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Building a hair: tip growth in *Arabidopsis thaliana* root hairs

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The *Arabidopsis thaliana* root hair is used as a model for studying tip growth in plants. We review recent advances, made using physiological and genetic approaches, which give rise to different, yet compatible, current views of the establishment and maintenance of tip growth in epidermal cells. For example, an active calcium influx channel localized at the tip of *Arabidopsis* root hairs has been identified by patch-clamp measurements. Actin has been visualized *in vivo* in *Arabidopsis* root hairs by using a green-fluorescent-protein–talin reporter and shown to form a dense mesh in the apex of the growing tip. The *kojak* gene, which encodes a protein similar to the catalytic subunit of cellulose synthase, is needed in the first stages of hair growth. A role for *LRX1*, a leucine-rich repeat extensin, in determining the morphology of the cell wall of root hairs has been established using reverse genetics. The new information can be integrated into a general and more advanced view of how these specialized plant cells grow.

Keywords: root hair; *Arabidopsis*; mutant; tip growth

1. INTRODUCTION

Root hairs are long, thin tubular outgrowths from epidermal cells that are produced in the differentiating zone of the root (figure 1). In *Arabidopsis thaliana* the trichoblasts (hair-forming cells) and atrichoblasts (non-hair cells) are arranged in alternating files along the root surface so hairs are produced in a simple and invariant striped pattern (Dolan *et al.* 1994; Dolan & Costa 2001). The trichoblast undergoes diffuse longitudinal growth in the meristem and elongation zones. Elongation along this axis then ceases and growth switches direction, leading to the initiation and maintenance of a polarized outgrowth from the basal region of the cell—the root hair (figure 1). Root hairs of *Arabidopsis* can grow up to 800 μm long and 11 μm in diameter, depending on the ecotype and growth conditions (Galway *et al.* 1997).

Root hairs are not essential for plant growth and development and are convenient to study since they are on the exterior of the root. The simplicity of the patterning and the range of mutants with defects in hair pattern and morphology make the *Arabidopsis* root hair a useful model for the study of plant cell growth, and for tip growth in particular. There are likely to be many parallels between the growth of the different types of tip-growing cells, e.g. plant pollen tube growth, fungal hyphal growth and algal rhizoids (Schnepf 1986; Yang 1998; Palanivelu & Preuss 2000).

Root hair development in *Arabidopsis* can be conceptually separated into a number of phases.

(i) The specification of hair-producing cells: some of the genetic factors that specify the position-dependent fate of trichoblasts and atrichoblasts in alternating epidermal cell files are known (reviewed by Schiefelbein 2000; Dolan & Costa 2001).
(ii) The initiation of root hair growth: the establishment of trichoblast cell polarity, the selection of a site for hair growth at a basal position in the cell; the migration of the nucleus to the centre of the trichoblast, and then to the base of the developing hair; local cell-wall acidification, thinning and loosening, leading to the formation of a bulge (figure 2*a*).
(iii) The slow establishment of tip growth from the bulge, followed by rapid sustained elongation: organization of polarized cytoplasm (figure 2*b*); rapid exocytosis of cell wall and membrane materials localized at the hair tip; migration of the nucleus into the growing hair; vacuolation.
(iv) Cessation of growth in mature root hairs.

2. THE MORPHOLOGY OF TIP-GROWING ROOT HAIRS

The ultrastructure of root hair cells of *Arabidopsis* and other plants has been described (Galway *et al.* 1997, 1999; Schnepf 1986). An actively tip-growing root hair cell has a characteristically polarized organization (figure 3). At the hemispherical apex is an α-layer of cellulose cell wall, behind which is a dense cytoplasm filled mainly with secretory vesicles (figure 2*b*; shown schematically in figure 3). These may be clathrin-coated vesicles and pits, spherical and pleiomorphic vesicles derived from the Golgi and endoplasmic reticulum, and lipid bodies. There is evidence from *Vicia villosa* root hairs that subsets of the vesicles contain specific cell wall components (Sherrier &

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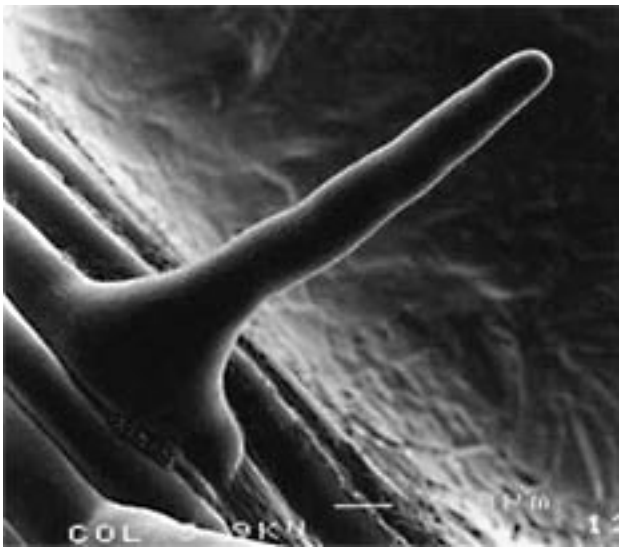

Figure 1. Scanning electron micrograph of an *Arabidopsis thaliana* root hair. Scale bar, 10 μm.

VandenBosch 1994). Vesicles in this region may be aligned along the axis of actin microfilaments or MTs (Galway *et al.* 1997, 1999). Other organelles are excluded from this extreme tip region (10 μm from the apex), but small organelles are found in a sub-apical region: mitochondria, Golgi stacks, rough and smooth endoplasmic reticulum, and plastids. The endoplasmic reticulum becomes compacted in trichoblasts at sites where root hairs are about to emerge and a compacted form is maintained throughout hair elongation (Ridge *et al.* 1999). Microfilaments, which are often bundled, are found throughout the cytoplasm, but MTs are found mainly at the periphery of the cytoplasm, and are oriented parallel to the axis of the extending hair. More basal than this (60 μm from the tip), hairs are highly vacuolated with the central vacuole taking up most of the diameter of the cell (Galway *et al.* 1997). The nucleus also migrates some way into the hair, following the advancing tip at a fixed distance (Galway *et al.* 1997; Grierson *et al.* 1997). In mature hairs that have reached their final length, the characteristic vesicle-containing cytoplasm at the tip disappears (Galway *et al.* 1997), a secondary cellulose β-layer is formed in the cell wall (Galway *et al.* 1999), and the endoplasmic reticulum takes on a more open reticulate form (Ridge *et al.* 1999).

3. PHYSIOLOGICAL PROCESSES DURING ROOT HAIR TIP GROWTH

(a) *The cell wall*

After nuclear migration and reorganization of the trichoblast cytoskeleton, one of the first detectable indications of imminent root hair initiation is the localized alkalinization of the cytoplasm and the respective acidification of the cell wall in the area where hair protrusion will begin (Bibikova *et al.* 1998). The local apoplastic acidification can be prevented by treatment with pH buffers and this also prevents subsequent hair initiation in a rapid and reversible manner. This suggests that the pH-responsive initiation mechanism is stable, and is possibly the cell wall polymer structure itself or plasma membrane ion channel activity (Bibikova *et al.* 1998). However, no pH gradient is detectable upon the establishment of tip growth, although high cytoplasmic pH can inhibit tip growth, and low extracellular pH causes growing hairs to burst (Bibikova *et al.* 1998).

For tip growth to occur, the cell wall of the hair must be restructured only at the apex to allow the localized exocytosis of new cell wall material (figure 4). Expansin—a protein that may be involved in pH-dependent loosening of cell wall polymers—localizes to bulges and growing tips of maize root hairs (Baluska *et al.* 2000; Cosgrove 2000). Regulated cellulose synthesis is required for tip growth since treatment of wild-type root hairs with the cellulose synthesis inhibitor 2,6-dichlorobenzonitrile causes cells to burst instead of elongating. This is similar to the phenotype observed in the roots of the *kjk* mutant (Favery *et al.* 2001; see § 4).

(b) *Calcium and other ions*

Extracellular Ca^{2+} in the medium is required for *Arabidopsis* root hair growth (0.3–3.0 μM Ca^{2+} is optimal) and an extracellular gradient has been detected at the growing tip of the hair reflecting a net influx at the tip of 4.4 pmol cm^{-1} s^{-1} Ca^{2+} (Schiefelbein *et al.* 1992). This gradient is not detected in the vicinity of non-growing tips or at the flanks of hairs. An inhibitor of plasma membrane calcium channel transport causes both the dissipation of the extracellular gradient and the inhibition of hair growth (Schiefelbein *et al.* 1992). The internal [Ca^{2+}] was found to increase after initiation of a bulge and prior to hair elongation. This increase leads to the development of a cytoplasmic [Ca^{2+}] gradient of 1 μM at the tip to 100 nM at the base of the hair (figure 4). The rate of growth is positively correlated with [Ca^{2+}] at the tip. Plasma membrane calcium channel blockers or manganese ions, which compete with Ca^{2+} for transport through the channel, prevented the formation of the intracellular gradient and inhibited hair growth (Wymer *et al.* 1997). Incidentally, the *rhd2* mutant, in which the hairs do not progress to the tip-growing phase but remain as bulges (see § 4), does not develop a high [Ca^{2+}] at the apex of the trichoblast bulges, indicating that RHD2 is required for the activation of Ca^{2+} channel activity. The precise role of Ca^{2+} that becomes established in the gradient is unknown but it may promote the exocytosis of secretory vesicles and/or affect the strength of the hair cell wall. The fate of the incoming Ca^{2+} is not known. There may be a non-localized efflux over the surface of the hair or it may be sequestered in the vacuole. Together these data indicate that an influx of Ca^{2+} is necessary to establish a tip-high Ca^{2+} gradient, which in turn is required for hair growth.

The artificial generation of Ca^{2+} gradients indicates that the establishment of a Ca^{2+} gradient is enough to initiate hair growth. Such artificial gradients were created using ultraviolet-activated caged ionophores. This had the effect of transiently shifting the direction of growth from the tip to the site of the induced gradient (Bibikova *et al.* 1997). When MT-disrupting drugs are added the new growing point is established permanently leading to growth from two points, generating a branched/forked hair morphology (Bibikova *et al.* 1999). This suggests that MTs may be necessary to maintain the calcium influx machinery at the tip of the hair (see below).

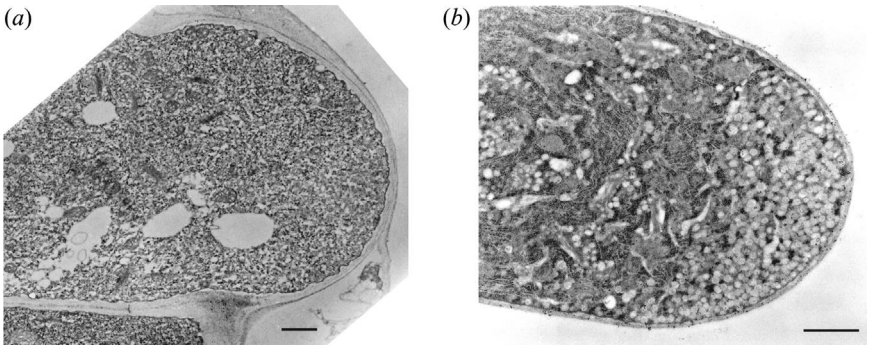

Figure 2. (*a*) Transmission electron micrograph of a longitudinal section through the base of a trichoblast, showing the cell wall thinning and bulge formation that occurs when hair growth is initiated. Scale bar, 1 μm. (*b*) Transmission electron micrograph of an oblique section through the apex of a tip-growing hair. The section was immunolabelled with M1 and a gold-conjugated secondary antibody. M1 recognizes a terminal fucose epitope present in xyloglucans. Scale bar, 1 μm.

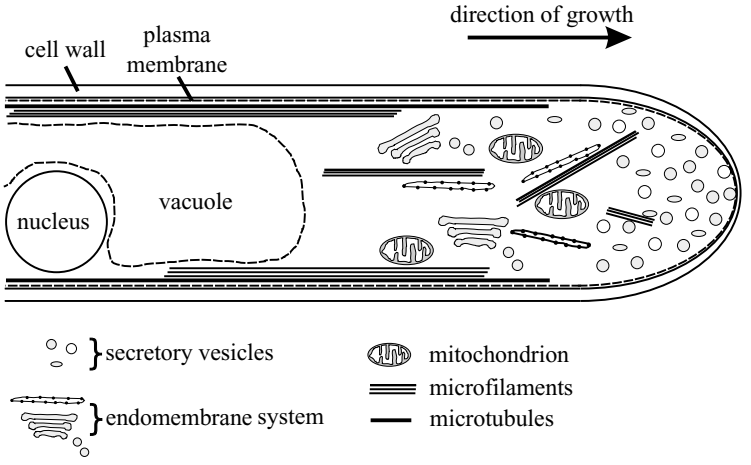

Figure 3. A schematic representation of a tip-growing root hair cell.

The nature of calcium channels present in *Arabidopsis* root hairs was recently assessed using patch-clamp methods (Véry & Davies 2000). An inward-rectifying Ca^{2+} conductance was detected, which probably represents the main transport system responsible for the apical Ca^{2+} influx that creates the tip-focused gradients. The channel activity is localized to the tip and was present only in the membranes of growing hairs (figure 4). The conductance of the channel was found to increase with high cytoplasmic [Ca^{2+}], so this could provide a mechanism of positive feedback for driving continued tip growth. Unlike other known *Arabidopsis* calcium channels, this channel was found to be active at the resting potential and at the [Ca^{2+}] that is found in root hairs.

Other ion channels and transporters have been identified in plant roots and hairs, e.g. those for K^+, Cl^- and proton transport. A combination of ion exchanges may be required for establishing hair growth (e.g. the potassium channel, TRH1, described in § 4). The availability of mineral nutrients can also affect the number, length and position of *Arabidopsis* root hairs.

The redox status of root hairs can affect their final length (Sánchez-Fernández *et al.* 1997). Glutathione and dithiothreitol (100 μM) cause a doubling of hair length and, when glutathione is depleted using an inhibitor of its synthesis, hair initiation is inhibited. So hair growth is highly responsive to the presence of active oxygen species in the environment.

(c) *The cytoskeleton*

Actin microfilaments have been observed *in vivo* in *Arabidopsis* root hairs by using GFP–talin as a reporter. F-Actin forms a densely staining mesh (cap) in the dome of the hair-forming bulge and at the apex of the tip-growing hair, with less-dense strands of filaments appearing in the more basal areas of the root hair (Baluska *et al.* 2000). The disruption of the F-actin microfilament network by the drug latrunculin B (which sequesters G-actin) arrests the tip growth of *Arabidopsis* and maize root hairs (Bibikova *et al.* 1999; Baluska *et al.* 2000). In *Arabidopsis*, hairs that grow in the presence of low concentrations of latrunculin B are straight and extend perpendicularly to the root axis (Bibikova *et al.* 1999). This suggests that actin is essential in maintaining the localized growth of hair cells but not in determining the morphology of the hair (figure 4). However, when F-actin is absent beneath the plasma membrane, hair bulges are mechanically unstable and the hair tip itself is slightly deformed (Baluska *et al.* 2000).

Profilins are small proteins that participate in the organization of the cytoskeleton by binding to actin and, in certain conditions, move the equilibrium in favour of

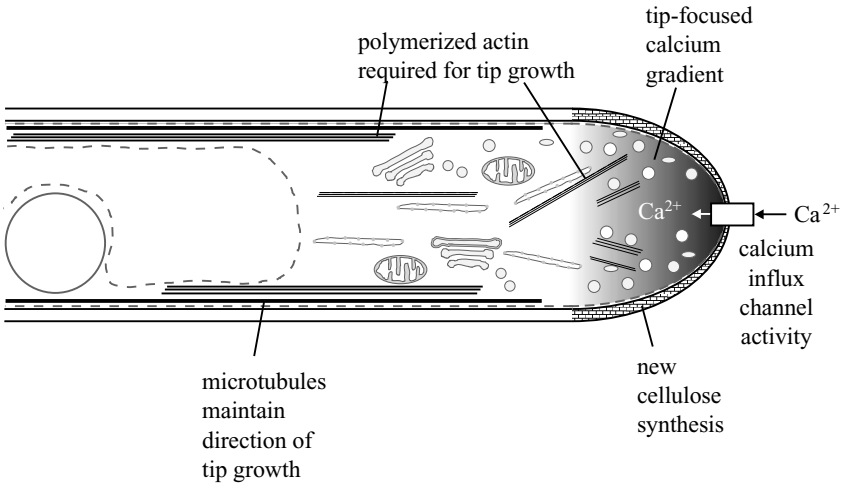

Figure 4. The physiology of the root hair. Some aspects of the physiological and biophysical basis of the tip growth of root hairs are represented.

polymerization (Staiger *et al.* 1997). *Profilin 1* (*PFN1*) is expressed in roots and root hairs of *Arabidopsis* (Ramachandran *et al.* 2000). Transgenic plants that overexpress *PFN1* develop longer root hairs than wild-type (Ramachandran *et al.* 2000). Both *PFN1* mRNA and protein accumulate in the bulges and tips of maize root hairs (Baluska *et al.* 2000). It is likely then that the *in situ* production of profilin is important for regulating actin polymerization at the tip of the hair. ADF also accumulates in the tips of root hairs (Jiang *et al.* 1997), probably facilitating depolymerization. It is likely that profilin and ADF act together in regulating the dynamics of the actin cytoskeleton necessary for tip growth to proceed. The effects of rho GTPases (e.g. Rop) and actin-related proteins (Arp) on the actin microfilament network have been studied in pollen tubes. Some of these molecules may be active in root hairs, but this has yet to be confirmed (for reviews, see Yang 1998; Palanivelu & Preuss 2000).

Unlike microfilaments, microtubules are initially absent from the hair-forming bulge, but as the nucleus moves towards the bulge and migrates into the growing hair, nuclear-associated MTs appear in the root hair and become longitudinally organized, parallel to the direction of growth (Baluska *et al.* 2000; Bibikova *et al.* 1999). Growth of roots in the presence of oryzalin, which depolymerizes MTs, and Taxol, which stabilizes MTs, results in the formation of wavy and branched hairs, while the growth rate is the same as for the controls (Bibikova *et al.* 1999). This suggests that dynamic MTs are required for the maintenance of the directionality of growth, but not for tip growth *per se* (figure 4). Interestingly, when directionality was disrupted by these drugs, new tip-focused calcium gradients appeared at the new growth points in the hairs, so there is possibly an intimate association between MTs and the apical positioning of the calcium influx (Bibikova *et al.* 1999).

4. THE GENETIC CONTROL OF ROOT HAIR GROWTH

Numerous *Arabidopsis* mutants have been identified that are affected in the outgrowth of root hairs from the trichoblast. Phenotypes observed range from roots with no hairs at all, through those with bulges or blebs, to those with short, wide, wavy, branched or forking hairs (Schiefelbein & Somerville 1990; Grierson *et al.* 1997; Ryan *et al.* 1998; Parker *et al.* 2000; Favery *et al.* 2001; Rigas *et al.* 2001; Masucci & Schiefelbein 1994; Schneider *et al.* 1997). Comparison of the morphological traits of the single and double mutants (which may have epistatic, additive or synergistic phenotypes) allows a dissection of the genetically controlled processes of hair growth (Schiefelbein & Somerville 1990; Grierson *et al.* 1997; Parker *et al.* 2000; Favery *et al.* 2001). These processes can be divided into those that affect the initiation of hair growth (the site and size of the hair-forming bulge and the number of hairs per site) and those involved in the later processes of the establishment and the maintenance of tip growth.

(a) *Initiation of hair growth*

rhl (*root hairless*) mutants show no sign of root hair initiation (and they have other severe growth defects; Schneider *et al.* 1997). RHL1 is a small nuclear protein of unknown function which is thought to be required for the initiation of root hair development (Schneider *et al.* 1998; figure 5). RHL1 may therefore be required for the promotion of hair development in trichoblasts, although this class of mutants is difficult to distinguish from the class of patterning mutants (Schiefelbein 2000).

The *rhd6* (*root hair defective*) mutant produces hairs at different positions along the outer face of the trichoblast. Similar phenotypes are observed in *axr2*, which is resistant to auxin and ethylene, and in *etr1*, which is resistant to ethylene (Masucci & Schiefelbein 1994). Ethylene can rescue the *rhd6* phenotype, and inhibition of ethylene biosynthesis in the wild-type produces an *rhd6*-like phenotype (Masucci & Schiefelbein 1994). This highlights the role of ethylene in the growth of root hairs. *rhd6* is epistatic to most other hair mutants. This suggests that *RHD6* acts earlier than the other genes and is involved in the selection of the position of hair initiation through a mechanism involving auxin and ethylene (Parker *et al.* 2000).

The site of hair initiation is much larger in the *rhd1* and *tip1* mutants than in wild-type. The *rhd1* mutant produces hairs with bulbous bases from bloated epidermal

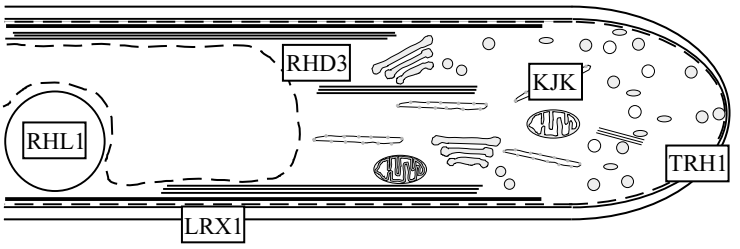

Figure 5. The molecular genetics of the *Arabidopsis* root hair. RHL1, a nuclear protein required to promote hair formation; RHD3, a GTP-binding protein (its localization is not known but it has a transmembrane domain and is possibly involved in vacuole biogenesis); KJK, an endoplasmic reticulum-localized cellulose synthase; LRX1, a cell wall-localized extensin, possibly anchored to the membrane; TRH1, a transmembrane potassium transporter, possibly in the plasma membrane.

cells (Schiefelbein & Somerville 1990). RHD1 is likely to be involved in determining the cell wall structure of trichoblasts and root hairs (G. Seifert, personal communication). *tip1* (*tip growth defective*) mutants form several short wide hairs from a wide initiation base (Ryan *et al.* 1998) and TIP1 requires RHD1 in order to restrict the size of the bulge (Parker *et al.* 2000). Roles for other genes at this stage of initiation were revealed in double-mutant analyses (e.g. *CEN2*, *SHV3*, *SCN1* and *RHD3*; see below) and further analysis will show whether they always play a role at this stage or only when the plants are genetically compromised.

The production of one hair per trichoblast initiation site is controlled by the genes *TIP1*, *COW1* (*CAN OF WORMS*), *SCN1* (*SUPERCENTIPEDE*), *BST1* (*BRISTLED*), *CEN1* (*CENTIPEDE*), *CEN2* and *CEN3.* When these genes are mutated, a proportion of the trichoblasts produce multiple hairs from a single initiation site (Grierson *et al.* 1997; Ryan *et al.* 1998; Parker *et al.* 2000).

(b) *Transition to tip growth*

The root hairs of the mutants *rhd2*, *shv1* (*shaven*), *shv2*, *shv3*, *trh1* (*tiny root hair*) and *kjk* (*kojak*) rarely elongate longer than 40 μm, and may form blebs or short, straight hairs. Although hairs are initiated correctly, the genes defined by these mutations are necessary for the establishment of tip growth (Schiefelbein & Somerville 1990; Parker *et al.* 2000; Rigas *et al.* 2001; Favery *et al.* 2001).

The *rhd2* mutant is unable to form the tip-focused calcium gradients required for hair growth (Wymer *et al.* 1997). From the epistasy observed in double mutants, *SHV3* is thought to act after *RHD2*, and *SHV2* acts after *SHV1* in establishing tip growth (Parker *et al.* 2000).

The *trh1* mutant produces small outgrowths that fail to make the transition to tip growth—several additional initiation sites may form on a single trichoblast (Rigas *et al.* 2001). The *TRH1* gene encodes a K^+ transporter. The *trh1* mutant is partially impaired in ^{86}Rb uptake and the *TRH1* gene complements a mutant of *Saccharomyces cerevisiae*, which is affected in high-affinity K^+ uptake. This complementation was abrogated when a C-terminal truncation of the intracellular domain of TRH1 was made (Rigas *et al.* 2001). A direct effect of external $[K^+]$ on root hair growth has not been demonstrated, although the blockage of inward-rectifying K^+ channels by tetraethylammonium was found to inhibit root hair growth transiently (Lew 1991). So it is possible that the TRH1 K^+ transporter is required to cooperate with other localized transporters in setting up the calcium gradient that signals the transition to tip growth.

kojak (*kjk*) mutants are so-called because the roots are 'bald' compared with those of the hairy wild-type. In fact, they form a bulge in the position on trichoblasts where a hair is anticipated to form, so neither specification of trichoblast cell fate nor hair initiation is defective (Favery *et al.* 2001). However, instead of initiating polarized growth perpendicular to the axis of the root, the bulge expands spherically to the point where it bursts. The *KJK* gene was cloned and it encodes a cellulose synthase-like gene, *AtCSLD3* (Favery *et al.* 2001). The protein has predicted transmembrane domains and a KJK–GFP protein fusion localizes to the endoplasmic reticulum (figure 5). Cellulose synthases are a large gene family. Some members of the family are known to encode proteins that catalyse the synthesis of cellulose or β-glycans, which are present in the cross-linked microfibrils of the plant cell wall. *KJK* is likely to be involved in the synthesis of cell wall polymers in root hairs. As no other phenotypes were obvious in the mutant, this activity may be specific to root hairs, although the *kjk* transcript was detected in other plant organs.

(c) *Tip growth*

rhd3, *rhd4*, *bst1*, *cen1*, *cen2*, *cen3*, *scn1*, *cow1* and *tip1* form a class of mutants where the root hairs grow longer than 40 μm, but they are always shorter than wild-type hairs and have an altered morphology (Grierson *et al.* 1997; Ryan *et al.* 1998; Galway *et al.* 1997; Parker *et al.* 2000). *rhd3* and *rhd4* have wavy, crooked hairs. *bst1* has short, straight hairs. The *cen* mutants all have curled hairs. *scn1* hairs are wide and curled. *cow1* and *tip1* hairs are wide and short. Of these mutants only *tip1* and *rhd3* have clear growth phenotypes in other parts of the plant, suggesting that the other gene products could be specifically required for root hair growth. *RHD4* is known to act before *COW1*, but the remaining genes act independently (Parker *et al.* 2000). The *rhd4* phenotype is a result of a variable hair diameter and growth rate. The primary and secondary cell wall *rhd4* hairs are thicker than wild-type hairs (Galway *et al.* 1997).

The *RHD3* locus was identified because the root hairs of the mutant are short and wavy compared with the long straight root hairs of the wild-type. The root itself is also shorter than the wild-type, suggesting that the *RHD3* gene product is involved not only in tip growth but also in diffuse growth. In the *rhd3* mutant hairs, the vacuole is

smaller and the arrangement of secretory vesicles at the tip of the hair is not focused (Galway *et al.* 1997). RHD3 is an 89 kDa GTP-binding protein with similarities to proteins from *Entamoeba histolytica*, yeast and other plants, but whose precise function is not known (Wang *et al.* 1997; figure 5).

5. REVERSE GENETICS

LRX1 is an *Arabidopsis* homologue of a chimeric protein (originally found in tomato roots) made up of a leucine-rich repeat domain and an extensin-like domain (Baumberger *et al.* 2001). LRX1 is expressed during the initiation and elongation of *Arabidopsis* root hairs and eventually becomes insolubilized in the hair walls (figure 5). In a reverse genetics approach, several mutant lines were found in which the *LRX1* gene was disrupted by the *En* transposon. The insertions created *lrx1*-null alleles as *LRX1* gene expression could not be detected. The root hairs in these mutants were deformed, having large orb-like bases with branched and irregular hairs (Baumberger *et al.* 2001). LRX1 may be important for the regulated and even deposition of cell wall material, or for determining the resistance of the cell wall to the turgor of the protoplast. Otherwise the protein may be important for establishing and stabilizing root hair polarization and tip growth, for example by connecting the cell wall to the plasma membrane.

6. CONCLUSION

Tip growth of root hairs has been studied in several species, e.g. maize and legumes such as *Vicia* and *Medicago*, but here the focus has been to review the complementary approaches that have been used to study this process in *Arabidopsis thaliana*. Integration of the information produced by the different approaches can identify useful areas for further study. For example, it may be possible to identify the gene encoding the Ca^{2+} transporter identified by biophysical measurements (Véry & Davies 2000) by finding a mutant where calcium influx at the hair tip is lost, or by reverse genetics of putative calcium channel genes known from the sequenced *Arabidopsis* genome. Conversely, the potassium channel activity of membranes taken from wild-type and *trh1* root hairs could be compared (Rigas *et al.* 2001). The functions of unknown proteins such as RHD3 and RHL1 may be further characterized, for example by finding molecular interactors (Wang *et al.* 1997; Schneider *et al.* 1998). The cloning of the *RHD* (Schiefelbein & Somerville 1990), *COW* (Grierson *et al.* 1997), *TIP* (Ryan *et al.* 1998), *CEN*, *SCN* and *BST* (Parker *et al.* 2000) genes will add further elements of molecular information to the model for tip growth in these cells.

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GLOSSARY

ADF: actin depolymerization factor
GFP: green fluorescent protein
MT: microtubule