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Review

Research review

ROP GTPase-dependent polarity establishment during tip growth in plants

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Summary

Polar cell growth in plants requires a cell peripheral region that undergoes membrane extension and cell wall remodeling. Since the 1990s, RHO-RELATED GTPASES FROM PLANTS (ROPs) have been identified as master regulators that determine the site of cell growth. ROPs function to regulate actin and microtubule cytoskeletons, calcium gradients, and exocytosis, thus directing the delivery of materials for membrane and cell wall extension. In recent years, our understanding of the regulatory mechanisms underlying polar localization and the activation of ROPs has greatly advanced. Evidence points to the crucial roles of membrane lipids, receptor-like kinases, and cell wall components. In this review, we provide updates on the mechanisms underlying polarity control in tip-growing cells, with a focus on ROP effectors and membrane-associated signals. By integrating knowledge from pollen tubes, root hairs, and findings in bryophyte protonema cells and rhizoids, we hope to offer important insights into a common conceptual framework on polarity establishment governed by intercellular and extracellular signals.

Introduction

Plant morphogenesis requires accurate spatiotemporal control of cell wall remodeling and cell growth, yet how these processes are guided is not fully understood. Tip-growing cells are valuable models for the study of plant cell growth. In these cells, cell expansion is highly directed and can be easily imaged and manipulated. Our knowledge about tip growth has mostly been gained from studies on pollen tubes, root hairs, and, more recently, moss protonema cells (Rounds & Bezanilla, 2013). As cell expansion depends on the directed delivery of materials for membrane extension and cell wall assembly at the growing apex, not surprisingly, actin and microtubule organization, vesicle trafficking, and exocytosis play fundamental roles. These processes are spatiotemporally controlled to enable cell expansion at a particular peripheral region and have been an active area in plant cell biology.

The RHO-RELATED GTPASES FROM PLANTS (ROPs) are CDC42/RHO/RAC-like small GTPases that exhibit polar membrane localization and regulate tip growth and other developmental processes (Feiguelman *et al.*, 2018). Recently, the tip growth of protonema cells and rhizoids in mosses has also been shown to rely on ROPs (Burkart *et al.*, 2015; Cheng *et al.*, 2020; Yi & Goshima, 2020). In tip-growing cells, the current model indicates that distinct effectors of ROPs act synergistically to regulate the cytoskeletal network and calcium gradient, thus eventually contributing to exocytosis and directed membrane extension, cell wall remodeling, and cell growth (Feiguelman *et al.*, 2018). ROPs undergo dynamic activation/inactivation cycles between the guanosine triphosphate (GTP)-bound active form and guanosine diphosphate (GDP)-bound inactive form (Feiguelman *et al.*, 2018; Smokvarska *et al.*, 2021). The activation of ROP is facilitated by ROP GUANINE NUCLEOTIDE EXCHANGE FACTORS (RopGEFs), while the conversion from the GTP-bound form to the GDP-bound form is stimulated by ROP GTPASE-ACTIVATING PROTEINS (RopGAPs). Another group of ROP regulators, ROP GUANINE NUCLEOTIDE DISSOCIATION INHIBITORS (RopGDIs), mediates the recycling of ROPs between the plasma membrane and cytoplasm by preferentially binding GDP-bound ROPs. ROP-mediated tip growth has been best studied in pollen tubes but is also under active investigation in other tip-growing cells, such as root hairs and moss protonemata. Given that pollen tubes, root hairs, and bryophyte protonema cells exhibit great similarities in cell structure and growth form but also differences in developmental contexts

(Rounds & Bezanilla, 2013), it is conceivable that conserved molecules and cell type-specific factors may be involved in ROP signaling. Recently, the regulation of ROPs has been linked to membrane lipids, receptor-like kinases, and cell wall components. These findings reveal the importance of self-organization and signaling control of ROP-dependent polarity establishment. In this review, we discuss how ROP signaling participates in and is regulated during directed cell expansion, with a focus on ROP effectors and membrane signals, hopefully providing updates on this key network.

ROP GTPases and effectors

The Arabidopsis genome contains 11 ROPs, of which at least seven and three are expressed in pollen tubes (ROP1/3/5/8/9/10/11) (Li *et al.*, 1998) and root hairs (ROP2/4/6) (Stanislas *et al.*, 2015; Denninger *et al.*, 2019), respectively. Owing to gene redundancy, functional studies in these cells have been mainly conducted by overexpressing wild-type or mutated ROPs. Recently, mutant analyses have clearly demonstrated the redundant function of ROP2/4/6 in regulating root hair growth (Denninger *et al.*, 2019; Gendre *et al.*, 2019). In contrast to Arabidopsis, there are only four ROPs in the moss *Physcomitrium patens* (previously known as *Physcomitrella patens*) (Eklund *et al.*, 2010) and one in the liverwort *Marchantia polymorpha* (Hiwatashi *et al.*, 2019). Loss of function analyses have revealed moderate growth defects in the single, double, and triple mutants but complete loss of tip growth and occurrence of round-shaped cells in the quadruple mutants of *P. patens* (Burkart *et al.*, 2015; Cheng *et al.*, 2020; Yi & Goshima, 2020), whereas there is no report of mutant phenotypes of ROP in *M. polymorpha*. The observed phenotypes in mosses suggest that ROPs play evolutionarily conserved roles in tip growth and cell shape regulation.

ROP signaling is conveyed by various effectors, which preferentially interact with the active forms of ROP, but not the inactive forms (Feiguelman *et al.*, 2018). In pollen tubes, ROP1 is the major regulator of polar growth (Fig. 1). Three effectors of ROP1, named ROP-INTERACTIVE CRIB MOTIF-CONTAINING PROTEIN RIC1/3/4, have been characterized. RIC4 functions to stimulate actin assembly and bundling (Gu *et al.*, 2005); RIC3 promotes F-actin disassembly by activating calcium signaling (Gu *et al.*, 2005); and RIC1 directly severs F-actin, thus promoting actin turnover (Zhou *et al.*, 2015). These pathways contribute to dynamic actin organization and vesicle targeting at the growing apex (Feiguelman *et al.*, 2018). In Arabidopsis, there are 11 RICs (Wu *et al.*, 2001). When overexpressed in tobacco pollen tubes, they display different subcellular localization patterns and effects on pollen tube growth. In contrast to RIC1/3/4, the function of other RICs is still not clear. Recently, a tobacco homolog of Arabidopsis RIC10/11, NtRIC11, was identified in a search for RAC-LIKE GTP-BINDING PROTEIN 5 (NtRAC5) interactors (Stephan, 2021). NtRIC11 interacts with C2-DOMAIN ABA-RELATED 4 (NtCAR4), a protein belonging to unconventional GAP proteins that contain a phospholipid-binding C2 domain. Phylogenetically, NtCAR4 is paralogous to POLLEN C2 DOMAIN-CONTAINING PROTEIN (NtPCCP) and is related to the ADP-RIBOSYLATION FACTOR GTPASE-ACTIVATING PROTEIN (ArfGAP) clade. These facts suggest a potential role of NtRIC11 and NtCAR4 in regulating vesicle biogenesis. In contrast to Arabidopsis, there is only one RIC in *P. patens* (Eklund *et al.*, 2010) and no closely related homologs in *M. polymorpha* (based on BLAST results against AtRIC1 to AtRIC11 and PpRIC in the Phytozome database https://phytozome-next.jgi.doe.gov/). Knockout of PpRIC does not reveal strong defects in growth or development (Bascom *et al.*, 2019). Thus, ROPs in basal land plants seem to employ distinct ROP effectors. Nevertheless, the dynamics of actin and microtubule networks and the deposition of cell wall materials are altered in the PpROP mutants (Burkart *et al.*, 2015), indicating that PpROPs may control cytoskeletal organization and cell wall remodeling, as they do similarly in flowering plants.

Another group of ROP effectors is INTERACTOR OF CONSTITUTIVE ACTIVE ROPs (ICRs, also known as RIPS FOR ROP INTERACTIVE PARTNERS). ICRs/RIPs are coiled-coil domain-containing proteins, of which there are five members in Arabidopsis (Lavy *et al.*, 2007; Li *et al.*, 2008). Overexpression of ICR1 leads to depolarized pollen tube growth (Li *et al.*, 2008) and swollen root hairs (Lavy *et al.*, 2007), indicating a critical role of ICR1 in tip growth. However, its loss of function does not exhibit discernible defects in root hairs or pollen tubes, which may be explained by gene redundancy or the absence of expression (e.g. publicly available transcriptomics indicate that ICR1 is not expressed in pollen tubes), although partial male sterility is observed (Lavy *et al.*, 2007). ICR1 plays a potential role in exocytosis as it physically interacts with the exocyst subunit SEC3 (Lavy *et al.*, 2007). ICR2/RIP3 and ICR5/RIP4 (also known as MIDD1), which are expressed in root hairs but not in pollen tubes, redundantly regulate root hair elongation (Fig. 2). Mechanistically, ICR2 and ICR5 recruit the cytoplasmic kinase AGC1.5 to the apical membrane, where AGC1.5 phosphorylates RopGEF4/10 and activates ROP2 (Li *et al.*, 2021). In another report, no redundancy was observed between ICR2, ICR5, and ICR3. Instead, the loss of function of ICR2 alone induced branched root hairs, implying a specific role of ICR2 in restricting the growth point (Feiguelman *et al.*, 2021). ICR2 is a microtubule-associated protein and has been proposed to recruit ROPs from membrane to microtubules, thus restricting their localization (Feiguelman *et al.*, 2021). ICRs seem to be absent in nonvascular plants, as no homologs are found in *Physcomitrium* or *Selaginella* (Eklund *et al.*, 2010).

In addition to RICs and ICRs, a *trans*-Golgi-associated protein, RAC5 INTERACTING SUBAPICAL POLLEN TUBE PROTEIN (RISAP), has been identified as an effector of NtRAC5 in tobacco (Stephan *et al.*, 2014). RISAP is a putative myosin receptor and can bind myosin XI. This notion suggests that ROPs may activate myosin-dependent vesicle trafficking via RISAP. Homologs of RISAP are present in basal land plants, including mosses (Stephan *et al.*, 2014). Thus, the function of RISAP may be conserved. In root hairs, a recent study characterized the Qa-SOLUBLE N-ETHYLMALEIMIDE-SENSITIVE FACTOR ATTACHMENT PROTEIN RECEPTOR (SNARE) SYP121 as an effector of ROP2 (Cui *et al.*, 2022). Active ROP2 promotes SYP121 accumulation at root hair tips and facilitates its association

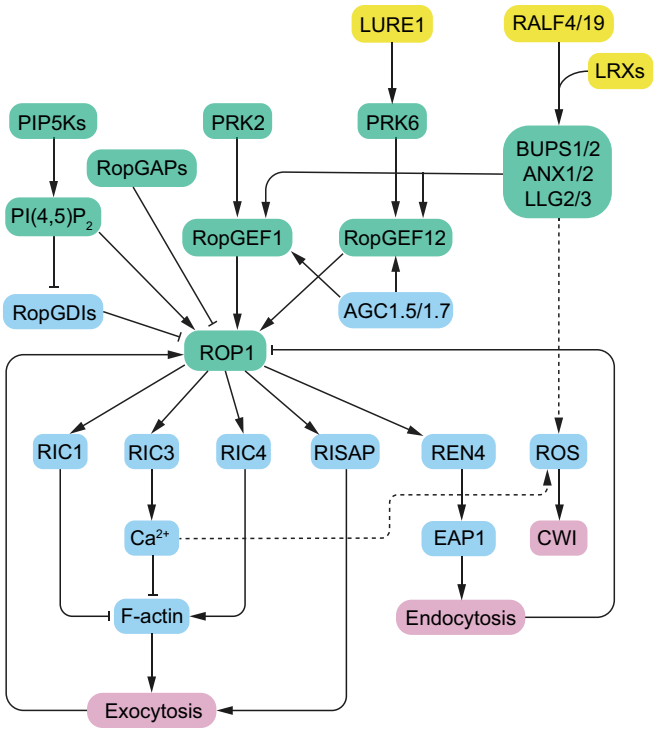

Fig. 1 ROP signaling in pollen tubes. ROP1 is the major ROP GTPase member that regulates polarity establishment during pollen tube growth. Other ROPs may also be involved, but their function has not been well studied, likely owing to gene redundancy. Redundancy has also been reported for other components, including PIP5Ks, PRKs, LRXs, and RopGEFs. Thus, only representative proteins whose function is well understood are shown. ROP1 can bind and regulate a variety of downstream proteins. The presented network shows only ROP effectors that preferentially bind active ROPs. ROP effectors eventually target exocytosis, endocytosis, and cell wall integrity (CWI) via reactive oxygen species (ROS), thus leading to highly directed pollen tube expansion. The establishment of ROP1 polarity depends on the anionic membrane lipid PI(4,5)P_2 and RopGEFs. RopGEFs are associated with and likely activated by two groups of receptor-like kinases, PRKs and BUPS1/2-ANX1/2, which respond to female-secreted peptide LURE1, and secreted peptide RALF4/19 and cell wall component LRXs, respectively. The polar distribution of ROP1 is also regulated by antagonistic actions of exocytosis and endocytosis in feedback loops and is restricted by negative regulators including RopGDIs and RopGAPs. Arrows indicate positive regulation, while blunt-ended arrows indicate negative regulation. Secreted components, membrane-associated proteins and lipids, and cytoplasmic proteins and ROS are colored yellow, green, and blue, respectively. Dashed lines represent genetic but not direct interactions.

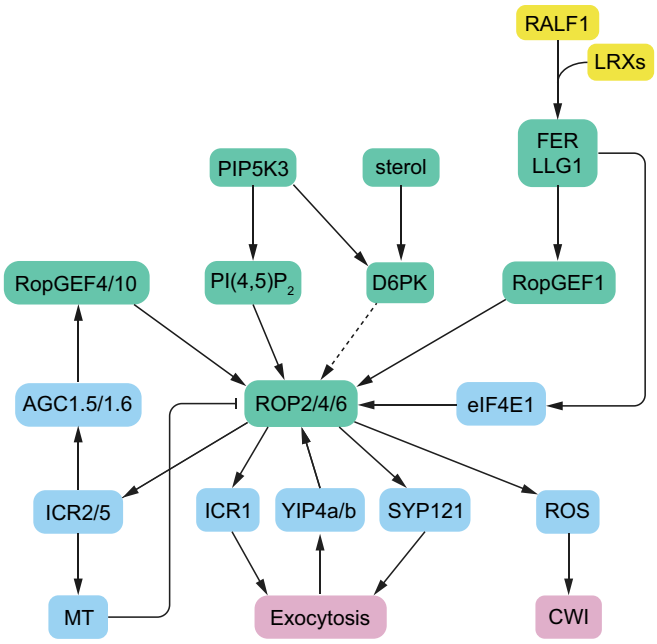

Fig. 2 ROP signaling in root hairs. ROP2/4/6 are the major ROPs for polarity establishment during root hair growth. They are positively regulated by RopGEF1/4/10, membrane-associated kinases PIP5K3 and D6PK, and membrane lipids sterol and PI(4,5)P_2. ROP2/4/6 target ICR2/5 to activate RopGEF4/10 and recruit ICR1 and SYP121 to promote exocytosis, thus forming three positive feedback loops. The secretion-related proteins YIP4a/b promote membrane localization and activation of ROPs. ICR2 also associates with the microtubule (MT) network and negatively regulates the membrane localization of ROPs. Additionally, ROP2/4/6 govern cell wall integrity by inducing reactive oxygen species (ROS) production in response to the RALF1/LRXs-FER/LLG1-RopGEF1 module. The FER/LLG1 membrane receptors phosphorylate the translation initiation factor eIF4E1 and upregulate ROP2 expression. Arrows indicate positive regulation, while blunt-ended arrows indicate negative regulation. Secreted components, membrane-associated proteins and lipids, and cytoplasmic proteins and ROS are colored yellow, green, and blue, respectively. Dashed lines represent genetic but not direct interactions. CWI, cell wall integrity.

with the R-SNARE VAMP722, likely promoting vesicle fusion with the plasma membrane. The known effectors have revealed versatile roles of ROPs in governing exocytosis by directly regulating cytoskeleton organization, vesicle trafficking, and vesicle fusion (Figs 1, 2). Conceivably, more effectors will be identified in the future.

Formation of the polar membrane domain

In tip-growing cells, ROPs typically display an enrichment at the apical membrane. This polar distribution is established by interactions with lipids via the polybasic hypervariable region and a carboxyl-terminal CAAX prenylation signal (Smokvarska *et al.*, 2021). In root hairs, sterol (Ovecka *et al.*, 2010) and phosphatidylinositol-4,5-bisphosphate (PI(4,5)P_2) (Denninger *et al.*, 2019) are enriched at the tip of growing root hairs. Perturbation of the function of PHOSPHATIDYLINOSITOL PHOSPHATE 5-KINASE 3 (PIP5K3), a kinase that converts phosphatidylinositol-4-phosphate (PI4P) to PI(4,5)P_2, decreases root hair growth (Kusano *et al.*, 2008; Stenzel *et al.*, 2008). PIP5K3 is localized at the apical membrane and likely functions to synthesize PIP$_2$ at the tip (Kusano *et al.*, 2008; Stenzel *et al.*, 2008). Disruption of PI(4,5)P_2 or sterol biosynthesis causes mislocalization of ROP2/6, indicating their important roles in specifying the polar localization of ROPs (Stanislas *et al.*, 2015). Additionally, the AGCVIII KINASE D6 PROTEIN KINASE (D6PK), which binds phosphatidylinositol phosphates, labels the emerging tip and is genetically downstream of PIP5K3 and sterol biogenesis but upstream of ROPs (Stanislas *et al.*, 2015). In pollen tubes, PI(4,5)P_2 also accumulates at the apical membrane and is locally synthesized by PIP5Ks (Fig. 1) (Ischebeck *et al.*, 2008; Sousa

et al., 2008). PIPKs such as PIP5K10/11 genetically compete with RopGDIs to positively regulate ROP activity (Ischebeck *et al.*, 2011). Recently, AtPIP5K2 has been shown to genetically and physically interact with NtRAC5 (Fratini *et al.*, 2021). Both AtPIP5K2 and PI(4,5)P_2 form membrane nanodomains, as NtRAC5 does. It is proposed that PI(4,5)P_2 functions as a GDI displacement factor, thus promoting ROP activation (Fratini *et al.*, 2021). However, whether this function is achieved by a directed action of PIP5Ks or PI(4,5)P_2 on ROPs remains undetermined. In root cells, another anionic lipid phosphatidylserine (PS) can recruit ROP6 into membrane nanoclusters and limit its lateral diffusion (Platre *et al.*, 2019). Notably, PS exhibits polar localization in pollen tubes, and disruption of its localization impairs pollen tube growth (Zhou *et al.*, 2020). These findings suggest that active ROPs are clustered into nondiffusive membrane domains by interacting with anionic membrane lipids. In mosses, little is known about the membrane–ROP interaction, although the prenylation signal is necessary for membrane localization of ROPs (Yi & Goshima, 2020). Nevertheless, PpPIPK1/2 control the tip growth of rhizoids and protonema cells (Saavedra *et al.*, 2011). A common pathway may exist in the land plant lineage (Fig. 3a).

In addition to membrane association, the activity status of ROPs is critical for polarity establishment. In general, RopGEFs promote ROP activation and membrane accumulation; RopGAPs restrict the active ROP domain (Feiguelman *et al.*, 2018). In plants, a noncanonical group of RopGAPs, the PLECKSTRIN HOMOLOGY (PH) domain-containing RenGAPs (REN1 and PHGAP1/2 in Arabidopsis), also negatively regulate ROP localization: REN1 functions in the apical cytoplasm of pollen tubes to globally inhibit ROPs (Hwang *et al.*, 2008), while PHGAP1/2 restrict ROP localization at the plasma membrane in pavement cells (Lauster *et al.*, 2022; Zhang *et al.*, 2022). Interestingly, the membrane-associated ARMADILLO REPEAT ONLY (ARO) proteins interact with REN1 and PHGAP1/2 to limit tip localization of ROPs in root hairs (Kulich *et al.*, 2020), suggesting the involvement of additional factors and complex interactions between ROP activity and membrane domain formation. Moreover, different members of ROPs and RopGEFs could play distinct roles in polarity establishment. For example, ROP10 localizes at the root hair shank and mediates cell wall hardening to support persistent tip growth (Hirano *et al.*, 2018); RopGEF3 plays a prominent role in polarization during root hair initiation, but RopGEF4 regulates tip growth during root hair elongation (Denninger *et al.*, 2019). Despite these diversifications, the prevailing model suggests that ROPs are self-organized into membrane domains in the presence of activity regulators (Smokvarska *et al.*, 2021). This model is supported by the formation of membrane domains when ROP11/RopGEF4/RopGAP3 or ROP2/RopGEF7^{PRONE}/RopGAP3 are co-expressed in tobacco leaves (Nagashima *et al.*, 2018). Based on the stable and dynamic associations of RopGEFs and RopGAPs with the membrane, a reaction–diffusion mechanism was developed. More recently, Sternberg *et al.* (2021) expressed RopGEF3, RopGAP1, and different ROPs in tobacco pavement cells. When all the components were co-expressed, ROPs formed nanodomains (< 1 μm in size) and microdomains (> 1 μm in size) and exhibited colocalization with RopGEF3 but not GAP1. In the absence of RopGAP1 or when constitutively active ROPs are used, only nanodomains could form. These findings demonstrate that active ROPs are clustered to

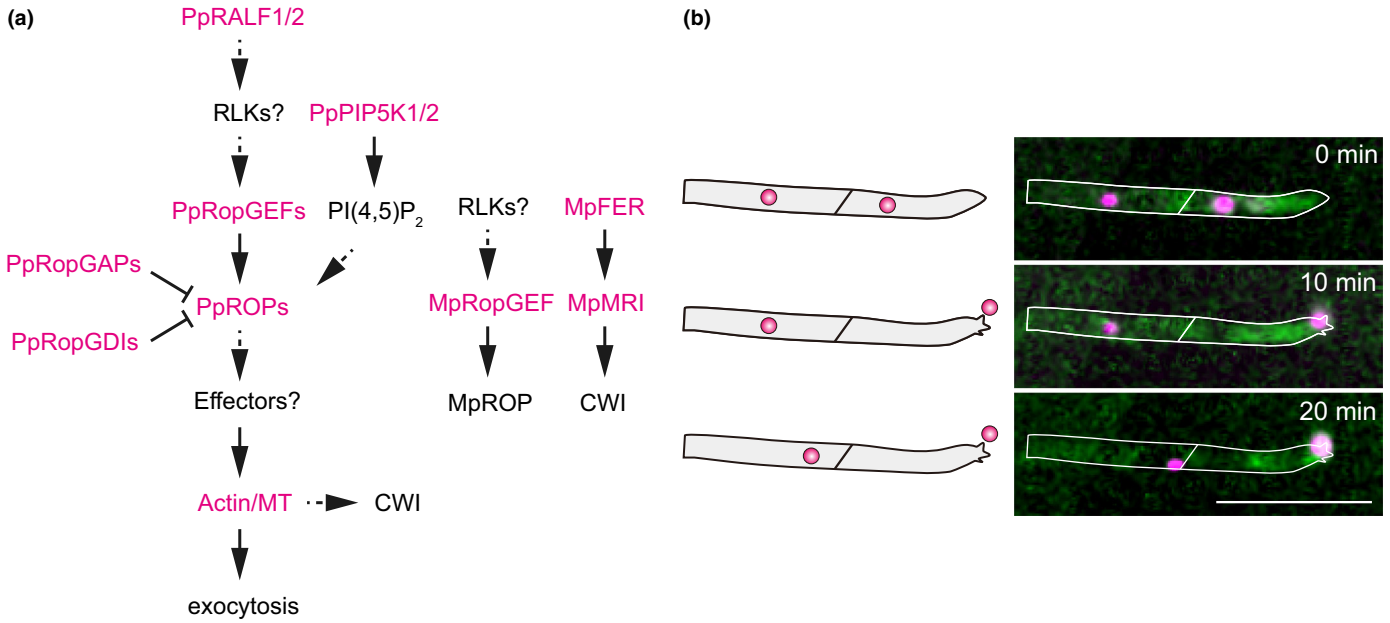

Fig. 3 ROP signaling in bryophyte protonemata and rhizoids. (a) Putative ROP signaling pathways in the moss *Physcomitrium patens* (left) and liverwort *Marchantia polymorpha* (right). The FERONIA (FER)-mediated cell wall sensing pathway may be separate from the ROP signaling in *M. polymorpha* rhizoids. Components that have been characterized are colored in magenta. Arrows indicate positive regulation, while blunt-ended arrows indicate negative regulation. Dashed lines represent putative interactions. (b) Latrunculin A induces tip rupture of protonema cells in the moss *P. patens*. The apical and subapical cells (white outline) and their nuclei (magenta) are labeled with GFP-tubulin and H2B-RFP, respectively. Bar, 100 μm. MT, microtubule.

form small nanodomains and must be dynamically inactivated to form larger membrane domains. When the lipid-binding region is mutated, ROPs also only form nanodomains. Therefore, membrane association additionally promotes nanodomain clustering and microdomain formation. These mechanisms nicely explain how the size of the polar membrane localization of ROPs could be established. However, present studies have exclusively used fluorescent protein tags to visualize ROP localization. ROP activity and localization could be severely impaired by fluorescent protein tagging at either the amino or the carboxyl terminus (Cheng *et al.*, 2020; Yi & Goshima, 2020). These mechanisms should be carefully analyzed. Moreover, although ROPs such as NtRAC5 have been shown to form nanoclusters in pollen tubes (Fratini *et al.*, 2021), whether and how ROP nanoclusters contribute to polarity formation and tip growth require in-depth investigation.

ROPs are posttranslationally modified and translocated from the site of synthesis to the plasma membrane. The intracellular route of ROP translocation has not been studied in depth. Emerging evidence implies the involvement of endomembrane trafficking. For example, overexpression of ICR1, a ROP1 effector that regulates exocytosis (Lavy *et al.*, 2007), could enhance membrane accumulation of ROP1 in pollen tubes (Li *et al.*, 2008). Conversely, perturbation of exocytosis results in depolarized pollen tubes and broader distribution of active ROPs (Luo *et al.*, 2017). Polar accumulation of ROPs at pollen tube tips also depends on clathrin-mediated endocytosis (CME) at the flank region, as evidenced by interactions between REN4, a novel WD40 domain protein, and active ROP1 and ENDOCYTOSIS ADAPTOR OF POLLEN TUBE (EAP1) (Fig. 1) (H. Li *et al.*, 2018). In root hairs, YPT-INTERACTING PROTEIN 4a and 4b (YIP4a/b), the *trans*-Golgi network-localized proteins that regulate secretory trafficking, recruit and activate ROPs at the plasma membrane (Gendre *et al.*, 2019). As ROPs are master regulators of exocytosis (Feiguelman *et al.*, 2018), these findings indicate a positive feedback mechanism of polar localization of ROPs via vesicle trafficking (Figs 1, 2).

Receptor-like kinases

Another layer of ROP regulation comes from membrane-associated receptor-like kinases (RLKs), which have been shown to interact with RopGEFs (Figs 1, 2). In Arabidopsis, at least six leucine-rich repeat RLKs (PRK1,2,3,5,6,8) and four RopGEFs (RopGEF1/9/12/14) redundantly control pollen tube growth (Chang *et al.*, 2013; Takeuchi & Higashiyama, 2016). PRK2 phosphorylates RopGEF1 and forms a complex with RopGEF1 and ROP1, thus providing strong evidence for an activation mechanism (Fig. 1) (Chang *et al.*, 2013). However, although PRK6 is polarly localized and functions to recruit RopGEF12 in response to the female-secreted peptide LURE1 (Takeuchi & Higashiyama, 2016), most PRKs are uniformly present on the membrane, which does not fit the model that PRKs activate ROPs at the tip. Moreover, direct phosphorylation of RopGEFs by PRKs has not been widely proven. Alternatively, other kinases may phosphorylate RopGEFs and regulate the polar localization of RopGEFs and ROPs. For example, cytoplasmic kinase AGC1.5, which functions redundantly with AGC1.7, phosphorylates RopGEF1/12 and specifies their membrane localization (E. Li *et al.*, 2018). This mechanism also exists during root hair growth, which requires overlapping roles between AGC1.5 and another paralog, AGC1.6 (Li *et al.*, 2021).

In root hairs, FERONIA (FER), which belongs to the *Catharanthus roseus* receptor-like kinase (CrRLK1L) family, activates ROPs by directly binding RopGEF1 (Fig. 2) (Duan *et al.*, 2010). Conceivably, FER may phosphorylate RopGEF1 to promote its activity. However, such a mechanism has not been characterized to date. As a membrane receptor, FER is activated by the secreted peptide RALF1, which requires a GPI-anchored protein, LLG1, as a co-receptor (Li *et al.*, 2015). Recently, the RALF1-FER/LLG1 complex has been shown to phosphorylate the eukaryotic translation initiation factor eIF4E1 (Zhu *et al.*, 2020). As a consequence, the synthesis of proteins including ROP2 is upregulated. Apart from its roles in regulating gene expression, the RALF1-FER/LLG1 module plays a major role in cell wall integrity maintenance because the *fer* and *llg1* mutants exhibit strong root hair rupture phenotypes, and the downstream RopGEF1/4/10-ROP2/6-reactive oxygen species (ROS) pathway has been revealed (Duan *et al.*, 2010; Li *et al.*, 2015). A similar cell wall-sensing mechanism is critical for pollen tube development, in which a distinct ligand-receptor-co-receptor module is involved. In pollen tubes, two subgroups of CrRLK1Ls BUPS1/2 and ANX1/2 interact with each other and are activated by RALF4/19 peptide ligands (Fig. 1) (Ge *et al.*, 2017). LLG2/3, which are molecular chaperones for ANX/BUPS, act as co-receptors of the BUPS1/2-ANX1/2 complex, similarly to LLG1 in root hairs (Feng *et al.*, 2019). Analogously, this receptor module interacts with RopGEF1/12 (Zhu *et al.*, 2018) and activates ROP1 in response to mechanical stimuli when pollen tubes penetrate the style and enter the transmitting tract in pistils (Zhou *et al.*, 2021). Reactive oxygen species production is also important for pollen tube growth and is genetically downstream of calcium signaling and the cell wall sensing module (Boisson-Dernier *et al.*, 2013; Kaya *et al.*, 2014), but how it directly interacts with ROP signaling remains unclear.

In flowering plants, pollen tube growth is guided by the female-secreted AtLURE1 peptide (Takeuchi & Higashiyama, 2016). However, pollen tubes can undergo tip growth without attractants *in vitro*, suggesting that the mechanism underlying tip growth is mostly intrinsic. In mosses, three RALF-encoding genes have been characterized and shown to regulate tip growth (Ginanjar *et al.*, 2022). Interestingly, PpRALF1 and PpRALF2 are secreted but differently localized. PpRALF1 is uniformly distributed across the membrane, while PpRALF2 is enriched at the apical region. These observations suggest that secreted peptides may act on intrinsic polarity modules to regulate tip growth (Fig. 3a). Such autocrine signaling has been well known for RALF4/19 and RALF1 in pollen tubes and root hairs, respectively (Li *et al.*, 2015; Ge *et al.*, 2017; Mecchia *et al.*, 2017). To date, the functional characterization of downstream RLKs and RopGEFs in basal land plants has been limited (Fig. 3a). In *M. polymorpha*, mutant screening has identified MpTHE (also known as MpFER), the sole homolog of Arabidopsis THESEUS and FER, in rhizoid growth and cell wall integrity (Honkanen *et al.*, 2016; Mecchia

et al., 2020). The cytoplasmic kinase MARIS (MpMRI), a downstream component of FER, has also been characterized (Westermann *et al.*, 2019). In mosses, the characterization of RLKs has not been reported. However, the knockdown of RopGEFs causes a significant loss of tip growth (Bascom *et al.*, 2019), supporting a critical role of ROP signaling in tip growth. Interestingly, at high concentrations (≥ 25 μM) the actin inhibitor latrunculin A induces tip rupture (Fig. 3b), implying the presence of a mechanism of cell integrity maintenance that may interact with the ROP–actin pathway. Moreover, most RLK subfamilies have evolved in all land plants (Dievart *et al.*, 2020). Thus, the cell wall sensing mechanism may be conserved and contribute to polarity control and tip growth in bryophytes. Intriguingly, mutation of the sole RopGEF in *M. polymorpha* does not affect rhizoid growth (Hiwatashi *et al.*, 2019). How cell wall sensing mechanisms are linked to polarity control in these cells remains unknown (Fig. 3).

Cell wall–RLK interaction

The CrRLK1L family contains a putative carbohydrate-binding malectin-like domain in the extracellular region (Dievart *et al.*, 2020). Two recent studies demonstrate that FER exhibits preferential binding capacity toward demethylesterified pectins and senses mechanical stresses to activate RopGEF14 and ROP6 in pavement cells (Lin *et al.*, 2022; Tang *et al.*, 2022). A similar mechanism may be involved in tip growth because pectins are abundant at the growing tip (Dehors *et al.*, 2019). In pollen tubes, the cell wall component leucine-rich repeat extensin proteins (LRXs), LRX8/10/11, also accumulate at the apical tip (Fabrice *et al.*, 2018; Wang *et al.*, 2018). LRXs can directly bind RALF4 and participate in the regulation of RALF4/19-ANX1/2 mediated growth and cell wall integrity control (Fig. 1) (Mecchia *et al.*, 2017). Direct binding between RALF1, LRXs, and FER is also important for tip growth in root hairs (Fig. 2) and LRXs are supposed to be RALF1 receptors (Herger *et al.*, 2019) as they associate with the plasma membrane (Fabrice *et al.*, 2018). The CrRLK1L family comprises a large number of members (Dievart *et al.*, 2020). It is unclear how many CrRLK1L members are involved in tip growth and whether they have different activities in relation to distinct cell wall components. Nevertheless, emerging evidence points to a critical role of CrRLK1L kinases in cell wall sensing and tip growth. As tip-growing cells display a polar distribution of cell wall polymers at the apex, we hypothesize that polar-secreted cell wall components such as pectin and extensin may act as an intrinsic signal to activate CrRLK1L kinases (Fig. 4), which subsequently leads to the activation of ROP signaling. Thus, cell wall materials are not only cargo to be delivered to the tip, but also may reinforce cell polarity in a positive feedback loop.

Concluding remarks

Playing a central part in establishing polarity, ROP GTPases function as membrane-anchored molecular switches that direct plant cell expansion (Feiguelman *et al.*, 2018). Besides the essential roles of ROPs in signal transduction, complex regulatory mechanisms underlying ROP localization and activation are emerging. These include activity-dependent self-organization of membrane domains, interactions with membrane lipids, intracellular trafficking via exocytosis and endocytosis, and activity control by RLKs and extracellular signals (Fig. 4). These pathways provide a

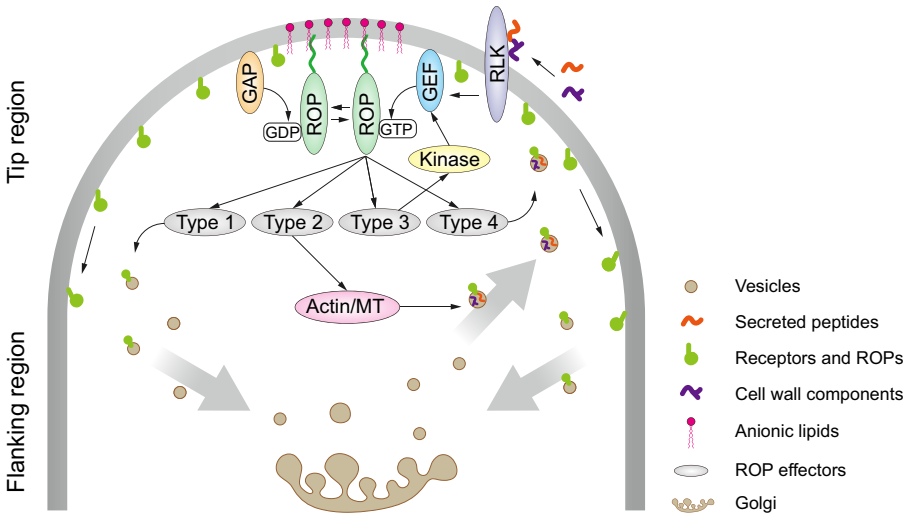

Fig. 4 A simplified model of the ROP-dependent polarity pathway in tip-growing cells. ROPs are anchored to the plasma membrane via carboxyl-terminal lipid modification. They are dynamically activated and inactivated by RopGEFs and RopGAPs, respectively. Activity control and the association of the carboxyl-terminal polybasic region with anionic membrane lipids (e.g. $PI(4,5)P_2$) mediate the self-organization of ROPs into membrane microdomains at the tip region. RopGEFs are phosphorylated and activated by receptor-like kinases (RLKs, e.g. PRKs and CrRLK1L family members) which bind secreted peptides (e.g. RALFs) and cell wall components (e.g. extensins and pectins). We classified ROP effectors into four groups that ultimately regulate ROP-dependent cell polarity in feedback loops: Type 1 effectors regulate endocytosis and recycle active ROPs from the flanking region; type 2 effectors act on actin and microtubule networks to promote exocytosis of cell wall materials, membrane lipids, secreted peptides, membrane receptors, and ROPs; type 3 effectors target cytoplasmic regulators (e.g. AGC kinases) to regulate RopGEFs and ROP activity; type 4 effectors directly link ROPs to vesicle trafficking and fusion and promote exocytosis independent of cytoskeletons. MT, microtubule.

comprehensive view of polarity establishment during tip growth and appear to be conserved in the land plant lineage. However, more work will be needed to understand the detailed mechanism in each pathway and the interactions between them. First, RLKs have been implicated in phosphorylating RopGEFs and releasing their autoinhibitory tail (Chang *et al.*, 2013). Whether this mechanism is widely conserved requires more evidence. In addition, RLKs may play distinct roles in tip growth and cell wall integrity, as evidenced by the presence of PRKs and BUPS1/2-ANX1/2 in pollen tubes. Whether such a division of labor exists in other tip-growing cells remains unknown. Alternatively, some RLKs such as FER in root hairs may be involved in both processes. The use of more cell models, such as bryophyte protonema cells and rhizoids, and the characterization of specific mutations that influence each pathway will help to answer these questions. Second, how do cell wall components activate RLKs? RLKs are activated by secreted ligands such as RALFs and can bind pectins and LRXs (Mecchia *et al.*, 2017; Herger *et al.*, 2019; Lin *et al.*, 2022). However, the precise roles of pectins and LRXs in activating RLK–ROP signaling are unclear. Third, how do membrane lipids influence ROP localization? ROPs may be clustered by interacting with asymmetrically distributed anionic lipids (Platre *et al.*, 2019; Sternberg *et al.*, 2021). However, lipids such as $PI(4,5)P_2$ may also activate ROP (Ischebeck *et al.*, 2011; Fratini *et al.*, 2021). It is as yet unknown how lipid interaction and the activity control of ROPs are coordinated to establish polar ROP localization. Fourth, how do ROPs translocate from the site of synthesis to the apical membrane? Emerging evidence implies the involvement of vesicle trafficking. A direct interaction between ROPs and secretory vesicles has not been characterized. Finally, feedback regulation appears to be a common strategy for ROP-mediated polarity establishment. To date, a variety of ROP effectors have been characterized, most of which can be classified into four groups (Fig. 4). These effectors ultimately contribute to ROP-dependent polarity formation in positive or negative feedback loops. Whether such a strategy is commonly involved in tip-growing cells remains an open question. Further work will be necessary to answer the above questions for a better understanding of polarity-dependent cell expansion. The use of high-resolution live-cell imaging, careful genetic manipulation, and comparative studies from distinct plant cell models will be helpful.

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Data availability

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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