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 the soil into the plant and help to anchor the plant body in the soil. They are exploratory tubes that grow exclusively at

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...
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\( \_1 \) (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\( \_1 \) 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

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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).
The state of the actin cytoskeleton might regulate the ARP2/3 complex, ADFs or AIPs (Figure 2). Alternatively, with actin-binding proteins such as profilins, proteins of actin cytoskeleton via direct interaction with actin and/or root hair tips and can be involved in rearrangements of the ROS [11], calcium ions [3] and MAPKs [17] are found in work [11,13,23,24], an intriguing possibility is that actin cytoskeleton [17]. In light of recently published MAPK) is involved in root hair tip growth. Its distribution development.

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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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 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 [15,16].

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].
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