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The tip of the iceberg: ROP2 directly interacts with SYP121 to regulate root-hair polarization, elongation, and exocytosis

Root hairs (RHs) grow in a polar manner as cylindrical protrusions from the root epidermis. They significantly expand the root volume in the rhizosphere to search for water and nutrients, anchor the plant, and promote interactions with several kinds of microorganisms in the soil. Trichoblasts, the epidermis cell type responsible for RH development, switch from anisotropic cell elongation to tip growth through polarization of the growth machinery to a predefined RH initiation domain (RHID) at the plasma membrane. The first step known in RH initiation is the recruitment of guanine nucleotide exchange factors (GEFs) to the RHID, specifically GEF3 and GEF4 (and to a lesser extent, also GEF12 and GEF14), being that GEF3 is the earliest known landmark to the RHID and that GEF4 is involved in the downstream activation of RH growth during bulging (Denninger et al., 2019) (Figure 1). GEFs are able to interact with plasma membrane-bound receptor-like kinases such as FERONIA (FER) (Duan et al., 2010). Once localized to the RHID, the GEFs recruit the RHO-like GTPase from plants (ROPs) required from the earliest stages of polar growth. ROPs are regulated in time and space by GEFs by controlling the GTP-/GDP-dependent activation/inactivation cycles that facilitate the release of GDP. On the other hand, GAPs enhance GTP hydrolysis and GDP dissociation inhibitors that recycle ROPs to and from the plasma membrane. ROPs bind plasma membranes through post-translational lipid modifications and interaction with membrane lipids (Feiguelman et al., 2018). In addition, all ROPs possess in their carboxy-terminal tail a polybasic region, which interacts with anionic phospholipids like $PI(4,5)P_2$, highly enriched in the plasma membrane (Hirano et al., 2018), and also, vesicle-mediated ROP targeting may occur. In RH, FER-GEFs-ROP2 are able to control RH growth by controlling ROS production (Duan et al., 2010). ROP2 is one of the main ROPs responsible for the early polarization in RHs. Together with ROP2, ROP4 and ROP6 are also important for RH polar growth at the initial stage of tip polarization in the epidermal cells as well as for tip-cell elongation (Denninger et al., 2019). In addition, several proteins such as PIP5K3 (that produces $PI(4,5)P_2$) and root hair defective 2 (RHD2), a NADPH oxidase C (that produces ROS) have both been shown to directly interact with ROPs during tip-growth regulation (Wong et al., 2007) and modulate vesicle traffic to the plasma membrane and cell-wall assembly, respectively.

Actively fast polar growing cells like RHs depend on active vesicle trafficking that involves vesicle tethering (via tethering complexes), docking, and fusion (via *trans*- Soluble N-ethylmaleimide-sensitive factor attachment protein receptors Q [SNARE] complex and N-ethylmaleimide-sensitive factors) of vesicles with target membranes. SNAREs SYP123 and SYP132 (both SYP121 homologs) coordinately mediated tip-focused vesicle trafficking for RH elongation by forming complexes with vesicle-membrane-localized R-SNARE VAMP721/722/724 (Ichikawa et al., 2014). Cui et al. (2022) recently reported in *Molecular Plant* that active ROP2 promotes SYP121 accumulation during RH elongation by a physical interaction and confirms the previously suspected relevance of SYP121 in the process (Ichikawa et al., 2014) (Figure 1). In addition, ROP2 promotes the interaction between SYP121 and the R-SNARE VAMP722 to form a SNARE complex, possibly by counteracting with the SEC1/MUNC18 protein SEC11, which suppresses SYP121's function. The authors demonstrate all this using classical genetic tools like mutant crosses and over-expressing lines and showing very solid interaction data. The mild phenotype observed raises some questions of whether SYP123 or SYP132 might also interact with ROP2. In addition, numerous environmental signals such as nutrient availability, changes in temperature, or microbial partners that impact RH development may enhance or trigger ROP's activation and modulate SYP-VAMP interactions. In this direction, recently, it was shown that a mutant of FER (*fer-8*) with reduced ROS levels or plants treated with RALF23 peptide (as ligand of FER) that inactivates the FER-ROP2 related pathway including ROS production were both able to enrich beneficial *Pseudomonas* in the complex rhizosphere microbiome (Song et al., 2021). This exemplifies how tightly interlinked is the interface between cell surface in the root cells and the soil environment including the microbiome.

Plants have greatly expanded their SNARE protein families when compared with other species (Sanderfoot 2007), and there is an active discussion on their degree of redundancy and specialization. For instance, SYP121 and SYP122 seem to have strong redundancy from a general point of view but are also somehow divergent in their cargo specificity (Waghmare et al., 2018). The same goes for the R-SNAREs VAMP721 and VAMP722, which have a higher degree of redundancy to the point that both, VAMP721 or VAMP722, can rescue the double-mutant phenotype (which is seedling lethality) (Kwon et al., 2008). Future studies with both, VAMP721 and SYP122, with ROP2 could enrich this discussion on general versus specialized secretion of these highly similar proteins. In addition, since there are at least three ROPs clearly involved in RH initiation and tip-growth process, this opens the question if there is any type of specificity in the interaction between ROP2 and

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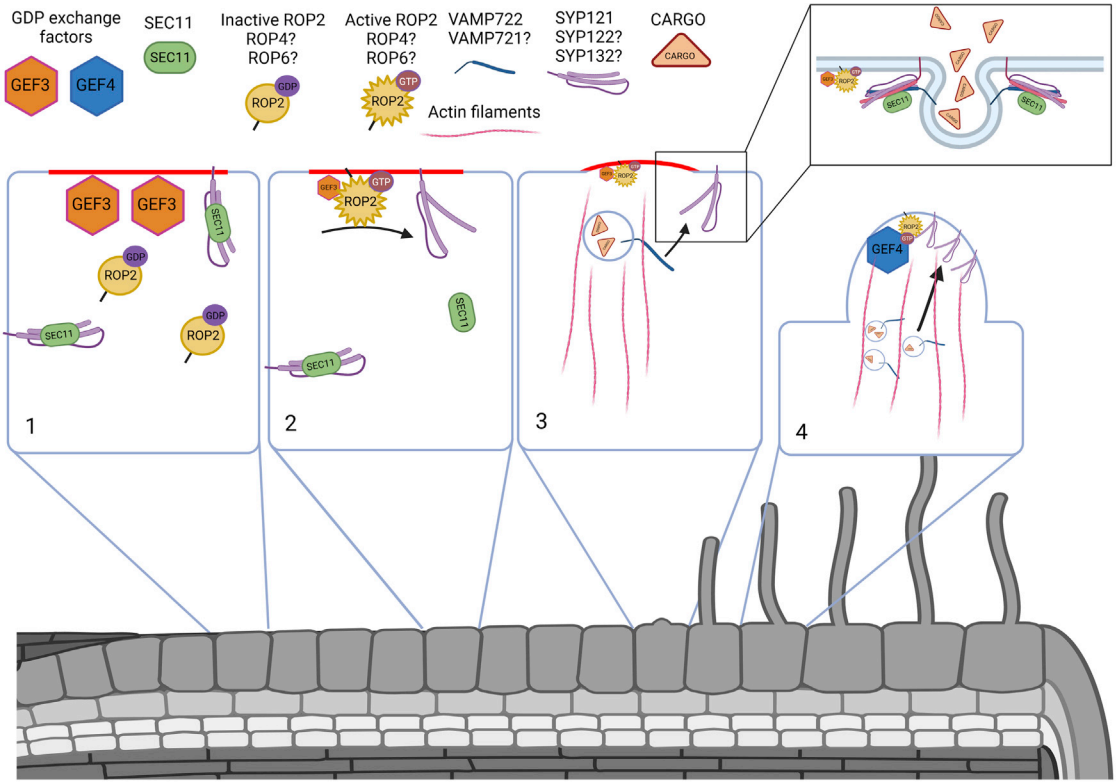

Figure 1. Developmental phases of RH initiation and outgrowth.
Roles of GEF3/4, ROP2, and VAMP722-SYP121 to regulate root hair polarization, elongation, and exocytosis. In gray, RH developmental stages at the root epidermis and timeline of recruitment of polar proteins to the RH initiation domain (RHID, in red). Interplay between GEF3/4 and ROP2. The recruitment of ROPs at the RHID requires the presence of GEF3/GEF4. ROPs are synthesized as soluble proteins in the cytosol, then prenylated and subsequently targeted to the plasma membrane. The sequential steps are the following: (1) GEF3 recruitment to the RHID (the earliest known landmark of the RHID). (2) GEF3 participates in the recruitment of ROP2 to the RHID. It also stimulates GDP-to-GTP exchange resulting in ROP2 activation. Active ROP2 (and possibly other ROPs) recruits and physically interacts with SEC11–SYP121 complexes, dissociating them and leaving SYP121 ready for interaction with SNARE partners (other Qa-SNAREs like SYP122 or SYP132 may be also involved). (3) SYP121 interacts with Q-SNARE VAMP722 (and probably VAMP721) and additional Q-SNAREs containing Qbc domains to form *trans*-SNARE complexes further stabilized by SEC11 binding and drive membrane fusion, releasing the cargo to the apoplast and incorporating new proteins and membrane components to the emerging tip of the root hair. Cytoskeleton starts to rearrange, preparing for the RH polar growth. (4) After RH initialization, GEF4 takes control of ROP2 activation to keep high levels of secretion.

other ROPs (e.g., ROP4 and ROP6) and SYP121 (and other SYPs) and how this process is regulated during RH polar growth initiation and tip-growth stages. Still, several interesting questions remain to be answered.

Could ROP2–SYP121 direct interaction lead to the discovery of another control point in plant secretion? Is this mechanism specific for RH polarized growth or could it become true for other types of elongating cells such as anisotropic growing cells (e.g., roots, hypocotyl, etc.), isotropic (e.g., meristems), hybrid growing cells (e.g., epidermis in leaves and cotyledons), etc.? Could this be revealed as a general mechanism for exocytosis regulation by ROP2 or similar proteins? What about the regulation of other specialized secretion events like the response to pathogens or pollen-tube growth? Will the RH tips become the tip of the iceberg? Only future discoveries will tell. One of the major future challenges in understanding cell polarity in plant cells, and specifically in roots, will be to elucidate how cell elongation, polarization, and exocytosis are coordinated with environmental signals in the soil.

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