Root Hair Development

Tatiana Bibikova and Simon Gilroy*

Biology Department, The Pennsylvania State University, 208 Mueller Lab, University Park, Pennsylvania 16802, USA

ABSTRACT

Root hairs are projections from the epidermal cells of the root that are thought to increase its effective surface area for nutrient and water uptake, enlarge the volume of exploited soil, and aid in anchoring the plant to the soil. Their formation occurs as a series of developmental processes starting with cell fate specification in the meristem. The root-hair-forming epidermal cell, or trichoblast, then participates in the diffuse growth phase associated with the elongation of the main root axis. After the fully elongated trichoblast exits the elongation zone, growth is reorganized and localized to the side in the process of root hair initiation. Initiation is then followed by a sustained phase of tip growth until the hair reaches its mature length. Thus, root hairs provide insight into a range of developmental processes from cell fate determination to growth control. The theme emerging from the molecular analysis of the control of root hair formation is that many regulators act at several stages of development. Root hair formation is also responsive to a multitude of nutrient and other environmental stimuli. Therefore, one explanation for the presence of the complex networks that regulate root hair morphogenesis may lie in the need to coordinate their highly plastic developmental program and entrain it to the current soil microenvironment being explored by the root.

Key words: *Arabidopsis*; Auxin; Calcium; Cytoskeleton; Root hair; Tip growth

ROOT HAIR FORM AND FUNCTION

Root hairs are specialized projections from modified epidermal cells of the root. They have been proposed to aid the root in nutrient and water acquisition by increasing both the surface area of the root and the volume of soil the root can access. In addition, they may aid in anchoring the root system more closely to the soil. In most plant species (most ferns, some monocots, and nearly all dicots), all epidermal cells of the root appear capable of producing a hair (so called Type I plants, Figure 1), whereas in others there is a mix of cells with the potential to make root hairs (trichoblasts) and those incapable of initiating this developmental program (atrichoblasts). Those species showing this mixed pattern are further divided on the basis of where the root hairs form. In Type II plants—*Lycopodium*, *Selaginella* and *Equisetum*, some monocots, and the dicot family Nymphaeaceae—root hairs form from the smaller cell produced by an asymmetric cell division in the meristem (Cutter and Feldman 1970; Cutter and Hung 1972; Cormack 1937). The Type III pattern of root hair formation is found in the Brassicaceae and is characterized by the root epidermal cells occurring in files composed of either atrichoblasts or trichoblasts (Cormack 1935, 1949; Figure 1A). Thus, the first phase of root hair production is cell fate specification at the meristem (either trichoblast or atrichoblast).
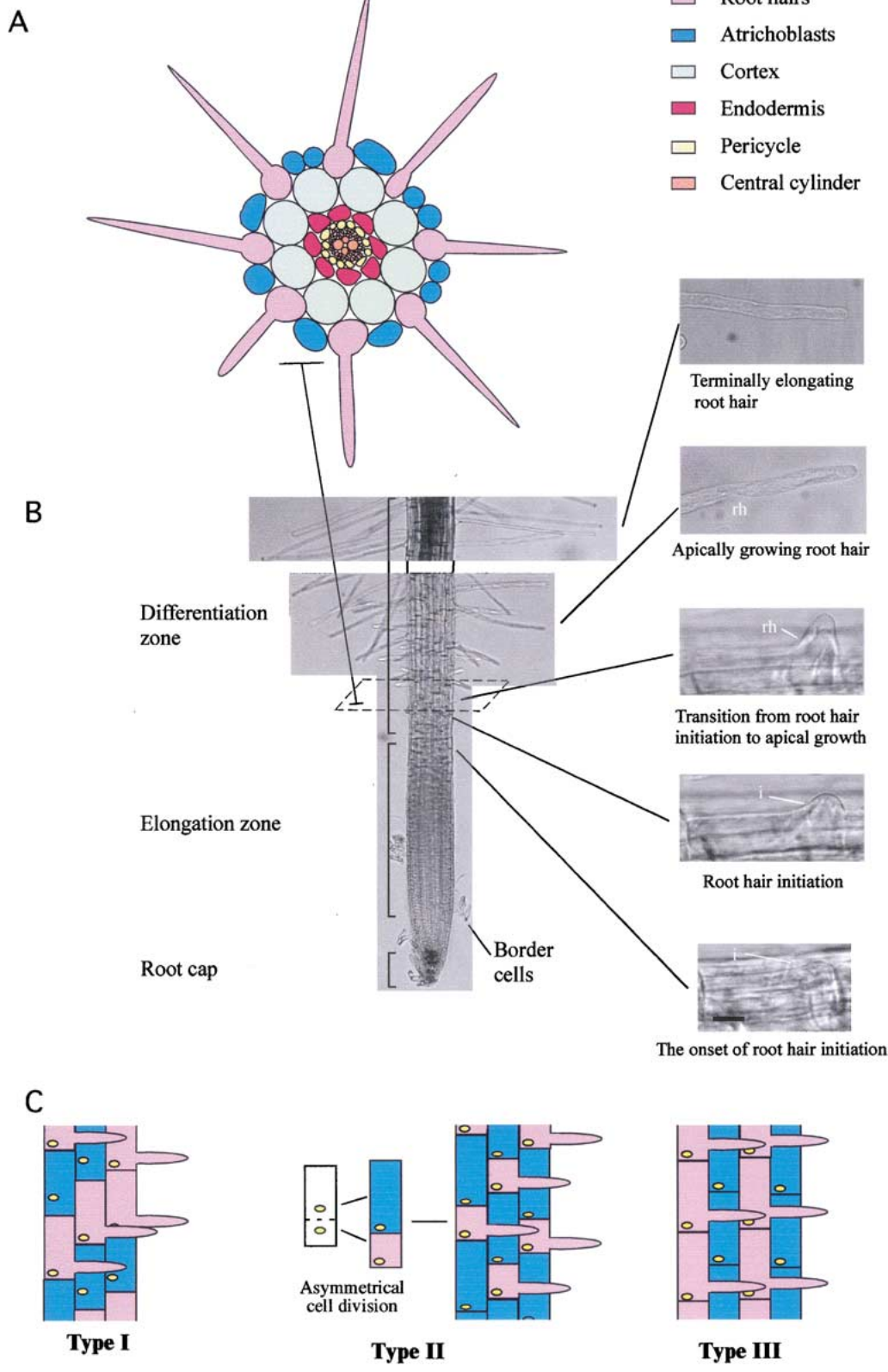
Figure 1. Patterns of root hair development. (**A**) Cross section of an *Arabidopsis* primary root showing spatial relationships of cell types to root-hair-forming cells (trichoblasts). Note that trichoblasts form over the junction of two underlying cortical cells. (**B**) Bright field images of the *Arabidopsis* primary root showing zones of development and developmental progression of a trichoblast as a root hair emerges, scale bar = 10 μm. i-initiation site; rh-root hair. (**C**) The three themes of root epidermal differentiation in plants: Type I, any cell can form a root hair; Type II, the trichoblast is the product of an asymmetrical cell division; Type III, differentiation producing files of either trichoblasts or atrichoblasts. Purple, atrichoblast; blue, trichoblast.

Upon exiting the elongation zone, the trichoblast initiates localized growth processes that lead to the emergence of a hairlike projection from the epidermal cell wall. The root hair is produced in the differentiation region of the root (Figure 1B) after diffuse elongation growth of the cell has ceased. Thus, when the root epidermal cell initiates a lateral bulge that will form a root hair, it must reorganize and redirect its axis of growth to a localized point where the outgrowth of the root hair will form. This site is precisely controlled along the lateral wall of the cell. For example, in *Arabidopsis*, in the absence of other factors, initiation occurs at the apical end of the trichoblast (closest to the root tip; Figure 1B, Dolan and others 1993, 1994). Thus, there must be a cellular mechanism to mark this position and act as a nucleation site for the growth machinery that will form the root hair. Once initiated, the root hair undergoes tip growth through which the tubelike form of the hair is generated until, at maturation, tip growth stops. Figure 1B shows this developmental progression in the model plant *Arabidopsis*.

All the developmental activities from cell fate specification to termination of root hair tip growth are plastic, being entrained to a range of endogenous and environmental signals, not the least of which appear to be the availability of the nutrients the root hairs will transport from the soil. In this review, we will outline our increasingly detailed understanding of the molecular and cellular basis of the complex developmental progression that generates a root hair. In addition, we will explore the role of root hairs in the nutrient status of the plant. Lastly, we will outline evidence supporting the idea that the complex plastic nature of root hair development is related to their specialized function in nutrient and water uptake.

DECIDING TO MAKE A ROOT HAIR: TRICHOBLAST CELL FATE SPECIFICATION

Trichoblasts can be distinguished from atrichoblasts even in the meristematic zone by differences in cytoplasmic structure (trichoblasts are smaller, show more dense cytoplasm, and reduced vacuolation; Dolan and others 1994; Galway and others 1997) in addition to showing unique patterns of gene expression (see below). Of the three basic schemes that describe root hair fate specification (Types I–III, Figure 1C), most is known about Type III from studies on *Arabidopsis*. In this plant, trichoblasts form from epidermal cells overlying the junction of two cortical cells, leading to alternating files of trichoblasts and atrichoblasts (Figure 1A; Dolan and

others 1994). This patterning relative to cell position suggests cell fate may be governed by cell-to-cell communication very early after cell formation in the meristem. The epidermal cells may therefore be responding to biochemical or perhaps even biophysical positional information from the cortical cells to make decisions as to cell fate. Although we are still far from a complete understanding of how such choices are made, a concerted effort from several labs to screen for mutants in root hair formation has helped reveal some of the transcriptional regulators responsible for specification of trichoblast and atrichoblast cell fate.

The Genetics of Root Hair Formation in *Arabidopsis*

Mutants in which the distribution of trichoblasts throughout the root epidermis is disrupted have proven very informative in unraveling molecular mechanisms responsible for epidermal cell differentiation (Table 1). At the extremes of the phenotypes of these cell-fate mutants are plants that produce either ectopic root hairs or completely lack root hairs. Mutants that form ectopic root hairs actually appear to be defective in gene products that promote atrichoblast cell fate, including defects in *ELP1*, *ERH1*, *ERH3*, *GL2*, *TTG1*, and *WER*. Conversely, mutants that lack root hairs appear to be defective in gene products that promote trichoblast cell fate such as *AXR2*, *AXR3*, *CPC*, *RHD6*, *RHL1*, *RHL2*, *RHL3*, and *SLR1* (Table 1).

GL2, CPC, TTG1, and WER are all transcription factors involved in cell patterning. For example, GL2 is a homeodomain leucine zipper transcription factor that is expressed in atrichoblast cells and is thought to promote hairless cell fate (Masucci and others 1996). *WER* encodes a Myb transcription factor and *TTG1* encodes a protein that contains four WD40 repeats and is part of the signaling pathway that likely regulates transcription factor activity (Walker and others 1999). Mutations in *TTG1* result in several pleiotropic phenotypes, including ectopic root hair formation and lack of leaf trichomes. Both WER and TTG1 positively regulate *GL2* expression and thus promote atrichoblast cell fate (Walker and others 1999). Conversely, the *CPC* gene encodes a Myb-related protein that promotes trichoblast cell identity (Wada and others 1997). There appears to be a complex network of transcriptional regulation between WER, CPC, and GL2. For example, position-dependent expression of *CPC* and *GL2* depends on the presence of the functional *WER* gene. In the *wer1* mutant background, the ectopic expression of *WER* induces formation of root hairs on approxi-

Table 1. *Arabidopsis* Mutants Affecting Root Hair Cell-fate Determination

Phenotype	Gene name	Cloned sequence	Expression pattern	Suggested mode of action	Other phenotypes	Rescued/ mimicked	References
Ectopic root hairs	*WER* werewolf	Myb transcription factor	Expressed in atrichoblasts	Determines root epidermal cell fate by regulating induction of CPC and GL2	All root epidermal cells have dense cytoplasm and small vacuoles		Lee and Schiefelbein 1999; Lee and Schiefelbein 2002
	TTG1 Transparent Testa Glabra	Protein with four WD 40 repeats	All major organs	Prevents atrichoblasts from forming a root hair	Defective formation of trichomes, integument development, secretion of seed mucilage, and anthocyanin synthesis. Few root epidermal cells have dense cytoplasm and small vacuoles		Galway and others 1994; Koornneef 1981; Walker and others 1999
	ERH1 Ectopic root hair 1			Negatively regulates trichoblast differentiation (or positively regulates atrichoblast differentiation)		Ectopic root hair phenotype is suppressed by application of AVG[a]	Schneider and others 1997
	ERH3 Ectopic root hair3	Katanin-p60 protein	All major organs	Severs microtubules and plays a role in cell wall organization, thus affecting cell fate determination	Abnormal trichomes, abnormal flowers, and bigger root diameter. Cytoplasmic density does not correlate with the position of epidermal cells in relation to cortical cells.		Schneider and others 1997; Webb and others 2002
	ELP1 Ectopic deposition of lignin in pith	Chitinase-like protein (AtCTl1).	All major organs	Overproduction of ethylene in the mutant causes the root hair phenotype	Ectopic lignin deposition and abnormal cell shapes. Enhanced production of ethylene/ethylene related phenotypes	Root hair phenotype can be suppressed by treatment with AVG[a] and Ag+.	Zhong and others 2002
	GL2 Glabra2	Homeodomain leucine zipper transcription factor.	Expressed in atrichoblasts, developing leaves, trichomes, and cells surrounding trichomes.	Prevents root hair formation in atrichoblasts	Abnormal trichome morphogenesis, no seed coat mucilage. Epidermal cell patterning in the root meristem is the same as in wt.		DiCristina and others 1996; Hung and others 1998; Koornneef 1981; Lin and Schiefelbein 2001; Masucci and others 1996; Szymanski and others 1998

Name	Gene product	Expression	Function	Phenotype	Root hair phenotype	Notes	References
POM1/ERH2 Ectopic root hair2			Negatively regulates root hair development	Show defects in shoot development. Epidermal cell patterning in the root meristem is the same as in wt.			Schneider and others 1997
CRT1 Constitutive triple response1	Serine–threonine protein kinase closely related to Raf family of protein kinases.	All major organs. mRNA present in all root cell types.	Negative regulator of the ethylene signaling pathway. Constitutively active ethylene signaling causes the mutant phenotype.	Ethylene-related phenotypes. Mutant does not produce more ethylene than wt. Epidermal cell patterning in the root meristem is the same as in wt.		Can be mimicked by WT plants treated ACC.[b]	Cao and others 1999; Dolan and others 1994; Kieber and others 1993; Roman and others 1995
TUA/antisense lines	Different antisense α-tubulin cDNA sequences were expressed under the control of the CaMV 35S promoter.	Ectopic expression.	Reduced levels of α-tubulin disrupts microtubule cytoskeleton dynamics thus affecting cell-fate determination.	No shoot phenotype, roots do not elongate normally after day 5 postgermination, big swelling at the root tip. Reduced root gravitropism.		Can be partially mimicked by treating wild-type plant with microtubule-disrupting drugs.	Bao and others 2001
CA-RoP2 Constitutively active GTP-bound mutated *Rop2*	Member of the RHO family of small monomeric GTPases.	Ectopic expression under control of the CaMV 35 S promoter[c]	May act as a common molecular switch in many different signal transduction pathways. Part of signaling network that controls polarized growth.	Pleiotropic phenotypes including abnormal light control of seedling development, more lateral roots, enhanced apical dominance, and abnormally shaped leaves.			Jones and others 2002; Li and others 2001
ETO1 Ethylene over production1			Overproduction of ethylene in the mutant causes the root hair phenotype.	Ethylene overproduction. Ethylene-related phenotypes.	More root hairs in dark-grown plants.	The phenotype is inhibited by application of ethylene antagonists.	Cao and others 1999; Dolan 2001; Guzman and Ecker 1990; Masucci and Schiefelbein 1996; Pitts and others 1998
ETO2 Ethylene over production2	Constitutively active ACC synthase 5.	In all major organs.	Overproduction of ethylene in the mutant causes the root hair phenotype.	Ethylene overproduction. Ethylene-related phenotypes.			Cao and others 1999; Vogel and others 1998; Woeste and others 1999
ETO4 Ethylene over production4			Overproduction of ethylene in the mutant causes the root hair phenotype.	Ethylene overproduction. Ethylene-related phenotypes.			Cao and others 1999

Continued

Table 1. *Continued*   Arabidopsis Mutants Affecting Root Hair Cell-fate Determination

Phenotype Gene name	Cloned sequence	Expression pattern	Suggested mode of action	Other phenotypes	Rescued/ mimicked	References
ETO3 Ethylene over production3			Overproduction of ethylene in the mutant causes the root hair phenotype.	100-fold ethylene overproduction. Ethylene-related phenotypes.		Cao and others 1999
Few root hairs *RHL1* Root hairless1	Small hydrophilic protein, localized to the nucleus.	In very low amounts throughout the plant.	Positively regulates trichoblast differentiation (or negatively regulate atrichoblast differentiation).	Similar to RHL1	Partially rescued by ethylene application.	Schneider and others 1997, 1998
RHL2 root hairless2			Positively regulates trichoblast differentiation (or negatively regulate atrichoblast differentiation).	Similar to RHL1	Partially rescued by the addition of ACC.	Schneider and others 1997
RHL3 Root hairless3				Similar to RHL1		Schneider and others 1997
RHD6 Root hair defective6			Positively regulate trichoblast differentiation (or negatively regulate atrichoblast differentiation). Interacts with ethylene and auxin signaling. Early epidermal cell specification is different from wild-type.	Epidermal cell patterning in the root meristem is abnormal.	Rescued by the addition of ethylene or auxin.	Masucci and Schiefelbein 1994, 1996 Parker and others 2000
HLQ Harlequin		Ectopically expressed under the ABA- and IAA-inducible transgenic carrot promoter Dc3-GUS.	Affects root hair development via disrupting normal hormone regulation of this process.	Epidermal cells are abnormal or collapsed, chlorotic leaves, callose accumulation, abnormal trichomes.	Not rescued by the application of hormones.	Subramanian and others 2002
SLR-1/IAA14 Solitary-root1	Member of Aux/IAA protein family. Localized to the nucleus.	All major organs. Not expressed in root meristematic region and in a root cap.	Abnormal auxin signaling causes the root hair phenotype.	No lateral roots, reduced root and shoot gravitropism.	Can be partially rescued by the addition of ACC.	Abel and others 1995 Fukaki and others 2002

Gene	Description	Expression	Function	Phenotype	Rescue	References
CPC Caprice	Protein with a Myb-like DNA binding domain.	Expressed in atrichoblasts and in trichoblasts at lower levels. Protein active in trichoblasts only.	Mediates inhibitory pathway originating in atrichoblasts preventing neighbors from adopting atrichoblast cell fate.		Partially rescued by the addition of ACC.	Lee and Schiefelbein 2002; Mendoza and Alvarez–Buylla 2000; Wada and others 1997
AXR2/IAA7 Auxin resistant2	Putative transcription regulator of auxin-responsive genes.	Broad expression in cells known to be affected by auxin. Less expression in leaves and siliques.	Positively regulates root hair differentiation. Acts downstream from the TTG/GL2, in a pathway separate from *rhd6*.	Auxin-related phenotypes. Epidermal cell patterning in the root meristem is abnormal.	Partially rescued by high levels of IAA and of low levels of phosphorus.	Abel and others 1995; Bates and Lynch 1996; Masucci and Schiefelbein 1996; Nagpal and others 2000; Wilson and others 1990
AXR3/IAA17 Auxin resistant3	Putative transcriptional regulator of auxin-responsive genes.	All major organs.	Abnormal auxin signaling causes the root hair phenotype.	Auxin-related phenotypes.	Rescued by application of high levels of cytokinin.	Leyser and others 1996; Worley and others 2000
DN-Rop2 Dominant negative GDP-bound mutated *Rop2*.	Member of the RHO family of small GTPases.	Transgene is expressed ectopically under control of the CaMV 35S promoter.[c]	May act as a common molecular switch in many different signal transduction pathways. Part of signaling network that controls polarized growth.	Multiple phenotypes, including delay in seed germination, long hypocotyls in the dark, reduced number of lateral roots, reduced apical dominance, and irregularly shaped leaves.		Jones and others 2002; Li and others 2001

[a] *AVG = aminoethoxyvinylglycine.*
[b] *ACC = 1-amino-cyclo propane-1-carboxylic acid.*
[c] *In wild-type plants, Rop2 is expressed in all vegetative tissues. Protein is localized to the root hair initiation site and root hair tip; wild-type Rop4 is expressed in root hairs and the protein localized to the initiation site and the growing apex.*

Table 2. Mutants of *Arabidopsis* Affecting the Root Hair Initiation Process

Phenotype	Gene name	Cloned sequence	Expression pattern	Suggested mode of action	Other phenotypes	Rescued/ mimicked	References
Wider root hair initiation site	*RHD1* Root hair defective1						Schiefelbein and Somerville 1990
	TIP1 Tip growth defective1				Pollen tubes fail to elongate normally, plants slightly dwarf.		Parker and others 2000 / Ryan and others 1998 / Schiefelbein and others 1993 / Parker and others 2000
	CEN3 Centipede3						Baumberger and others 2001
	LRX1 Lrr/extensin1	The protein contains both leucine-rich and extension protein domains.	Specifically in root hair cells. Protein is localized in cell wall.	Regulates swelling size by signaling between cell wall and plasma membrane. Extensin domain is essential for its function.			
	KEULE	Sec1 protein	All major organs. Enriched individing tissues.	The protein is a key regulator of vesicle trafficking. Interacts with KNOLLE.	Defective cytokinesis. Cell wallstubs, gapped walls, and multiple nuclei.		Assaad and others 2001 / Sollner and others 2002
	CLUB				Defective cytokinesis. Cell wall stubs, gapped walls, and multiple nuclei.		Sollner and others 2002
Initiation site shifted to apical side	*ETO1* Ethylene overproduction1			Ethylene overproduction promotes root hair formation at the apical side of the trichoblast.	Ethylene overproduction. Constitutive triple response phenotype.	Inhibitors of ACC synthase andethylene binding suppress roothair phenotype.	Dolan 2001 / Guzman and Ecker 1990 / Masucci and Schiefelbein 1994 / Pitts and others 1998
Initiation site shifted to basal side	*RHD6* Root hair defective6			Regulates assembly of cellular componentsat the initiation site. Interacts with ethylene and auxin signaling.		Rescued by the application of auxin or ethylene precursor.	Masucci and Schiefelbein 1994, 1996 / Parker and others 2000

Gene	Molecular function	Expression	Function	Phenotype	Comments	References
ETR1 Ethylene response1	Ethylene receptor. Member of the two component histidine protein kinase family. Predominantly localized to endoplasmic reticulum.	All major organs.	Regulates an assembly of cellular components at the initiation site via ethylene-dependent signaling.	Ethylene insensitivity.		Chang and others 1993; Chen and others 2002; Kieber 1997; Masucci and Schiefelbein 1994, 1996; Pitts and others 1998
AXR2/IAA7 Auxin resistant2	Putative transcriptional regulator of auxin-responsive genes.	Broad expression in cells known to be affected by auxin. Less expression in leaves and siliques.	Regulates assembly of cellular components at the initiation site via auxin-dependent signaling.	Auxin-related phenotypes.	Partially rescued by high levels of IAA and low levels of phosphorus.	Abel and others 1995; Bates and Lynch 1996; Couot-Gastelier and Vartanian 2001; Masucci and Schiefelbein 1994; Nagpal and others 2000; Wilson and others 1990
DER1 deformed root hair1	ACTIN2	Vegetative organs.	The actin cytoskeleton is required for organizing the initiation site.			Ringli and others 2002
SCN1 Supercentipede1, *BST1* Bristled1, *CEN2* & *CEN3* Centipede2 & 3						Parker and others 2000
TRH1 Tiny root hair1	K$^+$ transporter of the AtKKT/AtKUP/HAK$^+$ family.	All parts of the plant, higher level of transcript in the roots.	Defects in potassium transport prevent normal cell polarization.		Mutants are partially impaired in K$^+$ transport.	Rigas and others 2001
RHD6 Root hair defective6					Application of auxin or ethylene precursor rescues the phenotype.	Masucci and Schiefelbein 1994, 1996; Parker and others 2000
TUA6/Antisense lines	Antisense to α-tubulin cDNA under the control of 35S promoter.	Ectopic expression.	Reduction of α-tubulin level disrupts microtubule cytoskeletal dynamics thus affecting cell polarization.		No shoot phenotype, roots do not elongate normally after day 5 postgermination, swelling at the root tip. Reduced root gravitropism. Partially mimicked by treating wild-type with microtubule-disrupting drugs.	Bao and others 2001

Continued

Table 2. *Continued*   Mutants of *Arabidopsis* Affecting the Root Hair Initiation Process

Rop2 OX Overexpressing *Rop2*.	Member of the RHO family of small monomeric GTPases.	Ectopic expression under control of CaMV35S promoter.[a]	May act as a common molecular switchin many different signal transduction pathways. Part of signaling network that controls polarized growth.		Jones and others 2002
CA-Rop2 Constitutively active GTP-bound mutated *Rop2*.	Member of the RHO family of small GTPases.	Defects in potassium transport prevent normal cell polarization.	May act as a common molecular switchin many different signaltransduction pathways. Part of signaling network that controls polarized growth.	Pleiotropic phenotypes includingabnormal light control ofseedling development, more lateral roots, enhanced apical dominance, and abnormally shaped leaves.	Li and others 2001 Jones and others 2002

[a]In wild-type plants Rop2 is expressed in all vegetative tissues. Protein is localized to the root hair initiation site and root hair tip; wild-type Rop4 is expressed in root hairs and the protein localized to the initiation site and the growing apex.

mately 50% of root epidermal cells, but these cells are not necessarily located over the junction of two cortical cells as they would be in wild-type plants. This observation suggests that ectopic expression of WER1 induces epidermal cells to adopt cell fates that are not determined by their position, implying that *WER* gene regulation is critical for establishment of the normal epidermal cell pattern. CPC is a transcription factor that is expressed in trichoblast cells, but the encoded protein is active in atrichoblast cells. It has been suggested that CPC mediates a lateral inhibition pathway that originates in atrichoblast cells and prevents neighboring cells from adopting an atrichoblast cell fate (Lee and Schiefelbein 2002).

Hormonal Control of Cell Fate Specification

Physiological, biochemical, and anatomical studies indicate an important role of the plant hormones ethylene and auxin in establishing the epidermal patterning of the root epidermis (Table 1; Masucci and Schiefelbein 1994, 1996; Tanimoto and others 1995). For example, exposure of wild-type *Arabidopsis* plants to the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) induces the formation of ectopic root hairs, suggesting that ethylene is a positive regulator of trichoblast cell fate (Tanimoto and others 1995).

Consistent with this proposed role of ethylene is the observation that the ethylene-related *Arabidopsis* mutants *elp* and *ctr1* lead to ectopic hair production (Dolan and others 1994; Zhong and others 2002). *elp* is mutated in a gene that encodes a chitinase-like protein (AtCTL1; Zhong and others 2002) but this mutant's action on root hair formation is probably not directly through a role for chitinase in cell-fate determination but more likely from one of its pleiotropic effects, which are known to include increased ethylene production (Zhong and others 2002). *CTR1* encodes a serine–threonine protein kinase that acts as a negative regulator of ethylene response and so *ctr1* mutants behave as if they perceive ethylene all the time (Kieber and others 1993; Roman and others 1995). In *ctr1* the initial patterning of the root epidermis is identical to wild-type. For example, *GL2* expression patterns are unaltered in this mutant, implying both that *GL2* expression is not regulated by ethylene and that ethylene's effect on epidermal patterning is independent from GL2 (Masucci and Schiefelbein 1996). Therefore, it has been proposed that ethylene acts later in epidermal cell development after cell division has ceased. Consistent with this idea is the effect of ethylene on the *rhl1* mutant, *rhl1* is hairless

but the early differentiation of the root epidermis, as defined by wild-type-like *GL2* expression pattern, is normal. The mutant phenotype can be partially rescued by application of exogenous ethylene, implying that ethylene is acting to relieve a block in the cell-fate specification program after *GL2* has been expressed (Schneider and others 1998).

A further line of evidence for ethylene's action in fate determination comes from the effect of this hormone on dark-grown plants. Dark-grown *Arabidopisis* plants (sustained on sucrose-containing media) develop few root hairs but, if treated with ethylene, these plants develop root hairs in their normal (that is, over anticlinal cortical cell walls) position. The ethylene-overproducing mutants *eto1*, *eto2*, and *eto4* exhibit normal epidermal patterning, even in the dark, with only cells over cortical cell junctions forming root hairs (Cao and others 1999). However, the *eto3* mutant produces more ethylene than these other ethylene-overproducing mutants and this mutant forms root hairs on atrichoblasts, indicating that high ethylene levels can induce ectopic formation of root hairs (Cao and others 1999).

The observation that ethylene-signaling-deficient mutants such as *etr1* and *ein2* display normal root epidermal patterning might suggest that ethylene is not required for root hair formation. However, these mutants may not target the ethylene perception and response pathways associated with root hair function. Also, the multiple ethylene receptors known to exist in *Arabidopsis* (Bleecker and others 1998a) raise the possibility of functional redundancy and compensation in the ethylene regulatory pathway leading to root-hair-fate specification.

Even though application of auxin or auxin analogs does not affect epidermal cell patterning (Masucci and Schiefelbein 1996), genetic studies have implicated IAA in the process of epidermal cell-fate determination (Cernac and others 1997; Masucci and Schiefelbein 1996; Pitts and others 1998; Wilson and others 1990). Thus, mutations in genes that are known to alter auxin responses inhibit root hair formation (Tables 1–4). For example, AXR2 is a putative transcriptional regulator of auxin responsive genes (Nagpal and others 2000) and *axr2* mutants form very few root hairs. Similarly, *axr3* plants have reduced numbers of root hairs. *AXR3* encodes a transcription factor that needs to be degraded for auxin signal transduction to take place. The *axr3* protein is resistant to degradation and so inhibits auxin signaling (Leyser and others 1996; Worley and others 2000). The *solitary root* mutant (*slr*) is mutated in a member of the Aux/IAA protein family and also has reduced numbers of root hairs. *slr* is known to show reduced auxin sensitivity likely

through its function as an auxin-related transcriptional repressor (Fukaki and others 2002). The *axr2* and *axr3* root hairless phenotypes can be rescued by application of exogenous IAA, whereas the *slr1* root hair phenotype can be partially rescued by application of ethylene precursor ACC. Ethylene or auxin can also rescue the low-density root hair phenotype seen in the *rhd6* mutant (Masucci and Schiefelbein 1994), further suggesting that there may be a link between auxin and ethylene and root-hair-cell-fate determination. Indeed, there are many reports of crosstalk between auxin and ethylene response pathways in roots, for example, ethylene-induced inhibition of root growth requires auxin transport (reviewed in Swarup and others 2002). Characterizing root hair mutants such as *rhd6* at the molecular and cellular level will help determine if auxin and ethylene share a common signaling pathway to regulate cell-fate determination or if auxin operates via ethylene-mediated events (for example, through auxin-mediated ethylene production; Massuci and Schiefelbein 1996).

The Cytoskeleton and Cell Fate Determination

Recent evidence also points to the microtubule cytoskeleton as an important determinant of root epidermal cell fate. When A-tubulin levels were suppressed using *Arabidopsis* plants expressing antisense to A-tubulin, lines that showed a reduction in A-tubulin gene expression also exhibited ectopic root hair formation (Bao and others 2001). This observation indicates that disruption of microtubule cytoskeleton dynamics affects root hair development. Interestingly, the process of root hair formation once cell fate is specified (the initiation process, see below) appears unaffected by microtubule disrupting drugs (Bibikova and others 1999) suggesting that microtubules may be acting selectively in fate determination early in root hair development. Additional evidence for the role of microtubule-based cytoskeleton in trichoblast formation comes from the cloning of the *ERH3* gene. *erh3* is a mutant that forms ectopic root hairs and appears involved in specification of cell identities in the root cap, endodermis, and cortex. ERH3 appears to be required for both trichoblast and atrichoblast cell-fate determination and to act in the very early steps of the cell-fate specification process because *GL2* expression patterns in the meristem are disrupted in *erh3* plants. *ERH3* encodes a katanin-p60 protein. The katanin family of proteins is known to sever microtubules in animal cells (McNally and Vale 1993) and appears to be involved in plant cell wall assembly (Burk and others 2001). One possible

Table 3. Mutants of *Arabidopsis* Affecting the Transition from Root Hair Initiation to Tip Growth

Phenotype	Gene name	Cloned sequence	Expression pattern	Suggested mode of action	Other phenotypes	Rescued/mimicked	References
Growth stops as initiation site swells	*RHD2* Root hair development2			May regulate apical growth.			Parker and others 2000; Schiefelbein and Somerville 1990
	SHV1, SHV2, SHV3 Shaven1 & 3						Parker and others 2000
	TRH1 Tiny root hairs1	K^+ transporter of the AtKKT/AtKUP/HAK$^+$ transporter family.	All parts of the plant, higher level of the transcript in the roots.	Defects in K^+ transport is inhibiting the start of apical growth.	Mutants are partially impaired in K^+ transport	High external K^+ concentration does not rescue the phenotype.	Rigas and others 2001
	SOS4 Salt overly sensitive4	Pyridoxal kinase involved in pyridoxial-5-phosphate synthesis.	In all major organs	May regulate apical growth.	Enhanced sensitivity to NaCl. Slower root growth.	Can be partially rescued by the application of vitamin B6, ACC, or 2,4D.	Shi and others 2002; Shi and Shu 2002
	Rop7 Overexpressing *Rop7*	Member of the RHO family of small monomeric GTPases.	Ectopic expression under control of CaMV35S promoter.	May regulate apical growth.			Jones and others 2002
	LRX1 LRR/extensin1	Protein contains both leucine-rich and extensin domains.	Specifically in root hair cells. Protein localized to cell wall.	Regulates tip growth by signaling between cell wall and plasma membrane. Extensin domain is essential.			Baumberger and others 2001
Initiation site bursts instead of switching to apical growth.	*KJK/AtCSLD3* kojak	Cellulose synthase-like protein.	Preferentially in root hair cells, protein localized to endoplasmic reticulum.	Involved in the biosynthesis of β-glucan-containing polysaccharides that are required for root hair apical growth.			Favery and others 2001; Wang and others 2001
Sometimes root hairs are branched at the base.	*COW1* Can of worms1						Grierson and others 1997
	AUX1 Auxin1	Auxin permease. Putative auxin influx carrier.	All major organs.	Abnormal auxin signaling causes the root hair phenotype.	Resistant to both ethylene and auxin. Impaired gravitropism.		Cernac and others 1997; Picket and others 1990; Pitts and others 1998

AXR1 auxin resistant	A subunit of Rubr activating enzyme analogous to the E1 ubiquitin-activating enzyme.	All major organs.	Abnormal auxin signaling causes the root hair phenotype. Auxin is involved in the control of root hair development.	Auxin-dependent phenotypes.	Cernac and others 1997 Estelle and Somerville 1987 Pitts and others 1998 Ward and Estelle 2001
DER1 deformed root hair1.	ACTIN2	Vegetative organs.	The actin cytoskeleton is required for organizing the initiation site.		Ringli and others 2002 An and others 1996
Rop2 OX Overexpressing Rop2.	Member of the RHO family of small monomeric GTPases.	Ectopic expression under control of CaMV 35S promoter.[a]	May act as a common molecular switch in many different signal transduction pathways. Part of signaling network that controls polarized growth.		Jones and others 2002

[a]In wild-type plants Rop2is expressed in all vegetative tissues. Protein is localized to the root hair initiation site and root hair tip; wild-type Rop4 is expressed in root hairs and the protein localized to the initiation site and the growing apex.

Table 4. Mutants of *Arabidopsis* Impaired in Tip Growth

Phenotype	Gene name	Cloned sequence	Expression pattern	Suggested mode of action	Other phenotypes	Rescued/mimicked	References
Root hairs are short	*AUX1* Auxin1	Auxin permease. Putative auxin influx carrier.	Root epidema cells, apical tissues	Defects in auxin signaling affect root hair apical growth.	Resistant to ethylene and auxin. Impaired root gravitropism.		Pitts and others 1998 Cernac and others 1997 Bennett and others 1996
	EIN2 Ethylene insensitive2	Protein similar to Nramp family of metal transporters.	All major organs.	Defects in ethylene signaling affect root hair apical growth.	Ethylene insensitivity.		Alonso and others 1999 Guzman and Ecker 1990 Masucci and Schiefelbein 1996 Pitts and others 1998
	ETR1 Ethylene response1	Ethylene receptor. Member of the two-component histidine protein kinase family. Localized to ER.	All major organs.	Defects in ethylene signaling affect root hair apical growth.	Ethylene insensitivity.	Rescued by application of 2,4D.	Chang and others 1993 Chen and others 2002 Bleecker and others 1988 Pitts and others 1998
	IRE Incomplete root hair elongation	Protein with a serine/threonine protein kinase domain	All major organs	Possibly associates with microtubules to regulate tip growth via cytoskeleton.			Oyama and others 2002
	DN-Rop2 Dominant negative GDP-bound *Rop2*	Member of the RHO family of small, monomeric GTPases.	Ectopic expression under control of the CaMV 35S promoter.[a]	May act as a common molecular switch in many different signal transduction pathways. Part of signaling network that controls polarized growth.	Multiple phenotypes, including delay in seed germination, long hypocotyls in the dark, reduced number of lateral roots, reduced apical dominance, and irregularly shaped leaves.		Li and others 2001 Jones and others 2002
Root hairs are short and wide	*COW1, SCN1, CEN1, CEN2, CEN3, WAVY* Can of worms1, Supercentipede1, Centipede1, 2, & 3						Grierson and others 1997 Parker and others 2000
	Tip1 Tip growth defective1				Pollen tubes fail to elongate normally, plants slightly dwarf.		Parker and others 2000 Ryan and others 1998 Schiefelbein and others 1993

Root hairs are short and more variable in length	*AXR1* auxin resistant1	Protein with similarity to ubiquitin-activating enzyme	All major organs.	The defects in auxin signaling affect root hair apical growth.	Auxin-dependent phenotypes.	Rescued by application of 2,4D or ACC.	Cernac and others 1997; Leyser and others 1993; Pitts and others 1998
Root hairs are short and wavy	*RHD3* Root hair defective3	Putative GTP-binding protein.	All major organs.	The gene product is required for regulated cell enlargement.	Altered cell size in tissues throughout the plant.		Galway and others 1997; Parker and others 2000; Schiefelbein and Somerville 1990; Wang and others 1997, 2002
	CSLD3	Cellulose synthase-like protein.	All major organs.	Involved in the biosynthesis of β-glucan-containing polysaccharides that are required for root hair apical growth.			Wang and others 2001
	DN-Rop2 Dominant negative GDP-bound mutated *Rop2*	Member of the RHO family of small, monomeric GTPases.	Ectopic expression under control of CaMV 35S promoter.[a]	May act as a common molecular switch in many different signal transduction pathways. Part of signaling network that controls polarized growth.	Multiple phenotypes, including delay in seed germination, long hypocotyls in the dark, reduced number of lateral roots, reduced apical dominance, and irregularly shaped leaves.		Li and others 2001; Jones and others 2002
Short hairs with variable diameter	*RHD4* Root hair defectively						Galway and others 1999; Schiefelbein and Somerville 1990
	DER1 deformed root hairs 1	ACTIN2	Vegetative organs.	The actin cytoskeleton is required for organizing the intiation site and tip growth.			Ringli and others 2002
	KEULE	Sec1 protein.	All major organs. Enriched in dividing tissues.	The protein is a key regulator of vesicle trafficking. Interacts with KNOLLE. Abnormal vesicle trafficking affects apical growth.	Defective cytokinesis. Cells have cell wall stubs, gapped walls, and multiple nuclei in dividing cells. Trichomes with abnormal branching pattern.		Assaad and others 2001; Sollner and others 2002

Continued

Table 4. *Continued*   Mutants of *Arabidopsis* Impaired in Tip Growth

Phenotype	Gene name	Cloned sequence	Expression pattern	Suggested mode of action	Other phenotypes	Rescued/mimicked	References
	CLUB				Defective cytokinesis. Cells have cell wall stubs, gapped walls, and multiple nuclei in dividing cells.		Sollner and others 2002
Root hairs are sometimes branched	*TIP1* Tip growth defective1				Pollen tubes fail to elongate normally, plants slightly dwarf.		Parker and others 2000 Ryan and others 1998 Schiefelbein and others 1993 Parker and others 2000
	BST1, SCN1, CEN2, CEN3, Bristled1, Supercentipede1, Centepede 2 & 3						Galway and others 1999 Schiefelbein and Somerville 1990
	RHD4 Root hair defective4						
	RHD3 Root hair defective3	Putative GTP-binding protein.	All major organs.	The gene product is required for regulated cell enlargement.	Altered cell size in tissues throughout the plant.	Ethylene and auxin act independently or downstream from rhd3.	Galway and others 1997 Parker and others 2000 Schiefelbein and Somerville 1990 Wang and others 1997, 2002
	KNOLLE	Cytokinesis-specific syntaxin.	Expressed in dividing somatic cells but not during cytokinesis of male meiotic cells.	The protein affects membrane dynamics. Mutation disrupts normal regulation of apical growth.	Defective cytokinesis.		Lauber and others 1997 Lukowitz and others 1996 Sollner and others 2002
	HINKEL	Kinesin-related protein involved in cytokinesis.	All major organs.	The gene affects cytoskeletal dynamics required for apical growth.	Defective cytokinesis.		Sollner and others 2002 Strompen and others 2002
	BUBLINA			Regulation of apical growth is disrupted	Cytokinesis-defective mutant. Stout seedlings with reduced cotyledons.		Sollner and others 2002
	PLEAIDE			Regulation of apical growth is disrupted	Cytokinesis-defective mutant. Enlarged cells with large nucleus and multiple nucleoli.		Sollner and others 2002

Rop2 OX Overexpressing *Rop2*.	Member of the RHO family of small, monomeric GTPases.	Ectopic expression under control of CaMV 35S promoter.[a]	May act as a common molecular switch in many different signal transduction pathways. Part of signaling network that controls polarized growth.	Can be partially mimicked by treating wild-type plants with microtubule-disrupting drugs.	Jones and others 2002
TUA/antisense lines	Antisense to α-tubulin.	Ectopic expression under the control of 35S promoter.	Reduction of α-tubulin level disrupts cytoskeletal dynamics required for root hair growth and morphogenesis.	Can be partially mimicked by treating wild-type plants with microtubule-disrupting drugs.	Bao and others 2001
LRX1 LRR/Extensin1	Protein contains both leucine-rich and extensin protein domains.	Specifically in root hair cells. Protein localized to cell wall.	Regulates growth by communicating between cell wall and plasmalemma. Extensin domain is essential		Baumberger and others 2001
CSLD3	Cellulose synthase-like protein.	All major organs.	Involved in the biosynthesis of β-glucan-containing polysaccharides that are required		Wang and others 2001
SCN1, CEN1, CEN2, CEN3, WAVY Supercentipede1, Centipede1, 2, & 3				Root hairs are sometimes curved	Parker and others 2000
CA-Rop2 Constitutively active GTP-bound mutant *Rop2*.	Member of the RHO family of small, monomeric GTPases.	Ectopic expression under control of 35S promoter.[a]	May act as a common molecular switch in many different signal transduction pathways. Part of signaling network that controls polarized growth.	Pleiotropic phenotypes including abnormal light control of seedling development, more lateral roots, enhanced apical dominance, and abnormally shaped leaves.	Li and others 2001 Jones and others 2002

Continued

Table 4. *Continued*   Mutants of *Arabidopsis* Impaired in Tip Growth

Phenotype	Gene name	Cloned sequence	Expression pattern	Suggested mode of action	Other phenotypes	Rescued/mimicked	References
Hairs are longer than wild-type	*PHYB/HY3* PhytochromeB/ Long hypoocotyl3	Phytochrome B.	All major organs.		Abnormal elongation of stems, petioles, and hypocotyls. Earlier flowering time.	Root hair phenotype can be suppressed by putting the plants in the dark.	Reed and others 1993 Johnson and others 1991
	HY5 Long hypoocotyl5	Protein with a bZIP motif, localized to the nucleus.	All major organs.		Impaired root gravitropism and light-dependent hypocotyl elongation. Abnormal lateral root development.		Oyama and others 1997
	CEV1 Constitutive expression of vegetative storage protein1	Cellulose synthase CeSA3.	Predominantly in the root.	Control root hair apical growth by cell wall signaling.	Plants are smaller, with stunted roots, enhanced anthocyanin accumulation, enhanced pathogen resistance, increased production of jasmonate and ethylene.	Can be mimicked by treating wild-type plants with jasmonic acid[b] or cellulose biosynthesis inhibitors.	Ellis and Turner 2001 Ellis and others 2002 Feys and others 1994
	ETO1 Ethylene overproduction1			Overproduction of ethylene in the mutant causes the root hair phenotype.	Ethylene overproduction/ ethylene related-phenotypes.	The phenotype is inhibited by application of ethylene antagonists.	Cao and others 1999 Dolan 2001 Guzman and Ecker 1990 Masucci and Schiefelbein 1996 Pitts and others 1998
Hairs are longer and wider	CA-Rop2 Constitutively active GTP-bound mutant Rop2.	Member of the RHO family of small monomeric GTPases.	Ectopic expression under control of the CaMV 35S promoter.[a]	May act as a common molecular switch in many different signal transduction pathways. Part of signaling network that controls polarized growth.	Pleiotropic phenotypes including abnormal light control of seedling development, more lateral roots, enhanced apical dominance, and abnormally shaped leaves.		Li and others 2001 Jones and others 2002
	Rop2 OX Overexpressing Rop2.	Member of the RHO family of small monomeric GTPases.	Ectopic expression under control of the CaMV 35S promoter.[a]	May act as a common molecular switch in many different signal transduction pathways. Part of signaling network that controls polarized growth.		Can be partially mimicked by application of microtubule-disrupting drugs.	Bibikova and others 1999 Jones and others 2002

Root hair tip is swollen	*CA-Rop4* Constitutively active GTP-bound mutant *Rop4*.	Member of the RHO family of small monomeric GTPases.	Ectopic expression under control of the inducible dexamethasone responsive promoters.[a]	May act as a common molecular switch in many different signal transduction pathways. Part of signaling network that controls polarized growth.	Swollen epidermal cells of hypocotyl, cotyledons, and roots increase in root elongation zone diameter.	Molendijk and others 2001
	CA-Rop6 Constitutively active GTP-bound mutant *Rop6*.	Member of the RHO family of small monomeric GTPases.	Ectopic expression under control of the inducible dexamethanose responsive promoter.[a]	May act as a common molecular switch in many different signal transduction pathways. Part of signaling network that controls polarized growth.	Swollen epidermal cells of hypocotyl, cotyledons, and roots increase in root elongation zone diameter.	Molendijk and others 2001

[a] *In wild-type plants RoP2 is expressed in all vegetative tissues. Protein is localized to the root hair initiation site and root hair tip; wild-type Rop4 is expressed in root hairs, and the protein localized to the initiation site and the growing apex.*
[b] *All the other phenotypes, except for "longer root hairs" phenotype of this mutant, were rescued by treating the plant with high levels of JA.*

explanation of how microtubules might participate in the fate determination process is through a role in localizing positional cues that define the fate of cells once they divide and are exiting the meristem (Webb and others 2002).

ROOT HAIR INITIATION

After cell fate has been specified, the first morphological indication of root hair formation is the process of localized bulging in the trichoblast wall at the site of incipient root hair emergence, the process of root hair initiation. Initiation involves switching the growth habit of an epidermal cell from diffuse elongation to highly localized asymmetrical expansion on one side of the cell (Figure 1B; Leavitt 1904). Thus, in the course of root hair initiation, a new polarity axis is established within the trichoblast, while the old longitudinal polar axis is maintained (Figure 1B). This process of axis definition, fixation, and wall deformation is thought to involve the coordination of processes, including assembly of cytoskeleton-based machinery (Baluska and others 2000b; Emons and Derksen 1986), alteration of cell wall properties and the onset of localized exocytosis (Bibikova and others 1998; Vissenberg and others 2001), and activation of membrane transporters, possibly accomplished by a hormone-related control system (Masucci and Schiefelbein 1996; Rigas and others 2001).

Root hair initiation is best defined in *Arabidopsis*. In this plant, trichoblasts are arranged in files and the root hair is always formed at the apical (root tip) end of the cell (Figure 1, Type III). Consequently, it is possible to predict when and where an initiating root hair will form and so analyze cellular events at this particular site. Such analysis has allowed characterization of both transcriptional regulation (Schiefelbein 2000) and cell physiological events specifying the site of root hair formation (see for example, Baluska and others 2000a,b; Bibikova and others 1998; Dolan and others 1994; Vissenberg and others 2001).

The earliest morphological indication of the onset of root hair initiation is movement of the nucleus to the future initiation site (Meeks 1985). Such nuclear migration is seen in other plant cells undergoing localized growth, for example, budding in moss protonemata (Saunders and Helper 1982), but it is unclear why the nucleus should move. Such organelle relocalization may reflect the need for supplying elevated levels of transcripts at the growth initiation site, which is likely to represent an area of intense metabolic activity. However, although nuclear movement precedes the bulging of the cell wall at the root hair initiation site, it does not appear to be essential for initiation to take place. For example, preventing this nuclear movement with microtubule-depolymerizing drugs does not inhibit root hair formation (Baluska and others 2000a; T. Bibikova and S. Gilroy, unpublished results). In addition, current evidence indicates that the positional cues that locate the future initiation site are laid down prior to nuclear migration. Thus, proteins important to root hair initiation, such as the *RHO*-related, *p*lant-specific monomeric G-proteins ROP2 and ROP4, are localized soon after cell division (Jones and others 2002; Molendijk and others 2001), whereas nuclear migration occurs directly before wall bulging. ROP localization provides an important clue as to how the initiation site may be marked (that is, by G-protein-mediated events) and highlights just how early this site is specified (that is, in the meristem as cells exit mitosis). In addition, ROPs appear to be important elements in multiple phases of root hair development (discussed in more detail below), providing candidates for one class of proteins that might act to coordinate the developmental program that produces a root hair.

Although AtROP2 and AtROP4 remain the only clear molecular candidates as to how the initiation site is marked, our understanding of the molecular basis of how subsequent wall bulging occurs is more complete. For example, *lxr* mutants develop significantly wider initiation sites than wild-type plants. *LRX* encodes a wall protein that contains both leucine-rich and extensin domains. Leucine-rich domains are thought to mediate protein–protein interactions or possibly act in ligand binding in the apoplast, whereas extensins are wall proteins thought to be involved in modulating the physical characteristics of the cell wall (Baumberger and others 2001). Thus, LRX may well contain motifs for sensing and modifying the status of the wall essential for defining size of the localized outgrowth. This theme of control of wall properties has emerged as a key idea in understanding the process of wall bulging during initiation.

Role of Wall Structure and pH in Initiation

Cell biological evidence suggests that cell wall properties at the initiation site are altered in the course of root hair initiation and these changes are essential for initiation to take place. Thus, as soon as the initiation bulge starts to appear on the surface of a trichoblast, the pH of the cell wall at the initiation site drops from approximately 5 to 4.5. This local-

ized acidification persists throughout the initiation process (Bibikova and others 1998). Preventing localized cell wall acidification with strong pH buffers reversibly arrests root hair initiation, implying that the pH changes are an essential part of the mechanism that maintains and controls the local wall bulging. However, other factors (such as LRX or ROPs) must define the precise site and extent of bulge formation because buffering the entire trichoblast cell wall to pH 4.5 causes neither delocalized cell wall swelling nor formation of multiple bulges as might be expected if cell wall pH alone controlled wall deformation (Bibikova and others 1998). Possible mechanisms responsible for this spatially limited, asymmetrical change in trichoblast cell wall pH are localized activation of H^+–ATPases or localized secretion of weak acids at the initiation site. Because root cell walls possess a strong pH buffering capacity (Bibikova and others 1998; Fasano and others 2001), large amounts of protons must be moving to the wall to significantly alter its pH. This proton flux is perhaps most easily achieved by the activation of a plasma membrane pump. Consistent with this idea that local decreases in wall pH may mediate initiation, expansins have been detected in cell walls of initiating root hairs (Baluska and others 2000a). Expansins are cell-wall-loosening proteins that have a pH optimum of 4.5 (Cosgrove 2000). Local cell wall acidification would induce expansin activity and so facilitate cell wall modifications that ensure localized bulge formation.

Xyloglucan endotransferases (XETs) represent a further activity that might convert localized changes in wall pH to wall loosening. XETs are enzymes that cleave and rejoin xyloglucan chains and may thereby loosen the cell wall (Baydoun and Fry 1989; Fry and others 1992). During root hair initiation, XET action is elevated at the site of bulge formation (Vissenberg and others 2001). Unfortunately, which particular XET is active at the initiation site is not known, but XETs are more active at acid pH. Therefore, it is possible that localized cell wall acidification at the initiation site might contribute to localized activation of XET (Vissenberg and others 2001) and so promote turgor-driven bulge formation.

Role of the Cytoskeleton in Initiation

In *Equisetum hyemale*, root hair initiation is associated with localized rearrangement of microtubules from longitudinal to transverse orientation specifically at the initiation site (Emons and Derksen 1986). Although inhibitor studies suggest that disrupting the microtubule cytoskeleton does not inhibit the process of initiation (Bibikova and others 1999), *Arabidopsis* plants with reduced levels of A-tubulin expression sometimes form several initiation sites on one trichoblast implying that an intact microtubule cytoskeleton might play a role in defining the initiation site (Bao and others 2001).

The actin cytoskeleton also appears important for regulating root hair initiation. Actin depolymerizing and fragmenting drugs inhibit initiation (Bibikova and others 1999; Braun and others 1999; Miller and others 1999), and *der1* plants, which possess a point mutation in the gene encoding actin2, have enlarged or misplaced initiation sites (Ringli and others 2002). Interestingly, as yet there are few reports of dynamic rearrangements of the actin cytoskeleton associated with the initiation process. One possible mode of action of actin is suggested by the observation that expression of a constitutively active version of the ROP2 monomeric G-protein sometimes induces multiple or misplaced initiation sites. ROP2 is thought to be involved in regulation of F-actin assembly (Fu and others 2002). Therefore, it is tempting to speculate that ROP2 might be involved in positioning the root hair initiation site through localized regulation of actin-dependent processes.

Hormones and Regulating Initiation

As described above for cell-fate specification, auxin and ethylene signaling also appear to play critical roles in defining the exact location of the root hair initiation site. In *Arabidopsis*, root hairs normally form at the apical end of the trichoblast but excess ethylene causes a shift in this root hair initiation position. For example, the ethylene overproducing mutant *eto1* initiates hairs very close to the apical end of the trichoblast (Masucci and Schiefelbein 1994) whereas in *etr1*, a mutant deficient in ethylene perception, the initiation site is located more basally relative to wild-type (Masucci and Schiefelbein 1996; Dolan 2001).

Similar evidence suggests auxin action in regulating the site of initiation. In the auxin-insensitive mutant *axr2*, initiation is shifted toward the basal side of the trichoblast, and the basal shift in initiation seen in the *rhd6* mutant can be rescued by application of either ethylene or auxin analogs (Masucci and Schiefelbein 1994). Therefore, ethylene and auxin appear involved in the signaling cascade responsible for positioning the root hair initiation site along the length of the trichoblast. However, the precise molecular mechanism whereby auxin and ethylene might act to regulate
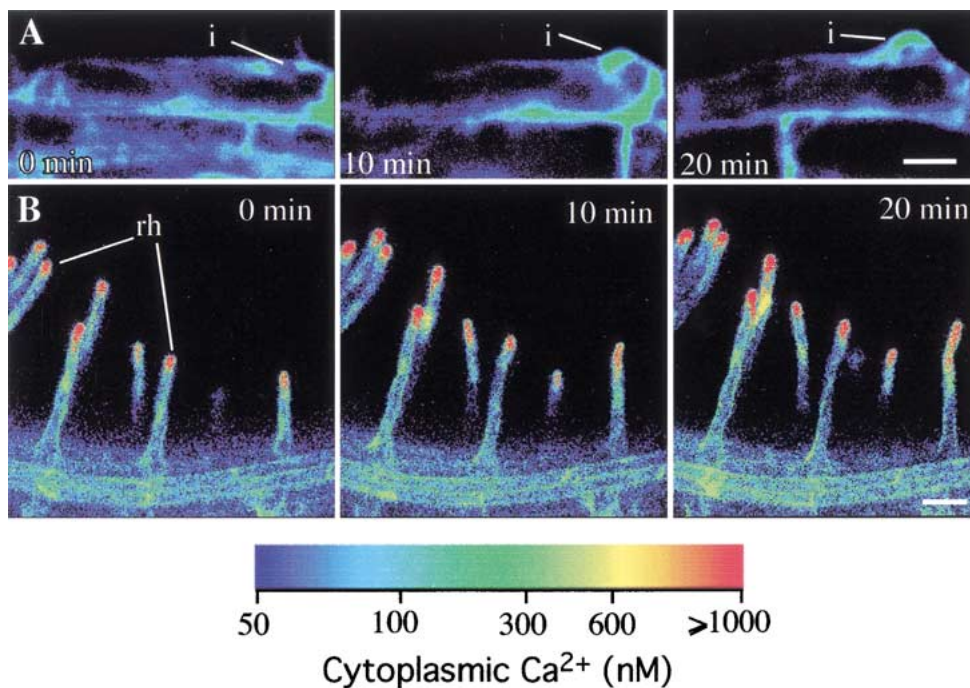
Figure 2. Tip-focused cytoplasmic Ca^{2+} gradients associated with tip growth of *Arabidopsis* root hairs but not the initiation process. (**A**) Ca^{2+} gradients are not evident during the initiation process. (**B**) A tip-focused Ca^{2+} gradient is associated with tip growth. Images are confocal ratio images of *Arabidopsis* root hairs expressing the fluorescent Ca^{2+} sensor Cameleon YC2.1 (Allen and others 1999) and monitored using a confocal microscope. Cytosolic Ca^{2+} levels have been color-coded according to the inset scale. i-initiation site; rh-root hair. Scale bar (**A**) -10 μm, (**B**) 20 μm.

positioning of the initiation of root hair remains to be determined.

Ionic Fluxes and the Regulation and Root Hair Initiation

During the process of initiation, although the levels of cytosolic Ca^{2+} remain unchanged at the initiation site (Figure 2; Wymer and others 1997), cytoplasmic pH is locally elevated (Bibikova and others 1998). This elevation persists throughout initiation and dissipates with the onset of tip growth. However, preventing the localized cytoplasmic alkalinization did not inhibit the initiation process (Bibikova and others 1998). Because the wall is locally acidified at this point in the initiation process, it is possible that the cytoplasmic pH change simply reflects H^+ efflux to the wall rather than a cytoplasmic regulatory event.

In addition to a role for H^+ fluxes in the initiation process, there is evidence that K^+ transport is important for setting up the correct growth machinery at the initiation site. Thus, *TRH1* encodes a potassium transporter belonging to the AtKKT/AtKUP/HAK^+ transporter family (Rigas and others 2001) and *trh1* mutants sometimes form several initiation sites. This observation may indicate that either a novel K^+-related signaling pathway linked to initiation may be operating in these cells or that K^+ transport, along with the associated control of membrane potential by K^+ fluxes, might play a regulatory role in the initiation process.

TRANSITION FROM INITIATION TO TIP GROWTH

Root hair initiation and subsequent tip growth are well recognized as morphologically, physiologically, and genetically distinct processes. However, after initiation but before the onset of actual tip growth, root hairs must organize and arrange the apical growth machinery at the tip of the now initiated bulge. It is not straightforward to distinguish mutants that are defective in the apical growth machinery from mutants that are defective in this process of transition from initiating bulge-to-tip growth. However, there are some indications that the transition process is distinct to the regulation of tip growth. For example, *Arabidopsis* root hairs do not simply exit initiation and begin tip growth, there is a small period of time where the initiated root hair does not increase in size, that is, initiation bulging has ended but rapid tip growth has yet to

commence (Dolan and others 1994; Wymer and others 1997). It seems likely that this pause in visible growth represents the initiated root hair recruiting and organizing elements of the tip growth machinery. The phenotype of some mutants also implies that there may be genetically distinct components of this transition period (Table 3). Thus, the *der1*, *cow1*, *aux1*, and *axr1* mutants have root hairs that are sometimes branched at the base, but these mutants do not form branches once tip growth has commenced. This observation suggests lesions in the organization of the machinery that controls the transition from initiation to tip growth rather than tip growth itself.

TIP GROWTH

Once the root hair is initiated and the tip growth transition completed, the genetically and physiologically independent process of tip growth occurs. During this apical growth phase, deposition of new plasma membrane and cell wall material is confined to the expanding tip. Such localized growth generates the hairlike morphology of the mature root hair. As outlined below, many insights about the machinery of tip growth come from studies on pollen tubes. Numerous features of tip growth are likely shared between pollen tubes and root hairs, for example, the *tip1* mutant affects elongation growth in both these cell types (Parker and others 2000; Ryan and others 1998; Schiefelbein and others 1993). On the other hand, there are undoubtedly unique aspects to growth control in each of these systems, not the least of which will be related to the divergent wall structure of the pollen tube and root hair.

Role of Ca^{2+} Gradients in Tip Growth

Elongation in a variety of tip-growing cells seems intimately associated with a tip-focused gradient in cytosolic Ca^{2+}. Plant cells usually maintain a cytosolic $[Ca^{2+}]$ of approximately 100 nM but several μM are found in the apical few micrometers of tip growing cells ranging from fungal hyphae (Garril and others 1993) and algal rhizoids (Brownlee and Pulsford 1988) to pollen tubes (Pierson and others 1994) and root hairs (Figure 2B; Bibikova and others 1997, 1999; de Ruijter and others 1998; Felle and Hepler 1997; Jones and others 1998; Wymer and others 1997). In addition, faster-growing root hairs appear to have a more pronounced gradient, whereas root hairs that have stopped elongating, either through attaining a mature length or due to

some experimental manipulation, show a uniform Ca^{2+} level of about 100 nM with no detectable Ca^{2+} gradient toward the tip (Bibikova and others 1997, 1999; de Ruijter and others 1998; Felle and Hepler, 1997; Jones and others 1998; Wymer and others 1997). Measurements with the self-referencing (vibrating) microelectrode have also shown that Ca^{2+} influx is higher at the tip than at the base or sides of growing root hairs (Herrmann and Felle 1995; Jones and others 1995; Schiefelbein and others 1992).

This tip-focused Ca^{2+} gradient appears important for driving growth. When either new tip growth is induced or the direction of root hair elongation is misdirected, for example, through treatments such as Nod factor application (de Ruijter and others 1998), touch stimulation (Bibikova and others 1998), or interference with the operation of the microtubule cytoskeleton (Bibikova and others 1999), the new growing tip always exhibits a tip-focused gradient centered on the new direction of growth. Similarly, treatments that disrupt the gradient in normally elongating root hairs, such as addition of Ca^{2+} ionophores, Ca^{2+} channel blockers, or the microinjection of Ca^{2+} buffers that diffuse the Ca^{2+} gradient, all arrest tip growth. Lastly, imposing an artificial $[Ca^{2+}]$ gradient specifically at the tip of the hair leads to growth directed by the new gradient (Bibikova and others 1998). All these observations are consistent with the tip-focused Ca^{2+} gradient localizing and directing growth to the apical dome at the very tip of the elongating root hair. Interestingly, in *Arabidopsis*, redirection of tip growth by treatment with Ca^{2+} ionophore leads to only a transient alteration in growth direction (Bibikova and others 1998). Thus, root hairs normally grow at about 90° to the *Arabidopsis* root surface, but, if reoriented to grow parallel to the root, this direction is maintained for only 5–10 min before the original growth direction is resumed (Bibikova and others 1998). This observation suggests that some other as yet unidentified factor is orienting the Ca^{2+} gradient that directs growth away from the surface of the root.

The tip-focused Ca^{2+} gradient and the associated localized Ca^{2+} influx at the apex of the hairs (Herrmann and Felle 1995; Schiefelbein and others 1992) implies an accumulation or localized activation of Ca^{2+} channels at the growing apex of the root hair. Although the positive identification of a growth-related Ca^{2+} transporter has yet to be reported, a strong candidate for this activity is a hyperpolarization-activated Ca^{2+}-permeable channel which has been electrophysiologically identified in root hair protoplasts and may be localized to the

apical part of the hair (Very and Davies 2000). Fluorescence staining for Ca^{2+} channels also tentatively implies a gradient of channels to the growing root hair tip (Bibikova and Gilroy 2000). However, we must await localization of defined channel proteins and spatial characterization of their activity to identify the elusive tip-growth-related Ca^{2+} influx channel.

Even though the precise nature of a root hair growth-related Ca^{2+} channel has yet to be determined, we have some models about how the Ca^{2+} influx might be regulated at the tip of root hairs from studies on tip-growing fungal hyphae. The hyphae of Saprolegnia and Neurospora show a tip-focused Ca^{2+} gradient that appears to drive growth (Garrill and others 1993; Knight and others 1994; Levina and others 1994, 1995). Stretch-activated Ca^{2+}-permeable channels are concentrated at the growing tip of S. ferax hyphae (Garrill and others 1993) and treatment with cytochalasin disrupts this asymmetrical distribution and inhibits hyphal growth. These observations imply that an actin network maintains the channel gradient in the tip and that the mechanical strain at the growing apex may regulate channel gating (Levina and others 1994). In contrast, the plasma membrane throughout the Neurospora hypha contains Ca^{2+}-activated Ca^{2+} channels (Levina and others 1995). In this case, once a tip-localized Ca^{2+} influx is initiated it may be self-sustaining, with the high apical Ca^{2+} recruiting more open channels to the hyphal tip. However, as a note of caution, the mechanisms of tip growth in plant and fungal cells are unlikely to be identical, for example, a secretion-related apical body called the Spitzenkorper (Girbardt 1969) is present in fungal but not plant cells, and the chitin-rich hyphal cell wall is markedly different in structure to the cellulosic root hair cell wall.

Monomeric G-Proteins and Tip Growth

Small, monomeric GTPases (M_r-21–30 kD) have recently emerged as important regulators of the tip growth process, perhaps through regulation of the Ca^{2+} gradient itself. Many insights into how these G-proteins regulate apical growth have emerged from studies on elongating pollen tubes, but, in this case, as opposed to growth localization, there seem to be very strong parallels between tip growth in pollen tubes and root hairs. Importantly, both appear to utilize Ca^{2+} gradient-driven growth that, as described below, is strongly influenced by the activity and/or localization of monomeric G-proteins and actin. Therefore, we will first discuss how G-proteins are thought to regulate pollen tube growth in order to develop some models about how the analogous processes might occur in root hairs.

The Arabidopsis genome likely encodes 93 monomeric G-proteins (Wu and others 2001). Of the 5 major classes of eukaryotic monomeric G-proteins (ras, rho, rab, arf, and ran), ras has not been found in plants and (in Arabidopsis) the rab family members predominate with 53 putative proteins (Wu and others 2001). Rabs and arfs are classically thought to mediate secretory processes and vesicle trafficking and so are obvious candidates for elements of the tip growth machinery. However, it is the plant Rho homologs (ROPS) that have emerged as key regulators of tip growth, as well as a host of other plant processes. Among 11 ROPs present in Arabidopsis genome, ROP1, ROP3, and ROP5 are expressed in pollen (Li and others 1998; Zheng and Yang 2000). Expression of dominant negative versions of ROPs 1 and 5 inhibited pollen tube growth, whereas expression of constitutively active versions of these proteins delocalized growth, causing swollen tubes to form. These ROPs preferentially localize to the apex of the growing pollen tube (Lin and others 1996). However, it appears that it is the GTP-bound ROP 1, but not its GDP-bound form, that localizes to the apical plasma membrane, leading to a proposed model where GTP-bound ROP forms a self-reinforcing recruitment system to the tip membrane (Li and others 1998). Regulation of ROP activity by proteins that promote or inhibit GTP turnover by the G-protein may be localized in the lateral membrane behind the tip and so also serve to focus the active ROPs to the apex of the pollen tube. Disrupting ROP 1 and 5 activity disrupts the localization of the growth-related, tip-focused Ca^{2+} gradient (Fu and others 2001), implying that ROPs may regulate formation of the gradient and so orient secretion and pollen tube growth.

G-proteins also seem to be part of the tip growth machinery in root hairs. For example, in Arabidopsis, RHD3 encodes a putative GTP-binding protein (Wang and others 2002) and the rhd3 mutant shows disrupted tip growth, exhibiting short, wavy, or branched root hairs. However, as in pollen tubes, the clearest link of G-protein activity to tip growth in root hairs has emerged from the identification of ROP2, 4, and 6 as potentially important regulatory players of root hair tip growth. Thus, ROP2 and ROP4 have been shown to localize to the root hair apex by immunofluorescence or GFP tagging (Jones and others 2002; Molendijk and others 2001). Constitutively active ROPs 2, 4, or 6 make root hairs swell as they grow or cause longer, wider, and sometimes curled root hairs to form, whereas a

dominant negative *ROP2* inhibits root hair growth and makes root hairs wave (Jones and others 2002; Molendijk and others 2001). Expression of constitutively active ROP2 depolarized root hair tip growth, whereas ROP2 overexpression resulted in hairs with multiple tips (Jones and others 2002). Disrupting ROP4 activity caused a delocalized Ca^{2+} gradient concomitant with swelling of the root hairs and also altered the structure of the microtubule cytoskeleton (Molendijk and others 2001), all effects consistent with altering the activity of a critical regulator of the tip growth machinery.

How might these ROPs be targeted to the plasma membrane? ROPs are thought to be generally associated with membranes due to posttranslational modification such as prenylation. However, treatment with the membrane-trafficking inhibitor brefeldin inhibited localization of ROP4 in trichoblasts (Moledijk and others 2001), suggesting that either membrane trafficking or a secreted protein might be responsible for targeting the protein to the plasma membrane. In pollen tubes, overexpression of a ROP GDP dissociation inhibitor (GDI) abolished the tip localization of ROP. ROPs are activated by binding GTP and inactivated as they convert the GTP to GDP. GDIs prevent subsequent exchange of GDP for GTP and so lock the G-protein in the GDP-bound form. Thus, the prevention of ROP localization by a GDI implies that ROP activity may be required to recruit itself to the membrane (Takahashi and others 1997), that is, the self-reinforcing model described above. Interestingly, a model implying a self-reinforcing tip growth machinery has also been proposed from observations on using Ca^{2+} gradients to redirect root hair tip growth in *Arabidopsis*. Thus, local ionophore activation reoriented Ca^{2+} influx in these root hairs and so imposed a new Ca^{2+} gradient that directed growth (Bibikova and others 1998). Although the Ca^{2+} ionophore activation in these experiments lasted only 0.7 s, the reorientation of the Ca^{2+} gradient and associated tip growth was sustained for several minutes. This observation led to the proposal that the Ca^{2+} gradient might be recruiting tip growth machinery and Ca^{2+} channels and pumps in a self-reinforcing/stabilizing system. One possible mechanism for this system would be ROP promoting the formation and stabilization of a Ca^{2+} gradient that then promotes vesicle fusion at the apex of the hair. Secretory vesicles may then help deliver more of both Ca^{2+} influx channels and ROP to the apex. Coupled to the self-recruiting nature of ROP–GTP, this interplay of Ca^{2+} and ROP would form a positive feedback loop that would stabilize the activities at the growing tip. A more detailed understanding of the interrelationships of

ROPs and Ca^{2+} transporters should help us to define how this self-reinforcing network might be regulated.

Tip Growth and the Cytoskeleton

Along with ROPs, the cytoskeleton has emerged as a key component of the apical growth machinery. Treatment of root hairs with drugs that disrupt actin filaments arrests apical growth, and F-actin is well recognized as playing an essential role in tip growth (Baluska and others 2000b; Braun and others 1999; Fu and others 2001; Miller and others 1999). Similarly, point mutations in the *ACTIN2* gene either arrest or alter tip growth patterns (Ringli and others 2002). At least two forms of F-actin have been observed in tip-growing cells in both pollen tubes and root hairs: actin cables that are aligned to the growth axis and dynamic fine F-actin localized to the tip (Baluska and others 2000b; Gibbon and others 1999; Fu and others 2001; Miller and others 1999). Thus, in mature root hairs, actin bundles run longitudinally to the very apex of the hair, but in actively elongating root hairs, the actin flares into fine bundles subapically and these bundles are excluded from the vesicle-rich apex where tip growth occurs (Miller and others 1999). How the subapical fine bundles of actin operate is poorly defined at present but they may help target Golgi and secretory vesicles to the apical clear zone (Fu and others 2001; Miller and others 1999) where the tip-focused Ca^{2+} gradient may facilitate secretory vesicle fusion to the plasma membrane (Carroll and others 1998). Elevated $[Ca^{2+}]$ is known to inhibit cytoplasmic streaming, fragment F-actin, and depolymerize microtubules (Cyr 1994; Staiger 2000), and, when the $[Ca^{2+}]$ gradient is lost, actin microfilaments protrude to the root hair tip (Miller and others 1999) and growth ceases. Thus, it is possible that the Ca^{2+} gradient may both promote formation of an apical zone devoid of cytoskeletal structures and facilitate the localized exocytosis in this region. A key observation may be that in pollen tubes *ROP1* overexpression leads to stabilization of tip F-actin and causes depolarized tip growth (Fu and others 2001). Thus, there seems an intimate link between actin, ROPs, and the apical Ca^{2+} gradient in maintaining the dynamic structure of the apical growth machinery of tip-growing cells.

Interestingly, if the microtubule cytoskeleton is disrupted, either by drug treatment or by using plants expressing antisense to A-tubulin, root hair tip growth is not arrested. These treatments result in the root hairs adopting either a waving or branching growth habit (Bao and others 2001; Bibikova and

others 1999). These observations suggest that although the microtubule cytoskeleton is not required for tip growth to proceed, it is involved in stabilizing the site of the apical growth machinery. How this stabilization occurs is unknown, but disruption of kinesin-like microtubule motors leads to waving growth patterns in tip-growing fungal hyphae (Wu and others 1998) and altered branching patterns in trichomes (Oppenheimer and others 1997) and root hairs (*hinkel*; Strompen and others 2002), thereby providing one candidate for a microtubule-associated protein that could help stabilize the tip growth machinery. Although microtubules have been proposed to direct growth through their control of cellulose deposition in diffuse growing cells (Baskin 2001), this is less likely to occur in root hairs where microtubules do not protrude to the localized site of growth at the hair's apex. However, it is clear that the unique helical structure of the root hair wall (Emons 1986) plays a critical role in regulating tube growth, as described in the next section.

The Role of Cell Wall in Tip Growth

During the process of apical growth, cell wall material is deposited at the growing tip where it stretches as the apical dome expands. This process of wall deformation appears to last for only a few minutes because as the wall migrates below the growing apex, due to the continued apical expansion, it loses its ability to yield (Shaw and others 2000). Therefore, apical growth requires coordination of cell wall deposition and its subsequent rigidification. Not surprisingly, therefore, several tip growth-related mutants are in cell wall-related genes. *CEV1* encodes cellulose synthase and *cev1* mutants form long root hairs, indicating that cellulose synthesis is important for determining root hair growth rate (Ellis and others 2002). In contrast, either the *kojak* mutation of another cellulose synthase-like protein, KJK, or a T-DNA insertional mutation in *AtCSLD3*, a cellulose synthase-like (subfamily D) gene family member, inhibit apical growth and the root hairs often burst when they should switch their developmental program to apical growth (Favery and others 2001; Wang and others 2001). Thus, it appears root hairs normally tightly regulate wall structure to permit expansion of the apical dome while retaining enough structural integrity to resist turgor. As well as an initiation phenotype, the previously described *lrx* mutant also has a tip-growth-related phenotype, sometimes forming branched root hairs, suggesting that signaling between cytoplasm and cell wall might provide some of the fine tuning of wall properties needed to sustain these tip growth activities (Baumberger and others 2001).

ROOT HAIR DEVELOPMENT IN CONTEXT: COMPLEXITY, PLASTICITY, AND NUTRIENT UPTAKE

The previous sections highlight just how complex the control of root hair formation is, and, although we have discussed each phase of development in isolation, Table 5 illustrates the extent of how intertwined the entire process of root hair development actually is. Thus, a huge proportion of root hair mutants describe regulatory components common to many of the developmental phases that give rise to a functional root hair. Likewise, auxin and ethylene seem intimately linked to the regulation of root hair development at all levels, from cell-fate specification to tip growth. How then can such a complex developmental network be placed in context? One answer might lie in the link between root hair function and its developmental control. The developmental processes outlined in the sections above lead to the formation of tubelike projections from the epidermal cells that should increase the surface area of the root for nutrient uptake as well as increase the effective volume of the root in contact with the soil. There is a wealth of data indicating that root hairs are important for the success of the plant in nutrient acquisition. For example, root hairs have been directly shown to take up phosphorus from the soil (Gahoonia and Nielson 1998), and, under conditions of low phosphorus availability, wild-type *Arabidopsis* roots acquire more phosphorus per unit of carbon respired than the hairless *rhd2* and *rhd6* mutants (Bates and Lynch 2000). Both *rhd2* and *rhd6* mutants do not have any other obvious phenotypes other than their lack of root hairs, and so it appears that root hairs provided an advantage in nutrient acquisition to the wild-type plants (Bates and Lynch 2000). There are also reports suggesting a relationship between root hair development and the levels of nutrients such as nitrate (Jungk 2001), calcium, and cobalt (Werner and others 1985) in the soil. We are only just beginning to identify the transporters in the root hair responsible for nutrient acquisition processes, but Table 6 outlines some of these activities that have been defined to the molecular level.

In addition to direct nutrient transport, root hairs are known to be essential to the nodulation process associated with rhizobial symbiosis and nitrogen fixation, a process where root hair growth is manipulated to allow successful bacterial colonization,

Table 5. *Arabidopsis* Mutants Involved in Multiple Phases of Root Hair Development

	Cell-fate determination (CFD)	Root hair initiation (RHI)	Transition from RHI to tip growth	Tip growth	CFD and RHI	RHI and transition from RHI to tip growth	RHI, transition from RHI to tip growth, and tip growth	RHI and tip growth	Transition from RHI to tip growth and tip growth	zCFD, RHI, and tip growth	CFD, RHI, transition from RHI to tip growth, and tip growth
Putative transcription factors	WER, TTG, GL2, CPC										
Putative hormone-regulated genes	ELP1, SLR/IAA14, CTR1, ETO2, ETO3, ETO4, HLQ, AXR3		SOS4	EIN2	RHD6, AXR2/IAA7			ETR1	AUX1, AXR1	ETO1	
Cytoskeleton-related	ERH3			IRE			DER1			TUA/AS lines	
Ion transporters						TRH1					
Light-related				PHYB/HY3, HY5							
RHO family of small GTPases			ROP7	ROP4, ROP6							ROP2
Cell wall				CEV1							
Cytokinesis				KNOLLE, HINKEL, PLEAIDE, BUBLINA		KJK	LRX1	KEULE, CLUB	CSLD3		
Unidentified function	ERH1, RHL1, RHL2, RHL3, POM1/ERH2	RHD1	SHV1, SHV2, SHV3, RHD2	CEN1, WAVY, RHD3, RHD4				TIP1, CEN3, CEN2, SCN1, BST1	COW1		

Table 6. Examples of Molecularly Identified Nutrient Transporters in Root Hairs for the Major Macronutrients N, P, and K

	Plant species	Transporters	References
Potassium	*Arabidopsis thaliana*	Inward-rectifying K$^+$ channel: AKT1 ATKC1	Reintanz and others 2002
		Outward-rectifying K$^+$ channel:	Rigas and others 2001 Ache and others 2000
		GORK	
		AtKT/AtKUP/HAK K+ transporter family member: TRH1	Ivashikina and others 2001
Phosphorous	*Lycopersicon esculentum*	High-affinity phosphorous transporter: LePT1	Daram and others 1998
Nitrogen	*Lycopersicon esculentum*	Ammonium transporter: LeAMT1	Lauter and others 1996
		Nitrate transporters: LeNrt1-1 LeNrt1-2	Ludewig and others 2002

nodule formation, and subsequently nitrogen fixation (Lhuissier and others 2001). Similarly, root hair density directly correlates with mychorrhizal infection; the more mychorrhizal associations, the fewer root hairs (Schweiger and others 1995). The theme emerging from these symbioses is that root hair development is altered in response to nutrient acquisition status, an idea that may be fundamental to understanding root hair development in general.

Thus, the available ecophysiological, molecular, and electrophysiological data indicate that root hairs are indeed specialized to improve nutrient acquisition by the plant. How then might this help explain the almost bewildering complexity of root hair development? Root hairs appear to be remarkably plastic in their development in response to environmental stimuli, not the least of which are the levels of the nutrients they are to transport. For example, light regime (Cao and others 1999), pH of the soil (Ewens and Leigh 1985), and availability of nutrients such as phosphorus (Bates and Lynch 1996), boron (Goldbach and others 2001), and iron (Schikora and Schmidt 2001) can profoundly affect root hair growth and development. At first glance, Figure 3 could be showing root hair developmental mutants. In reality, the figure shows the morphology of *Arabidopsis* root hairs in response to water stress, indicating just how the plasticity of root hair development can be entrained to the environment.

Understanding root hair development will undoubtedly need an appreciation of how developmental plasticity relates to the functions of the root hair. The observation that many of the defined molecular regulators and mutants affect many levels of root hair formation (Table 5) may reflect a highly coordinated control mechanism entraining the whole process of root hair development to ambient environmental conditions. The root hair phenotype of nutrient transporter mutants, for example, the tiny root hair K$^+$ transporter mutant *trh1*, suggests nutrient fluxes might play a role in regulating these control points. However, it is currently unclear how the transport activities outlined in Table 6 relate to the signaling of nutrient status to the root.

Clearly, root hair development is a complex and plastic process but the hormones auxin and ethylene appear to be strong candidates for integrators of the various phases of root hair formation. Our increasing knowledge of, for example, the transcriptional regulators of cell fate or the role of the cytoskeleton in mediating tip growth provide clear candidates for the sites of action of the regulators of this developmental program. However, the critical question of the molecular mechanism(s) through which auxin or ethylene exert their coordinating action remains to be answered.

The ROP family of monomeric G-proteins also appear involved in multiple steps of the root hair developmental program and so could play a role in the coordination of these processes. In addition, the identification of AtROPs as being localized to the initiation site represents an important advance in providing a molecular marker for site specification. However, there are many other tantalizing indications of the complexity of the initiation/site specification process. For example, cytokinesis-related mutants such as *keule* and *club* are incapable of forming a normal root hair initiation site (Tables 1–4). Considering the indications from the work on
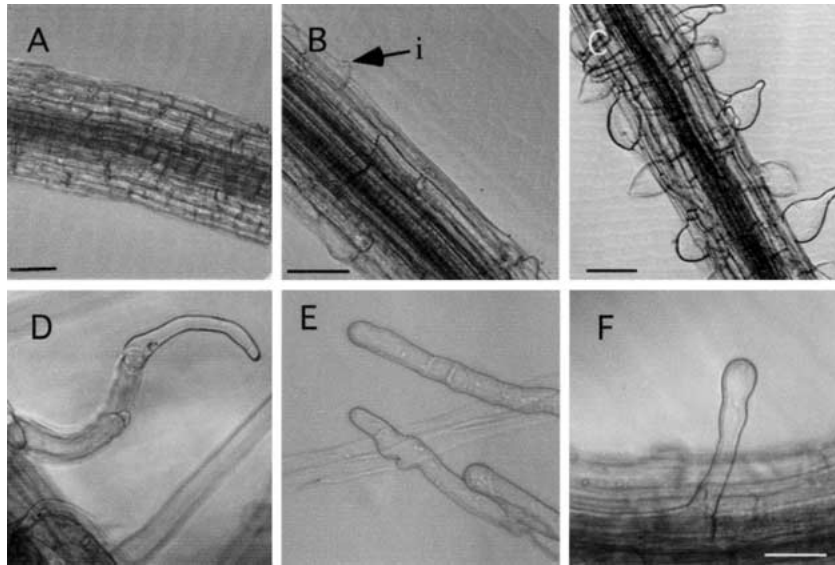
Figure 3. Root hair development in *Arabidopsis* plants exposed to water stress. Note the responses to this stress phenocopy many classes of developmental mutants that have been described: (**A**) hairless, (**B**) arrest during initiation, (**C–F**) branching, waving, and bulging during tip growth. i-initiation site. Scale bar (**A–C**) 100 μm. (**D–F**) 25 μm.

the AtROPs that site specification occurs very early in the production of the trichoblast in the meristem, it is tempting to speculate that the initial polarizing event in the trichoblast may be closely linked to the polarizing axis of cytokinesis itself. However, mutations in these cytokinesis-related genes cause a plethora of pleiotropic phenotypes and at present it is difficult to argue the effect of these mutations on the root hair initiation process is specific (Sollner and others 2002).

Clearly, we are far from a comprehensive description of the molecular basis of root hair formation. There are still a host of mutants that are characterized to affect all phases from root hair development from cell-fate determination to tip growth, but we still have very little idea of how most of them act (Tables 1–4). For example, even though *rhl1* has helped in highlighting the interaction between auxin and ethylene in root hair formation, all we know at present about its mode of action is that *RHL1* encodes a small hydrophilic protein that contains a nuclear localization signal and causes reduced root hair numbers. It is unclear whether it plays a regulatory, structural, or enzymatic role in plant development, although its low expression level may very tentatively hint at a regulatory activity. Other genes that are involved in root epidermal patterning include *POM/ERH2*, *RHL2*, *RHL3*, and *HLQ* and, for these, we have no cell or molecular information about what they encode or their mode of action. Fortunately, this wealth of uncharacterized mutants in all phases of root hair formation indicates that we have a rich set of potential regulatory genes that, once cloned and characterized, should help advance our understanding of the remarkably plastic developmental program that characterizes root hair formation.

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