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The Rop GTPase switch turns on polar growth in pollen

Zhi-Liang Zheng and Zhenbiao Yang

Pollen-tube growth not only represents an essential stage of plant reproduction but also provides an attractive model for studying cell polarity and morphogenesis. For many years, pollen-tube growth has been known to require a tip-focused Ca^{2+} gradient and dynamic F actin, but the way that these are controlled remained a mystery until recently. Rop appears to be activated at growth sites by a tip-localized growth cue, acting as a central switch that controls the polar growth of pollen tubes, probably having its effect through phosphoinositides and Ca^{2+}. These findings have begun to shed light on the molecular basis of pollen-tube growth and cell morphogenesis in plants.

As the male gametophyte of higher plants, pollen provides some fascinating biology. To deliver sperms to the ovule, pollen grains first land on the stigma surface, where they choose a germination aperture. A tube protrudes from the chosen aperture, penetrates the stigma surface, extends rapidly within the transmitting track (as fast as 1 cm h^{-1}), emerges from the septum and is finally targeted into the ovule.

This growth behavior, known as pollen-tube guidance, bears a remarkable resemblance to neuronal-axon guidance in animals: both involve attractants, repellents, matrix-mediated cell adhesion and competition for target sites[1–3]. Extracellular signals (e.g. lipid molecules, lipid-transfer proteins and glycoproteins) are implicated in the modulation of this growth behavior[4–6]. As well as this compatible interaction, an incompatible interaction is also common, in which self-pollen is prevented from germinating or pollen tubes are arrested on their way to the ovule by specific molecules present in self-stigma[7–10]. Consequently, understanding the mechanism of pollen-tube growth has important practical implication in agriculture, such as in hybrid-crop production. However, the way that signals modulate pollen-tube growth remains poorly understood.

As in axon-growth guidance, the growth behavior of pollen tubes is associated with an internally controlled polar growth, known as tip growth. Tip growth requires the specification of a cortical site in the cell to which Golgi vesicles are targeted and fused[11,12]. This localized exocytosis at the defined site results in a polar extension of the plasma membrane (PM; derived from the vesicle membrane) and the cell wall (derived from the contents of the vesicle), leading to the formation of a cylindrical tube.

Polar growth is critical for the morphogenesis of the non-motile plant cells. A central problem of polar growth and cell morphogenesis is how the cell defines a PM domain for growth. Owing to their capacity to grow synchronously in cultures as a

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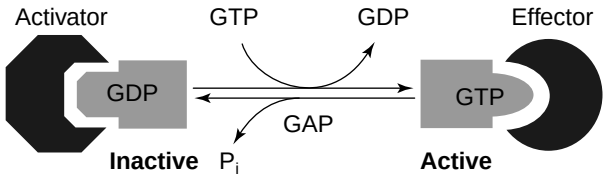

Dominant mutations:

Dominant negative	Constitutively active
Locked in GDP form	Locked in GTP form
Sequester activators	Constitutively activate effectors
T20N, D121A	G15V, Q64L

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Fig. 1. Regulation of RHO GTPases and dominant mutations of *Arabidopsis* Rop1At. T2ON, D121A, G15V and Q64L are specific point mutations created on Rop1At to produce the indicated effects. Abbreviations: GAP, GTPase activating protein; P_i, inorganic phosphate.

haploid cell, pollen tubes provide an attractive model system for studying the mechanisms of cell-polarity formation and cell morphogenesis in plants[11,12]. Using this simple system, specific questions about polar growth can be addressed, including the following:

- What are the internal cues that determine pollen to be a tip-growing cell?
- How do these cues specify the PM domain for tip growth?
- Are the signaling pathways that lead to tip growth used by the external cues that guide pollen-tube growth?

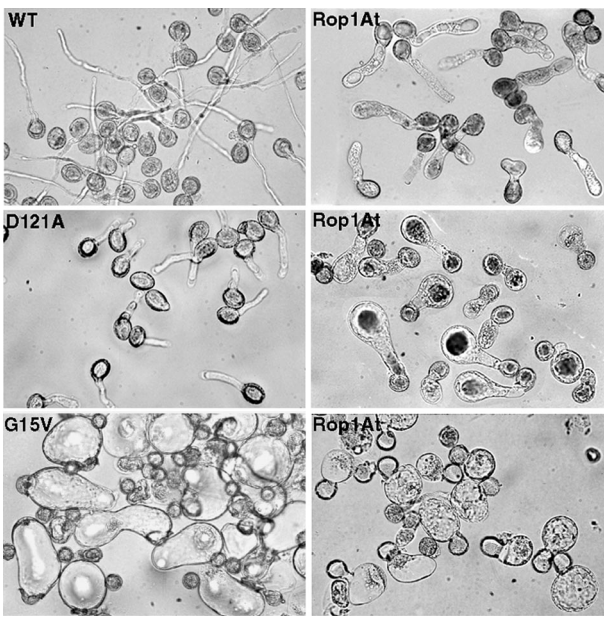

Fig. 2. Rop controls both the specification of tip growth sites and the rate of pollen-tube growth. Wild-type *Arabidopsis* pollen tubes (WT) are compared with transgenic tubes expressing dominant-negative DN-*rop1At* (D121A), constitutively active CA-*rop1At* (G15V) and wild-type Rop1At under the control of the *LAT52* promoter (Rop1At)[22]. Wild-type-Rop1At overexpression causes various degrees of depolarized growth, depending on the level of Rop1At expression (three independent transgenic lines are shown).

Recent research has centered on several aspects of pollen-tube growth: signals that regulate pollen-tube growth, actin dynamics, tip-focused Ca^{2+} gradients and Ca^{2+} influxes, and signaling proteins including G proteins and protein kinases[11,13,14]. In particular, the evidence suggests that a Rop (Rho-related GTPase from plants) GTPase plays a critical role in coordinating the temporal and spatial controls of pollen-tube growth, probably by modulating tip-focused Ca^{2+} gradients and possibly actin dynamics. Here we discuss the mechanism by which Rop regulates pollen-tube growth. For more-comprehensive reviews of pollen-tube growth, see Refs 11–15.

Rop GTPase is a central switch for pollen-tube growth

The importance of Rop in the control of pollen-tube growth was first indicated by the observation that a pea Rop (Rop1Ps) is preferentially localized to the apical region of the pollen tube PM (Ref. 16). Rop is a plant-specific subfamily of the RHO family of monomeric G proteins (GTPases), which includes Cdc42, Rac and Rho subfamilies from animals and fungi[17–19], which belongs to the Ras superfamily of low molecular weight monomeric guanine nucleotide-binding proteins. Because some RHO GTPases (e.g. Cdc42) control the establishment of cell polarity in yeast and mammalian cells via the asymmetric organization of cortical F actin, it was speculated that the tip-localized Rop might also play a similar role in pollen tubes[16]. This hypothesis was supported by the observation that the *Arabidopsis* pollen-specific Rop protein Rop1At and a root-hair Rop (Rop6At) caused depolarized cell expansion, when overexpressed in the tip-growing fission yeast[19,20].

The story about Rop in pollen-tube growth took a twist when it was shown that injected anti-Rop1Ps antibodies inhibit pollen-tube growth but do not affect growth polarity[21]. That Rop is critical for cell growth per se is further demonstrated using dominant-negative (DN) mutants of Rop GTPases[22,23]. Single amino acid replacements of critical residues within the nucleotide-binding domains of small GTPases allow them to bind GDP or GTP permanently. Those that only bind GDP become DN, as their accumulation in the cell blocks a specific GTPase from signaling by sequestering the GTPase activators; by contrast, those that bind GTP permanently are constitutively active (CA; Fig. 1).

The transgenic expression of DN-*rop1At* using the pollen-specific *LAT52* promoter in *Arabidopsis* or the transient expression of DN mutants of an *Arabidopsis* homolog of Rop1At, Rop5At (also known as At-Rac2), in tobacco pollen causes drastic inhibition of pollen-tube growth but has no obvious effects on pollen-tube morphology[22,23] (Fig. 2). The transgenic expression of an antisense *rop1At* gene in *Arabidopsis* also causes tube-growth inhibition but the inhibition is much less than the effect of DN mutants, probably because of the expression of functionally redundant Rop genes in pollen[22]. Indeed, at least three closely related Rop genes (*Rop1At*, *Rop3At* and *Rop5At/At-rac2*) are expressed in *Arabidopsis* pollen[19]. These genes will have to be knocked out in order to determine whether they are indeed functionally redundant in tube growth. Nonetheless, these results clearly show that one or more Rop GTPases and their activation are critical for the temporal control of pollen-tube growth.

Is Rop only required for pollen-tube elongation, not for the establishment of cell polarity? Either of the following models could account for the above-described role for Rop. First, a Rop-dependent pathway might be involved in the temporal control of tube growth (i.e. the timing of growth activation and the rate of growth), whereas a Rop-independent pathway establishes the site of growth. Second, a single Rop-dependent pathway might couple the specification of growth sites with exocytosis (i.e. growth does not occur unless the pathway is activated to specify sites of growth).

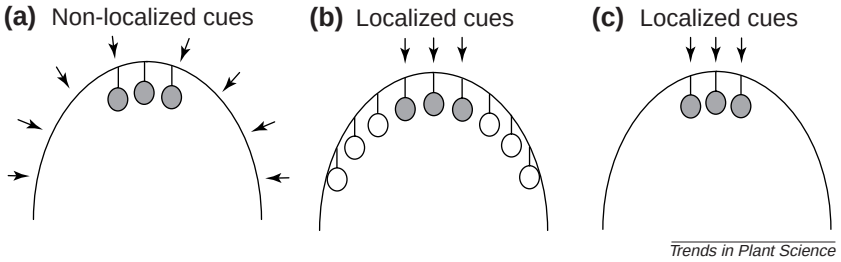

Fig. 3. Models for the spatial control of Rop activation to couple the specification of growth sites with the control of growth rates during pollen-tube growth. (a) Polar localization of Rop controls the site of growth. This model implies that tip-localized Rop is activated by a non-localized cue to initiate tip growth. (b) Localized activation of randomly distributed Rop determines the site of growth. This model implies the presence of a localized cue that activates Rop, leading to tip growth. (c) Both polar localization and localized activation of Rop are required to specify the site of growth. This implies the presence of a localized cue to activate Rop, which in turn regulates its own localization (a positive-feedback loop; Fig. 4). Rop-GDP is represented in white and Rop-GTP in gray.

To distinguish between these two models, a gain-of-function approach was used that involved overexpressing wild-type Rop1At (WT-Rop1At) or CA-*rop1At* mutants[22]. In each case, the rate of *in vitro* pollen germination was promoted, confirming the role of Rop in the temporal control of tube growth (H. Li *et al*., unpublished). More importantly, CA-*rop1At* or high-level expression of WT-Rop1At also causes isotropic growth in pollen tubes (Fig. 2), and similar results were obtained when wild-type Rop5At/At-Rac2 or its CA mutants were transiently expressed in tobacco pollen[23]. These results indicate that Rop also plays an important role in the establishment of cell polarity and thus provide evidence for the model that Rop couples the spatial control with the temporal control of pollen-tube growth. This is in contrast with the yeast RHO GTPase Cdc42 that is necessary for specifying budding sites but not growth *per se*; cdc42 null mutations cause enlarged unbudded cells[24]. Nonetheless, these studies indicate that Rop is a central molecular switch that controls tip growth in pollen tubes.

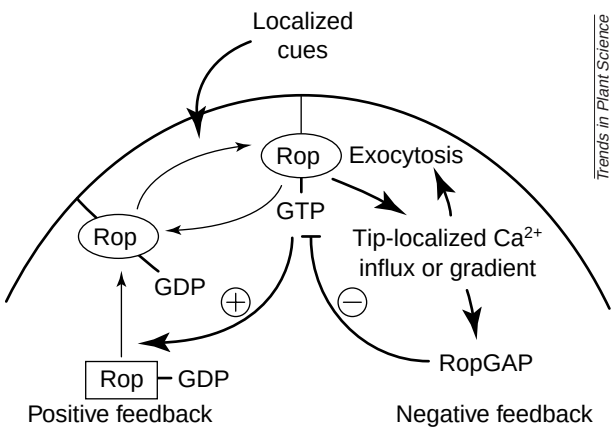

Fig. 4. Model for the control of pollen-tube growth by a combination of the Rop-dependent positive- and negative-feedback loops. The correlation of ectopic accumulation of Rop at the tip with depolarization of pollen tubes supports the model that the tip-localized activated-Rop regulates its own localization. This positive feedback loop could enable the rapid activation of localized exocytosis through the tip-localized calcium influx and the tip-focused calcium gradient (see Fig. 5). The accumulation of intracellular calcium at the tip above a threshold level could initiate the negative Rop–calcium feedback loop, in which high levels of intracellular calcium can hyper-activate Rop-GTPase-activating proteins (RopGAPs), leading to deactivation of Rop and growth inhibition. The negative feedback loop could allow the cell to coordinate the rate of exocytosis with other activities of the cell such as remodeling of the cell wall. The alternating operation of the two feedback loops might account for pulsatory growth commonly observed in pollen tubes.

How is Rop regulated during polar growth: polar localization or localized signaling?

Because of its critical role in signaling to tip growth, understanding how Rop is regulated might shed some light on tip-growth mechanisms. Several models might explain how Rop is regulated to control tip growth (Fig. 3). First, Rop might be restricted to the site of growth, where its activity is regulated by non-localized growth cues. Second, the regulation of Rop activity by localized growth cues might be sufficient to activate tip growth, regardless of its distribution. Third, both polar localization of Rop and the regulation of its activity by localized cues might be critical for tip growth. Current evidence favors the third model.

Biochemical analyses suggest that a fraction of total cellular Rop protein is localized to the PM in pea pollen tubes[16]. Furthermore, Rop1At and its homologs produced in pollen are subject to isoprenylation, which is required for their membrane localization; non-isoprenylated *rop* mutants are non-functional[16,22,23]. These results suggest that Rop must be localized to the PM for its biological function.

Both immunolocalization and the localization of Rop tagged with green fluorescent protein (GFP) show that Rop is localized to the apical domain of PM (Refs 16,22,23). Does this polar localization play a regulatory role in the specification of tip growth sites? A GFP–Rop1At fusion protein was localized in transgenic plants that accumulate different levels of GFP–Rop1At in their pollen tubes[22]. Higher levels of GFP–Rop1At accumulation led to more-severe depolarized growth, which was associated with a greater abundance of GFP–Rop1At distributed to a wider apical domain of the PM. These results imply that the proper polar localization of Rop to the apex of pollen tubes is important for defining the site of tip growth.

However, polar localization alone appears not to be sufficient for Rop to specify the site of tip growth, because Rop is not restricted to the extreme tip of the cell, where growth occurs, but is also distributed to a lesser extent in the subapical region[22,23]. To assess whether the regulation of Rop activity by localized cues is also needed for the Rop-mediated specification of growth sites, the severity of the phenotypes induced by WT-Rop1At and CA-rop1At overexpression were compared[22]. When expressed to a similar level in transgenic pollen tubes, CA-rop1At causes a complete loss of cell polarity whereas WT-Rop1At only induces partial loss of polarity. These results indicate that the localized activation of Rop at the growth site and/or the deactivation of Rop away from the growth site are also critical for Rop to regulate the site of tip growth. How, then, could the increased accumulation of Rop proteins at the apex be associated with depolarized growth? An attractive explanation is that polar localization and localized activation of Rop forms a positive-feedback loop[22] (Fig. 4). Thus, ectopic Rop accumulation at the tube tip would activate Rop signaling in a larger area, leading to depolarized growth and further ectopic Rop localization. This feedback loop might explain why Rop signaling can couple the specification of growth sites with the control of growth rates[22].

How does Rop control tip growth?

Tip-localized Ca^{2+} signaling acts downstream of Rop to activate tip growth

A tip-focused intracellular Ca^{2+} gradient in pollen tubes is ubiquitous in plants and is essential for tip growth, probably because of its role in exocytosis[13–15,25–27]. Tip-localized Ca^{2+} entry is at least partially responsible for the formation of the Ca^{2+} gradient, but other mechanisms, such as inositol trisphosphate [Ins(1,4,5)P_3]-dependent Ca^{2+} release from intracellular stores, might also play a role[28–31]. Importantly, both the tip-localized Ca^{2+} entry and the Ca^{2+} gradient also define the site of tip growth and thus control the orientation of pollen-tube elongation[29,32]. Therefore, this dual role for the localized Ca^{2+} activity parallels that for Rop, suggesting a possible connection between Rop and Ca^{2+}.

A Rop–Ca^{2+} interaction was first suggested by the observation that low concentrations of extracellular Ca^{2+} ($[Ca^{2+}]_{ex}$) and caffeine treatments potentiate tube-growth inhibition by injected anti-Rop1Ps antibodies[21]. Recent studies suggest that Ca^{2+} acts downstream of Rop in tip growth[22]. First, the tip-focused Ca^{2+} gradient is eliminated within 1–2 min after microinjection of an anti-Rop1Ps antibody into pea pollen tubes; this timing coincides with that of antibody-induced growth arrest[21]. Second, increasing $[Ca^{2+}]_{ex}$ (from 0.5 mM to 20 mM) reverses the growth inhibition caused by DN-*rop1At* expression in transgenic *Arabidopsis* pollen tubes. By contrast, the growth of *Arabidopsis* wild-type tubes requires an optimal $[Ca^{2+}]_{ex}$ of 2 mM, whereas high $[Ca^{2+}]_{ex}$ (10–20 mM) inhibits wild-type tube growth. Third, the expression of an antisense *rop1At* gene in transgenic *Arabidopsis* pollen only inhibits pollen-tube growth at low $[Ca^{2+}]_{ex}$ ($\leq$0.5 mM), probably owing to a partial suppression of Rop protein accumulation. Interestingly, the antisense gene has no effects at the optimal $[Ca^{2+}]_{ex}$ and stimulates tube growth at higher $[Ca^{2+}]_{ex}$ (10–20 mM). These results provide evidence that Rop regulates the formation of the tip-focused Ca^{2+} gradient and the tip-localized Ca^{2+} influx, leading to the activation of tip growth.

When $[Ca^{2+}]_{ex}$ reaches a threshold level, Ca^{2+} influxes and the steepness of the Ca^{2+} gradient could be reduced via a negative Rop–Ca^{2+} feedback loop (Fig. 4), that is, accumulation of intracellular Ca^{2+} beyond a threshold level could deactivate Rop, leading to reduced Ca^{2+} influxes and growth inhibition[22]. This negative Rop–Ca^{2+} feedback loop and the Rop-dependent Ca^{2+} influx explain why a high $[Ca^{2+}]_{ex}$ inhibits the growth of wild-type tubes but not tubes expressing the antisense *rop1At* gene.

Additional evidence for the negative Rop–Ca^{2+} feedback loop came from the studies of Rop-GTPase-activating proteins (RopGAPs) from *Arabidopsis* pollen (G. Wu *et al*., unpublished). By stimulating the intrinsic GTPase activity of Rop proteins to increase GTP hydrolysis, RopGAPs deactivate Rop signaling. Unlike the antisense *rop1At* gene, extra-copies of genes of RopGAP sensitize growth inhibition caused by high $[Ca^{2+}]_{ex}$. This implies that RopGAP is regulated by Ca^{2+} and participates in the negative feedback loop. When $[Ca^{2+}]_{ex}$ rises above a threshold level, an increased Ca^{2+} influx or intracellular Ca^{2+} level could enhance the activity of RopGAPs, which in turn deactivate Rop, leading to a lower Ca^{2+} influx and thus growth inhibition (Fig. 4). Nonetheless, further work is needed to clearly demonstrate whether the tip-localized intracellular Ca^{2+} is indeed involved in the feedback loop and the regulation of RopGAP activity, and to identify other potential components in this loop.

Does Rop regulate actin organization?

Inhibitor studies suggest that F actin is critical for pollen-tube growth[33]. Two major forms of F actin have been found in pollen tubes: a ring of fine actin near the apex, which defines the apparent actin-free zone (AFZ) at the tip, and cortical actin cables that extend along the growth axis to the actin ring[33–35]. The cortical actin cables are involved in cytoplasmic streaming, which facilitates the movement of post-Golgi vesicles to the apical region. The function of the actin ring is unclear, although similar actin structures called fine actin bundles, which are thought to transport vesicles to the extreme apex, are found at the apex of root hairs and are implicated in tip growth[36]. Alternatively, fine actin bundles or the actin ring might be important for maintaining the AFZ. An AFZ is found in all tip-growing cells examined, including axon and fungal hyphae in addition to pollen tubes and root hairs. The AFZ might be essential for exocytosis to occur (supported by the observation that treatments that cause the AFZ to disappear arrest pollen-tube growth[35]).

Because RHO GTPases control the organization and remodeling of actin cytoskeleton in yeast and animals, it is reasonable to speculate that Rop might play a similar role in pollen tubes. Using a talin–GFP fusion as an F-actin marker[33], transient expression of CA-*rop5At/At-rac2* was found to cause extensive spiral cortical actin cables in balloon-shaped tobacco pollen tubes, whereas DN-*rop5At/At-rac2* reduced actin bundling[23]. It was suggested that Rop regulates the organization of cortical actin bundles in pollen tubes[23]. However, it remains to be determined whether the observed changes in the cortical actin cables were due to a direct effect of the mutant proteins or resulted indirectly from the changes in pollen-tube growth. Furthermore, these changes in actin organization alone do not account for the dramatic changes in pollen-tube growth induced by the *rop5At/At-rac2* mutants[23]. Thus, further studies are needed to clarify the role of Rop in the regulation of actin dynamics in pollen tubes.

A potential interplay between Rop, Ca^{2+} and the actin cytoskeleton

As well as playing a role in the formation of the Ca^{2+}gradient and a potential role in the organization of the cortical actin cables, Rop might also be involved in the generation of the AFZ directly and/or indirectly through the actin ring or the Ca^{2+} gradient. In rat mast cells, Rho controls exocytosis and actin disassembly at the cell cortex in a manner that depends on, and/or is synergistic with, Ca^{2+} (Ref. 37). A similar mechanism could also operate during pollen-tube growth. Thus, it would be interesting to determine whether overexpression of Rop or *rop* dominant mutants alters actin ring and AFZ in pollen tubes.

Based on the observation that various treatments that disrupt the Ca^{2+} gradient also cause the disappearance of the AFZ, it has been proposed that the tip-focused Ca^{2+} gradient is critical for AFZ formation[35], although direct evidence for the Ca^{2+} gradient causing the AFZ is lacking. Furthermore, it is possible that the AFZ, which could be directly controlled by Rop, might be important for the establishment of the Ca^{2+} gradient. Finally, a potential interplay among Rop, Ca^{2+} and actin dynamics might be linked through a putative Rop effector, as discussed below.

Phosphatidylinositol monophosphate kinase: a Rop effector regulating localized Ca^{2+} signaling and actin organization?

One class of effectors for animal RHO GTPases are phospholipid kinases: phosphatidylinositol 3-kinase (a Rac effector) or phosphatydylinositol-4-phosphate 5-kinase (a Rho effector)[38]. Recombinant Rop5At/At-Rac2 physically associates with a phospholipid-kinase activity that specifically generates phosphatydylinositol-4,5-bisphosphate[23] [PtdIns(4,5)P_2]. Furthermore, a mammalian pleckstrin homology (PH) domain, which specifically binds PtdIns(4,5)P_2, is localized to the apical region of the PM in tobacco pollen tubes. The PH domain also specifically inhibits pollen-tube growth (an effect similar to that of DN Rop mutants)[23]. These are important and interesting observations, because they provide evidence that at least

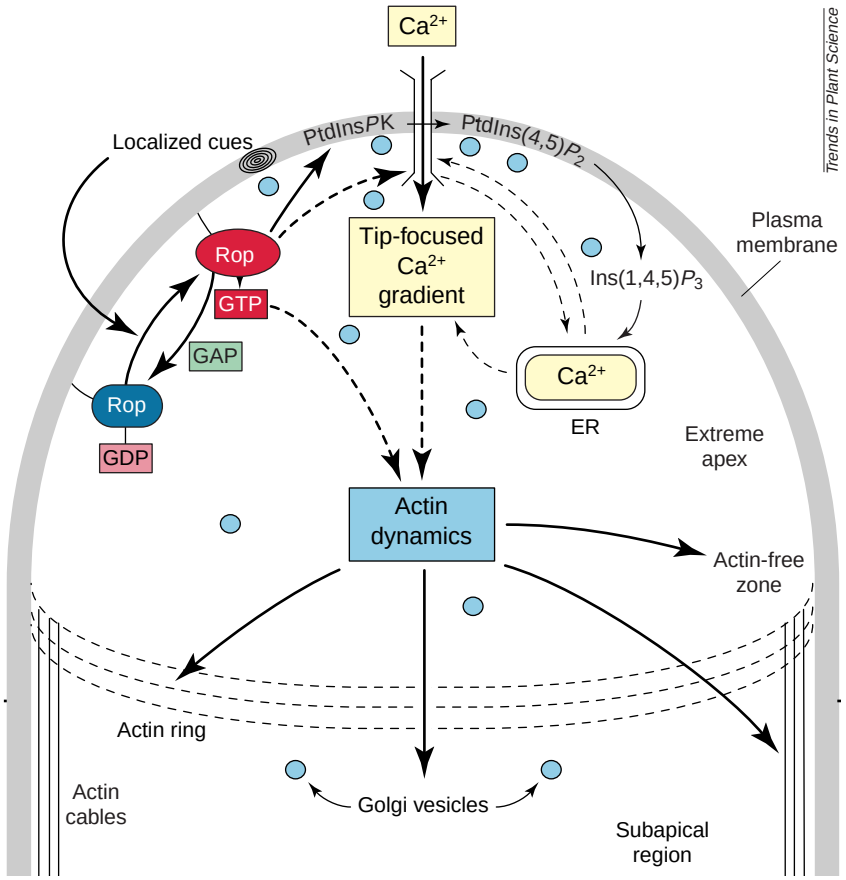

Fig. 5. A Rop-mediated signaling network controls pollen-tube-tip growth. The available data indicate that a Rop-dependent pathway is central to the control of pollen-tube growth. Components of the pathway include one or more Rop GTPases, a Rop-GTPase-activating protein (GAP) and a putative Rop effector (PtdIns*P*-kinase) and its product (PtdIns(4,5)P_2). The localized Ca^{2+} signaling is probably downstream of this pathway, and might involve both Ca^{2+} release from endoplasmic reticulum (ER) stores and tip-localized Ca^{2+} entry. The Rop-dependent pathway might also regulate actin dynamics directly or indirectly (the actin ring, and actin cables) and the formation of the actin-free zone at the tip. Arrows with a bold solid line indicate steps suggested by existing data, whereas arrows with a non-bold solid line or a broken line indicate steps that are supported by some experimental evidence or are speculative, respectively. Abbreviations: Ins(1,4,5)P_3, inositol triphosphate; PtdIns*P*K, phosphatidylinositol monophosphate kinase; PtdIns(4,5)P_2, phosphatydylinositol-4,5-bisphosphate.

one Rop effector is a phosphatidylinositol monophosphate kinase (PtdIns*P* kinase).

Even though which PtdIns*P* kinase (PtdIns5*P* 4-kinase or PtdIns4*P* 5-kinase) is associated with Rop and whether this association involves a direct interaction remain to be determined, this PtdIns*P*-kinase is a probable Rop-signaling component that links Rop with Ca^{2+} signaling and/or actin dynamics. PtdIns(4,5)P_2 generated by the Rho effector PtdIns4*P* 5-kinase promotes the assembly of the focal adhesion complex and the activation of ezrin/radixin/moesin (ERM)-type actin-binding proteins in mammalian cells[38]. The pollen PtdIns*P*-kinase might have a similar role but this does not seem to be consistent with the specific accumulation of PtdIns(4,5)P_2 at the tip of pollen tubes, as suggested by PH–GFP localization[23]. Alternatively, the tip-localized PtdIns(4,5)P_2 could participate in the formation of the AFZ by activating actin-depolymerizing factors such as profilins and gelsolin, whose activities are regulated by PtdIns(4,5)P_2 in mammalian cells.

Another likely role for the pollen PtdIns*P*-kinase and the localized PtdIns(4,5)P_2 is regulating the establishment of the Ca^{2+} gradient. PtdIns(4,5)P_2 is hydrolyzed by phospholipase C to Ins(1,4,5)P_3, which is an agonist responsible for Ca^{2+} release from intracellular stores. There is evidence that Ins(1,4,5)P_3 might play a role in the accumulation of cytosolic Ca^{2+} at the tip of pollen tubes and thus in directing pollen-tube growth[28]. The Ins(1,4,5)P_3-triggered release of Ca^{2+} could then activate a putative capacitative PM Ca^{2+} channel to replenish Ca^{2+} stores or to contribute to the formation of the Ca^{2+} gradient. Such agonist-activated Ca^{2+} channels are ubiquitous in animals[39] and might also exist in plant cells[28]. A role for Ins(1,4,5)P_3 in the formation of the Ca^{2+} gradient is also consistent with the observed lag of Ca^{2+} influx behind the Ca^{2+} gradient and the presence of endoplasmic reticulum at the tip in pollen tubes[40].This Rop–Ins(1,4,5)P_3–Ca^{2+} model might be a key mechanism by which Rop regulates the Ca^{2+} gradient and influx (Fig. 5). However, this does not rule out the possibility of other distinct Rop effectors that might regulate the tip-localized Ca^{2+} influx more directly. A tip-localized protein-kinase-C-like activity in pollen tubes[41] might be a potential Rop target. In addition, Rop-modulated localized accumulation of PtdIns(4,5)P_2 might be involved in both Ca^{2+} signaling and the regulation of the actin status at the tip of pollen tubes.

Conclusions

Recent studies have provided compelling evidence for a Rop-dependent signaling network that controls polar growth in pollen tubes (Fig. 5). Pollen-tube growth probably involves a tip-localized growth cue that activates one or more Rop GTPases at the site of growth. On the one hand, Rop appears to modulate the recruitment of Rop to the tip of pollen tubes, forming a localization–activation positive-feedback loop. On the other hand, Rop probably regulates the formation of the tip-focused Ca^{2+} gradient and possibly also the organization of actin in the apex. Tip-localized Ca^{2+} probably activates exocytosis, leading to tip growth, and also appears to regulate a negative-feedback loop when cytosolic Ca^{2+} reaches a threshold level. These two Rop-dependent feedback loops could be central to the behavior of growing pollen tubes (i.e. pusatile polar growth)[31,40,42]. The positive-feedback loop might enable the cell rapidly to activate Ca^{2+}-dependent localized exocytosis rapidly, whereas the negative- feedback loop might allow the coordination of exocytosis with cell-wall remodeling[40].

The demonstration of a central role for Rop in the control of pollen-tube growth has revealed only the ‘tip of the iceberg’ and further investigation is needed to determine the molecular mechanism for polar growth of pollen tubes. Already this has led to the identification of a putative Rop effector (PtdIns*P*-kinase) and a negative Rop regulator (RopGAP). Clearly, the next few years will be an exciting time, as the answers to many important questions about Rop-mediated polar growth become clear. For example:

- What is the tip-localized cue that activates Rop? How does this cue activate Rop?

- What factors control the localization of Rop to the tip and how are they linked to Rop signaling?
- Does Rop regulate multiple effectors that control the formation of the Ca^{2+} gradient and actin organization, and how do Rop effector(s) modulate these processes?
- Is the Rop-dependent signaling also involved in pollen-tube-growth guidance in the pistil?

Elucidating the Rop-dependent signaling to polar growth in the 'single cell' pollen model system probably has a broader implication in the understanding of cell morphogenesis, cell-polarity control and development in plants. Preliminary results indicate that Rop also controls morphogenesis and polar growth in other cell types, including root hairs (H. Li *et al*., unpublished). This suggests that Rop might be a common switch in the signaling to cell morphogenesis in plants. Further studies will be necessary to determine whether the Rop-dependent signaling pathway in pollen tubes can be a paradigm for understanding cell morphogenesis and cell-polarity formation determined by other specific developmental cues in plants.

Acknowledgements

We thank Chris Staiger and Vernonica E. Franklin-Tong for stimulating discussions. Our work was supported by National Science Foundation (No. MCB-9724047).

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