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ROP/RAC GTPase signaling

Zhenbiao Yang and Ying Fu

Center for Plant Cell Biology, Institute of Integrative Genome Biology, Department of Botany and Plant Sciences, University of California, Riverside, CA 92521, United States

Abstract

ROP/RAC GTPases are versatile signaling molecules in plants. Recent studies of ROP/RAC regulators and effectors have generated new insights into the molecular basis of their functional versatility. Significant progress has also been made in our understanding of the mechanism for the localization of ROP/RAC signaling to specific domains of the plasma membrane.

Introduction

ROP/RAC is the sole plant subfamily of the conserved Rho family of small GTPases [1,2]. These GTPases act as a simple binary switch (the ‘off’ GDP-bound and the ‘on’ GTP-bound states), capable of receiving a wide variety of inputs and accordingly generating a multitude of specific outputs. Consequently, Rho is known to orchestrate a great number of signaling networks across eukaryotic kingdoms. The large number of interacting partners and their variants is the key to the functional versatility of Rho GTPases in plants, as in other systems. Many of their interacting partners are able to associate with specific phospholipids, providing them with the capacity to localize their signaling in specific membrane domains. Several excellent reviews have covered different aspects of ROP/RAC signaling [3–6]. The current review will emphasize the latest work not analyzed in these reviews.

Emerging roles of ROP/RAC signaling

ROP signaling is known to regulate polarized cell growth, cell morphogenesis, hormone signaling, defense, and responses to oxygen deprivation [1,3,4,6]. Several new roles for ROP signaling are emerging. Tobacco Nt-Rac2 was reported to regulate auxin-induced assembly of nuclear protein bodies containing IAA/AUX proteins, the SCF-E3 ubiquitin ligase complex, the COP9 signalsome, and the 26S proteasome [7]. As a negative regulator of ABA responses, ROP10 regulates ABA induction of a subset of genes associated with stress responses [8]. ROP11/AtRac10 has been suggested to modulate endocytosis and membrane recycling [9]. ROP7 expression is associated with xylem differentiation, and several ROPs in brassica are associated with microspore development [10–12]. Knockdown of a ROP-interacting protein, ICR1, caused defect in root meristem, implying a possible role for ROP signaling in meristem maintenance [13••].

Message from new effectors: ROP/RAC coordination of multiple pathways

ROP2 promotes lobe outgrowth during the formation of the jigsaw-puzzle appearance of pavement cells by coordinately regulating lobe-associated actin microfilaments (F-actin) and indentation-associated microtubules (MTs) through two effectors, RIC4 and RIC1,

respectively [14,15]. The latest report from Yalovsky's group reveals a new ROP/RAC interacting protein termed ICR1 (interactor of constitutively active ROPs 1) in the mechanism behind the jigsaw-puzzle shape formation [13••]. ICR1 interacts with active but not with inactive ROPs. Knocking down ICR1 caused the jigsaw-puzzle pavement cells to become square-shaped. Thus ICR1 seems to act as a new effector of ROPs in the regulation of this process. Interestingly, yeast two-hybrid screens identified SEC3 homolog as an ICR1-interacting protein. In yeast, SEC3 is an effector of Cdc42 (a yeast relative of ROPs) that recruits exocyst (a protein complex that tethers exocytic vesicles to the exocytic site). Although a role for ICR1 in exocytosis remains to be determined, it is tempting to propose that ICR1 recruits exocyst to the lobe tip. In this case, lobe tip-localized active ROP2 would coordinate the ICR1 pathway with the two cytoskeletal pathways (Figure 1A). ROP2 suppression of the RIC1 pathway generates a MT-free lobe-forming region. ROP2 activation of the RIC4 pathway promotes the accumulation of fine F-actin in the growing lobe, which could target exocytic vesicles to the lobe tip. These vesicles may be tethered by the ICR1/exocyst complex to the lobe tip. At least one additional ROP2 downstream pathway would be expected to promote vesicle fusion to complete localized exocytosis. In its control of tip growth in pollen tubes, similarly coordinates multiple pathways. ROP1 activates two effectors, RIC4 and RIC3, that act coordinately to regulate the dynamics of tip F-actin [16]. Exocyst components play an important role in tip growth [17], and it would not be surprising if the ICR1-like pathway also exists in pollen tubes to activate vesicle tethering in coordination with the regulation of vesicle targeting and fusion by the F-actin dynamics (Lee *et al.* unpublished).

Work from Shimamoto's group suggests that OsRAC1 activates defense responses by coordinating multiple pathways as well. OsRAC1 activates the NADPH-mediated production of H₂O₂ by directly binding to RBOH protein (the catalytic subunit of NADPH oxidase) [18, 19••,20]. ROP regulation of NADPH-mediated H₂O₂ production is also involved in root hair tip growth and hypoxia tolerance [21,22]. An elegant study from the same group provides evidence that OsRAC1 activates another effector, cinnamoyl CoA reductase 1 (CCR1) [19••]. CCR1 catalyzes the first committed step of monolignol biosynthesis. Monolignols are cross-linked by H₂O₂ to form lignin polymers, which guard off pathogens' attack (Figure 1B). Additional OsRAC1-dependent pathways may also be involved in defense responses [23,24].

Novel RhoGEF: why bother reinventing the wheel?

One manifestation of functional versatility for Rho GTPase in animals is their activation by a large number of Rho guanine nucleotide exchange factors (GEFs). Surprisingly, only a single homolog of RhoGEF (SPK1) is present in Arabidopsis [25]. However, a family of plant-specific novel RhoGEFs named RopGEFs has been recently discovered [26,27••].

Overexpression (OX) analysis supports a role for RopGEF1 in the activation of ROP1 to control pollen tube growth [27••], though the function of the remaining 13 RopGEFs in Arabidopsis is unexplored. RopGEFs contain a central core catalytic domain (DUF315 domain or PRONE) that does not share any sequence similarity to any known RhoGEFs [26,27••,28•]. However, X-ray crystallographic analysis shows that the RopGEF-ROP-GDP tertiary structure is highly similar to other GEF-GTPase complexes and that RopGEF shares the universal mechanism by which GEF activates GDP dissociation from small GTPases [12,28•]. The N-terminal and C-terminal variable regions were shown to contain regulatory elements that auto-inhibit GEF activity [27••]. The C-terminal region of RopGEF1 binds to the catalytic domain [27], and this binding might prevent the RopGEF dimerization to form the active state [28•]. This auto-inhibition is also analogous to RhoGEF regulation in other systems [29].

Why do plants need a unique family of novel RopGEFs? The answer might lie in a potential connection to receptor-like kinases (RLKs). McCormick's group identified a tomato protein termed KPP that the intracellular kinase domain of a pollen-expressed LRR RLK, LePRK1

[30]. KPP is a homolog of RopGEF1 [26,27••]. KPP interacts with LePRK1 and is phosphorylated *in vivo* [30]. These observations support a potential functional interaction between RopGEF1/KPP and LePRK1. Interestingly, a stigma cysteine-rich protein (STIG1) interacts with the extracellular LRR domain of PRK1 and promotes pollen tubes [31]. Thus STIG1 may be a ligand for PRK1 in the regulation of ROP1-dependent pollen tube growth. ROP was reported to associate with the active but not inactive complex of the CLV1 RLK [32], raising the possibility that RLK–RopGEF interactions might have a broader role in relaying RLK signals. RLKs are the predominant cell surface receptors in plants (more than 400 RLKs in Arabidopsis alone) [33], as compared to G protein-coupled receptors and receptor tyrosine kinases that are predominant membrane receptors in animals. Given the versatility of ROP signaling, this potential RLK–RopGEF–ROP module could be a powerful invention of plant-specific signaling mechanisms.

A positive role for ROP/RAC-negative regulators

Excessive activation of ROPs causes cell depolarization [34–37], implying a critical role for their negative regulation in cell polarity control. Indeed, knocking out RhoGDI1 (guanine nucleotide dissociation inhibitor) induces a root hair phenotype similar to that caused by ROP2 OX [34,38]. RhoGDIs sequester Rho in the cytosol and prevent its activation by GEFs that occurs in the PM. Consequently, loss of RhoGDI1 function induced depolarized localization of ROP2 to the PM in root hair forming cells [38]. Consistent with its sequestering role, GFP-tagged Nt-RhoGDI2 was localized to the cytosol in tobacco pollen tubes, and Nt-Rac5 OX suppressed growth depolarization induced by Nt-Rac5 OX [39]. *RhoGDI2a RNAi* expression induced growth depolarization and delocalization of ROPs in Arabidopsis pollen (Hwang *et al.* submitted). Thus RhoGDIs play an essential role in cell polarity.

Another class of Rho-negative regulators is GTPase activating proteins (GAPs) that promote its intrinsic GTPase activity. An important role for RhoGAPs in ROP signaling has been implicated by much more dramatic phenotypic changes induced by expressing a constitutively active form of ROP compared to those induced by OX of a WT ROP [35,36]. When co-overexpressed in pollen, tobacco Nt-RopGAP3 suppressed growth depolarization induced by Nt-Rac5 OX [40•]. Similarly pollen-expressed Arabidopsis RopGAPs suppressed growth depolarization induced by ROP1 OX [41]. However, the role of Rop-GAPs in cell polarity needs to be confirmed by loss of function analyses. RhoGAPs are also important in ROP signaling to other processes, such as seedling tolerance to oxygen deprivation in Arabidopsis [21].

The value of subcellular location of ROP signaling

A most interesting aspect of Rho signaling is the significance of its subcellular localization, in many cases, to a specific domain of the PM. Several ROPs and ROP-interacting proteins are localized to specific PM domains, consistent with their roles in the control of cell polarity, polar growth, and cell morphogenesis [15,16,27••,34–36,42,43]. The localization of ROPs along the cortex of trichoblasts in the root epidermis determines the site of root hair formation [34]. In pollen tubes, the activation of ROP1 exhibits spatiotemporal dynamics required for polarized growth and growth oscillation [44••]. The development of a live ROP1 activity marker, GFP-RIC4ΔC, allows the visualization of the spatiotemporal changes in ROP1 activity [44••]. RIC4ΔC, in which the C-terminal effector domain is deleted, binds specifically active but not inactive ROP1, as did WT RIC4, a ROP1 effector [16,44••]. GFP-RIC4ΔC localization to the PM apex as an apical cap marks active ROP1 in pollen [44••]. The apical ROP1 activity exhibited oscillation during pollen tube growth oscillation. Increase in the apical ROP1 activity temporally precedes an increase in pollen tube growth rates, and its position of localization spatially predicts the new direction of pollen tube growth [44]. Disrupting the dynamics of the

apical ROP1 activity by RIC4 OX induces growth depolarization and abolishes growth oscillation, suggesting that the dynamics of the apical ROP1 activity couples the spatial and temporal control of tip growth.

How to achieve the localization and dynamics of ROP signaling in a specific PM domain? A study from Grebe's group suggests that auxin gradient is required for ROP2 localization near the basal end of the trichoblast where a single root hair is initiated [45••]. Exogenous auxin induced a basal shift, but mutations in AXR2 (auxin-resistant 2) or NPA and BFA treatments caused an apical shift in ROP2 localization and the formation of multiple hairs, which is mimicked by ROP2 OX and loss of RhoGDI1 function [34,38]. Thus auxin may regulate ROP2 activity and the position of ROP2 localization along the trichoblast to determine root hair initiation sites [45••]. Earlier work from Alice Cheung's group suggests that ROPs participate in auxin signaling to IAA/AUX protein degradation [7,46]. Other studies also implicate ROP in regulation of auxin transport. ROP2 was reported to affect PIN2 localization [47]. Auxin regulates PIN localization to the PM by a positive feedback loop involving auxin inhibition of endocytosis that internalizes PINs [48]. CA-rop11 OX appears to inhibit endocytosis [9], raising the possibility that auxin could regulate PIN localization through its effect on ROP signaling. Clearly, future studies are needed to explore the exciting possible connection between auxin gradient, localized ROP signaling, and cell polarity.

ROP association with membranes requires the C-terminal modification by prenyl and/or acyl lipid groups. A recent study from Yalovsky's group suggests that acylation may regulate ROP signaling in specific PM domains [49•]. GDP-bound and GTP-bound forms of ROP6 were partitioned into Triton X-100-soluble and Triton X-100-insoluble fractions, respectively. The former is only prenylated, but the latter is both prenylated and S-acylated. Lipid rafts are Triton-insoluble and contain acylated proteins. Thus it appears that only the active form of ROP6 is specifically associated with lipid rafts, which are usually found in membrane microdomains. ROPs might be activated in lipid rafts and stabilized there by acylation, forming a self-sustaining/self-organizing mechanism. A self-organizing Rho GTPase signaling mechanism that involves positive feedback loops is proposed to establish cell polarity in yeast and animal systems, and is also proposed to control cell polarity for tip growth in pollen tubes and root hairs [44••,50]. RIC4-dependent F-actin has been implicated in the feedback activation of ROP1 [16,44••]. F-actin targets exocytic vesicles to the tip (Lee *et al.* unpublished data), which may carry proteins or lipids that are important for ROP activation at the PM. A direct feedback loop could involve recruitment of ROP activators to the PM apex by active ROP1 or ROP1 effector. RopGEF1 can bind to active ROP1, suggesting that it might also be an effector of ROP1 [16]. When transiently expressed in tobacco pollen tubes, RopGEF1 and ROP1 mutually promoted their localization to the tube PM [16]. Thus, active ROP1 may recruit and stabilize RopGEF1 to the tube apex.

Positive feedback loops allow a rapid generation of a PM domain for localized ROP signaling, but need to be down regulated to restrict the ROP signaling domain. In the absence of inhibition, the ROP signaling domain would expand indefinitely, causing depolarization as seen with CA-rop expression [34-36]. There are two possible mechanisms for restricting ROP signaling to a localized PM domain: global and lateral inhibitions. In the former, down regulation of ROP activation would result in overall reduction of ROP signaling, preventing it from excessive lateral spreading. In the latter, down regulation is spatially restricted to the region flanking the domain of ROP activation. Kost's group showed that Nt-Rac5 (R69A) mutation, which abolishes Nt-RhoGDI2 binding, causes GFP-tagged Rac5 to be localized to the flank of the pollen tube PM in contrast to the tip localization of GFP-tagged wild type Nt-Rac5 [39]. It was proposed that Nt-RhoGDI2 preferentially removes Nt-Rac5 from the region flanking tip-localized Nt-Rac5 [39]. The same group also found that GFP-tagged Nt-RopGAP3 is preferentially localized the region flanking the pollen tube PM apex [40•]. These observations

are consistent with a role for both Nt-RhoGDI2 and Nt-RopGAP3 as lateral inhibitors for restricting ROP signaling to the apical domain of the PM in tobacco pollen tubes [39,40•]. Future studies using loss of function mutants and the localization of the native forms of these negative regulators will be necessary to further test this hypothesis.

Future prospects

Recent progress in the investigation of ROP/RAC interactors support the notion that ROPs/RACs are ‘master regulators’ in cellular signaling, orchestrating multiple pathways and navigating them in space and time. Much has yet to be learned about the roles of ROPs and their partners and how ROP signaling is linked to other known pathways. In particular, establishing a broad functional connection between RLKs and ROPs would be most exciting given how little is known about intracellular signaling mechanisms for the superfamily of RLKs in plants.

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Figure 1. ROP/RAC signaling coordinates multiple downstream pathways. **(A)** ROP2 promotes lobe outgrowth of pavement cells by coordinately regulating several downstream pathways required for localized exocytosis. **(B)** OsRac1 activates defense responses coordinately by activating lignin biosynthesis and other pathways.