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## Plant Cell Biology: How to Give Root Hairs Enough ROPs?

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**Root hairs are precisely positioned close to the rootward end of epidermal cells. A new study shows that the successful production of root hairs is a two-step process with different molecular players driving the initial polarization and subsequent hair outgrowth.**

Planar polarity is the coordination of cell polarity across a two-dimensional tissue layer [1]. Two classical examples of planar polarity are the polar emergence of hairs on epidermal cells of either the *Drosophila* wing or *Arabidopsis* root [1, 2]. However, the establishment of hair planar polarity is regulated by distinct mechanisms in animals and plants. In animals, the core planar polarity pathway (also known as the Frizzled pathway) interprets positional cues and then positions and regulates Rho GTPases, which execute downstream cellular effects [1]. Plant genomes lack the Frizzled pathway, yet Rho-Of-Plants (ROPs) are polarized early at the root hair initiation domain (RHID), which is positioned close to the distal (rootward) end of hair-forming cells (Figure 1) [2]. How ROP proteins are precisely positioned at the RHID in the absence of the core planar polarity pathway is still unclear. A new study in this issue of *Current Biology* tackled this question and uncovered ROP GEF3 as a key protein driving the initial ROP polarization for the correct positioning of root hairs [3].

ROPs are highly conserved small GTPases, which function as molecular switches due to conformational differences between an inactive GDP-bound state and an active GTP-bound state. This activation/inactivation cycles are regulated in time and space by GUANINE NUCLEOTIDE EXCHANGE FACTORS (GEFs) that promote the GTP-bound state and GTPase-ACTIVATING PROTEINs (GAPs) that enhance GTP hydrolysis [4]. The *Arabidopsis thaliana* genome encodes for 11 ROPs, which control fundamental cellular processes including cytoskeleton dynamics and vesicular trafficking [5]. To determine the timing of ROP polarization at the RHID, Deniger, Reichelt et al., took advantage on the fact that cells along the root tip are ordered according to their developmental age, with younger cells located rootward and older cells shootward [3]. In this developmental timeline, the cell with the first visible root hair bulge is numbered as +1 (Figure 1). Using this staging system, the authors separated root hair formation in three phases: initiation (from cells -7 to -1, in blue), bulging (cells +1 and +2, in green), and polar growth/elongation (starting at cell +3 and onward, in orange) [3]. Using confocal imaging and fluorescently-tagged ROPs, Deniger, Reichelt et al., showed that three ROPs (ROP2, ROP4 and ROP6) polarize at the RHID much before root hair initiation (i.e. at cell -4 before root hair bulging, Figure 1). Accordingly, genetic analyses with double and triple *rop* mutants suggest that these three ROPs redundantly regulate root hair development [3, 6].

Strikingly, all the known downstream or upstream ROP regulators polarize much later, at the stage of root hair bulging or after (Figure 1) [3]. This suggests a two-phase assembly of the tip growth machinery: an initiation phase, during which the RHID is positioned and predefined, followed by the tip growth phase. To uncover whether GEF could participate in ROP membrane targeting, Deniger, Reichelt et al., localized, via translational fusion reporters, all the GEF that are expressed in the root epidermis (6 GEFs in total). This analysis uncovered two GEFs (GEF3, GEF4 and to a lesser extent also GEF12 and GEF14) with polar localization in root hair cells. Interestingly, GEF3 polarizes early to the RHID, while GEF4 localizes at the root hair initiation site during bulging. *gef3* and *gef4* mutant analyses confirmed that *gef3* is involved in root hair initiation, while *gef4* is involved in root hair polar growth, suggesting that distinct GEFs are responsible for polar initiation and polarized growth, respectively.

Elegant live imaging analyses using microfluidics suggested that GEF3 localizes to the RHID prior to ROP proteins (i.e. at cell -5) and could therefore be an early landmark for root hair positioning acting upstream of ROPs [3]. Biochemical interactions, structure/function analyses and over-expression studies validated that GEF3 polarizes first to the RHID and in turn recruits ROP proteins to this polar domain. Strikingly, upon ectopic expression in cells that are not normally forming hair (in the root and the hypocotyl) GEF3 accumulates into plasma membrane domains that resembled those of the RHID. Ectopic accumulation of GEF3 in these RHID-like domains subsequently drove the ectopic ROP2 localization in the same membrane patches. Together, the GEF3/ROP interplay appears critical to position the RHID, which is latter translated into root hair tip growth via the recruitment of GEF4.

Meanwhile, Gendre and collaborators studied the mechanisms and factors underlying ROP localization at the root hair plasma membrane with a focus on membrane trafficking [6]. They demonstrated that the membrane trafficking machinery acts as a central component in ROP-dependent root hair formation. Indeed, the *trans*-Golgi Network (TGN)-localized YPT-INTERACTING PROTEIN (YIP)4a and YIP4b are required for the activation and plasma membrane accumulation of ROPs at the RHID (figure 1). Another important factor that regulates ROP localization and signaling activity is its interaction with lipids. ROP2, 4 and 6 C-termini are prenylated (i.e. geranylgeranylation, a covalently linked lipid anchor), which provides hydrophobic anchoring and is required for membrane association [5]. ROP6 is also S-acylated (i.e. palmitoylation), which is a reversible lipid modification required for ROP6 function [7]. Furthermore, all ROPs possess in their C-terminal tail a polybasic region, which interacts with anionic phospholipids, at least in the case of ROP6 [8]. The plasma membrane is highly enriched in anionic phospholipids and hence is strongly electronegative [9, 10]. Consequently, ROP6 polybasic tail interacts with this plasma membrane electrostatic field and is required for the proper targeting of ROP to the cell surface [8]. At the bulging stage, the RHID is enriched in the anionic phospholipid PI(4,5)P<sub>2</sub>, and ROP2 colocalizes and interacts with PIP5K3, the kinase that produces PI(4,5)P<sub>2</sub> [3, 11, 12] (Figure 1). Interestingly, this situation is not restricted to ROP2, but also ROP10, which localizes in a complementary domain at the shank of growing root hairs [11] (Figure 1). ROP10 colocalizes and interacts with both the anionic phospholipids PI(3,5)P<sub>2</sub> and the lipid kinase that produces it (i.e. FAB1) [11] (Figure 1). This suggests that ROP activity may regulate lipid synthesis, while in turn lipids themselves control ROP activity and/or localization. Therefore, ROP/anionic lipids may form a self-organizing system for the focal recruitment and activation of Rho GTPases in plants. However, given that PIP5K3 and PI(4,5)P<sub>2</sub> polarize relatively late at the RHID [3] (Figure 1), it is likely that they act as an amplifying system rather than the initial polarizing cue. Taken together, this suggests that ROP localization and focal activation in root hairs may require multiple factors, including enzymatic activation, lipid modification, direct interaction with lipids and vesicular

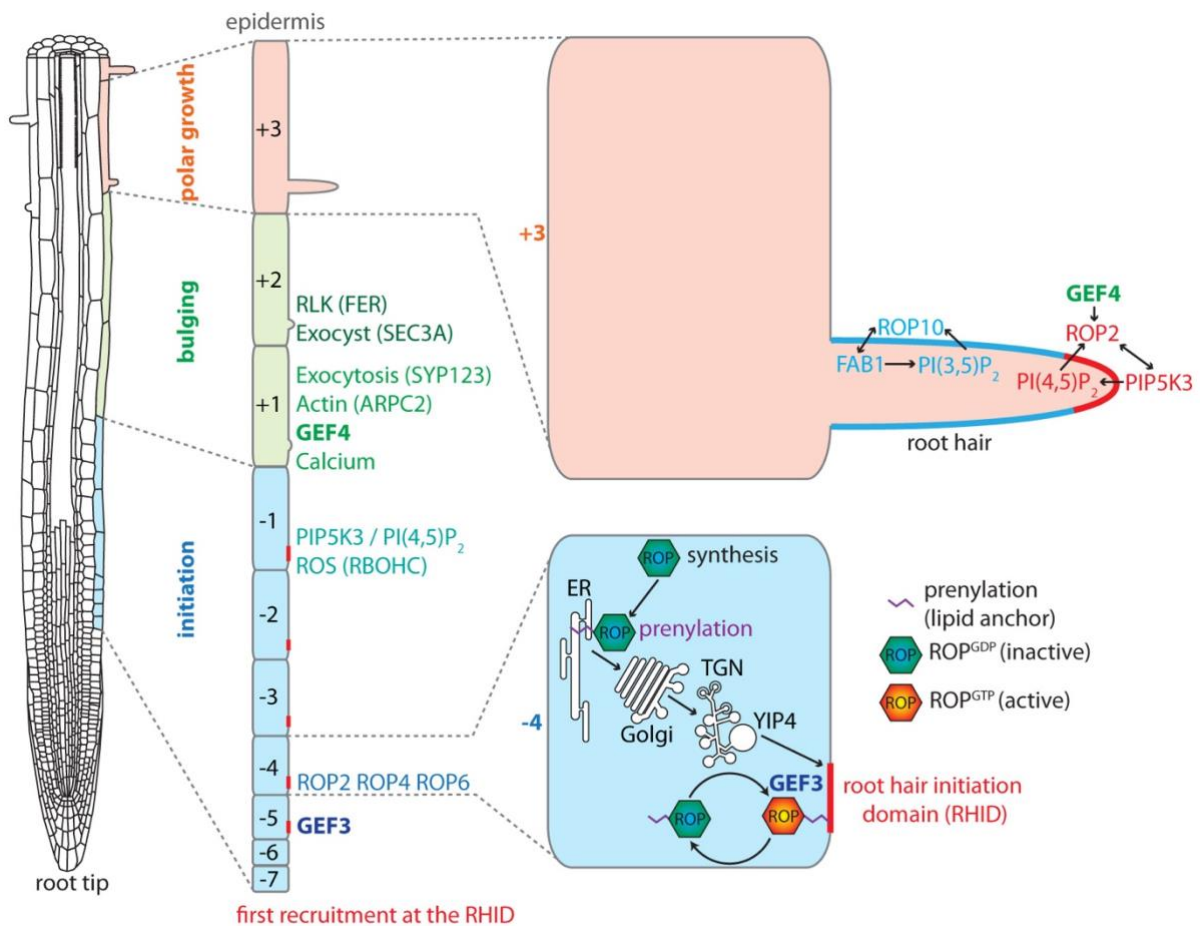
trafficking.

An open question is to understand what is(are) the primary signal(s) that defines the position of the RHID; i.e. what are the factors that initially drive GEF3 polarity? In other contexts, ROP GEFs act downstream of Receptor-Like Kinases (RLK), which are membrane receptor able to translate signal(s) across the plasma membrane [5]. One may speculate that there are root hair-expressed receptors that localize at the RHID. Alternatively, such receptors would not necessary have to be polarized themselves but could rather perceive a polarized signal. Such signal could include the concentration gradient of the plant hormone auxin at the root tip, which is critical to determine ROPs localization at the RHID [13]. In addition, auxin promotes ROP2 and ROP6 activation [14], but the machinery that perceives and transduces the auxin signal across the plasma membrane is still elusive [15]. One of the future challenge in understanding planar cell polarity in plants will be to elucidate how ROP polarization is linked with extracellular signals, such as for example auxin.

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**Figure 1: Successive phases in root hair initiation and outgrowth.**

Left, Schematic drawing of an Arabidopsis root (drawing by Benjamin Peret: [https://figshare.com/articles/Primary\\_and\\_lateral\\_root\\_ai/5143987](https://figshare.com/articles/Primary_and_lateral_root_ai/5143987)). Middle, root hair staging and timeline of recruitment of polar proteins to the root hair initiation domain (RHID, in red). Top right, interplay between ROP, anionic lipids and lipid kinases during root hair growth. Bottom right, the recruitment of ROPs at the RHID requires the presence of GEF3 [3]. Note that ROPs are synthesized as soluble proteins in the cytosol. They are then prenylated at the surface of the Endoplasmic Reticulum (ER) and are subsequently targeted to the plasma membrane via YIP4-dependent secretion from the TGN [6].