ROP2 (Carol et al., 2005; Jones and Smirnoff, 2006).

Genes affecting the location of hairs on the cell. Root hairs of many species including Arabidopsis emerge at the root-ward end of the cell (Leavitt, 1904; Masucci and Schiefelbein, 1994). The Arabidopsis *RHD6* gene affects this positioning. Hairs on *rhd6* mutant roots emerge at a more shoot-ward position (further away from the root tip; Figure 17), implicating *RHD6* in mechanisms that promote 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. The location of hairs on the cell is also affected by auxin and ethylene (*hyperlink to the section "Auxin and ethylene regulate root hair growth" below*).

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). Mutations in the TIP GROWTH DEFECTIVE1 (*TIP1: At5g20350*) and *ROOT HAIR DEFECTIVE1* (*RHD1: At1g64440*) genes generate mutant plants with 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, presumably by re- stricting 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 affect swelling size unless the RHD1 gene product is present (Parker et al., 2000). *RHD1* encodes a UDP-glucose 4-epimerase, which is involved in cell wall synthesis, suggesting that swelling formation on *rhd1* mutants is due to altered cell wall properties (Seifert et al., 2002). *TIP1* encodes a palmitoyl (or S-acyl) transferase (Hemsley et al. 2005). Palmitoyl trans- ferases regulate protein localization and activity at cell mem- branes, so it is likely that the *tip1* mutant phenotype is due to the mislocalization or misregulation of one or more unidentified proteins in root hair cells.

Genes establishing tip growth. Tip growth is established by the time hairs reach 40 μm long (Dolan et al., 1994). Root hairs without functional *RHD2* (Figure 19), *SHAVEN1* (*SHV1: un- known gene*), *SHV2/MRH4/COBL9* (*At5g49270*), *SHV3/MRH5*
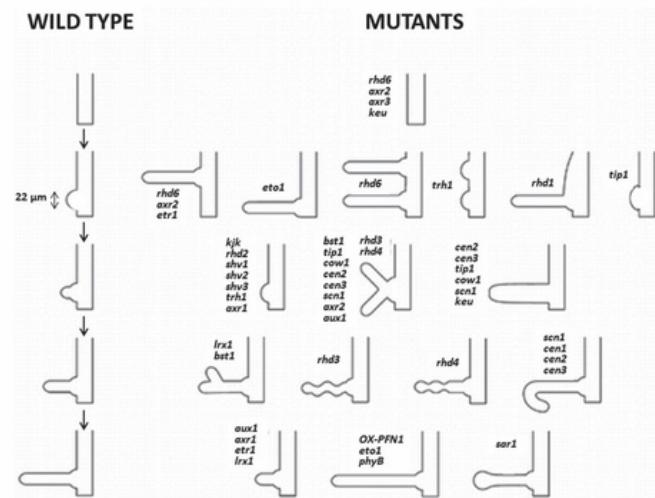
Figure 16. Genetics of root hair morphogenesis.

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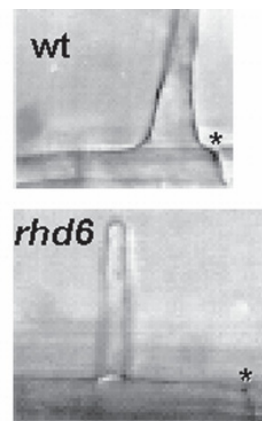
Figure 17. Hairs on the *rhd6* mutant emerge at a more basal position on the hair cell than wild type hairs (wt). An asterisk (*) indicates the apical wall of each cell.

(At4g26690), TRH1, or 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 affect- ing the CENTIPEDE1 (CEN1: unknown gene), CEN2 (unknown gene), CEN3 (unknown gene), SCN1, BRISTLED1 (BST1/MRH3: At5g65090), 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 im- portant for tip growth to be successfully established.

Sustained root hair tip growth involves oscillations of extra- cellular pH, reactive oxygen species, and cytosolic calcium. In- terfering with these oscillations can prevent root hair elongation or induce bursting at the tip (Monshausen et al. 2007, 2008). Hairs can also burst because wall loosening does not stop, or the balance between wall deposition and protoplast growth fails. For example, the hairs of kjk mutants burst after swelling forma- tion, killing the cells (Favery et al., 2001; Wang et al., 2001). As described earlier in this chapter, the KJK/CSLD3/RHD7 gene encodes a cellulose synthase- related protein. Interestingly, cel- lulose 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. SHV2 and SHV3 also encode proteins that affect cell wall synthe- sis. The SHV2/COBL9/MRH4 gene encodes a glucosylphospha- tidylinositol (GPI)-anchored protein related to COBRA, which af- fects cellulose deposition (Parker et al. 2000; Jones et al., 2006). SHV3 encodes a glycerophosphoryl diester phosphodiesterase- like protein that affects cellulose content and pectin modification. Adding borate to the growth medium of shv3 mutants prevents the root hairs from bursting, possibly by altering the cross-linking of pectic polysaccharides (Hayashi etl a. 2008).

Root hair growth may also stop if a crucial part of the machin- ery supporting tip growth fails. The TRH1 potassium transporter is required for tip growth, and supplementing trh1 mutant roots with high levels of potassium does not restore tip growth (Rigas et al., 2001). These results suggest that tip growth depends on potassium transport that is precisely localized within the cell or coordinated with other events. The expression pattern of the
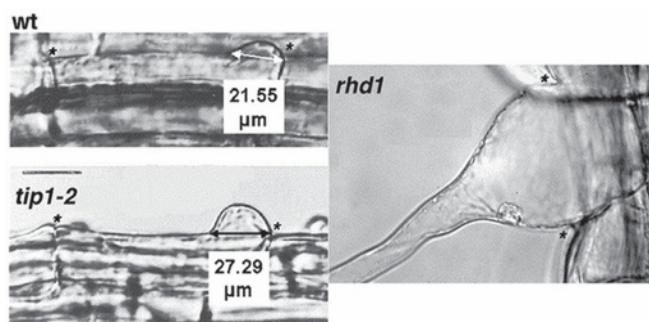
DR5::GUS auxin reporter and measurements of auxin concen- tration in wild type and mutant roots reveal that trh1 mutant roots have altered auxin distribution, further supporting the idea that auxin is required for root hair growth (Rigas et al. 2013) (hyperlink to "Auxin and ethylene regulate root hair growth" section below).

Genes that prevent branching. Wild-type root hairs rarely branch. Plants with mutations in the SCN1, CAN OF WORMS1 (COW1: At4g34580), TIP1, CEN1, CEN2, CEN3, BST1, ROOT HAIR DEFECTIVE3 (RHD3: At3g13870), 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 preventing branching. Plants homozygous for the loss-of-function scn1-1 allele exhibit a high percentage of branched hairs, and, in some double mutant com- binations that include scn1-1, every hair is branched (Parker et al., 2000). SCN1 encodes a negative regulator of ROP small GT- Pases, suggesting that the scn1 phenotype is due to ROP over- activity. This is supported by observations of root hair branching in ROP overexpression lines (Jones et al. 2002). As discussed above (hyperlink to the "Genes restricting swelling size" section),
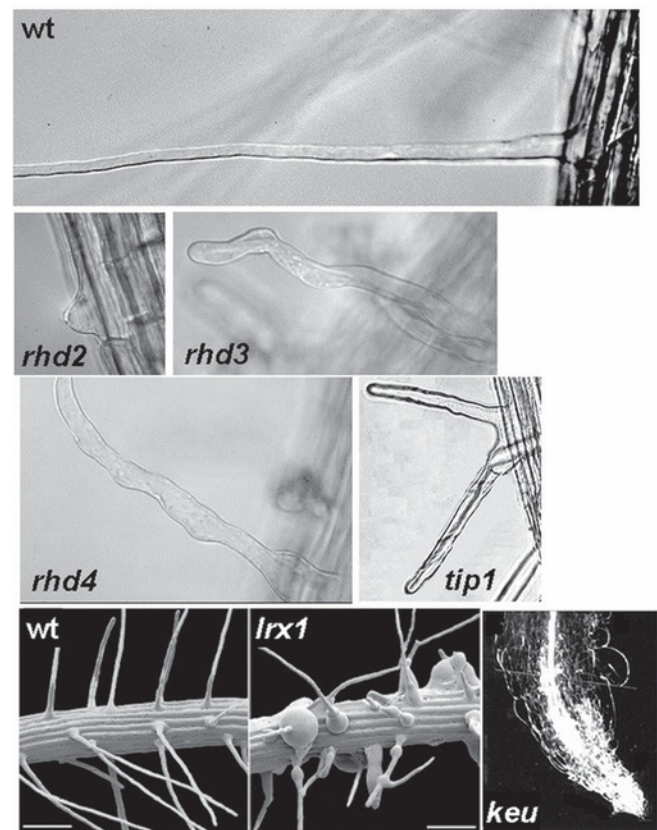
Figure 19. Phenotypes of wild type and mutant root hairs. Light mi- crographs of single hairs of wild type (wt), rhd2, rhd3, and rhd4, and a branched hair from a tip1 plant. Scanning electron micrographs of wild type (wt) and lrx1 roots. Light micrograph of a keule root tip (keu) with no root hairs. See Supplemental Table 1 for references.

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).

TIP1 probably regulates the localization or activity of one or more unidentified proteins in root hair cells. Several genes that affect branching encode proteins with roles in the secretory pathway. COW1 is a phosphatidylinositol transfer protein that localizes to the site of root hair growth (Böhme et al. 2004). The *rhd3* mutants have short and curled/wavy root hairs, as well as cell expansion defects throughout the plant (Schiefelbein and Somerville, 1990; Wang et al., 2002). *RHD3* encodes a protein with GTP binding domains (Wang et al., 1997) that contributes to the formation of the tubular ER network (Chen et al. 2011), likely by mediating ER fusion events (Zhang et al., 2013), and therefore affects trafficking through the secretory network. RHD4 is a phosphatidylinositol-4-phosphate phosphatase that regulates the accumulation of PI(4)P on membrane compartments at growing root hair tips (Thole et al. 2008).

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

The *LEUCINE-RICH REPEAT/EXTENSIN 1* (*LRX1: At1g12040*) encodes 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 localizes 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.

Potential regulators of root hair tip growth include INCOMPLETE ROOT HAIR ELONGATION1 (*IRE1: At5g62310*) and ROOT HAIR DEFECTIVE6-LIKE2 (*RSL2: At4g33880*). Mutations in either of these genes lead to short root hairs and each gene is preferentially expressed in growing root hairs (Oyama et al., 2002; Yi et al., 2010), which implies that the *IRE1*-encoded AGC kinase and the RSL2-encoded bHLH transcription factor regulate tip growth activities.

Consistent with the important role of actin in tip growth, mutations in the *ACTIN2* (*ACT2: At3g18780*) gene exhibit defects in root hair initiation and tip growth, with the severity dependent on the nature of the mutation and double-mutant partner genes (Gilliland et al., 2002; Ringli et al., 2002).

PFN1 encodes one of four Arabidopsis profilin actin-binding proteins, and is expressed in Arabidopsis root hairs (Huang et al., 1996). Transgenic plants overexpressing *PFN1* 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).

The GLOVEN (GLV) family of small modified peptides includes two members (*GLV4: At3g02240* and *GLV8: At3g02242*) that are required for complete root hair elongation (Fernandez et al., 2013). Although their precise biochemical role is not clear, it is possible that the GLV4 and GLV8 peptides participate in signaling events during root hair tip growth.

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). See above for information about the proteins encoded by many of these genes (*hyperlink to* "Genes establishing tip growth" and "Genes that prevent branching".)

The Sec1 protein KEULE (*KEU: At1g12360*) is involved in secretory trafficking at the growing root hair tip. 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 and/or tip growth, but it is likely that KEULE contributes to root hair development by facilitating targeted vesicle fusion (Assaad et al., 2001; Karnik et al. 2013). 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* mutant 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* mutants all have wide hairs (Schiefelbein and Somerville, 1990; Parker et al., 2000), suggesting that the *RHD2, SCN1, TIP1* and *COW1* genes 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 (Schiefelbein and Somerville, 1990; Galway et al., 1997). The *rhd4* mutant hairs have inconsistent di- ameters (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 (Schiefelbein and Somerville, 1990; Galway et al., 1999). Perhaps surprisingly, light signaling can influence root hair length. Mutations in either the *PHYTOCHROME A* (*PHYA: At1g09570*) or *PHYB (At2g18790)* 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; DeSimone et al., 2000).

Auxin and ethylene related genes. The plant hormones auxin and ethylene act in overlapping and independent ways to influence many aspects of root hair development, as outlined in Supplemental Table 1 and Figure 16.

AXR2 (Nagpal et al., 2000*)* and *AXR3* (Leyser et al., 1996*)* are transcriptional regulators of auxin-responsive genes that affect root hair initiation. The degradation of AXR2 and AXR3 proteins is stimulated by auxin (Gray et al. 2001). Mutations in *AXR2* and *AXR3* that reduce auxin-responsive degradation reduce root hair production, suggesting that auxin-responsive changes in gene expression are required for root hair initiation. As well as permitting root hair initiation, auxin regulates cell and tissue polarity, and hence the locations of root hairs on cells. This was first noted in *axr2-1* and *rhd6* mutants, in which both the number and location of root hairs is altered (Masucci and Schiefelbein, 1994). The nor- mal site of hair emergence is restored to *rhd6* mutants by treat- ment with auxin (Masucci and Schiefelbein, 1994). Treating *rhd6* mutants with an ethylene precursor also restores the position of hair emergence, implicating ethylene in this process. This is sup- ported by the mutant phenotypes of the *ETHYLENE RESPONSE 1* (*ETR1: At1g66340*) and *ETHYLENE OVERPRODUCER 1* (*ETO1: At3g51770*) mutants. The *etr1* mutants perceive eth- ylene poorly because they have a damaged ethylene receptor. Like *rhd6* hairs, *etr1* hairs emerge nearer to the basal end of the hair cell. The *eto1* plants produce more ethylene than wild type and root hairs form closer to the apical end of the cell (Masucci and Schiefelbein, 1996). Genes affecting auxin transport, such as *AUXIN1* (*AUX1: At2g38120*), which encodes an auxin influx car- rier, and *GNOM (GN: At1g13980)*, which affects PIN auxin efflux

carriers, also alter the locations of root hairs on cells, with root hairs forming towards the end of the cell where the auxin concen- tration is likely to be highest (Fischer et al., 2006). This suggests that auxin gradients govern where on each cell a root hair forms. The formation of these auxin gradients is regulated by ethylene (Ikeda et al., 2009), A computational model that assumes ROP activation is increased by intracellular auxin can account for the location of ROP patches and hence root hair initiation in various wild-type and mutant plants (Payne and Grierson, 2009).

Auxin and ethylene continue to contribute to root hair develop- ment after the initiation site has been selected. The phenotype of *rhd1* mutants is suppressed by ethylene, implicating ethylene in the regulation of root hair cell wall synthesis (Seifert et al., 2004). Evidence for a role for auxin in the establishment of tip growth comes from mutants of the *AUXIN RESISTANT 1* (*AXR1: At1g05180*) gene, which produce some root hairs that stop grow- ing after elongation begins (Pitts et al., 1998). *AXR1* encodes a subunit of the RUB1-activating enzyme that is necessary for pro- tein degradation required for responses to auxin. Similarly, the putative ethylene signal transduction component ETHYLENE INSENSITIVE2 (*EIN2: At5g03280*) is required for full-length root hairs (Pitts et al., 1998).

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 maintain unidirec- tional tip growth and prevent branching.

Mutations in the *AXR1, ETR1*, and *AUX1* genes have short root hairs, so these genes are required for root hairs to achieve wild- type length (Pitts et al., 1998). As discussed above *ETR1* encodes an ethylene receptor, *AXR1* encodes a component of the auxin sig- naling machinery, and *AUX1* encodes an auxin influx carrier, so the short hairs on *etr1, axr1,* and *aux1* mutant plants suggest that eth- ylene and auxin stimulate elongation (Pitts et al., 1998). In the case of ethylene this is supported by the phenotype of *eto1* mutants, which synthesize more ethylene and have longer root hairs than wild-type plants (Pitts et al., 1998). Overexpressing a PIN auxin ef- flux carrier or the PIN regulator PINOID (*PID: At2g34650*) reduces root hair growth (Lee and Cho 2006; Cho et al., 2007), consistent with the idea that intracellular auxin sustains growth. Auxin levels may be influenced by strigolactone, to alter root hair length (Kapul- nik et al., 2011). Computation of auxin flow based on amounts and locations of auxin carrier proteins suggests that auxin is supplied to developing root hair cells by neighboring non-hair cells, and that *aux1* and *wer* mutants have short root hairs because the auxin supply to developing root hairs fails prematurely, curtailing root hair growth (Jones et al., 2009).

Auxin and ethylene likely act through transcription factors to control root hair length. ACC does not increase root hair length in loss-of-function mutants of the C2H2 zinc-finger protein *ZINC FINGER PROTEIN 5* (*ZPF5: At1g10480*), suggesting that eth- ylene may stimulate root hair growth by activating transcription through ZPF5 (An et al., 2012). Manipulating the levels and sta- bility of the AUX/IAA transcription factors AXR3 and SHY2 alters root hair initiation and root hair length (Knox et al. 2003). Further evidence that auxin acts on root hair growth via a transcriptional mechanism comes from mutants lacking the basic helix-loop-he- lix transcription factor RSL4, in which auxin cannot stimulate root hair growth (Yi et al. 2010). Figure 20 summarizes the effects of auxin and ethylene signaling on root hair phenotypes.
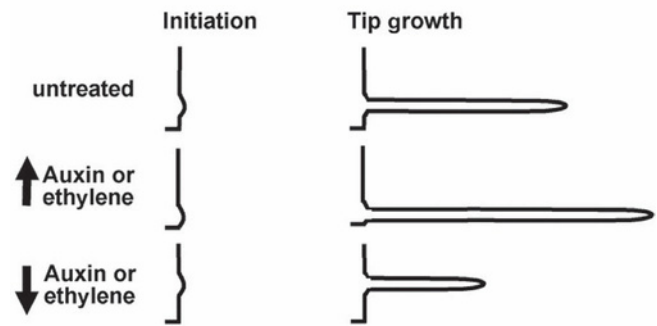

Figure 20. Roles of auxin and ethylene signaling in root hair growth.

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.

Genes affecting coordination of tip growth cessation. One mutant affects coordination of events at the end of tip growth (*hy- perlink to section on the end of tip growth above*). Mutations in *SUPPRESSOR OF AUXIN RESISTANCE1* (*SAR1: At1g33410*) cause expanded (fat) tips to form at the end of root hairs (Figure 16). SAR1 acts downstream of AXR1 in the auxin response, sug- gesting that auxin signaling plays a coordinating role at the end of root hair growth (Cernac et al., 1997).

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 or 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 plants are 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 concentra- tion. When nutrients are sparse the density and the length of root hairs generally 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). Phos- phate has the strongest and best characterized effect. Root hair density on Columbia roots grown in low phosphorous (1 μM) is up

to five times greater than on roots grown in high phosphorous (1000 µM) (Bates and Lynch, 1996; Savage et al., 2013). 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). The basic helix-loop-helix transcription factor RSL4 is required for the increase in root hair growth that occurs when roots are grown in low phosphate conditions (Yi et al. 2010). 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 pro- vided a useful and simple model to uncover new insights into gen- eral 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 differ- entiation 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 nutri- ent deficiency to produce ectopic hairs. These mechanisms may ensure proper root-hair cell production in the event of extreme envi- ronmental conditions or rare abnormal cell divisions.

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