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New signalling molecules regulating root hair tip growth

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Root hairs are tip-growing tubes that emerge from trichoblasts (hair-forming epidermal cells) along the length of the root. Signalling events involved in the formation of root hairs are largely unknown. However, two recent studies have revealed that signalling enzymes such as NADPH oxidase and phospholipase D are crucial for root hair growth and development. Reactive oxygen species (ROS) produced by NADPH oxidase activate calcium ion channels in the apical plasma membrane leading to the tip-focused calcium gradient, an inherent feature of growing root hairs.

Root hairs facilitate water and nutrient uptake from the soil into the plant and help to anchor the plant body in the soil. They are exploratory tubes that grow exclusively at their apical domes (tip growth). Initiation and maintenance of polarity in growing root hairs is under genetic, hormonal and environmental control. During the past decade, root hairs have emerged as an exciting model system for uncovering general principles underlying cell polarity and driving polar growth in plants and other higher eukaryotes. Because root hairs are not essential for plant growth under laboratory conditions, mutant lines with root hair phenotypes can survive – a major advantage for genetic studies [1]. In contrast to root hairs, pollen tubes, another important cell model displaying tip growth, are more difficult to study because mutations of essential gametophytic genes are usually lethal. Here we highlight the recent discoveries about root hair tip growth and focus on emerging signalling pathways that regulate this process.

Cellular basis of root hair tip growth

A tip-focused cytoplasmic calcium ion gradient, the actin cytoskeleton and polarly targeted vesicular traffic are crucial components of the tip-growth machinery in root hairs and pollen tubes [1–3]. Continuous actin polymerization is required for their growth, as revealed by experiments with the potent actin filament-disrupting drug latrunculin B [4,5]. The phenotype of aberrant root hairs in the *crooked* mutant is caused by a mutation in the smallest subunit of the *arp2/3* complex, resulting in impaired branching of actin filaments [6], supporting the view that the actin cytoskeleton is crucial for tip growth. Additionally, overexpression of two actin-binding proteins regulating the dynamic turnover of actin filaments, profilin and actin-depolymerizing factor ADF1, caused longer or shorter root hair phenotypes, respectively [7]. Moreover, recent genetic studies unambiguously demonstrated that ACTIN2 is essential for root hair initiation and growth [8,9]. Small Rho GTPases of plants called ROPs are believed to generate tip-focused F-actin and calcium ion gradients [10].

Lessons from root hair mutants: NADPH oxidase and phospholipase D are essential for tip growth

Studies on root hair mutants have significantly improved our knowledge of the molecular components involved in root hair development [1] (Figure 1). Recently, Julia Foreman and colleagues [11] showed that *root hair defective 2* (*rhd2*), a mutant forming root hair bulges but no elongated root hairs (Figure 1), has a mutation in a *NADPH OXIDASE/RHD2* gene. NADPH oxidase/RHD2 is a key enzyme involved in intracellular signalling that produces reactive oxygen species (ROS) as second messengers. The authors localized ROS by fluorescent indicator dyes in the growing tips of root hairs of wild-type plants

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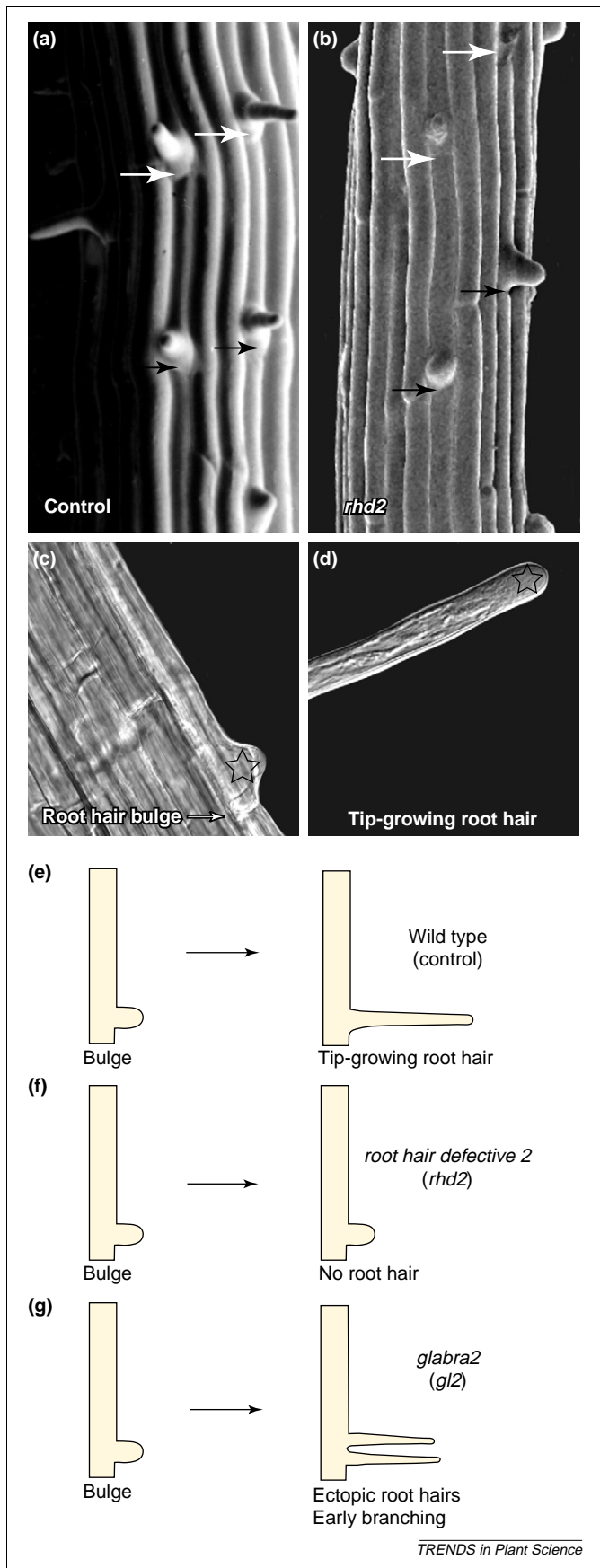


Figure 1. Root hair formation and tip growth. The *Arabidopsis* root epidermis shows a clear patterning into separate cell files of trichoblasts (hair-forming cells) and atrichoblasts (non-hair cells). The black arrows indicate the apical ends of selected trichoblasts and the white arrows indicate their opposite basal ends (a).

but not in the root hair bulges of the *rhd2* mutant. Foreman *et al.* demonstrated that ROS activate a specific type (hyperpolarization activated) of calcium ion channel localized on root hair tips [11], which leads to calcium ion influx (Figure 2). This study provides the first convincing evidence that the tip-focused calcium ion gradient in root hairs [2] is regulated by local production of ROS. Cytoplasmic calcium ions are known to regulate cytoskeletal rearrangements and vesicular trafficking in tip-growing systems [3]. Foreman *et al.* propose that NADPH oxidase activity might be controlled via small GTPases of the Rop family in direct analogy to mammalian cells, where the small GTPase Rac regulates NADPH oxidase and ROS production [11]. Future localization studies should reveal whether this is the case and, if so, how NADPH oxidase accumulates and persists in growing root hair tips. Similar calcium ion gradients are present in pollen tubes and other tip-growing eukaryotic systems [3], but it is not known how these gradients are established and maintained in these systems. It was reported that ROS modulate calcium signalling-dependent neuronal plasticity by activating calcineurin and altering the activity of calcium ion channels [12]. However, signalling cascades involved in ROS generation during neuronal polar growth have not been studied yet.

In another recent study, Yohei Ohashi and colleagues [13] report that in *glabra2* mutants, the phenotype of ectopic and branched short root hairs (Figure 1) is caused by the loss of *GLABRA2* (*GL2*) activity resulting in overexpression of phospholipase D ζ 1 (*PLD 1*). *GL2* is a transcription factor involved in the development of trichomes, root hairs and seed coat in *Arabidopsis*. Ohashi *et al.* localized *PLD* ζ 1 to accumulating vesicles in root hair tips and showed that its inducible overexpression resulted in ectopic and branched root hairs phenotypically similar to the *gl2* mutant. The authors propose that *GL2* acts as a transcriptional repressor of the *PLD 1* gene. In addition, root hair formation was abolished in *Arabidopsis* seedlings germinated in the presence of 1-butanol, an agonist of *PLD* that inhibits phosphatidic acid formation [13]. Interestingly, phospholipase D produces phosphatidic acid, a second messenger, which is required for pollen tip-growth [14]. However, it remains to be shown whether *PLD 1* produces phosphatidic acid and triggers phosphoinositide-dependent signalling in growing root hairs as well.

Emerging signalling networks in plant tip growth

ROS, calcium ions and phosphatidic acid, as well as the phytohormones auxin and ethylene, can activate signal

Both of these epidermal cell files elongate parallel to the apical–basal axis of the root body. The site of root hair formation is genetically predetermined in *Arabidopsis* to the distal end of the outer cell wall of a trichoblast cell (a,c) (black arrows). (c) The growth axis of the trichoblast becomes perpendicular to the root surface leading to the emergence of the bulge (star), which further elongates by tip-growth (d). Star in (d) indicates a vesicle-rich zone at the growing tip of the root hair. (a) Wild-type root (control) with root hair bulges and tip-growing root hairs appearing within the root hair formation zone. (b) *rhd2* mutant showing root hair bulges and short root hairs exclusively, but no elongated tip-growing root hairs within the mature part of the root. Trichoblasts in wild-type root (control) (e) and root hair mutants *rhd2* (f) and *gl2* (g). The left row of (e–g) shows the root hair initiation stage, the right row shows root hair of wild-type plant (control) and altered root hair phenotypes in *rhd2* and *gl2* mutants.

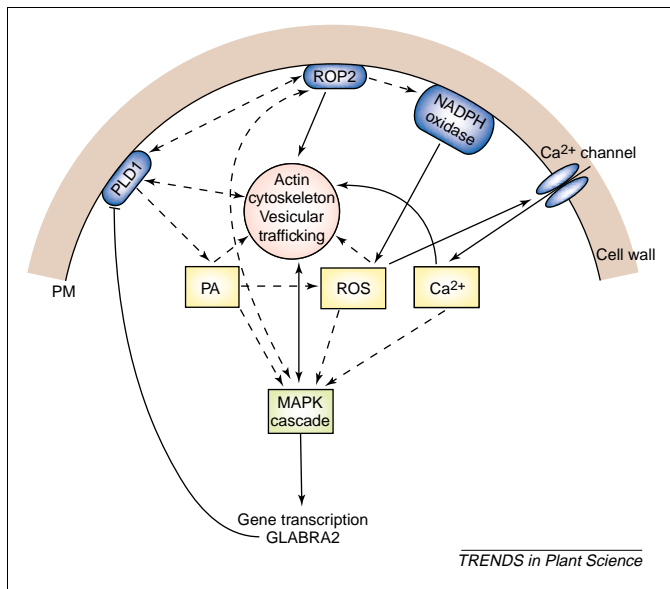


Figure 2. Emerging signalling pathways regulating root hair tip growth. Molecules associated with the plasma membrane (PLD1, ROP2, NADPH oxidase/RHD2 and the calcium channel) are depicted in blue, second messengers (PA, ROS and calcium) are depicted in yellow, and signal transduction pathways (MAPK cascades) are depicted in green. Black arrows indicate links established in root hairs and broken arrows represent putative links. ROS produced by RHD2 activate calcium channels and calcium signalling. GLABRA2 is a negative transcriptional regulator of PLD1-dependent signalling. MAPK cascades are suggested to participate and integrate signal transduction of diverse signals. Abbreviations: MAPK, mitogen-activated protein kinase; NADPH oxidase, nicotinamide adenine dinucleotide phosphate oxidase; PA, phosphatidic acid; PLD1, phospholipase D ζ 1; ROP2, small Rho-like GTPase of plants 2; ROS, reactive oxygen species.

transduction pathways involving mitogen-activated protein kinases (MAPKs) in plant cells [15,16]. The same pathways are triggered by abiotic stress or during pathogen infection in plants, a response that also involves polarization of the actin cytoskeleton and targeted vesicular trafficking. These findings indicate that during evolution plants might have adopted some components of stress-related signalling pathways for root hair development.

Recently, it was shown that SIMK (stress-induced MAPK) is involved in root hair tip growth. Its distribution and function correlates with the organization of the actin cytoskeleton [17]. In light of recently published work [11,13,23,24], an intriguing possibility is that MAPKs are involved in the transduction of second messenger signals including phosphatidic acid, ROS and calcium ions in root hairs (Figure 2). AtMPK6, the *Arabidopsis* orthologue of SIMK, is activated by ROS as well as by diverse abiotic and biotic factors [15,18].

In view of the close connection between the actin cytoskeleton and signal transduction, it will be fundamental to learn how all these components of the signalling cascades impinge on the actin-based tip-growth machinery. Signalling molecules, including phospholipase D [13], ROS [11], calcium ions [3] and MAPKs [17] are found in root hair tips and can be involved in rearrangements of the actin cytoskeleton via direct interaction with actin and/or with actin-binding proteins such as profilins, proteins of ARP2/3 complex, ADFs or AIPs (Figure 2). Alternatively, the state of the actin cytoskeleton might regulate the

activity of signalling proteins including MAPKs [17] and PLDs because polymerized filamentous actin was recently shown to enhance the activity of plant PLD *in vitro*, whereas G-actin has an inhibitory effect [19].

Conclusions and outlook

In many aspects, root hairs resemble other tip-growing cell systems, particularly pollen tubes and neurons [3,20,21], highlighting the general role of actin polarization and signalling via rho proteins, calcium ions, phosphoinositides and MAPKs in tip growth [2,4,6,8–11,13,17]. The major contribution of the Foreman *et al.* report and our model (Figure 2) is that for the first time it explains a mechanism by which ROS are locally generated and involved in the regulation of tip growth via the activation of specific tip-localized calcium channels driving calcium-dependent signalling [11].

Many questions, particularly those related to the establishment of root hair polarity, remain to be answered. It is not known which initial cues determine the position of the root hair outgrowth and how these cues are spatio-temporally stabilized. For a long time, auxin has been implicated in trichoblast polarization and root hair formation [22] but molecular details of auxin perception and function during selection of root hair position within a trichoblast are largely unknown. Now that some of the major players involved in the signalling process during root hair tip growth have been identified, such as NADPH oxidase/RHD2, PLD1, ROP2 and SIMK, the next major challenge will be to reveal their modes of direct or indirect interactions. There are several questions that need to be answered. How are these signalling components regulated at the molecular and cellular level? Do they form protein complexes with other signalling, regulatory and cytoskeletal proteins? How are they targeted to and maintained at proper cellular destinations?

In the near future, we need to clone the genes of additional root hair mutants and elucidate their roles, as well as undertaking reverse genetics and mutant complementation studies to add to our current picture of the structural dynamics, regulatory factors and signalling networks involved in root hair initiation and tip growth.

Note added in proof

Two recent reports [23,24] have revealed that a protein kinase belonging to the AGC family (AGC2-1 and OXI1 are two different names for the same kinase) is activated by phosphatidic acid [23] and by hydrogen peroxide [24], and that it is involved in root hair growth. Because the *oxi1* null T-DNA mutant shows down-regulated activities of two MAPKs (AtMPK3 and AtMPK6) upon oxidative and cell wall stress [24], it is likely that OXI1 kinase is upstream of the MAPK module.

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