Root Hairs

Authors: Grierson, Claire, Nielsen, Erik, Ketelaarc, Tijs, and Schiefelbein, John

Root Hairs

Claire Grierson[a], Erik Nielsen[b], Tijs Ketelaar[c], and John Schiefelbein[d,1]

[a] School of Biological Sciences, University of Bristol, Bristol, UK BS8 1UG.
[b] Department of Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, MI, USA 48109.
[c] Laboratory of Cell Biology, Wageningen University, 6708 PB Wageningen, The Netherlands.
[d] Department of Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, MI, USA 48109.
[1] Address correspondence to schiefel@umich.edu

Roots hairs are cylindrical extensions of root epidermal cells that are important for acquisition of nutrients, microbe interactions, 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 epidermal cells. In Arabidopsis, root hairs are approximately 10 μm in diameter and can grow to be 1 mm or more in length (Figure 1). Because they vastly increase the root surface area and effectively increase the root diameter, root hairs are generally thought to aid plants in nutrient acquisition, anchorage, and microbe interactions (Hofer, 1991).

Root hairs in Arabidopsis have attracted a great deal of attention from plant biologists because they provide numerous advantages for basic studies of development, cell biology, and physiology (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 cuticle layer allows physical and chemical probes to be applied with ease. Root hairs grow rapidly, at a rate of more than 1 μm/min, which facilitates studies of cell expansion. Perhaps most importantly, root hairs are not essential for plant viability, which permits the recovery and analysis of all types of mutants that alter root hair development and function. Also, root hairs become visible on seedling roots shortly after seed germination, which enables genetic screens and physiological tests to be performed rapidly with large numbers of individuals 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 produced 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 (Figure 3). This provides the opportunity for detailed analysis of the cellular changes that occur during the entire process of root hair formation.
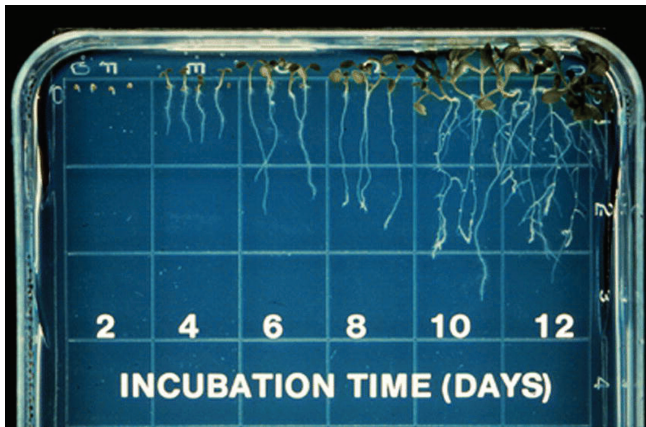
Figure 2. Development of Arabidopsis seedlings growing on agarose-solidified nutrient medium in vertically-oriented Petri plates. The roots grow along the surface of the medium, and root hairs are visualized easily using a low-magnification microscope.

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 specification of a newly-formed epidermal cell to differentiate as a root hair cell rather than a non-hair cell. This process has been studied intensively during the past several years because it serves as a simple model for understanding the regulation of cell-type patterning in plants.

The Arabidopsis root epidermis is generated from a set of 16 initial (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). Furthermore, anticlinal longitudinal divisions occasionally occur and result in an increase in the number of epidermal cell files; this activity causes the observed number of epidermal cell files in the seedling root to vary from 18 to 22 (Galway et al., 1994; Baum and Rost,
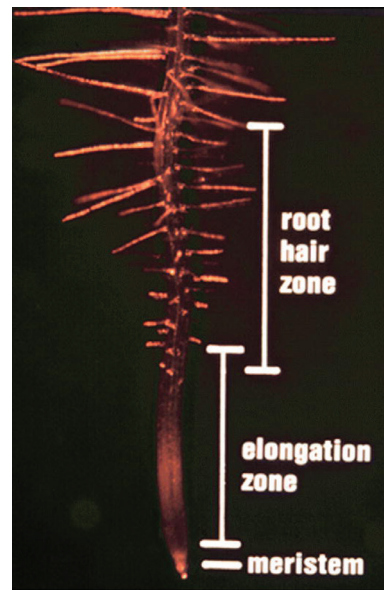
1996; Berger et al., 1998b). The epidermal cells are symplastically connected during much of their development (Duckett et al., 1994).

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 cortical 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 cortical 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 differentiation 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 junction, in a region containing 3-7 tiers of cells called the collet (Parsons, 2009). Here, every epidermal cell forms a root-hair-like extension during early seedling growth (Scheres et al., 1994; Lin and Schiefelbein, 2007; Sliwinska et al., 2012). Consistent with this exceptional 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).
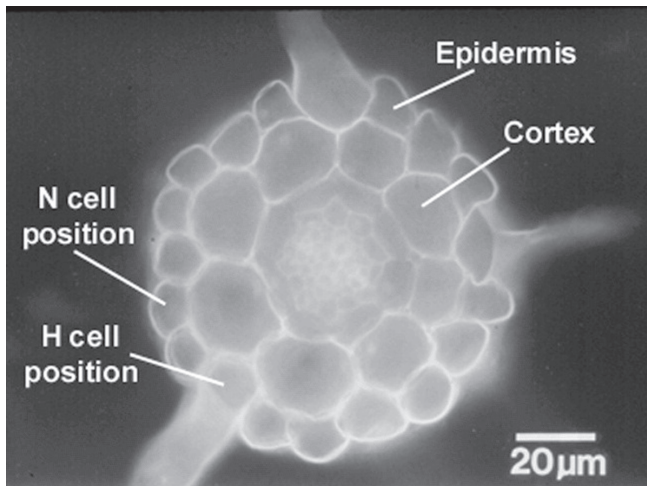
Figure 4. Transverse section of an Arabidopsis root, showing the position-dependent pattern of hair cells and non-hair cells. The hair-bearing cells are located outside the space separating two cortical cells (the H cell position), whereas the non-hair cells are located outside a single cortical cell (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 differentiating root-hair cells display a greater rate of cell division (Berger et al., 1998b), a reduced cell length (Dolan et al., 1994; Masucci et al., 1996), greater cytoplasmic density (Dolan et al., 1994; Galway et al., 1994), a lower rate of vacuolation (Galway et al., 1994), unique cell surface ornamentation (Dolan et al., 1994), and distinct cell wall epitopes (Freshour et al., 1996).

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

The presence of differential gene expression in the early meristem 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
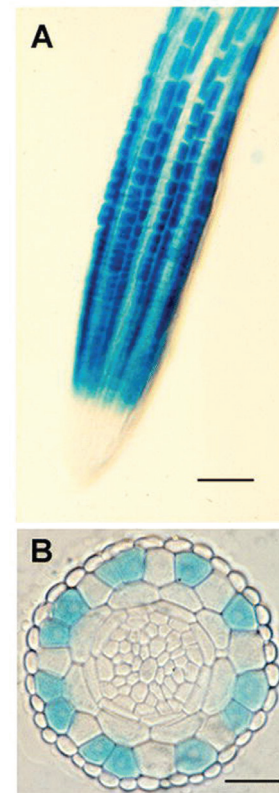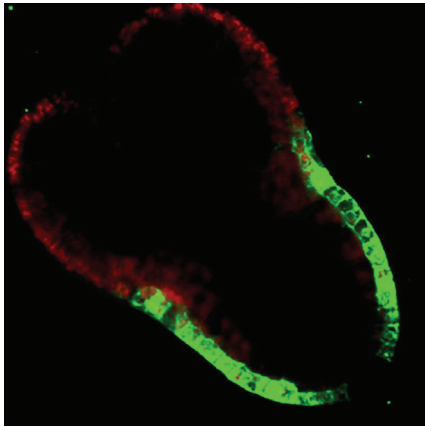

Figure 5. Expression of the *GL2::GUS* reporter fusion during root development.

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

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

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 pattern (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 resulting daughter cells to occupy different positions relative to the underlying cortical cells. The cells within these clones expressed marker genes and exhibited cellular characteristics that are appropriate for their new position (Figure 7). In a second set of experiments, specific differentiating epidermal cells were subjected to laser ablation such that neighboring epidermal cells were able

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

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-embryonically, not only embryonically, to ensure appropriate cell specification 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 epidermal neighbors, maintained the same reporter gene expression and differentiated according to their original position (Berger et al., 1998a). In a second set of cell ablations, 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 ablation of the underlying cortical cell(s) had no effect on their future GFP expression or morphogenesis (Berger et al., 1998a). These results imply that continuous signaling between living cortical and/or epidermal cells is not required to maintain the appropriate cell fate decision. However, it is still unclear whether signaling between cortical and epidermal cells may be required to establish cell fates.

Molecular Genetics of Root Hair Cell Specification

Several mutants have been identified in Arabidopsis that possess a disrupted pattern of root epidermal cell types (Supplemental Table 1; Figure 8). Mutations in the *WEREWOLF (WER: At5g14750), TRANSPARENT TESTA GLABRA (TTG: At5g24520), GLABRA3 (GL3: At5g41315)/ENHANCER OF GLA-*
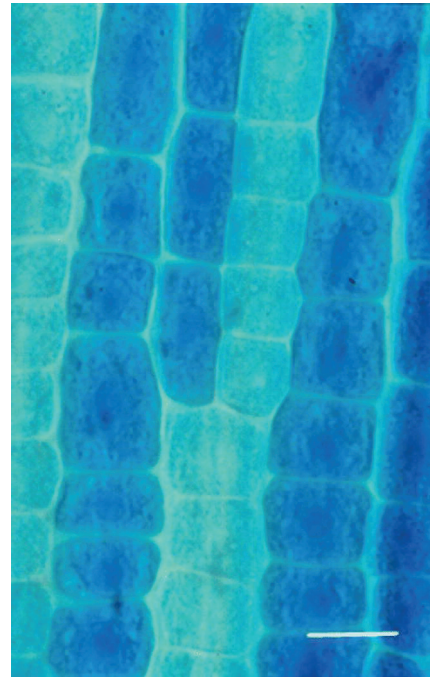
Figure 7. Expression of the *GL2::GUS* reporter fusion in an epidermal cell clone derived from a rare longitudinal division. Note that only one set of cells in the clone expresses the *GL2* marker. Bar = 10 μm.
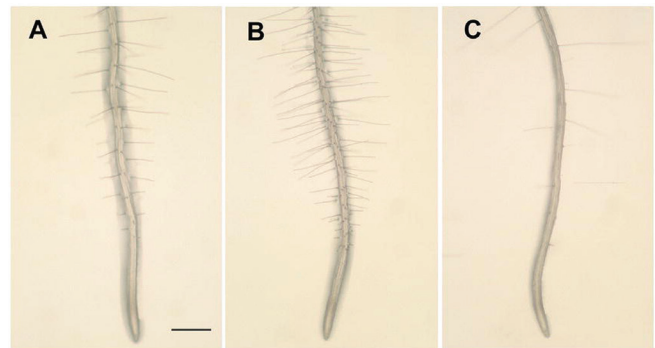
Figure 8. Root hair production in wild-type and cell specification mutants.

(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 differentiation (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 differentiation; 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 cellular phenotypes (Galway et al., 1994; Masucci et al., 1996; Berger et al., 1998b; Lee and Schiefelbein, 1999). Thus, WER and TTG may be earlier (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 mucilage, or anthocyanin production. A closely related gene, *MYB23* (*At5g40330*), encodes a protein with a similar biochemical function 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) transcriptional activators GL3 and EGL3, which act in a partially functionally 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* expression initiates during the early heart stage of embryogenesis, where it rapidly assumes its N-cell-specific expression pattern (Lin and Schiefelbein, 2001). The embryonic and post-embryonic *GL2* gene expression is influenced by the *WER, 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 Schiefelbein, 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*), affects root epidermis cell specification in a different manner. Rather than causing ectopic root hair cells, the *cpc* mutant produces a reduced number of root hair cells (Wada et al., 1997). This implies that *CPC* is a positive regulator of the root hair cell fate. Interestingly, the *gl2* mutation is epistatic to *cpc*, which suggests that CPC acts in the WER/TTG/GL3/EGL3/GL2 pathway as a negative regulator of *GL2*. A possible explanation for CPC's negative action is provided by the nature of its gene product; *CPC* encodes a small protein with a single R3 Myb repeat with the bHLH- and

DNA-binding domains but without a typical transcriptional activation 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 competitive 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, enabling 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 proposed 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 accumulate 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 plasmodesmata; 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 eliminate one of the epidermal cell types but merely alters the distribution 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. Interestingly, the *SCM* gene is itself under the transcriptional feedback 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 Schiefelbein, 2002; Figure 9). In this model, the root hair cell fate is proposed to represent the default fate for a root epidermal cell. The pattern of hair and non-hair cell types relies on the relative activity of two competing 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, respectively, 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 epidermal cells located in the H position are proposed to accumulate
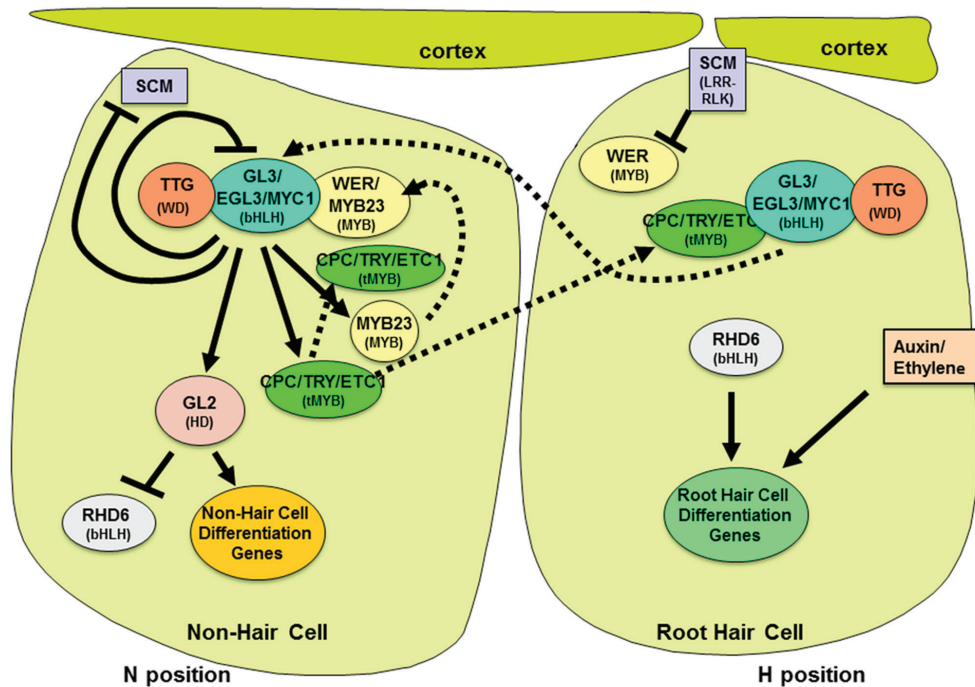
Figure 9. Model for the specification of the root hair and non-hair cell types in the Arabidopsis root epidermis. The proposed accumulation and interaction of cell fate regulators is shown within root epidermal cells destined to be root hair cells (in the H position) or non-hair cells (in the N position). 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 transcriptional 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 Arabidopsis epidermal patterning system. The efforts thus far have centered on modeling the accumulation of the central transcriptional complex, largely based on the classic "reaction-diffusion" mechanisms described by Turing and others (Turing, 1952). These studies 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 undergo analogous position-dependent cell differentiation to generate a common pattern of cell types throughout the apical-basal axis of the Arabidopsis seedling.

The similarity in cell specification in the root and hypocotyl epidermis is also apparent in the molecular components employed. The *wer*, *ttg*, and *gl2* mutants exhibit alterations in the patterning of the hypocotyl cell types, causing a greater proportion of ectopic stomata (stomata located outside a periclinal cell wall) (Hung et al., 1998; Berger et al., 1998c; Lee and Schiefelbein, 1999), whereas *cpc* and *try* mutants have reduced stomata formation (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 beginning during embryogenesis to ensure that cells located outside a periclinal cortical cell wall differentiate into non-hair cells in the root and non-stomatal cells in the hypocotyl epidermis.

In addition to affecting the hypocotyl epidermis, the *TTG, GL3/EGL3, CPC/TRY* and *GL2* genes are known to also affect trichome formation in the shoot epidermis of Arabidopsis (Koorn-
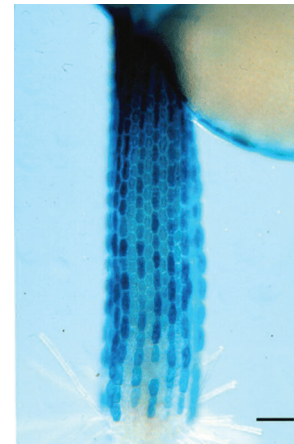
Figure 10. Expression of the *GL2::GUS* reporter fusion in the hypocotyl epidermis. Cells expressing the *GL2::GUS* marker are located in the N position. Bar = 100 μm.

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 mechanism relies on the positional relationship between epidermal cells and underlying cortical cells whereas the leaf epidermis mechanism relies on sensing trichome density. A further interesting aspect of this relationship is that the 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 experiments indicate that auxin and ethylene help promote root hair cell differentiation in Arabidopsis. For example, treatment of Arabidopsis 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 *CONSTITUTIVE 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 inclusion 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. Results 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 together, the results suggest that the WER/TTG/GL2/CPC pathway acts upstream of, or independently from, the ethylene/auxin pathway to define the pattern of cell types in the root epidermis.

In addition to auxin and ethylene, other plant hormones influence 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 brassinosteroids act at an early stage to promote the hair cell fate (Kuppusamy et al., 2009). Furthermore, the synthetic strigolactone GR24 increases root hair length, perhaps by interfering with auxin-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 process is termed root hair initiation and is summarized in Figure 12.
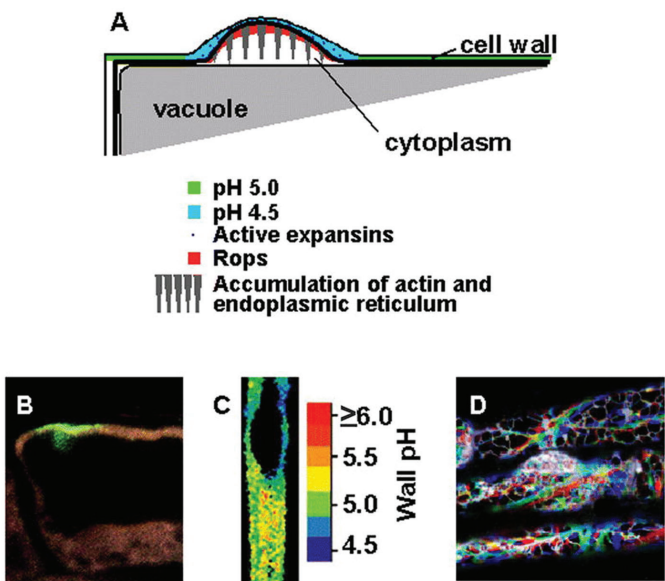
Figure 12. Root hair initiation.

(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 imaged using NERF/Texas Red and pseudo-color coded according to the inset scale (see Bibikova et al., 1998).

(D) Local accumulation of endoplasmic reticulum (ER) in an initiating hair flanked by two non-hair cells. Red, blue and green images were taken 30 seconds apart. White indicates that ER was present in the same location in all three images. (see Ridge et al., 1999).
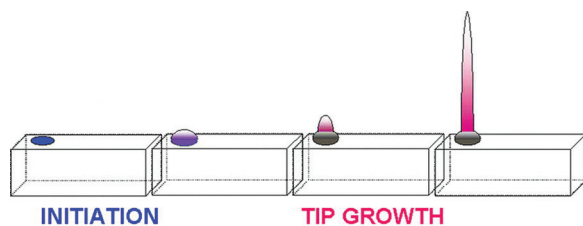
Figure 11. Stages of root hair formation. Root hairs form in two main stages: initiation, when a small, disc-shaped area of the cell wall loosens to form a swelling, and tip growth, when the remainder of the hair grows by targeted secretion.

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 incorporated 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 support tip growth accumulate. Together, they are referred to as the
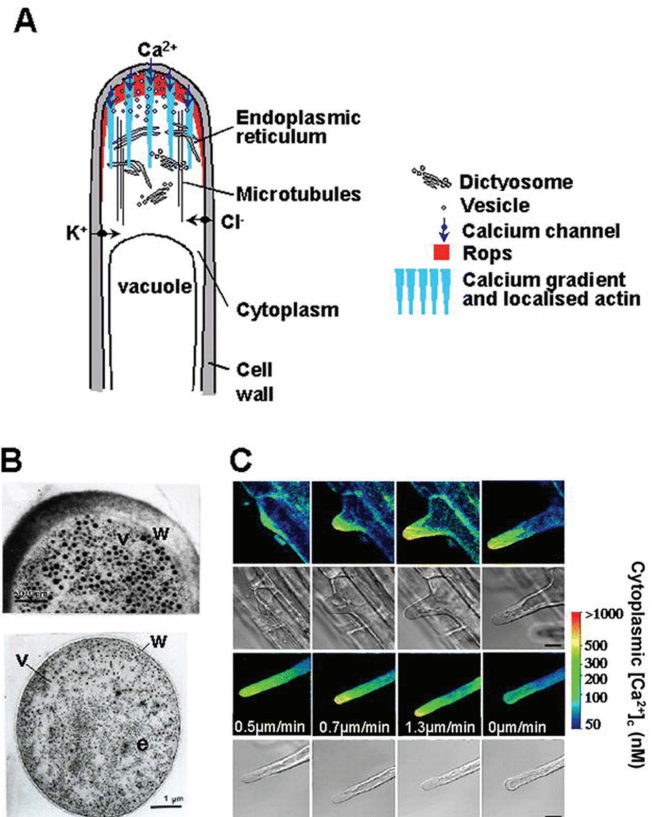
Figure 13. Root Hair Tip Growth.

(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 ($[Ca^{2+}]_c$) was imaged using indo-1 and pseudo-color coded according to the inset scale. $[Ca^{2+}]_c$ 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 structures 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 calcium (Schiefelbein et al., 1992). When Arabidopsis root hairs are 5-10 µm long the Ca^{2+} concentration at the tip of the swelling increases from about 200 nM to at least 1 µM, and remains very high throughout tip growth (Figure 13C; Wymer et al., 1997; Bibikova et al., 1999). 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 apical 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 fusion 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 membrane H+ ATPase. The source of calcium for root hair tip growth is uncertain. When hairs are growing through a solution, calcium is apparently imported from liquid surrounding the growing tip. However, root hairs grow well in moist air and are abundant in air pockets in soil (C. Grierson, unpublished observations; Ryan et al., 2001). Under these conditions, calcium for tip growth must be either (1) released from the newly deposited wall, or (2) transported through the apoplast and deposited near the tip of the hair, or (3) released from intracellular stores (Ryan et al., 2001).

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 reactive 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 maintained 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, although 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 formation 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)apical 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 actin 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 specifically depolymerized; cytoplasmic streaming continues and thick F-actin bundles loop through the tip. This results in the disappearance 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 observations 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 growing 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 different 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 nucleating 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 Ca^{2+} 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, Ca^{2+}/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 Ca^{2+} concentrations, they increase actin turnover at higher Ca^{2+} 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. Ca^{2+} 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 Ca^{2+} gradient, it is likely that the Ca^{2+}-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 myosin 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 Ca^{2+} 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 Ca^{2+} 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 Ca^{2+} concentrations (Cyr, 1991, 1994), the high Ca^{2+} 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).

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 membrane 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 membrane 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 recruitment of cytosolic "effector proteins" to their target membranes. During tip-growth in Arabidopsis root hairs, RAB GTPASE HOMO-
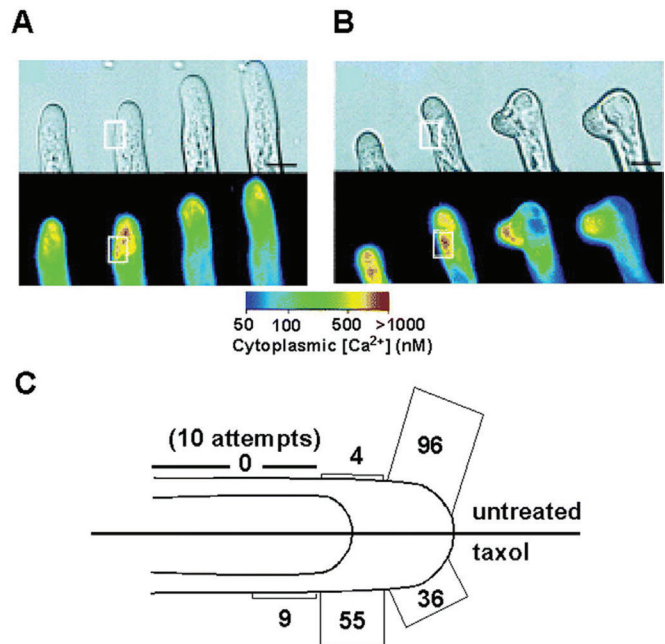
Figure 15. The direction of tip growth is controlled by calcium and microtubules.

(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 ($[Ca^{2+}]_c$) 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).

LOG A4b (*RAB-A4b: At4g39990*) recruits the lipid kinases, PHOSPATIDYLINOSITOL 4-OH KINASE BETA1 (*PI4Kβ1: At5g64070*) and PHOSPHATIDYLINOSITOL 4-OH KINASE BETA2 (*PI4Kβ2: At5g09350*), which phosphorylate the inositol head group of phosphatidylinositol (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 regulation and delivery of secretory cargo to the tips of growing root hairs (Preuss et al., 2004, 2006; Thole et al., 2008). Additionally, phosphorylation of PI-4P to PI-4,5P_2 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-4,5P_2 likely participate 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-4,5P_2 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 layers 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 enriched 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 catalytic 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 required for tip-growth in root hair cells. Xyloglucan is a β-1,4 linked glucan polymer with extensive side chain modifications containing 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 *XYLOGLUCAN XYLOSYL TRANSFERASE*1 (*XXT1: At3g62720*), *XXT2* (*At4g02500*), and others. *Arabidopsis xxt1/xxt2* double mutants grew normally, but root hairs in these mutants are shorter and possess bulged bases (Cavalier et al., 2008). Recently, XYLOGLUCAN-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 pectin 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-REDUCTASE1 (*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 proteins are post-translationally modified to form hydroxyproline, in a process catalyzed by a family of membrane-bound prolyl 4-hydroxylases (P4Hs) (Velasquez et al., 2011; Mohnen and Tierney, 2011). Elimination of the root-expressed *PROLYL 4-HYDROXYLASE* (*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 actively 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 using 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 (Schiefelbein et al., 1992; Very and Davies, 2000) are lost from the tip.
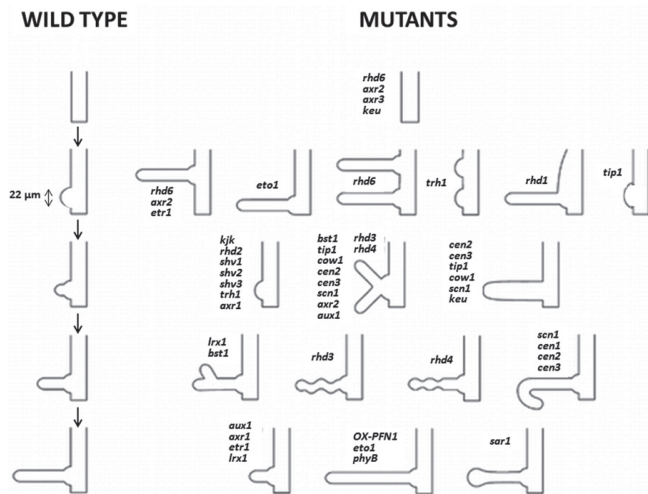
Molecular Genetics of Root Hair Formation

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 number 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 swellings from forming on wild type hair cells. *TRH1* encodes a potassium transporter (Rigas et al., 2001). Further research is required to discover how this affects swelling number. 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 restricting the area of wall that is loosened (Parker et al., 2000; Ryan et al., 1998; Schiefelbein et al., 1993; Schiefelbein and Somerville, 1990). The *tip1 rhd1* double mutants have similar swelling dimensions to *rhd1* single mutants, suggesting that *TIP1* cannot 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 transferases regulate protein localization and activity at cell membranes, 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: unknown gene*), *SHV2/MRH4/COBL9 (At5g49270)*, *SHV3/MRH5*
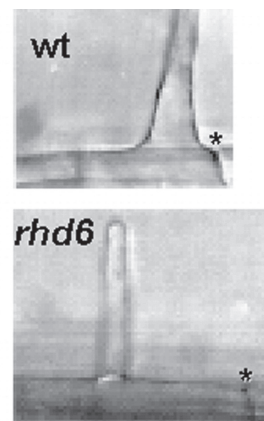

Figure 16. Genetics of root hair morphogenesis.

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.
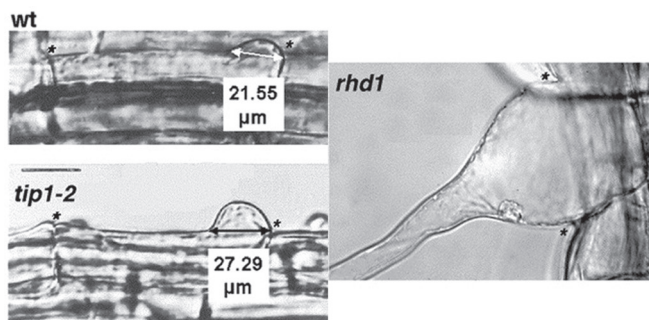

Figure 17. Hairs on the *rhd6* mutant emerge at a more basal position on the hair cell than wild type hairs (wt). An asterisk (*) indicates the apical wall of each cell.

(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 affecting 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 important for tip growth to be successfully established.

Sustained root hair tip growth involves oscillations of extracellular pH, reactive oxygen species, and cytosolic calcium. Interfering 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 formation, 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, cellulose is formed at the plasma membrane whereas KJK is found in the endoplasmic reticulum. KJK probably contributes to the synthesis of polysaccharides such as beta-xylans, mannans or xyloglucan (Favery et al., 2001). The *kjk* mutant hairs have weak cell walls that cannot contain the growing protoplast and burst. *SHV2* and *SHV3* also encode proteins that affect cell wall synthesis. The *SHV2/COBL9/MRH4* gene encodes a glucosylphosphatidylinositol (GPI)-anchored protein related to COBRA, which affects 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).
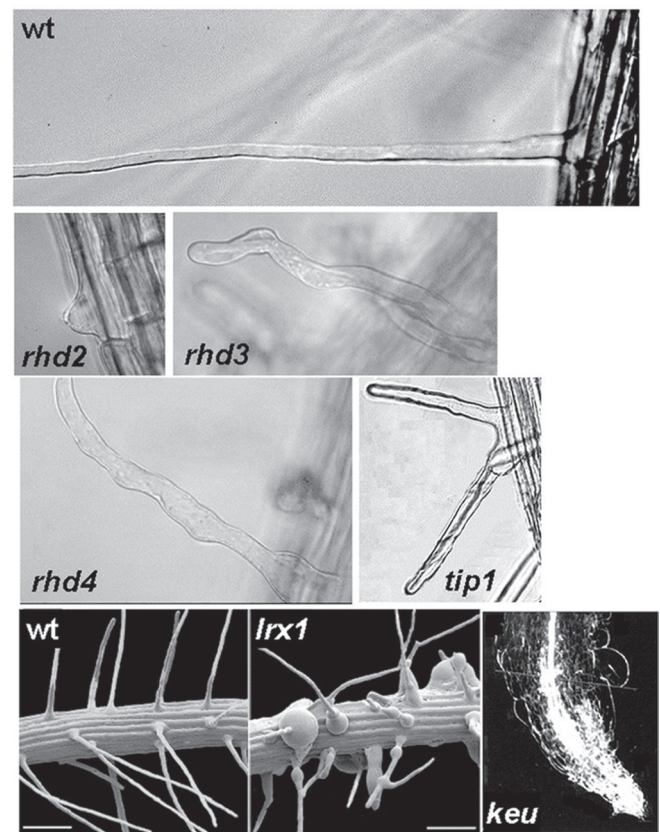
Root hair growth may also stop if a crucial part of the machinery supporting tip growth fails. The TRH1 potassium transporter is required for tip growth, and supplementing *trh1* mutant roots with high levels of potassium does not restore tip growth (Rigas et al., 2001). These results suggest that tip 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 concentration 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 combinations that include *scn1-1*, every hair is branched (Parker et al., 2000). SCN1 encodes a negative regulator of ROP small GTPases, suggesting that the *scn1* phenotype is due to ROP overactivity. 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 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).

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

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 diameters (Figure 19) and patches of thick cell wall, suggesting that the amount of material that is deposited varies as the tube grows, producing local constrictions and expansions along the length of the hair (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 normal site of hair emergence is restored to *rhd6* mutants by treatment 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 supported by the mutant phenotypes of the *ETHYLENE RESPONSE 1* (*ETR1: At1g66340*) and *ETHYLENE OVERPRODUCER 1* (*ETO1: At3g51770*) mutants. The *etr1* mutants perceive ethylene poorly because they have a damaged ethylene receptor. Like *rhd6* hairs, *etr1* hairs emerge nearer to the basal end of the hair cell. 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 carrier, 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 concentration 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 development 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 growing after elongation begins (Pitts et al., 1998). *AXR1* encodes a subunit of the RUB1-activating enzyme that is necessary for protein 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 unidirectional 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 signaling machinery, and *AUX1* encodes an auxin influx carrier, so the short hairs on *etr1*, *axr1*, and *aux1* mutant plants suggest that ethylene 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 efflux 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 (Kapulnik 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 ethylene may stimulate root hair growth by activating transcription through ZPF5 (An et al., 2012). Manipulating the levels and stability 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-helix 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.
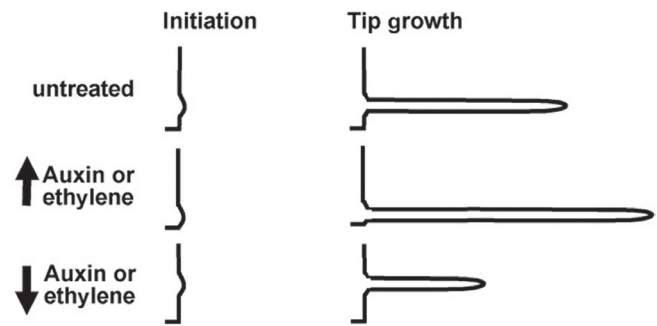
Figure 20. Roles of auxin and ethylene signaling in root hair growth.

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

ACKNOWLEDGMENTS

REFERENCES

Allwood, E.G., Smertenko, A.P., and Hussey, P.J. (2001). Phosphorylation of plant actin-depolymerising factor by calmodulin-like domain protein kinase. FEBS Lett **499:** 97-100.

Allwood, E.G., Anthony, R.G., Smertenko, A.P., Reichelt, S., Drobak, B.K., Doonan, J.H., Weeds, A.G., and Hussey, P.J. (2002). Regulation of the pollen-specific actin-depolymerizing factor LiADF1. Plant Cell **14:** 2915–2927.

An, L., Zhou, Z., Sun, L., Yan, A., Xi, W., Yu, N., Cai, W., Chen, X., Yu, H., Schiefelbein, J., and Gan, Y. (2012). A zinc finger protein gene ZFP5 integrates phytohormone signaling to control root hair development in Arabidopsis. Plant J. **72:** 474-490.

Anderson, C.T., Wallace, I.S., and Somerville, C.R. (2012). Metabolic click-labeling with a fucose analog reveals pectin delivery, architecture, and dynamics in Arabidopsis cell walls. Proc. Natl. Acad. Sci. USA **109:** 1329-1334.

Assaad, F. F., Huet, Y., Mayer, U., and Jurgens, G. (2001). The cytokinesis gene KEULE encodes a Sec1 protein that binds the syntaxin KNOLLE. J. Cell Biol. **152:** 531-543.

Baluska, F., Salaj, J., Mathur, J., Braun, M., Jasper, F., Samaj, J., Chua, N. H., Barlow, P. W., and Volkmann, D. (2000). Root hair formation: F-actin-dependent tip growth is initiated by local assembly of profilin-supported F-actin meshworks accumulated within expansin-enriched bulges. Dev. Biol. **227:** 618-632.

Bates, T. R., and Lynch, J. P. (1996). Stimulation of root hair elongation in *Arabidopsis thaliana* by low phosphorus availability. Plant Cell Environ. **19:** 529-538.

Bates, T. R., and Lynch, J. P. (2000a). Plant growth and phosphorus accumulation of wild type and two root hair mutants of *Arabidopsis thaliana* (Brassicaceae). Am. J. Bot. **87:** 958-963.

Bates, T. R., and Lynch, J. P. (2000b). The efficiency of *Arabidopsis thaliana* (Brassicaceae) root hairs in phosphorus acquisition. Am. J. Bot. **87:** 964-970.

Baum, S.F., and Rost, T.L. (1996). Root apical organization in *Arabidopsis thaliana*. 1. Root cap and protoderm. Protoplasma **192:** 178-188.

Baumberger, N., Ringli, C., and Keller, B. (2001). The chimeric leucine-rich repeat/extensin cell wall protein LRX1 is required for root hair morphogenesis in *Arabidopsis thaliana*. Genes Dev. **15:** 1128-1139.

Benitiz, M., Espinosa-Soto, c., Padilla-Longoria, P., Diaz, J., and Alvarez-Buylla, E.R. (2007) Equivalent genetic regulatory networks in different contexts recover contrasting spatial cell patterns that resemble those in Arabidopsis root and leaf epidermis: a dynamic model. Int. J. Dev. Biol. **51:** 139-155.

Benitiz, M., Monk, N.A., and Alvarez-Buylla, E.R. (2011) Epidermal patterning in Arabidopsis: models make a difference. J. Exp. Zool. B Mol. Dev. Evol. **316:** 241-53.

Berger, F., Haseloff, J., Schiefelbein, J., and Dolan, L. (1998a). Positional information in root epidermis is defined during embryogenesis and acts in domains with strict boundaries. Curr. Biol. **8:** 421-430.

Berger, F., Hung, C.-Y., Dolan, L., and Schiefelbein, J. (1998b). Control of cell division in the root epidermis of *Arabidopsis thaliana*. Dev. Biol. **194:** 235-245.

Berger, F., Linstead, P., Dolan, L., and Haseloff, J. (1998c). Stomata patterning on the hypocotyl of *Arabidopsis thaliana* is controlled by genes involved in the control of root epidermis patterning. Dev. Biol. **194:** 226-234.

Bernal, A.J., Yoo, C.M., Mutwil, M., Jensen, J.K., Hou, G., Blaukopf, C., Sorensen, I., Blancaflor, E.B., Scheller, H.V., and Willats, W.G. (2008). Functional analysis of the cellulose synthase-like genes CSLD1, CSLD2, and CSLD4 in tip-growing Arabidopsis cells. Plant Physiol. **148:** 1238-1253.

Bernhardt, C., Lee, M. M., Gonzalez, A., Zhang, F., Lloyd, A., and Schiefelbein, J. (2003). The bHLH genes GLABRA3 (GL3) and ENHANCER OF GLABRA3 (EGL3) specify epidermal cell fate in the Arabidopsis root. Development **130:** 6431-9.

Bernhardt, C., Zhao, M., Gonzalez, A., Lloyd, A., and Schiefelbein, J. (2005). The bHLH genes GL3 and EGL3 participate in an intercellular regulatory circuit that controls cell patterning in the Arabidopsis root epidermis. Development **132:** 291-8.

Bibikova, T. N., Zhigilei, A., and Gilroy, S. (1997). Root hair growth in *Arabidopsis thaliana* is directed by calcium and an endogenous polarity. Planta **203**: 495-505.

Bibikova, T. N., Jacob, T., Dahse, I., and Gilroy, S. (1998). Localized changes in apoplastic and cytoplasmic pH are associated with root hair development in *Arabidopsis thaliana*. Development **125**: 2925-2934.

Bibikova, T. N., Blancaflor, E. B., and Gilroy, S. (1999). Microtubules regulate tip growth and orientation in root hairs of *Arabidopsis thaliana*. Plant J. **17**: 657-665.

Böhme, K., Li, Y., Charlot, F., Grierson, C., Marrocco, K., Okada, K., Laloue, M., and Nogué, F. (2004). The Arabidopsis COW1 gene encodes a phosphatidylinositol transfer protein essential for root hair tip growth. Plant J. **40**:686-698.

Brady, S. M., Orlando, D. A., Lee, J. Y., Wang, J. Y., Koch, J., Dinneny, J. R., Mace, D., Ohler, U., and Benfey, P. N. (2007). A high-resolution root spatiotemporal map reveals dominant expression patterns. Science **318**: 801-806.

Brembu, T., Winge, P., Seem, M., and Bones, A.M. (2004). NAPP and PIRP encode subunits of a putative wave regulatory protein complex involved in plant cell morphogenesis. Plant Cell **16**: 2335-2349.

Bruex, A., Kainkaryam, R. M., Wieckowski, Y., Kang, Y. H., Bernhardt, C., Xia, Y., Zheng, X., Wang, J. Y., Lee, M. M., Benfey, P. et al. (2012). A gene regulatory network for root epidermis cell differentiation in Arabidopsis. PLoS Genet **8**: e1002446.

Bubb, M.R., Baines, I.C., and Korn, E.D. (1998). Localization of acto-bindin, profilin I, profilin II, and phosphatidylinositol-4,5-bisphosphate (PIP2) in Acanthamoeba castellanii. Cell Motil. Cytosk. **39**: 134-146.

Bunning, E. (1951). Uber die Differenzierungsvorgange in der Cruciferenwurzel. Planta **39**: 126-153.

Cao, X. F., Linstead, P., Berger, F., Kieber, J., and Dolan, L. (1999). Differential ethylene sensitivity of epidermal cells is involved in the establishment of cell pattern in the Arabidopsis root. Physiol. Plant. **106**: 311-317.

Carol, R.J., Takeda, S., Linstead, P., Durrant, M.C., Kakesova, H., Derbyshire P., Drea, S., Zarsky, V., and Dolan, L. (2005). A RhoGDP dissociation inhibitor spatially regulates growth in root hair cells. Nature **438**: 1013-1016.

Cavalier, D.M., Lerouxel, O., Neumetzler, L., Yamauchi, K., Reinecke, A., Freshour, G., Zabotina, O.A., Hahn, M.G., Burgert, I., Pauly, M., et al. (2008). Disrupting two *Arabidopsis thaliana* xylosyltransferase genes results in plants deficient in xyloglucan, a major primary cell wall component. Plant Cell **20**: 1519-1537.

Cernac, A., Lincoln, C., Lammer, D., and Estelle, M. (1997). The SAR1 gene of Arabidopsis acts downstream of the AXR1 gene in auxin response. Development **124**: 1583-1591.

Chant, J. (1999). Cell polarity in yeast. Ann. Rev. Cell Dev. Biol. **15**: 365-391.

Chaudhry, F., Guérin, C., von Witsch, M., Blanchoin, L., and Staiger, C.J. (2007). Identification of Arabidopsis cyclase-associated protein 1 as the first nucleotide exchange factor for plant actin. Mol Biol Cell **18**: 3002-3014.

Chen, J., Stefano, G., Brandizzi, F., and Zheng, H. (2011). Arabidopsis RHD3 mediates the generation of the tubular ER network and is required for Golgi distribution and motility in plant cells. J. Cell Sci. **124**: 2241-2252.

Cheung, A.Y., Niroomand, S., Zou, Y., and Wu, H.M. (2010). A transmembrane formin nucleates subapical actin assembly and controls tip-focused growth in pollen tubes. Proc. Natl. Acad. Sci. USA **107**: 16390-16395.

Cho, H.T., and Cosgrove, D.J. (2002). Regulation of root hair initiation and expansin gene expression in Arabidopsis. Plant Cell **14**: 3237-3253.

Cho, M., Lee, S.H., and Cho, H.T. (2007). P-glycoprotein4 displays auxin efflux transporter-like action in Arabidopsis root hair cells and tobacco cells. Plant Cell **19**: 3930-3943.

Cnops, G., Wang, X., Linstead, P., Van Montagu, M., Van Lijsebettens, M., and Dolan, L. (2000). *TORNADO1* and *TORNADO2* are required for the specification of radial and circumferential pattern in the *Arabidopsis* root. Development **127**: 3385-3394.

Cocuron, J.C., Lerouxel, O., Drakakski, G., Alonso, A.P., Liepman, A.H., Keegstra, K., Raikhel, N., and Wilkerson, C.G. (2007) A gene from the cellulose synthase-like C family encodes a beta-1,4 glucan synthase. Proc. Natl. Acad. Sci. USA **104**: 8550-8555.

Cormack, R.G.H. (1935). The development of root hairs by Elodea canadensis. New Phytol. **34**: 19-25.

Cormack, R. G. H. (1949). The development of root hairs in angiosperms. Bot. Rev. **15**: 583-612.

Cvrcková, F., Novotny, M., Pickova, D., and Zarsky, V. (2004). Formin homology 2 domains occur in multiple contexts in angiosperms. BMC Genomics **5**: 44.

Cyr, R.J. (1991). Calcium/calmodulin affects microtubule stability in lysed protoplasts. Journal of Cell Science **100**: 311–317.

Cyr, R.J. (1994). Microtubules in plant morphogenesis: role of the cortical array. Annual Review of Cell Biology **10**: 153–180.

Deeks, M.J., Cvrcková, F., Machesky, L.M., Mikitová, V., Ketelaar, T., Zársky, V., Davies, B., and Hussey, P.J. (2005). Arabidopsis group Ie formins localize to specific cell membrane domains, interact with actin-binding proteins and cause defects in cell expansion upon aberrant expression. New Phytol **168**: 529-540.

Deeks, M.J., Rodrigues, C., Dimmock, S., Ketelaar, T., Maciver, S.K., Malhó, R., and Hussey, P.J. (2007). Arabidopsis CAP1 - a key regulator of actin organisation and development. J. Cell Sci. **120**: 2609-2618.

DeSimone, S., Oka, Y., and Inoue, Y. (2000). Effect of light on root hair formation in *Arabidopsis thaliana* phytochrome-deficient mutants. J. Plant Res. **113**: 63-69.

DiCristina, M.D., Sessa, G., Dolan, L., Linstead, P., Baima, S., Ruberti, I., and Morelli, G. (1996). The Arabidopsis Athb-10 (GLABRA2) is an HD-Zip protein required for regulation of root hair development. Plant J. **10**: 393-402.

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

Dolan, L., Duckett, C., Grierson, C., Linstead, P., Schneider, K., Lawson, E., Dean, C., Poethig, S., and Roberts, K. (1994). Clonal relations and patterning in the root epidermis of Arabidopsis. Development **120**: 2465-2474.

Dong, C.H., Xia, G.X., Hong, Y., Ramachandran, S., Kost, B., and Chua, N.H. (2001). ADF proteins are involved in the control of flowering and regulate F-actin organization, cell expansion, and organ growth in Arabidopsis. Plant Cell **13**: 1333-1346.

Doree, M., and Picard, A. (1980). Release of Ca2+ from intracellular pools stops cytoplasmic streaming in Tradescantia staminal hairs. Experientia **36**: 1291–1292.

Duan, Q., Kita, D., Li, C., Cheung, A.Y., and Wu, H.M. (2010). FERONIA receptor-like kinase regulates RHO GTPase signaling of root hair development. Proc. Natl. Acad. Sci. USA **107**: 17821-17826.

Duckett, C.M., Oparka, K.J., Prior, D.A.M., Dolan, L., and Roberts, K. (1994). Dye-coupling in the root epidermis of Arabidopsis is progressively reduced during development. Development **120**: 3247-3255.

El-Assal Sel, D., Le, J., Basu, D., Mallery, E.L., and Szymanski, D.B. (2004). Arabidopsis GNARLED encodes a NAP125 homolog that positively regulates ARP2/3. Curr. Biol. **14**: 1405-1409.

El-Din El-Assal, S., Le, J., Basu, D., Mallery, E.L., and Szymanski, D.B. (2004). DISTORTED2 encodes an ARPC2 subunit of the putative Arabidopsis ARP2/3 complex. Plant J. **38:** 526-538.

Emons, A.M.C., and Ketelaar, T. (2009). Intracellular organization: a prerequisite for root hair elongation and cell wall deposition. In: A.M.C. Emons, T. Ketelaar (Eds.), Root Hairs. Plant Cell Monographs, **12**, Springer (2009), pp. 27–44.

Favery, B., Ryan, E., Foreman, J., Linstead, P., Boudonck, K., Steer, M., Shaw, P., and Dolan, L. (2001). *KOJAK* encodes a cellulose synthase-like protein required for root hair cell morphogenesis in Arabidopsis. Genes Dev. **15:** 79-89.

Fernandez, A., Drozdzecki, A., Hoogewijs, K., Nguyen, A., Beeckman, T., Madder, A., and Hilson, P. (2013). Transcriptional and functional classification of the GOLVEN/ROOT GROWTH FACTOR/CLE-like signaling peptides reveals their role in lateral root and hair formation. Plant Physiol. **161:** 954–970.

Fischer, U., Ikeda, Y., Ljung, K., Serralbo, O., Singh, M., Heidstra, R., Palme, K., Scheres, B., and Grebe, M. (2006). Vectorial information for Arabidopsis planar polarity is mediated by combined AUX1, EIN2, and GNOM activity. Curr. Biol, **16:** 2143–2149.

Foreman, J., Demidchik, V., Bothwell, J.H., Mylona, P., Miedema, H., Torres, M.A., Linstead, P., Costa, S., Brownlee, C., Jones, et al. (2003). Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. Nature **422:** 442-446.

Freshour, G., Clay, R.P., Fuller, M.S., Albersheim, P., Darvill, A.G., and Hahn, M.G. (1996). Developmental and tissue-specific structural alterations of the cell-wall polysaccharides of *Arabidopsis thaliana* roots. Plant Physiol. **110:** 1413-1429.

Frietsch, S., Wang, Y.F., Sladek, C., Poulsen, L.R., Romanowsky, S.M., Schroeder, J.I., and Harper, J.F. (2007). A cyclic nucleotide-gated channel is essential for polarized tip growth of pollen. Proc. Natl. Acad. Sci. USA **104:** 14531-14536.

Galway, M.E., Masucci, J.D., Lloyd, A.M., Walbot, V., Davis, R.W., and Schiefelbein, J.W. (1994) The TTG gene is required to specify epidermal cell fate and cell patterning in the Arabidopsis root. Dev. Biol. **166:** 740-754.

Galway, M.E., Heckman, J.W., and Schiefelbein, J.W. (1997). Growth and ultrastructure of Arabidopsis root hairs: the rhd3 mutation alters vacuole enlargement and tip growth. Planta **201:** 209-218.

Galway, M.E., Lane, D.C., and Schiefelbein, J.W. (1999). Defective control of growth rate and cell diameter in tip-growing root hairs of the rhd4 mutant of *Arabidopsis thaliana*. Can. J. Bot. **77:** 494-507.

Galway, M.E., Eng, R.C., Schiefelbein, J.W., and Wasteneys, G.O. (2011). Root hair-specific disruption of cellulose and xyloglucan in AtCSLD3 mutants, and factors affecting the post-rupture resumption of mutant root hair growth. Planta **233:** 985-999.

Gendreau, E., Traas, J., Desnos, T., Grandjean, O., Caboche, M., and Hofte, H. (1997). Cellular basis of hypocotyl growth in *Arabidopsis thaliana*. Plant Physiol. **114:** 295-305.

Gibbon, B.C., Zonia, L.E., Kovar, D.R., Hussey, P.J., and Staiger, C.J. (1998). Pollen profilin function depends on interaction with proline-rich motifs. Plant Cell **10:** 981-994.

Gibbon, B.C., Kovar, D.R., and Staiger, C.J. (1999). Latrunculin B has different effects on pollen germination and tube growth. Plant Cell **11:** 2349-2363.

Gilliland, L.U., Kandasamy, M.K., Pawloski, L.C., and Meagher, R.B. (2002). Both vegetative and reproductive actin isovariants complement the stunted root hair phenotype of the Arabidopsis act2-1 mutation. Plant Physiol. **130:** 2199-2209.

Gray, W.M., Kepinski, S., Rouse, D., Leyser, O., and Estelle, M. (2001). Auxin regulates SCF(TIR1)-dependent degradation of AUX/IAA proteins. Nature **414:** 271-276.

Grierson, C.S., Roberts, K., Feldmann, K.A., and Dolan, L. (1997) The COW1 locus of Arabidopsis acts after RHD2, and in parallel with RHD3 and TIP1, to determine the shape, rate of elongation, and number of root hairs produced from each site of hair formation. Plant Physiol. **115:** 981-990.

Guimil, S., and Dunand, C. (2006) Patterning of Arabidopsis epidermal cells: epigenetic factors regulate the complex epidermal cell fate pathway. Trends Plant Sci. **11:** 601-609.

Hassan, H., Scheres, B., and Blilou, I. (2010). JACKDAW controls epidermal patterning in the Arabidopsis root meristem through a non-cell-autonomous mechanism. Development **137:** 1523-1529.

Hayashi, S., Ishii, T., Matsunaga, T., Tominaga, R., Kuromori, T., Wada, T., Shinozaki, K., and Hirayama, T. (2008). The glycerophosphoryl diester phosphodiesterase-like proteins SHV3 and its homologs play important roles in cell wall organization. Plant Cell Physiol. **49:** 1522-1535.

Hemsley, P.A., Kemp, A.C., and Grierson, C.S. (2005). The TIP GROWTH DEFECTIVE S-acyl transferase regulates plant cell growth in Arabidopsis. Plant Cell **17:** 2554–2563.

Higgs, H.N., and Pollard, T.D. (2001). Regulation of actin filament network formation through ARP2/3 complex: activation by a diverse array of proteins. Annu Rev Biochem **70:** 649-676.

Hofer, R.-M. (1991). Root hairs. In: Plant Roots The Hidden Half, Y. Waisel, A. Eshel, and U. Kafkafi, eds., pp 129-148.

Hoffmann, A., and Nebenführ, A. (2004). Dynamic rearrangements of transvacuolar strands in BY-2 cells imply a role of myosin in remodeling the plant actin cytoskeleton. Protoplasma **224:** 201–210.

Huang, S., McDowell, J.M., Weise, M.J., and Meagher, R.B. (1996). The Arabidopsis profilin gene family. Evidence for an ancient split between constitutive and pollen-specific profilin genes. Plant Physiol **111:** 115-126.

Huang, S., Robinson, R.C., Gao, L.Y., Matsumoto, T., Brunet, A., Blanchoin, L., and Staiger, C.J. (2005). Arabidopsis VILLIN1 generates actin filament cables that are resistant to depolymerization. Plant Cell **17:** 486–501.

Huang, G.-Q., Li, E., Ge, F.-R., Li, S., Wang, Q., Zhang, C.-Q., and Zhang, Y. (2013). Arabidopsis RopGEF4 and RopGEF10 are important for FERONIA-mediated developmental but not environmental regulation of root hair growth. New Phytol. **200:** 1089-1101.

Hung, C.Y., Lin, Y., Zhang, M., Pollock, S., Marks, M.D., and Schiefelbein, J. (1998). A common position-depndent mechanism controls cell-type patterning and GLABRA2 regulation in the root and hypocotyl epidermis of Arabidopsis. Plant Physiol. **117:** 73-84.

Ikeda, Y., Men, S., Fischer, U., Stepanova, A.N., Alonso, J.M., Ljung, K., and Grebe, M. (2009). Local auxin biosynthesis modulates gradient-directed planar polarity in Arabidopsis. Nat. Cell Biol. **11:** 731-738.

Ishida, T., Hattori, S., Sano, R., Inoue, K., Shirano, Y., Hayashi, H., Shibata, D., Sato, S., Kato, T., Tabata, S. et al. (2007). Arabidopsis TRANSPARENT TESTA GLABRA2 is directly regulated by R2R3 MYB transcription factors and is involved in regulation of GLABRA2 transcription in epidermal differentiation. Plant Cell **19:** 2531-43.

Ishida, T., Kurata, T., Okada, K., and Wada, T. (2008). A genetic regulatory network in the development of trichomes and root hairs. Annu. Rev. Plant Biol. **59:** 365-86.

Johnson, C. S., Kolevski, B., and Smyth, D. R. (2002). TRANSPARENT TESTA GLABRA2, a trichome and seed coat development gene of Arabidopsis, encodes a WRKY transcription factor. Plant Cell **14:** 1359-75.

Jones, A.R., and Smirnoff, N. (2006). Nuclear dynamics during the simultaneous and sustained tip growth of multiple root hairs arising from a single root epidermal cell. J. Exp. Bot. **57:** 4269-4275.

Jones, A.R., Raymond, M.J., Yang, Z., and Smirnoff, N. (2007). NADPH oxidase-dependent reactive oxygen species formation required for root hair growth depends on ROP GTPase. J. Exp. Bot. **58:** 1261-1270.

Jones A.R., Kramer E.M., Knox K., Swarup R., Bennett M.J., Lazarus C.M., Leyser H.M., and Grierson C.S. (2009). Auxin transport through non-hair cells sustains root-hair development. Nat. Cell Biol. **11:**78-84.

Jones, M.A., Shen, J.J., Fu, Y., Li, H., Yang, Z., and Grierson, C.S. (2002). The Arabidopsis Rop2 GTPase is a positive regulator of both root hair initiation and tip growth. Plant Cell **14:** 763-776.

Jones, M.A., Raymond, M.J., and Smirnoff, N. (2006) Analysis of the root-hair morphogenesis transcriptome reveals the molecular identity of six genes with roles in root-hair development in Arabidopsis. Plant J. **45:** 83-100.

Kang, B.H., Nielsen, E., Preuss, M.L., Mastronarde, D., and Staehelin, L.A. (2011). Electron tomography of RabA4b- and PI-4Kbeta1-labeled trans Golgi network compartments in Arabidopsis. Traffic **12:** 313-329.

Kang, Y. H., Kirik, V., Hulskamp, M., Nam, K. H., Hagely, K., Lee, M. M., and Schiefelbein, J. (2009). The MYB23 gene provides a positive feedback loop for cell fate specification in the Arabidopsis root epidermis. Plant Cell **21:** 1080-1094.

Kang, Y.H., Song, S.K., Schiefelbein, J., and Lee, M.M. (2013) Nuclear trapping controls the position-dependent localization of CAPRICE in the root epidermis of Arabidopsis. Plant Physiol. **163:** 193-204.

Kapulnik, Y., Delaux, P.-M., Resnick, N., Mayzlish-Gati, E., Wininger, S., Bhattacharya, C., Sejalon-Delmas, N., Combier, J.-P., Becard, G., Belausov, E., et al. (2011). Strigolactones affect lateral root formation and root-hair elongation in Arabidopsis. Planta **233:** 209–216.

Karnik R., Grefen C., Bayne R., Honsbein A., Köhler T., Kioumourtzoglou D., Williams M., Bryant N.J., and Blatt, M.R. (2013). Arabidopsis Sec1/Munc18 protein SEC11 is a competitive and dynamic modulator of SNARE binding and SYP121-dependent vesicle traffic. Plant Cell **25:** 1368-1382.

Ketelaar, T. (2013). The actin cytoskeleton in root hairs: all is fine at the tip. Curr Op Plant Biol **16:** 749–756.

Ketelaar, T., Faivre-Moskalenko, C., Esseling, J.J., De Ruijter, N.C.A., Grierson, C.S., Dogterom, M., and Emons, A.M.C. (2002). Positioning of nuclei in Arabidopsis root hairs: an actin-regulated process of tip growth. Plant Cell **14:** 2941–2955.

Ketelaar, T., de Ruijter, N.C., and Emons, A.M. (2003). Unstable F-actin specifies the area and microtubule direction of cell expansion in Arabidopsis root hairs. Plant Cell **15:** 285-292.

Ketelaar, T., Allwood, E.G., Anthony, R., Voight, B., Menzel, D., and Hussey, P.J. (2004). The actin-interacting protein AIP1 is essential for acting organization and plant development. Curr. Biol. **14:** 145-149.

Ketelaar, T., Allwood, E.G., and Hussey, P.J. (2007). Actin organization and root hair development are disrupted by ethanol-induced overexpression of Arabidopsis actin interacting protein (AIP1). New Phytol. **174:** 57-62.

Ketelaar, T., Galway, M.E., Mulder, B.M., and Emons, A.M. (2008). Rates of exocytosis and endocytosis in Arabidopsis root hairs and pollen tubes. J Microsc. **231:** 265-273.

Khurana, P., Henty, J.L., Huang, S., Staiger, A.M., Blanchoin, L., and Staiger, C.J. (2010). Arabidopsis VILLIN1 and VILLIN3 have overlapping and distinct activities in actin bundle formation and turnover. Plant Cell **22:** 2727-2748.

Kieber, J.J., Rothenberg, M., Roman, G., Feldman, K.A., and Ecker, J.R. (1993). CTR1, a negative regulator of the ethylene response pathway in Arabidopsis, encodes a member of the Raf family of protein kinases. Cell **72**, 427-441.

Kirik, V., Simon, M., Huelskamp, M., and Schiefelbein, J. (2004). The ENHANCER OF TRY AND CPC1 gene acts redundantly with TRIPTY-CHON and CAPRICE in trichome and root hair cell patterning in Arabidopsis. Dev. Biol. **268:** 506-513.

Knox, K., Grierson, C.S., and Leyser, O. (2003). AXR3 and SHY2 interact to regulate root hair development. Development **130:** 5769-5777.

Kohno, T., and Shimmen, T. (1988). Accelerated sliding of pollen tube organelles along Characeae actin bundles regulated by Ca2+. J Cell Biol **106:** 1539–1543.

Koornneef, M. (1981). The complex syndrome of ttg mutants. Arabidopsis Inf. Serv. **18:** 45-51.

Koornneef, M., Dellaert, L.W.M., and van der Veen, J.H. (1982). EMS- and radiation-induced mutation frequencies at individual loci in *Arabidopsis thaliana*. Mutat. Res. **93:** 109-123.

Kuppusamy, K.T., Chen, A.Y., and Nemhauser, J.L. (2009). Steroids are required for epidermal cell fate establishment in *Arabidopsis* roots. Proc. Natl. Acad. Sci. USA **106:** 8073-8076.

Kurata, T., Ishida, T., Kawabata-Awai, C., Noguchi, M., Hattori, S., Sano, R., Nagasaka, R., Tominaga, R., Koshino-Kimura, Y., Kato, T. et al. (2005). Cell-to-cell movement of the CAPRICE protein in Arabidopsis root epidermal cell differentiation. Development **132:** 5387-98.

Kusano, H., Testerink, C., Vermeer, J.E., Tsuge, T., Shimada, H., Oka, A., Munnik, T., and Aoyama, T. (2008). The Arabidopsis phophatidylinositol phosphate 5-kinase PIP5K3 is a key regulator of root hair tip growth. Plant Cell **20:** 367-380.

Kwak, S. H., and Schiefelbein, J. (2008). A feedback mechanism controlling SCRAMBLED receptor accumulation and cell-type pattern in Arabidopsis. Curr. Biol. **18:** 1949-1954.

Kwak, S. H., Shen, R., and Schiefelbein, J. (2005). Positional signaling mediated by a receptor-like kinase in Arabidopsis. Science **307:** 1111-1113.

Larkin, J.C., Marks, M.D., Nadeau, J., and Sack, F. (1997). Epidermal cell fate and patterning in leaves. Plant Cell **9:** 1109-1120.

Leavitt, R.G. (1904). Trichomes of the root in vascular cryptograms and angiosperms. Proc. Boston Soc. Nat. Hist. **31:** 273-313.

Lee, M.M., and Schiefelbein, J. (1999). WEREWOLF, a MYB-related protein in Arabidopsis, is a position-dependent regulator of epidermal cell patterning. Cell **99:** 473-483.

Lee, M.M., and Schiefelbein, J. (2001). Developmentally distinct MYB genes encode functionally equivalent proteins in Arabidopsis. Development **128:** 1539-1546.

Lee, M.M., and Schiefelbein, J. (2002). Cell pattern in the Arabidopsis root epidermis determined by lateral inhibition with feedback. Plant Cell **14:** 611-618.

Lee, S.H., and Cho, H.T. (2006). PINOID positively regulates auxin efflux in Arabidopsis root hair cells and tobacco cells. Plant Cell **18:** 1604-1616.

Lee, Y.-R.J., and Liu, B. (2004). Cytoskeletal motors in Arabidopsis. Sixty-one kinesins and seventeen myosins. Plant Physiol **136:** 3877–3883.

Lew, R.R. (1991). Electrogenic Transport-Properties of Growing Arabidopsis Root Hairs - the Plasma-Membrane Proton Pump and Potassium Channels. Plant Physiol. **97:** 1527-1534.

Lew, R.R. (1996). Pressure regulation of the electrical properties of growing *Arabidopsis thaliana L* root hairs. Plant Physiol. **112:** 1089-1100.

Lew, R.R. (1998). Immediate and steady state extracellular ionic fluxes of growing *Arabidopsis thaliana* root hairs under hyperosmotic and hypoosmotic conditions. Physiol. Plant. **104:** 397-404.

Lew, R.R. (2000). Electrobiology of Root Hairs. In Root Hairs: Cell and Molecular Biology, R. W. Ridge and A. M. C. Emons, eds. (Tokyo: Springer-Verlag), pp. 115-139.

Leyser, H.M., Pickett, F.B., Dharmasiri, S., and Estelle, M. (1996). Mutations in the AXR3 gene of Arabidopsis result in altered auxin response including ectopic expression from the SAUR-AC1 promoter. Plant J.

10: 403-413.

Lin, C., Choi, H.S., and Cho, H.T. (2011). Root hair-specific EXPANSIN A7 is required for root hair elongation in Arabidopsis. Mol. Cells **31:** 393-397.

Lin, Y., and Schiefelbein, J. (2001). Embryonic control of epidermal cell patterning in the root and hypocotyl of Arabidopsis. Development **128:** 3697-3705.

Ma, Z., Bielenberg, D. G., Brown, K. M., and Lynch, J. P. (2001). Regulation of root hair density by phosphorus availability in *Arabidopsis thaliana*. Plant Cell Environ. **24:** 459-467.

Maciver, S.K., and Hussey, P.J. (2002). The ADF/cofilin family: actin-remodeling proteins. Genome Biol. **3:** 3007.1–3007.12.

Marc, J., Granger, C.L., Brincat, J., Fisher, D.D., Kao, T., McCubbin, A.G., and Cyr, R.J. (1998). A GFP-MAP4 reporter gene for visualizing cortical microtubule rearrangements in living epidermal cells. Plant Cell **10:** 1927–1939.

Masucci, J.D., and Schiefelbein, J.W. (1994) The rhd6 mutation of *Arabidopsis thaliana* alters root-hair initiation through an auxin- and ethylene-associated process. Plant Physiol. **106:** 1335-1346.

Masucci, J.D., and Schiefelbein, J.W. (1996) Hormones act downstream of TTG and GL2 to promote root hair outgrowth during epidermis development in the Arabidopsis root. Plant Cell **8:** 1505-1517.

Masucci, J.D., Rerie, W.G., Foreman, D.R., Zhang, M., Galway, M.E., Marks, M.D., and Schiefelbein, J.W. (1996). The homeobox gene GLABRA2 is required for position-dependent cell differentiation in the root epidermis of *Arabidopsis thaliana*. Development **122:** 1253-1260.

Mathur, J., Mathur, N., Kernebeck, B., and Hulskamp, M. (2003a). Mutations in actin-related proteins 2 and 3 affect cell shape development in Arabidopsis. Plant Cell **15:** 1632-1645.

Mathur, J., Mathur, N., Kirik, V., Kernebeck, B., Srinivas, B.P., and Hulskamp, M. (2003b). Arabidopsis CROOKED encodes for the smallest subunit of the ARP2/3 complex and controls cell shape by region specific fine F-actin formation. Development **130:** 3137-3146.

Menand, B., Yi, K., Jouannic, S., Hoffmann, L., Ryan, E., Linstead, P., Schaefer, D.G., and Dolan, L. (2007). An ancient mechanism controls the development of cells with a rooting function in land plants. Science **316:** 1477-1480.

Michelot, A., Guérin, C., Huang, S., Ingouff, M., Richard, S., Rodiuc, N., Staiger, C., and Blanchoin, L. (2005). The formin homology 1 domain modulates the actin nucleation and bundling activity of Arabidopsis FORMIN1. Plant Cell **17:** 2296-2313.

Miller, D.D., De Ruijter, N.C.A., Bisseling, T., and Emons, A.M.C. (1999). The role of actin in root hair morphogenesis: studies with lipochito oligosaccharide as a growth stimulator and cytochalasin as an actin perturbing drug. Plant J. **17:** 141–154.

Mizra, J.I., Olsen, G.M., Iversen, T.-H., and Maher, E.P. (1984). The growth and gravitropic responses of wild-type and auxin resistant mutants of *Arabidopsis thaliana*. Physiol. Plant. **60:** 516-522.

Mohnen, D., and Tierney, M.L. (2011). Plant science. Plants get Hyp to O-glycosylation. Science **332:** 1393-1394.

Molendijk, A. J., Bischoff, F., Rajendrakumar, C. S. V., Friml, J., Braun, M., Gilroy, S., and Palme, K. (2001). *Arabidopsis thaliana* Rop GT-Pases are localized to tips of root hairs and control polar growth. EMBO J. **20:** 2779-2788.

Monshausen G.B., Bibikova T.N., Messerli M.A., Shi C., and Gilroy S. (2007). Oscillations in extracellular pH and reactive oxygen species modulate tip growth of Arabidopsis root hairs. Proc. Natl. Acad. Sci. U S A. **104:** 20996-21001.

Monshausen G.B., Messerli M.A., and Gilroy S. (2008). Imaging of the Yellow Cameleon 3.6 indicator reveals that elevations in cytosolic Ca2+ follow oscillating increases in growth in root hairs of Arabidopsis. Plant

Physiol. **147:** 1690-1698.

Moog, P.R., Vanderkooij, T.A.W., Bruggemann, W., Schiefelbein, J.W., and Kuiper, P.J.C. (1995). Responses to Iron-Deficiency In Arabidopsis-Thaliana - the Turbo Iron Reductase Does Not Depend On the Formation Of Root Hairs and Transfer Cells. Planta **195:** 505-513.

Moutinho, A., Hussey, P.J., Trewavas, A.J., and Malhó. R. (2001). cAMP acts as a second messenger in pollen tube growth and reorientation. PNAS **98:** 10481-10486.

Mueller-Roeber, B., and Pical, C. (2002). Inositol phospholipid metabolism in Arabidopsis. Characterized and putative isoforms of inositol phospholipid kinase and phosphoinositide-specific phospholipase C. Plant Physiol. **130:** 22-46.

Nagpal, P., Walker, L. M., Young, J. C., Sonawala, A., Timpte, C., Estelle, M., and Reed, J. W. (2000). AXR2 encodes a member of the Aux/IAA protein family. Plant Physiol. **123:** 563-573.

Narang, R. A., Bruene, A., and Altmann, T. (2000). Analysis of phosphate acquisition efficiency in different arabidopsis accessions. Plant Physiol. **124:** 1786-1799.

Newcomb, E.H., and Bonnett, H.T. (1965). Cytoplasmic microtubule and wall microfibril orientation in root hairs of radish. J. Cell Biol. **27:** 575-589.

Nielsen, E., Cheung, A.Y., and Ueda, T. (2008). The regulatory RAB and ARF GTPases for vesicular trafficking. Plant Physiol. **147:** 1516-1526.

Ojangu, E.L., Järve, K., Paves, H., and Truve, E. (2007). *Arabidopsis thaliana* myosin XIK is involved in root hair as well as trichome morphogenesis on stems and leaves. Protoplasma **230:** 193-202.

Ovecka, M., Berson, T., Beck, M., Derksen, J., Samaj, J., Baluska, F., and Lichtscheidl, I.K. (2010). Structural sterols are involved in both the initiation and tip growth of root hairs in *Arabidopsis thaliana*. Plant Cell **22:** 2999-3019.

Oyama, T., Shimura, Y., and Okada, K. (2002). The IRE gene encodes a protein kinase homologue and modulates root hair growth in Arabidopsis. Plant J. **30:** 289-299.

Pang, C.Y., Wang, H., Pang, Y., Xu, C., Jiao, Y., Qin, Y.M., Western, T.L., Yu, S.X., and Zhu, Y.X. (2010). Comparative proteomics indicates that biosynthesis of pectic precursors is important for cotton fiber and Arabidopsis root hair elongation. Mol. Cell Proteomics **9:** 2019-2033.

Park, E., and Nebenfur, A. (2013). Myosin XIK of *Arabidopsis thaliana* accumulates at the root hair tip and is required for fast hair growth. PLoS One **8:** e76745.

Park, S., Szumlanski, A.L., Gu, F., Guo, F., and Nielsen, E. (2011). A role for CSLD3 during cell-wall synthesis in apical plasma membranes of tip-growing root-hair cells. Nat. Cell Biol. **13:** 973-980.

Parker, J.S., Cavell, A.C., Dolan, L., Roberts, K., and Grierson, C.S. (2000). Genetic interactions during root hair morphogenesis in Arabidopsis. Plant Cell **12:** 1961-1974.

Parsons, R.F. (2009). Hypocotyl hairs: an historical perspective. Aust. J. Bot. **57:** 106-108.

Payne, R.J., and Grierson, C.S. (2009). A theoretical model for ROP localisation by auxin in *Arabidopsis* root hair cells. PLoS ONE **4:** e8337.

Pei, W., Du, F., Zhang, Y., He, T., and Ren, H. (2012). Control of the actin cytoskeleton in root hair development. Plant Sci **187:** 10-18.

Peña, M.J., Kong, Y., York, W.S., and O'Neill, M.A. (2012). A galacturonic acid-containing xyloglucan is involved in Arabidopsis root hair tip growth. Plant Cell **24:** 4511-4524.

Peremyslov, V.V., Prohknevsky, A.I., Avisar, D., and Dolja, V.V. (2008). Two class XI myosins function in organelle trafficking and root hair development in Arabidopsis. Plant Physiol **146:** 1109-1116.

Peremyslov, V.V., Prokhnevsky, A.I., and Dolja, V.V. (2010). Class XI myosins are required for development, cell expansion, and F-Actin organization in Arabidopsis. Plant Cell **22:** 1883-1897.

Pitts, R.J., Cernac, A., and Estelle, M. (1998). Auxin and ethylene promote root hair elongation in Arabidopsis. Plant J. **16:** 553-560.

Preuss, M.L., Serna, J., Falbel, T.G., Bednarek, S.Y., and Nielsen, E. (2004). The Arabidopsis Rab GTPase RabA4b localizes to the tips of growing root hair cells. Plant Cell **16:** 1589-1603.

Preuss, M.L., Schmitz, A.J., Thole, J.M., Bonner, H.K., Otegui, M.S., and Nielsen, E. (2006). A role for the RabA4b effector protein PI-4Kbeta1 in polarized expansion of root hair cells in *Arabidopsis thaliana*. J. Cell Biol. **172:** 991-998.

Prokhnevsky, A.I., Peremyslov, V.V., and Dolja, V.V. (2008). Overlapping functions of the four class XI myosins in Arabidopsis growth, root hair elongation, and organelle motility. Proc. Natl. Acad. Sci. USA **105:** 19744-19749.

Qu, X., Zhang, H., Xie, Y., Wang, J., Chen, N., and Huang, S. (2013). Arabidopsis villins promote actin turnover at pollen tube tips and facilitate the construction of actin collars. Plant Cell **25:**1803-1817.

Ramachandran, S., Christensen, H.E., Ishimaru, Y., Dong, C.H., Chao-Ming, W., Cleary, A.L., and Chua, N.H. (2000). Profilin plays a role in cell elongation, cell shape maintenance, and flowering in Arabidopsis. Plant Physiol. **124:** 1637-1647.

Reddy, A.S.N., and Day, I.S. (2001). Analysis of the myosins encoded in the recently completed *Arabidopsis thaliana* genome sequence. Genome Biol. **2:** research0024.1-0024.17.

Reed, J.W., Nagpal, P., Poole, D.S., Furuya, M., and Chory J. (1993) Mutations in the gene for the red far-red light receptor phytochrome-b alter cell elongation and physiological responses throughout Arabidopsis development. Plant Cell **5:** 147-157.

Reisen, D., and Hanson, M.R. (2007). Association of six YFP-myosin XI-tail fusions with mobile plant cell organelles. BMC Plant Biol. **7:** 6.

Rerie, W.G., Feldmann, K.A., and Marks, M.D. (1994). The GLABRA2 gene encodes a homeodomain protein required for normal trichome development in Arabidopsis. Genes Dev. **8:** 1388-1399.

Ridge, R. W., Uozumi, Y., Plazinski, J., Hurley, U. A., and Williamson, R. E. (1999). Developmental transitions and dynamics of the cortical ER of Arabidopsis cells seen with green fluorescent protein. Plant Cell Physiol. **40:** 1253-1261.

Rigas, S., Debrosses, G., Haralampidis, K., Vicente-Agullo, F., Feldmann, K. A., Grabov, A., Dolan, L., and Hatzopoulos, P. (2001). TRH1 encodes a potassium transporter required for tip growth in Arabidopsis root hairs. Plant Cell **13:** 139-151.

Rigas, S., Ditengou, F.A., Ljung, K., Daras, G., Tietz, O., Palme, K., and Hatzopoulos, P. (2013). Root gravitropism and root hair development constitute coupled developmental responses regulated by auxin homeostasis in the Arabidopsis root apex. New Phytol. **197:** 1130–1141.

Ringli, C., Baumberger, N., Diet, A., Frey, B., and Keller, B. (2002). ACTIN2 is essential for bulge site selection and tip growth during root hair development of Arabidopsis. Plant Physiol. **129:** 1464-1472.

Ryan, E., Grierson, C.S., Cavell, A., Steer, M., and Dolan, L. (1998). TIP1 is required for both tip growth and non-tip growth in Arabidopsis. New Phytol. **138:** 49-58.

Ryan, E., Steer, M., and Dolan, L. (2001). Cell biology and genetics of root hair formation in *Arabidopsis thaliana*. Protoplasma **215:** 140-149.

Ryu, K.H., Kang, Y.H., Park, Y.H., Hwang, I., Schiefelbein, J., and Lee, M.M. (2005). The WEREWOLF MYB protein directly regulates CAPRICE transcription during cell fate specification in the Arabidopsis root epidermis. Development **132:** 4765-4775.

Ryu, K. H., Zheng, X., Huang, L., and Schiefelbein, J. (2013). Computational modeling of epidermal cell fate determination systems. Curr. Opin. Plant Biol. **16:** 5-10.

Sakai, T., van der Honing, H.S., Nishioka, M., Uehara, Y., Takahashi, M., Fujisawa, N., Saji, K., Seki, M., Shinozaki, K., Jones, M.A.,

Smirnoff, N., Okada, K., and Wasteneys, G.O. (2008). Armadillo repeat-containing kinesins and a NIMA-related kinase are required for epidermal-cell morphogenesis in Arabidopsis. Plant J. **53:** 157-171.

Savage, N.S., Walker, T., Wieckowski, Y., Schiefelbein, J., Dolan, L., and Monk, N.A. (2008) A mutual support mechanism through intercellular movement of CAPRICE and GLABRA3 can pattern the Arabidopsis root epidermis. PLoS Biol. **6:** e235.

Savage, N., Yang, T.J., Chen, C.Y., Lin, K.L., Monk, N.A., and Schmidt, W. (2013). Positional signaling and expression of ENHANCER OF TRY AND CPC1 are tuned to increase root hair density in response to phosphate deficiency in *Arabidopsis thaliana*. PLoS One **8:** e75452.

Schellmann, S., Schnittger, A., Kirik, V., Wada, T., Okada, K., Beermann, A., Thumfahrt, J., Jurgens, G. and Hulskamp, M. (2002). TRIPTYCHON and CAPRICE mediate lateral inhibition during trichome and root hair patterning in Arabidopsis. EMBO J. **21:** 5036-5046.

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

Schiefelbein, J.W., and Somerville, C. (1990). Genetic control of root hair development in *Arabidopsis thaliana*. Plant Cell **2:** 235-243.

Schiefelbein, J. W., Shipley, A., and Rowse, P. (1992). Calcium influx at the tip of growing root-hair cells of *Arabidopsis thaliana*. Planta **187:** 455-459.

Schiefelbein, J., Galway, M., Masucci, J., and Ford, S. (1993). Pollen tube and root-hair tip growth is disrupted in a mutant of *Arabidopsis thaliana*. Plant Physiol. **103:** 979-985.

Schiefelbein, J., Kwak, S.-H., Wieckowski, Y., Barron, C., Bruex, A. (2009) The gene regulatory network for root epidermal cell-type pattern formation in Arabidopsis. J. Exp. Bot. **60:** 1515-1521.

Schmidt, W., and Schikora, A. (2001). Different pathways are involved in phosphate and iron stress- induced alterations of root epidermal cell development. Plant Physiol. **125:** 2078-2084.

Schmidt, W., Tittel, J., and Schikora, A. (2000). Role of hormones in the induction of iron deficiency responses in Arabidopsis roots. Plant Physiol. **122:** 1109-1118.

Schneider, K., Wells, B., Dolan, L., and Roberts, K. (1997) Structural and genetic analysis of epidermal cell differentiation in Arabidopsis primary roots. Development **124:** 1789-1798

Schneider, K., Mathur, J., Boudonck, K., Wells, B., Dolan, L., and Roberts, K. (1998). The ROOT HAIRLESS1 gene encodes a nuclear protein required for root hair initiation in Arabidopsis. Genes Dev. **12:** 2013-2021.

Seifert, G.J., Barber, C., Wells, B., Dolan, L., and Roberts, K. (2002). Galactose biosynthesis in Arabidopsis: genetic evidence for substrate channeling from UDP-D-galactose into cell wall polymers. Curr. Biol. **12:** 1840–1845.

Seifert, G.J., Barber, C., Wells, B., and Roberts, K. (2004). Growth regulators and the control of nucleotide sugar flux. Plant Cell **16:** 723–730.

Serna, L. (2008). CAPRICE positively regulates stomatal formation in the Arabidopsis hypocotyl. Plant Signal Behav. **3:** 1077-1082.

Sieberer, B., and Emons, A.M.C. (2000). Cytoarchitecture and pattern of cytoplasmic streaming in root hairs of Medicago truncatula during development and deformation by nodulation factors. Protoplasma **214:** 118–127.

Sieberer, B.J., Ketelaar, T., Esseling, J.J., and Emons, A.M. (2005). Microtubules guide root hair tip growth. New Phytol **167:** 711-719.

Simon, M., Lee, M. M., Lin, Y., Gish, L., and Schiefelbein, J. (2007). Distinct and overlapping roles of single-repeat MYB genes in root epidermal patterning. Dev Biol **311:** 566-78.

Simon, M., Bruex, A., Kainkaryam, R.M., Zheng, X., Huang, L., Woolf,

P.J., and Schiefelbein, J. (2013). Tissue-specific profiling reveals transcriptome alterations in Arabidopsis mutants lacking morphological phenotypes. Plant Cell **25:** 3175-85.

Sliwinska, E., Mathur, J., and Bewley, J.D. (2012). Synchronously developing collet hairs in *Arabidopsis thaliana* provide an easily accessible system for studying nuclear movement and endoreduplication. J. Exp. Bot. **63:** 4165-4178.

Smertenko, A.P., Jiang, C.J., Simmons, N.J., Weeds, A.G., Davies, D.R., and Hussey, P.J. (1998). Ser6 in the maize actin-depolymerizing factor, ZmADF3, is phosphorylated by a calcium-stimulated protein kinase and is essential for the control of functional activity. Plant J. **14:** 187-193.

Song, S.K., Ryu, K.H., Kang, Y.H., Song, J.H., Cho, Y.H., Yoo, S.D. Schiefelbein, J., and Lee, M.M. (2011). Cell fate in the Arabidopsis root epidermis is determined by competition between WEREWOLF and CAPRICE. Plant Physiol. **157:** 1196-208.

Staiger, C.J., Poulter, N.S., Henty, J.L., Franklin-Tong, V.E., and Blanchoin, L. (2010). Regulation of actin dynamics by actin-binding proteins in pollen. J. Exp. Bot. **61:** 1969-1986.

Stenzel, I., Ischebeck, T., Konig, S., Holubowska, A., Sporysz, M., Hause, B., and Heilmann, I. (2008). The type B phosphatidylinositol-4-phosphate 5-kinase 3 is essential for root hair formation in *Arabidopsis thaliana*. Plant Cell **20:** 124-141.

Sugimoto-Shirasu, K., Stacey, N.J., Corsar, J., Roberts, K., and Mc-Cann, M.C. (2002). DNA topoisomerase VI is essential for endoreduplication in Arabidopsis. Curr. Biol. **12:** 1782-1786.

Sugimoto-Shirasu K, Roberts GR, Stacey NJ, McCann MC, Maxwell A., and Roberts K. (2005). RHL1 is an essential component of the plant DNA topoisomerase VI complex and is required for ploidy-dependent cell growth. Proc. Natl. Acad. Sci. USA **102:** 18736-18741.

Takagi, S., and Nagai, R. (1986). Intracellular Ca2+ concentration and cytoplasmic streaming in Vallisneria mesophyll cells. Plant Cell Physiol. **27:** 953–959.

Tanimoto, M., Roberts, K., and Dolan, L. (1995). Ethylene is a positive regulator of root-hair development in *Arabidopsis thaliana*. Plant J. **8:** 943-948.

Thole, J.M., Vermeer, J.E., Zhang, Y., Gadella, T.W., and Nielsen, E. (2008). Root hair defective4 encodes a phosphatidylinositol-4-phosphate phosphatase required for proper root hair development in *Arabidopsis thaliana*. Plant Cell **20:** 381-395.

Tominaga, M., Yokota, E., Vidali, L., Sonobe, S., Hepler, P.K., and Shimmen, T. (2000). The role of plant villin in the organization of the actin cytoskeleton, cytoplasmic streaming and the architecture of the transvacuolar strand in root hair cells of Hydrocharis. Planta **210:** 836–843.

Tominaga, M., Kojima, H., Yokota, E., Nakamori, R., Anson, M., Shimmen, T., and Oiwa, K. (2012). Calcium-induced mechanical change in the neck domain alters the activity of plant myosin XI. J. Biol. Chem. **287:** 30711-30718.

Tominaga, R., Iwata, M., Okada, K. and Wada, T. (2007). Functional analysis of the epidermal-specific MYB genes CAPRICE and WEREWOLF in Arabidopsis. Plant Cell **19:** 2264-77.

Tominaga-Wada, R., Ishida, T. and Wada, T. (2011). New insights into the mechanism of development of Arabidopsis root hairs and trichomes. Int. Rev. Cell Mol. Biol. **286:** 67-106.

Turing, A. (1952). The chemical basis of morphogenesis. Phil. Trans. R. Soc. Lond. B **237:** 37-72.

Valenta, R., Ferreira, F., Grote, M., Swoboda, I., Vrtala, S., Duchêne, M., Deviller, P., Meagher, R.B., McKinney, E., Heberle-Bors, E., Kraft, D., and Scheiner, O. (1993). Identification of profilin as an actin-binding protein in higher plants. J. Biol. Chemistry **268:** 22777-22781.

Van Bruaene, N., Joss, G., and Van Oostveldt, P. (2004). Reorganization and in vivo dynamics of microtubules during Arabidopsis root hair development. Plant Physiol. **136:** 3905–3919.

Van der Honing, H.S., de Ruijter, N.C., Emons, A.M., and Ketelaar, T. (2010). Actin and myosin regulate cytoplasm stiffness in plant cells: a study using optical tweezers. New Phytol. **185:** 90-102.

Van Gisbergen, P.A., and Bezanilla, M. (2013). Plant formins: membrane anchors for actin polymerization. Trends Cell Biol. **23:** 227-233.

Velasquez, S.M., Ricardi, M.M., Dorosz, J.G., Fernandez, P.V., Nadra, A.D., et al. (2011). O-glycosylated cell wall proteins are essential in root hair growth. Science **332:** 1401-1403.

Vernoud, V., Horton, A.C., Yang, Z., and Nielsen, E. (2003). Analysis of the small GTPase gene superfamily of Arabidopsis. Plant Physiol. **131:** 1191-1208.

Very, A. A., and Davies, J. M. (2000). Hyperpolarization-activated calcium channels at the tip of Arabidopsis root hairs. Proc. Natl. Acad. Sci. USA **97:** 9801-9806.

Vidali, L., Van Gisbergen, P.A.C., Guérin, C., Franco, P., Li, M., Burkart, G.M., Augustine, R.C., Blanchoin, L., and Bezanilla, M. (2009). Rapid formin-mediated actin-filament elongation is essential for polarized plant cell growth. PNAS **106:** 13341-13346.

Wada, T., Tachibana, T., Shimura, Y., and Okada, K. (1997). Epidermal cell differentiation in Arabidopsis determined by a Myb homolog, CPC. Science **277:** 1113-1116.

Walker, A.R., Davison, P.A., Bolognesi-Winfield, A.C., James, C.M., Srinivasan, N., Blundell, T.L., Esch, J.J., Marks, M.D., and Gray, J.C. (1999). The TRANSPARENT TESTA GLABRA1 locus, which regulates trichome differentiation and anthocyanin biosynthesis in Arabidopsis, encodes a WD40 repeat protein. Plant Cell **11:** 1337-1349.

Wang, H.-Y., Lockwood, S.K., Hoeltzel, M.F., and Schiefelbein, J.W. (1997). The ROOT HAIR DEFECTIVE3 gene encodes an evolutionarily conserved protein with GTP-binding motifs and is required for regulated cell enlargement in Arabidopsis. Genes Dev. **11:** 799-811.

Wang, H., Lee, M.M., and Schiefelbein, J.W. (2002). Regulation of the cell expansion gene RHD3 during Arabidopsis development. Plant Physiol. **129:** 638-649.

Wang, S., Barron, C., Schiefelbein, J., and Chen, J.G. (2010). Distinct relationships between GLABRA2 and single-repeat R3 MYB transcription factors in the regulation of trichome and root hair patterning in Arabidopsis. New Phytol. **185:** 387-400.

Wang, X., Cnops, G., Vanderhaeghen, R., De Block, S., Van Montagu, M., and Van Lijsebettens, M. (2001). AtCSLD3, a cellulose synthase-like gene important for root hair growth in Arabidopsis. Plant Physiol. **126:** 575-586.

Wei, N., Kwok, S.F., von Arnim, A.G., Lee, A., McNellis, T.W., Piekos, B., and Deng, X.-W. (1994). Arabidopsis COP8, COP10, and COP11 genes are involved in repression of photomorphogenic development in darkness. Plant Cell **6:** 629-643.

Wilson, A., Pickett, F.B., Turner, J.C., and Estelle, M. (1990). A dominant mutation in Arabidopsis confers resistance to auxin, ethylene, and abscisic acid. Mol. Gen. Genet. **222:** 377-383.

Won, S.K., Lee, Y.J., Lee, H.Y., Heo, Y.K., Cho, M., and Cho, H.T. (2009). Cis-element- and transcriptome-based screening of root hair-specific genes and their functional characterization in Arabidopsis. Plant Physiol. **150:** 1459-1473.

Woods, C.M., Polito, V.S., and Reid, M.S. (1984). Response to chilling stress in plant cells. II. Redistribution of intracellular calcium. Protoplasma **121:** 17–24.

Wymer, C. L., Bibikova, T. N., and Gilroy, S. (1997). Cytoplasmic free calcium distributions during the development of root hairs of *Arabidopsis thaliana*. Plant J. **12:** 427-439.

Xu, C. R., Liu, C., Wang, Y. L., Li, L. C., Chen, W. Q., Xu, Z. H., and Bai, S. N. (2005). Histone acetylation affects expression of cellular patterning genes in the Arabidopsis root epidermis. Proc. Natl. Acad. Sci. U S A **102:** 14469-14474.

Yang, G., Gao, P., Zhang, H., Huang, S., and Zheng, Z.L. (2007). A mutation in MRH2 kinesin enhances the root hair tip growth defect caused by constitutively activated ROP2 small GTPase in Arabidopsis. PLoS One **2:** e1074.

Yao, H., Wang, G., Guo, L., and Wang, X. (2013). Phosphatidic acid interacts with a MYB transcription factor and regulates its nuclear localization and function in Arabidopsis. Plant Cell **25:** 5030-5042.

Ye, J., Zheng, Y., Yan, A., Chen, N., Wang, Z., Huang, S., and Yang, Z. (2009). Arabidopsis formin3 directs the formation of actin cables and polarized growth in pollen tubes. Plant Cell **21:** 3868-3884.

Yi, K., Guo, C., Chen, D., Zhao, B., Yang, B., and Ren, H. (2005). Cloning and functional characterization of a formin-like protein (AtFH8) from Arabidopsis. Plant Physiol. **138:** 1071-1082.

Yi, K., Menand, B., Bell, E., and Dolan, L. (2010). A basic helix-loop-helix transcription factor controls cell growth and size in root hairs. Nature Genet. **42:** 264–267.

Yin, L., Verhertbruggen, Y., Oikawa, A., Manisseri, C., Knierim, B., Prak, L., Jensen, J.K., Knox, J.P., Auer, M., Willats, W.G., and Scheller, H.V. (2011). The cooperative activities of CSLD2, CSLD3, and CSLD5 are required for normal Arabidopsis development. Mol. Plant **4:** 1024-1037.

Yokota, E., Tominaga, M., Mabuchi, I., Tsuji, Y., Staiger, C.J., Oiwa, K., and Shimmen, T. (2005). Plant villin, lily P-135-ABP, possesses G-actin binding activity and accelerates the polymerization and depolymerization of actin in a Ca2+-sensitive manner. Plant Cell Physiol. **46:** 1690-1703.

Yoo, C.M., Quan, L., Cannon, A.E., Wen, J., and Blancaflor, E.B. (2012). AGD1, a class 1 ARF-GAP, acts in common signaling pathways with phosphoinositide metabolism and the actin cytoskeleton in controlling Arabidopsis root hair polarity. Plant J. **69:** 1064-1076.

Zerial, M., and McBride, H. (2001). Rab proteins as membrane organizers. Nat. Rev. Mol. Cell Biol. **2:** 107-117.

Zhang, H., Qu, X., Bao, C., Khurana, P., Wang, Q., Xie, Y., Zheng, Y., Chen, N., Blanchoin, L., Staiger, C.J., and Huang, S. (2010). Arabidopsis VILLIN5, an actin filament bundling and severing protein, is necessary for normal pollen tube growth. Plant Cell **22:** 2749-2767.

Zhang, Z., Zhang, Y., Tan, H., Wang, Y., Li, G., Liang, W., Yuan, Z., Hu, J., Ren, H., and Zhang, D. (2011a). RICE MORPHOLOGY DETERMINANT encodes the type II formin FH5 and regulates rice morphogenesis. Plant Cell **23:** 681-700.

Zhang, Y., Xiao, Y., Du, F., Cao, L., Dong, H., and Ren, H. (2011b). Arabidopsis VILLIN4 is involved in root hair growth through regulating actin organization in a Ca2+-dependent manner. New Phytol. **190:** 667–682.

Zhang, M., Wu, F., Shi, J., Zhu, Y., Zhu, Z., Gong, Q., and Hu, J. (2013). ROOT HAIR DEFECTIVE3 family of dynamin-like GTPases mediates homotypic endoplasmic reticulum fusion and is essential for Arabidopsis development. Plant Physiol. **163:** 713-720.

Zhu, C., Gan, L., Shen, Z., and Xia, K. (2006). Interactions between jasmonates and ethylene in the regulation of root hair development in Arabidopsis. J. Exp. Bot. **57:** 1299-1308.