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Rapid tip growth: Insights from pollen tubes

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Abstract

Pollen tubes extend rapidly in an oscillatory manner by the extreme form of polarized growth, tip growth, and provide an exciting system for studying the spatiotemporal control of polarized cell growth. The Rho-family ROP GTPase is a key signaling molecule in this growth control and is periodically activated at the apical plasma membrane to spatially define the apical growth region and temporally precede the burst of growth. The spatiotemporal dynamics of ROP GTPase is interconnected with actin dynamics and polar exocytosis that is required for tip-targeted membrane and wall expansion. Recent advances in the study of the mechanistic interlinks between ROP-centered signaling and spatiotemporal dynamics of cell membrane and wall remodeling will be discussed.

Keywords

ROP signaling; vesicular trafficking; cell wall mechanics; actin dynamics; oscillation

1. Introduction

As a male gametophyte, pollen germinates on the flower stigma into a single pollen tube, which navigates through several female tissues to reach the ovule (Figure. 1A). This guided tip growth, which is remarkably similar to neuronal guidance, involves signal-mediated attraction to female tissues as well as repulsion and competition among pollen tubes [1–3]. In addition to the fascinating biology, the pollen tube provides an excellent experimental system. Unlike most plant cells, which dedifferentiate and lose polarity upon *in vitro* culture, cultured pollen maintains its polarity and developmental identity. *In vitro* pollen tubes grow synchronously and uniformly, and exhibit highly polarized cytoplasmic organization with the apical region packed with exocytic vesicles (Figure. 1B). Although each pollen tube contains two sperm cells embedded in the vegetative cytoplasm, the sperm genome does not seem to contribute to the genetic control of pollen tube growth. Instead, it is controlled by the haploid genome carried by the vegetative nucleus, and thus lethal mutations affecting tube growth can be maintained in heterozygous plants, which facilitates genetic analysis of essential genes involved in polarity and growth control. These advantages, combined with

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the ease with which live imaging is performed with pollen tubes, make it one of the most exciting systems for the studies of polarity and tip growth.

To efficiently reach their target in the ovary, pollen tubes elongate at an astonishing rate (up to 1 cm/hr) to an extraordinary length (e.g., the length of corn silk) by polarized tip growth, which is strictly dependent on polar exocytosis that delivers cell membrane and wall materials to the growing tip as in other tip growing systems [4–8]. An intriguing question is how pollen tubes design their structural and molecular machineries to achieve such rapid polar growth. At the sub-cellular level, pollen tube growth requires a highly polarized cytoplasmic organization [5, 6]. As the pollen tube grows, periodic cross-wall callose deposition isolates the pollen protoplast, which contains the biosynthetic machinery and the male germ unit, in the tip region of the elongating tube. This process is analogous to septum formation in fungal hyphae. The tip region displays four distinct zonings: an apical zone essentially packed with exocytic vesicles accumulated as a typical V shape to facilitate massive tip-targeted exocytosis, a subapical organelle-rich zone, a nuclear zone, and a vacuolated zone that may extend toward the grain [4]. Cytoplasmic streaming drives organelles moving rapidly back and forth along the main axis of the pollen tube in a reverse fountain pattern, which maintains the distribution of membranous structures and releases exocytic vesicles to the apical zone [9]. A complete picture of the cytoskeletal elements and dynamics that regulate the polar organization of the tube cytoplasm and targeted exocytosis is emerging [7, 9–15]. Due to a high global turgor pressure, the exocytosis-based membrane and wall extension needs to be coupled with the spatiotemporal regulation of cell wall mechanics. Both experimental and computational approaches have recently provided important insights into the cell wall mechanics during pollen tip growth.

At the molecular level, recent studies have uncovered a Rho GTPase-based self-organizing signaling network that controls tip growth in pollen tubes *via* its inter-connection with the cytoskeletal elements and the polarized exocytosis [13, 16–20]. Several excellent recent reviews provide important insights into the molecular mechanisms under pollen tube tip growth [6–8, 21–24]. Our current review will focus on the latest advances in the structural basis of this process and its interface with the Rho GTPase-based signaling network. Emphasis will be given to the comparison and contrast of the mechanisms for tip growth of pollen tubes with those of other systems.

2. The structural system: Roles in structure and regulation

2.1. The cytoskeleton

Pollen tubes contain two major cytoskeletal elements, microtubules (MTs) and actin microfilaments (F-actin), which are highly organized and dynamic through their interaction with various actin-binding proteins and microtubule-associated proteins [22, 25–27]. MTs are involved in the organization of spitzenköper (the exocytosis organizing center) and organelle movement and regulate the efficiency of tip growth but not essential for this growth [28–30]. In contrast, F-actin structures are quintessential for tip growth in pollen tubes. Multiple forms of F-actin in different regions of pollen tube support its pivotal role for tip growth. Abundant long actin cables axially aligned in the shank provide the main tracks for movement of organelles and vesicles and regulate cytoplasmic streaming [9, 10]. The formin 3 (FH3) actin nucleation factor is responsible for the formation of these actin cables [15]. RNAi-mediated *FH3* down-regulation abolished the actin cables and altered cytoplasmic streaming pattern [15]. *FH3RNAi* tubes were much shorter and wider than wild type tubes [15], suggesting that the actin cables play an important role in tip growth. Although the mechanism by which the axial actin cables regulate tip growth is unclear, cables-dependent cytoplasmic streaming may rapidly bring exocytic vesicles to the subapical zone. In this zone, a collection of shorter and thinner actin cables constitutes a

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collar, ring- or funnel-like F-actin structure. This highly dynamic subapical F-actin structure is assembled by the tip-located, cell membrane-anchored formin 5 (FH5) in pollen tubes [14]. *FH5RNAi* pollen tubes were devoid of the subapical F-actin and exhibited abnormal twists and turns [11, 14]. Thus the subapical F-actin apparently maintains the direction of tip growth. It was proposed that the subapical F-actin participates in vesicular trafficking in the apical region [11, 14]. However, the vesicular trafficking mediated by the subapical F-actin alone cannot satisfactorily explain how exocytic vesicles accumulate in the apical zone in a V-shape with the highest density of vesicles in the cortex of the extreme apex (Figure. 1B). The subapical F-actin could capture vesicles released from the axial actin cables and then transport them to the apical zone. In the apical zone, a population of fine and less abundant microfilaments is present in the extreme apex [11, 31]. Evidence suggests these fine F-actin filaments promote the accumulation of exocytic vesicles to the extreme apex in a V-shape pattern [13]. The dynamic apical F-actin is regulated by Rho-related GTPase of plants (ROP) (see below) [11–13, 31], but the nature of the nucleation factor responsible for the assembly of this F-actin remains unknown.

2.2. Exocytosis and endocytosis

The rapid and continuous tip growth has to rely on efficient and ample supply of cell wall components, membrane materials, enzymes, and signaling molecules, which is dependent on polarized exocytosis. The quintessential role of exocytosis in pollen tube tip growth has been inferred from the massive apical accumulation of vesicles and the cessation of tip growth by treatments with brefeldin A and chemicals that disrupt F-actin [32, 33]. Tobacco and Arabidopsis homologs of Rab11 are associated with vesicles accumulated in the extreme apex [34, 35]. Subsequently, the octameric exocyst complex will facilitate the exocytic vesicles targeting and tethering to the PM [36]. In yeast, it is proposed that six of the eight exocyst subunits (Sec5p, Sec6p, Sec8p, Sec10p, Sec15p and Exo84p) ride the exocytic vesicle along actin cables to exocytic sites [37], while the other two components Sec3p and Exo70p bind to plasma membrane (PM) localized Rho1-GTP and Rho3-GTP respectively and define the location of exocytic sites [38, 39]. Thus, the assembly of the eight subunits of exocyst determines where and when vesicles are tethered in preparation for fusion [40]. Mutations of the homologs of exocyst subunits, such as SEC3, SEC8 and Exo70, in Arabidopsis result in defective root hairs or pollen tube growth [41–43]. Moreover, Sec3 is shown to interact with ROP effector, ICR1 [44], suggesting that exocyst plays crucial roles in polar tip growth through integration with ROP regulation of polar exocytosis.

Two different views of vesicular trafficking in pollen tube tips have been proposed. Recently it was proposed that exocytosis occurs at the shoulder of the tip while the apical vesicles are endocytic vesicles [45, 46]. This contrasts with the long-standing prevalent model, which proposes that exocytosis occurs at the extreme apex, whereas endocytosis occurs preferentially in the subapical area [47–49]. The localization of exocyst to the tip of pollen tubes supports the prevalent model [42]. To definitely distinguish these two models, a method for direct measurements of exocytic activity in the tip of pollen tubes is needed. Recently a novel strategy involving fluorescence recovery after photobleaching (FRAP) of receptor-like kinase (RLK)-GFP, whose targeting to the PM completely depends on exocytosis, was developed to visualize exocytosis in pollen tube tips and led to the conclusion that exocytosis of the RLK-GFP tracer protein was indeed restricted to the PM apex with a tip-high gradient, which corresponds to the gradient of active ROP1 that has been shown to promote the tip-targeted exocytosis [13]. The polarized exocytosis is dependent upon the dynamics of the apical F-actin that is regulated by the ROP1 Rho-family GTPase *via* two counteracting RIC4 and RIC3 downstream pathways that promote actin assembly and disassembly, respectively [11, 13, 31].

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By monitoring the changes in the amount of exocytic wall material and the surface appearance of a direct exocytosis marker, pectin methylesterase (PME), it was shown that exocytosis in pollen tubes oscillates temporally and serves as key factor in the initiation and regulation of oscillatory pollen tube growth [50]. Evidence suggests that exocytosis delivers to the apical PM PRK2 receptor kinase that recruits and activates ROP1 upstream activators, RopGEFs, *via* phosphorylation regulation to release the C-terminal inhibition of RopGEFs [51–53]. Therefore, exocytosis participates in the positive feedback activation of ROP1 and might create an autocrine signaling mechanism underlying the speedy establishment and regeneration of the active ROP1 cap. Moreover, exocytosis also participates in the negative feedback loop to restrict the positive feedback-based lateral amplification of ROP1 activation to the tip growth domain through targeting ROP1 negative regulators, such as REN1 RhoGAP, to the tube apical PM [17–19]. In addition to the spatiotemporal regulation of the dynamic apical cap of ROP1, exocytosis might also regulate cell wall mechanics (see below).

Exocytosis in growing pollen tubes is balanced by the retrieval of excess PM and wall materials and associated signaling molecules by endocytosis [54]. Clathrin-dependent internalization is the predominant endocytic system in plants and is required for the generation of cell polarity [55]. Clathrin-dependent endocytosis occurs preferentially in the shoulder (or subapical) area of the pollen tube tip [47, 56], which also coincides with the localization of the subapical F-actin (see above). Thus it would be reasonable to speculate that rapid exocytosis that occurs at the extreme apex is coordinated with clathrin-dependent endocytosis in the subapical area to maintain the identity of the tube apex, and that endocytic vesicles are transported *via* the subapical F-actin to the apex for recycling. This notion is supported by several observations. First, FM dyes that stain endocytic vesicles accumulate as the canonical V-shaped pattern in the tip as exocytic vesicles [48, 56]. Second, inhibition of clathrin-dependent endocytosis disrupted canonical V-shaped fluorescence in the tip region and uptake of negatively charged nanogold [57]. Third, the depletion of the subapical F-actin by FH5RNAi also leads to the collapse of the V-shaped vesicle accumulation [14]. Interestingly, clathrin-dependent endocytosis also occurs in the subapical area of fungal hyphae [58, 59] implying that the complementary apical exocytosis and subapical endocytosis may be a conserved design principle underlying rapid tip growth.

The machinery for clathrin-dependent endocytosis appears to be conserved [56]. For example, phosphatidylinositol 4,5-bisphosphate (PIP_2) localized in the inner lea et of the PM is known to promote the early stages of clathrin-dependent endocytosis in yeast and animal cells [60], and has also been shown to do so in pollen tubes [56]. Earlier studies in yeast suggest that the hydrolysis of PIP_2 to phosphatidylinositol 4-phosphate (PI4P) is important for the late stage of endocytosis [60]. Our work in pollen tubes suggests that it is actually the hydrolytic product PI4P that is required for the completion of clathrin-dependent endocytosis [56].

How does the pollen tube control the site of clathrin-dependent endocytosis in the subapical area? Given PIP_2's role in initiating the recruitment of clathrin coat onto the inner leaflet of the PM site for endocytosis, it would be important to know the mechanism for the polar PIP_2 distribution. Consistent with the site of endocytosis, in growing pollen tube PIP_2 is preferentially compartmentalized in the subapical area of the PM through the subapical distribution of PI4P-5 kinases that generate PIP_2 from PI4P, which is distributed throughout the whole apical region [56, 61, 62]. In non-growing tubes, PIP_2 appears to be compartmentalized in the apical PM [63], implying a potential role for the subapical endocytosis in the regulation of tip growth. Thus the mechanism underlying PI4P5K localization and activation is anticipated to be the signaling events governing the spatial control of clathrin-dependent endocytosis. Interestingly, a ROP/Rac GTPase was reported to

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be physically associated with a PIP5K activity in tobacco pollen tubes, although direct evidence for ROP/Rac regulation of clathrin-dependent endocytosis is missing [63]. In the budding yeast, clathrin-dependent endocytosis is regulated by the Cdc42 member of the Rho GTPase family *via* its effect on actin dynamics [64]. Rho-family GTPase regulation of PIP signaling has also been reported in other systems [65, 66]. Thus, Rho GTPase signaling may provide a conserved mechanism for the modulation of endocytosis during polarized cell growth.

2.3. Cell wall composition and mechanics

Because of high turgor pressure in pollen tubes as in other plant cells, biosynthesis-based growth *via* exocytic delivery of cell wall materials has to be coordinated with the mechanics of the cell wall that facilitate localized cell expansion at the tip. It was reported that the growing region of pollen tubes spatially coincides with a region of lower stiffness and that the growth rate oscillations in pollen tubes are correlated with spatially confined dynamic changes in the mechanical properties of the apical cell wall [67–69]. Mathematical simulations of cell wall mechanics combined with experimental manipulation of cell wall composition provide an important approach for understanding the complex tip growth process not only in pollen tube but also other tip-growing walled cells, such as root hairs and fungal hyphae [70, 71]. Given that globally acting turgor pressure drives a walled cell to expand in the wall region that is “soft” or extensible [72], the spatiotemporal regulation of cell wall mechanics learned from pollen tubes may provide insights into the mechanisms of shape formation and polar growth for all walled cells. The apical exocytosis could regulate the wall mechanics through its targeting of methylated soft pectin and PME inhibitors to the tip. Likewise the subapical endocytosis might also contribute to the wall mechanics by retrieving specific wall components or their modifiers, e.g., demethylated pectin [73, 74]. Therefore we speculate that intracellular signaling pathways leading the localized exocytosis and endocytosis may coordinate the spatiotemporal control of cell wall growth with that of cell wall mechanics.

3. The regulatory system: Self-organization and structural regulation

Pollen grains generate pollen tubes *in vitro* in the absence of external signals, suggesting that their tip growth is controlled by a self-organizing system. Studies over the last decade have demonstrated the existence of a self organizing signaling network, which is centered on a tip-localized Rho GTPase (ROP1) and tip-focused calcium gradients and their interactions with the actin cytoskeleton and vesicular trafficking [11, 13, 16–18, 31, 56, 75–79].

3.1. ROPs

As in all other polar growth systems, Rho-family GTPases play an essential role in the control of pollen tube growth. All plant Rho-like GTPases fall into a single subfamily named ROP, which was first identified from pea [80]. Arabidopsis has 11 ROPs, six (ROP1 to ROP6) of which are known to regulate cell polarity formation and polar cell growth [6, 81–84]. ROP1 protein exhibits polar localization to the apical domain of the PM of pollen tubes [76]. Active ROP1 is distributed as an apical cap, which corresponds to the site of exocytosis [11, 12, 77, 85]. Blocking ROP1 signaling by expressing DN-ROP1 or by microinjecting anti-ROP1 antibody inhibited pollen-tube tip growth, whereas expression of a CA-ROP1 and overexpression of WT-ROP1 induced a dramatically enlarged active ROP1 cap and growth depolarization [63, 77, 86–88]. These observations led to the proposal that the apical cap of active ROP1 defines the tip growth region, termed tip growth domain, and activates tip growth, and that efficient tube elongation requires an optimum level of apical ROP1 activity that is dynamically self-organized to maintain an optimal cap size [18, 63, 77, 86–88].

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Apical ROP1 signaling must be regulated by a self-organizing mechanism because spontaneous polar growth occurs *in vitro* in the absence of external spatial cues. To understand the spatial regulation of ROP1, a GFP-based reporter, ΔRIC4-GFP, in which the CRIB (Cdc42/Rac-interactive binding) domain of the RIC4 ROP1 effector is fused with GFP, was developed to monitor the distribution of active ROP1 [16]. ΔRIC4-GFP was localized to the PM as an apical cap in a ROP1 activation-dependent manner, and ΔRIC4-GFP imaging reveals an oscillation of ROP1 activity along with the growth oscillation of the pollen tubes [16, 18]. The maximal cap of active ROP1 defines the tip growth domain [16, 18]. The apical ROP1 cap is generated by lateral propagation of a localized ROP activity and is regulated by downstream events including tip F-actin and Ca^{2+} [16–18, 31]. The stabilization of tip F-actin causes dramatic enlargement of the apical cap, generating a ballooned tip, as does knocking out the REN1 RhoGAP [16, 17, 19, 31]. Elevation of Ca^{2+} or actin depolymerization reduces the apical ROP1 activity [16, 18, 19, 31]. Thus tip F-actin and Ca^{2+} feedback activate and inhibit ROP1, respectively [19].

Exocytosis in pollen tubes is tightly regulated in time and space. In oscillating pollen tube, polar exocytosis oscillates in a phase just slightly behind ROP1 activity, but well ahead of tip growth [13]. Tip F-actin oscillates in the same phase with ROP1 activity and is required for exocytosis [13, 16]. The effect of tip F-actin on the positive ROP1 feedback regulation may be due to actin-mediated exocytosis by targeting of ROP1 activators such as PRK2 receptor kinase that could activate ROP1 upstream activators RopGEFs, to the tip [51–53]. In addition, ROP1 interacts with its effecter RIP1/ICR1, which subsequently targets to exocyst subunit SEC3, to regulate polarized exocytosis [20, 44, 89]. This exocytosis-dependent positive feedback loop coupled with diffusion may be responsible for the formation and regeneration of the apical ROP1 cap as well as the lateral propagation of active ROP1 cap (Figure. 2) [18].

To maintain an optimal apical ROP1 cap and generate the apical ROP1 activity oscillation required for efficient pollen-tube elongation, a negative feedback mechanism is required to limit the positive feedback-based lateral propagation of apical ROP1 activity [18]. A global inhibition mechanism governed by ROP1 negative regulators, RopGAPs (Rop GTPase activating proteins) and RhoGDIs (Rho guanine nucleotide dissociation inhibitors), is shown to restrict the lateral amplification once the apical ROP1 cap reaches a certain size, thus preventing excess lateral propagation and finally terminating one cycle of ROP1 activity increase (Figure. 4) [18]. A screen for mutations that enhance ROP1-overexpression-induced depolarization of pollen tube growth identified a novel RhoGAP, termed REN1, which was demonstrated to play a primary role in restricting active ROP1 to the pollen tube tip, by globally inhibiting ROP1 GTPase at the tube apex [17]. REN1 localizes to the apical cap and exocytic vesicles in the pollen tube tip, implying that exocytosis is also involved in the negative feedback inhibition of ROP1 activity [17]. In addition to exocytosis, Ca^{2+} signal is also involved in the negative feedback inhibition of ROP1 to balance the positive feedback of ROP1 activity and to generate the oscillation of the apical ROP1 activity [19] (see below).

3.2. Calcium gradients

Calcium has long been recognized as a critical intracellular signal regulating tip growth in pollen tubes. Growing pollen tubes display a tip-focused Ca^{2+} gradient [79, 90], which oscillates with the same periodicity as pollen tube growth, but the calcium pulses slightly lag behind those of growth rates [91]. The dissipation of the gradients using Ca^{2+} channel blockers or applying extra Ca^{2+} into growth media leads to growth arrest [79]. Manipulations that alter the focal point of the gradients reorient the direction of pollen tube growth [92]. These observations suggest that calcium gradients are essential for pollen tube growth and regulate tip growth polarity, a role that parallels that of ROP1. Indeed several

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recent studies suggest that ROP1 and calcium are interwoven in the control of tip growth (Figures 2 and 3). It was shown that apical ROP1 activity oscillates ahead of growth rate and the apical calcium [16]. Tip-localized ROP1 GTPase activates downstream effector RIC3, which mediates the formation of the calcium gradients [31]. By promoting F-actin depolymerization, calcium regulates the F-actin dynamics at the tip, which in turn promotes exocytosis (Figure. 3) [13, 31]. This role for calcium is also supported by the observation that low Ca^{2+} concentrations caused an excessive quantity of vesicles to accumulate at the tip, while high Ca^{2+} concentrations accelerates vesicle fusion at the tip [93]. Calcium might also play a direct role in the regulation of exocytosis as in neuronal cells, although evidence for this is lacking.

Adding to the complexity of calcium-ROP1 interaction is the finding that increased rate of calcium accumulation actually suppresses ROP1 activity and balances the RIC4- and F-actin-dependent apical ROP1 activation [19]. Thus calcium functions in the negative feedback regulation of the apical ROP1 activity necessary for growth oscillation [19]. The complex roles of calcium and its interaction with ROP1 are consistent with the large number of calcium sensors expressed in pollen, including actin-binding proteins, calcium-dependent protein kinases, and calcineurin-interacting proteins [27, 75, 94, 95]. It will be important to elucidate the functions of these calcium sensors in the regulation of tip growth in pollen tubes and their relationship with ROP1 signaling. Calcium also regulates tip growth in root hairs of plants and likely in other rapid tip growth systems such as fungal hyphae [96, 97]. Thus, the Rho-calcium interplay might provide a universal mechanism underpinning rapid tip growth.

The mechanisms regulating the generation and the oscillation of the Ca^{2+} gradients are complex as well, probably involving multiple pathways and transport systems. The ROP1 effector RIC3 is proposed to promote calcium influxes through the plasma membrane [31], but the underlying mechanism is unknown. The cyclic nucleotide-gated channel (CNGC) 18 localized to the PM at the growing tip of pollen tube is shown to function as a Ca^{2+}-permeable channel and regulate pollen tube growth, but it seems not be the target of RIC3 signaling, even though its tip PM expression pattern is affected by ROP1 signaling [98, 99]. An important breakthrough is the newly uncovered function of glutamate receptor-like (GLR) family in the generation of Ca^{2+} gradients and oscillations through facilitating Ca^{2+} influx across the apical PM [100]. Regulation of intracellular calcium stores and PM-localized calcium pumps is expected to participate in Ca^{2+} gradient formation and oscillations, but their connection to ROP1 signaling has yet to be explored.

3.3. ROS

NADPH oxidase (NOX)-dependent reactive oxygen species (ROS), which is localized in the pollen tube tip, is also implicated in pollen tube growth [101], and is likely linked to ROP1 signaling. NOX directly interacts with and appears to be a ROP/Rac effector in cultured rice cells and Arabidopsis during stress responses [102, 103]. ROS' function and linkage with ROP signaling has been well studied with regards to tip growth in root hair, a process that share many mechanistic similarities with pollen tubes. In root hair, its regulation of tip growth is tightly connected with calcium signal. ROS activates Ca^{2+} permeable channels required for generation of tip-focused Ca^{2+} gradient in root hairs [104]. Establishment and maintenance of tip growing site in root hair is regulated by ROS produced by RHD2, a PM localized NOX, and Ca^{2+} mediated positive feedback loop [105]. SCN1, a RhoGDI isoform spatially regulates RHD2-catalyzed production of ROS to hair tips, implying that ROP may play a pivotal role in the spatial regulation of ROS production [106]. In addition to spatial regulation, ROP controls ROS production through regulation of the enzyme activity of NOX in root hair. CA-rop2 expression promotes ROS production in root hairs in a RHD2-dependent manner, whereas DN-rop2 decreases ROS formation [102, 107]. ROPs regulate

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ROS production most likely through both direct activation of NOX and ROP-dependent Ca^{2+}, which in turn increases the enzyme activity of RHD2 [105]. ROP regulation of ROS may also occurs in pollen tubes [101]. Similarly Cdc42 has been shown to modulate tip-localized ROS production in fungal hyphae, which is required for hyphal morphgenesis and growth [108, 109]. Thus the Rho GTPase-ROS signaling module appears to provide a new universal mechanism regulating tip growth in various systems.

4. A working model for the interface between intracellular signaling and cellular structures

Based on the recent findings discussed above, we propose a working model for the mechanisms behind pollen tube tip growth that overarch the intracellular signaling pathways, cellular structures, and vesicular trafficking (Figures 2 and 3). ROP1 is locally activated in the PM to determine the site of exocytosis and activates multiple pathways leading to polar exocytosis [11, 12, 63, 76, 77, 85, 87]. The RIC4 pathway promotes F-actin assembly and induces the accumulation of exocytic vesicles to the tip, and promotes positive feedback loops to increase the area of active ROP1 probably by targeting ROP1 upstream components such as RopGEFs and PRK2 [13, 31, 51–53]. Positive feedbacks coupled with diffusion rapidly generate the apical cap of active ROP1 that defines the tip growth domain. Meanwhile, ROP1 also activates the RIC3–calcium pathway, which promotes the depolymerization of tip F-actin, allowing exocytic vesicles to tether on and fuse with the PM [13, 31]. The tethering is promoted by another potential ROP1 effector, RIP1/ICR1, which may regulate tip targeting of the SEC3 exocyst subunit [20, 44, 89]. Polarized exocytosis brings the REN1 RhoGAP to the apical PM [17]. The PM-localized REN1 may not be active until Ca^{2+} is further increased to a threshold level, which could then trigger REN1 activation to deactivate PM-localized active ROP1 [17, 19]. Thus the REN1-dependent global inhibition of ROP1 prevents excess ROP1 activation in the apical PM, and restricts the enlargement of the apical cap to the tip growth domain [18]. Following ROP1 inactivation, REN1 returns to the cytosol, thus allowing ROP1 activity to increase again. Consequently, tip F-actin and polar exocytosis oscillates in a phase just slightly behind ROP1 activity, but well ahead of tip growth, whereas the apical Ca^{2+} oscillation lags behind the growth rate [16, 18]. This interlinked positive and negative feedback loops explain the self-organization of the active ROP1 cap as well as the oscillation of the apical ROP1 activity. As more information becomes available, other signaling loops and pathways need to be integrated into this ROP1- and Ca^{2+}-based self-organizing framework. For example, ROP1 may also regulate a NOX-ROS-calcium signaling loop to boost calcium accumulation [105]. Other signaling factors such as actin-binding proteins (e.g., ADFs) [110], RopGAPs [111], PLC [112, 113] should also be incorporated. ROP signaling may also coordinate endocytosis and cell wall mechanics to drive rapid tip growth in pollen tubes.

5. Extracellular signals for directional and polarized tip growth

5.1. Potential signals for tip growth: An implication for autocrine signaling

Evidence suggests that the interaction between PRK2 and RopGEFs recruits RopGEFs to the PM, leading to the activation of ROP signaling [53]. PRK2 is required for pollen tube growth *in vitro* in the absence of exogenous signals, implying that PRK-based ROP activation is critical for pollen tube growth. As a trans-membrane cell surface receptor, PRK2 is expected to detect and transduce an extracellular signal(s) that is produced by pollen, constituting an autocrine signaling system. Potential PRK2 ligands include LTP5 and LAT52 [114–116]. LTP5 is a member of the lipid-transfer protein family and is presumably secreted from pollen [116]. Interestingly, dominant-active mutation of LTP5 causes depolarized swollen tip [116], resembling the abnormal morphology of pollen tubes

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overexpressing ROP1 GTPase or its upstream activator RopGEFs or PRK2 [11, 51, 53, 77]. The physical interaction between pollen secreted extracellular protein LAT52 and pollen receptor kinase LePRK2, suggests that ligand-receptor kinase system regulates pollen tube growth [114, 115].

5.2. Signals that direct tip growth

In vivo pollen tubes are precisely targeted to the ovule for sperm delivery, which is expected to require multiple guidance signals from the female tissues. To date, only two molecules, a cyanin-like small protein from lily stigma called chemocyanin [117] and a cysteine-rich small protein from Torenia synergids called LURE [118], have been implicated as guidance signals, and no receptors for any guidance signals have been identified. The RLK superfamily is the predominant receptors in plants, and is involved in sensing a wide range of signals including small molecules, peptides, and cell wall components. More than 100 RLKs are expressed in pollen, implying that they could perceive various guidance signals [119]. The pollen-expressed RLK, PRK2, interacts directly with RopGEFs in the control of polar tip growth in pollen tubes [53], and the localization of active ROP1 in the apical PM region predicts the future direction of pollen tube turning [16, 18]. Therefore it is likely that possible guidance signal-sensing RLKs also interact with RopGEFs to activate ROP1 signaling; consequently modulating the spatial distribution of ROP1 signaling and directing pollen tube growth in response to the guidance signals. Identification of various guidance signals and their receptors and determination of their intracellular signaling pathways in pollen tubes are in the forefront of exploration of guided tip growth in pollen tubes, a phenomenon that is shared by other tip growing systems such as fungal hyphae and neuronal axon.

6. Conclusion

Knowledge of regulatory and structural mechanisms underlying polarized tip growth in pollen tubes have expanded and evolved over the past several decades in virtue of extensive studies. The advent of innovative tools (e.g., FRAP-based visualization of exocytosis in growing pollen tube [13]) and new approaches (e.g., screen for the *ren* mutations that enhance ROP1 overexpression-induced depolarization [17]) combined with mathematical simulations significantly advances the investigation of tip growth processes. Systems-based and quantitative approaches are needed to produce a comprehensive view of the pollen tube tip growth system with regards to what all major structural and regulatory components and circuitries are and how they are integrated to achieve rapid and directional tip growth. Pollen tubes use conserved mechanisms such as conserved Rho family GTPase signaling to coordinate F-actin dynamics and polar exocytosis leading to rapid tip growth as observed in root hairs, yeasts, fungal hyphae and neuronal cells. Elucidations of the regulatory principles that govern polarized pollen tube growth may help to enlighten the mechanisms underlying tip growth across eukaryotic kingdoms.

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Abbreviations

CA	constitutively active
CNGC	cyclic nucleotide-gated channel

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CRIB	Cdc42/Rac-interactive binding
DN	dominant negative
ER	endoplasmic reticulum
F-actin	actin laments
FH3	formin3
FH5	formin 5
FRAP	fluorescence recovery after photobleaching
GAP	GTPase activating protein
GDI	guanine nucleotide dissociation inhibitor
GEF	guanine nucleotide exchange factor
GLR	glutamate receptor-like
GTPase	guanosine triphosphatase
ICR	interactor of constitutive active ROPs
IP3	1,4,5-trisphosphate
MTs	microtubules
NOX	NADPH oxidase
PI	phosphatidylinositol
PM	plasma membrane
PME	pectin methylesterase
PIP_2	phosphatidylinositol 4,5-bisphosphate
PI4P	phosphatidylinositol 4-phosphate
PI4PK	phosphatidylinositol-4-phosphate kinase
PRK	pollen receptor kinase
RLK	receptor like kinase
RIC	ROP-interactive CRIB motif containing protein
RIP	ROP interactive partner
ROP	Rho-related GTPase of plants
ROS	reactive oxygen species

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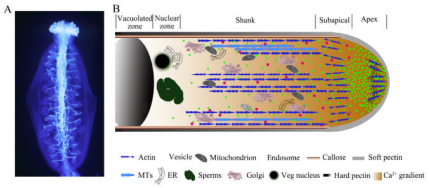

Figure 1. The pollen tube system: Directional and polarized cell growth

(A) Pollen tubes in the pistil, aniline blue staining of pollen tubes in the Arabidopsis pistil. (B) Schematic diagram of the internal zonation and structural elements of a growing pollen tube. The growing tube displays a tip-focused cytoplamic Ca^{2+} gradient and contains a single soft pectin apical wall and two layers shank wall, the inner sheath of callose and outer coating of hard pectin, which are non-plastic and able to resist turgor pressure. The apical clear zone is characterized by a V shaped accumulation of secretory vesicles that facilitate massive tip-targeted exocytosis. The subapical organelle-rich zone is followed by a nuclear and a vacuole zone. Microtubules (MTs) and long actin cables axially aligned in the shank and are excluded from the apical zone. A collar-like actin microfilaments (F-actin) structure is present in the subapical region and a population of fine and short F-actin is detected in the extreme apex.

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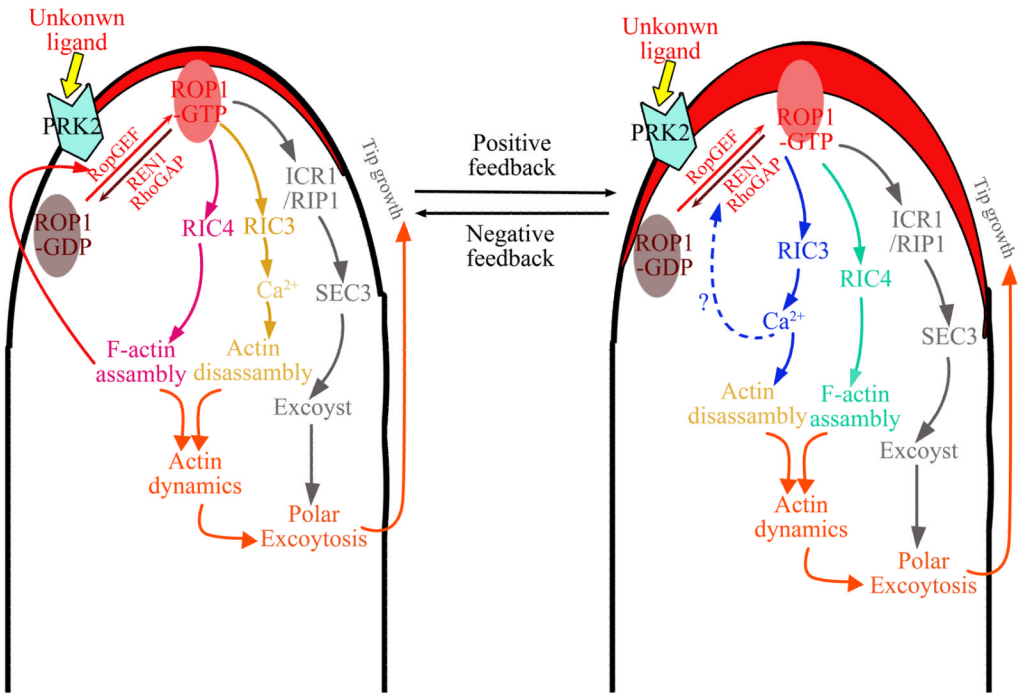

Figure 2. The ROP1 signaling network that control pollen tube tip growth

The network is composed of several pathways coordinately promoting tip-targeted exocytosis and positive and negative feedback loops, which may balance each other to maintain a certain size of the apical ROP1 cap that defines tip growth domain or may allow the oscillation of the ROP1 activity. ROP1 is locally activated in the PM to determine the site of exocytosis and activates multiple pathways leading to polar exocytosis. The RIC4 pathway promotes F-actin assembly and induces the accumulation of exocytic vesicles to the tip, and promotes positive feedback loops to increase the area of active ROP1 probably by targeting ROP1 upstream components such as RopGEFs and PRK2. Positive feedbacks coupled with diffusion rapidly generate the apical cap of active ROP1 that defines the tip growth domain. Meanwhile, ROP1 also activates the RIC3–calcium pathway. RIC3-dependent Ca^{2+} promotes tip F-actin disassembly and facilitates exocytosis. Polar exocytosis is also promoted by another likely ROP1 effector, RIP1/ICR1, which subsequently target recruits the SEC3 exocyst subunit that mediates the tethering of exocytic vesicles on the PM. Polarized exocytosis brings the REN1 RhoGAP to the apical PM, which deactivate PM-localized active ROP1. Thus the REN1-based negative-feedback globally inhibits ROP1, prevents excess ROP1 activation in the apical PM, and restricts the enlargement of the apical cap to the tip growth domain.

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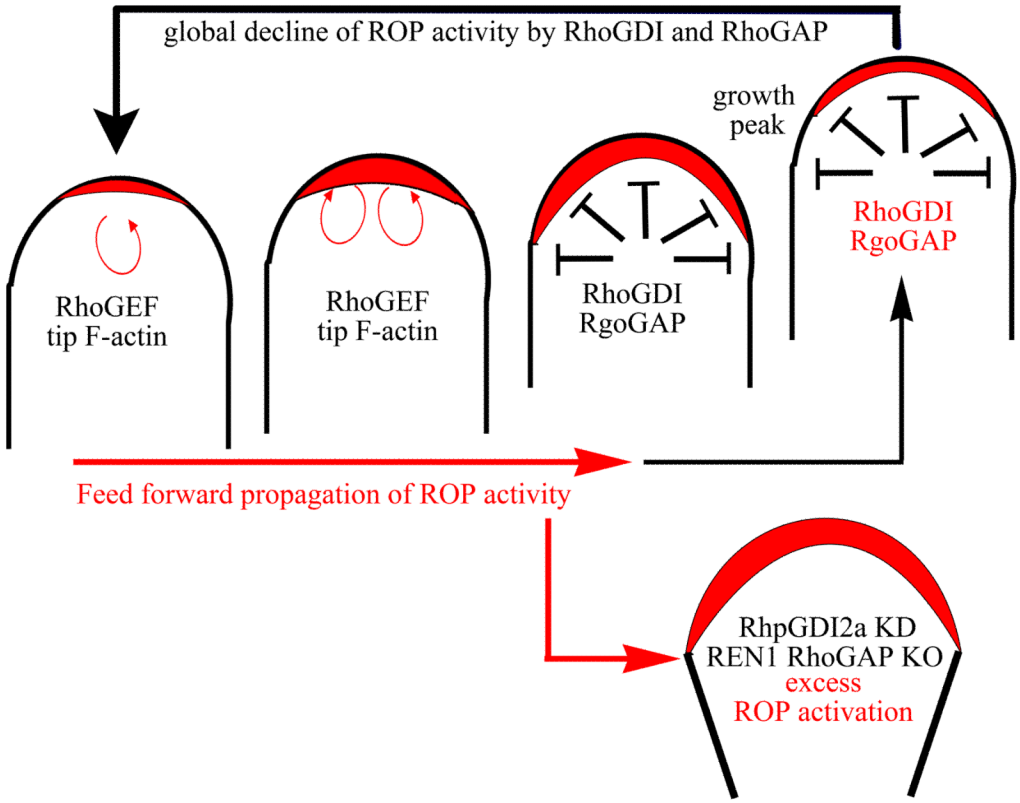

Figure 3. A model for the generation and maintenance of the apical cap of active ROP1 in growing pollen tubes

The localized ROP1 activity in the center of tube apical PM is amplified through a positive-feedback loop of ROP1 activation, such as recruitment of RhoGEF or other upstream ROP activator, which induces a rapid increase of local ROP1 activity and then its lateral propagation through the apex, generating the active ROP1 cap. The ROP1-mediated tip F-actin dynamic might also contribute to the rapid lateral propagation of ROP1 activity by facilitating the diffusion of ROP1 and its regulators in the PM through polarized exocytosis. RhoGAP and RhoGDI globally inhibit ROP1 in the apex, preventing excess lateral propagation and finally terminating one cycle of ROP1 activity increase. ROP1 activity starts to increase again, probably *via* positive feedback from the remnant of the previous active ROP1 cap. A tightly balanced interaction of ROP1 activation and inactivation might continuously generate the dynamic apical ROP1 activity for the continuous tip growth. When the balance is broken by loss of critical RhoGDI or RhoGAP activity (RhoGDI2a and REN1 RhoGAP in Arabidopsis pollen tube), ROP1 becomes activated, resulting in the depolarization of apical ROP1 cap and pollen-tube tip growth.