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*Mini-Review*

# The Rop GTPase: an emerging signaling switch in plants

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**Abstract**

G proteins are ubiquitous molecular switches in eukaryotic signal transduction, but their roles in plant signal transduction had not been clearly established until recent studies of the plant-specific Rop subfamily of RHO GTPases. Rop participates in signaling to an array of physiological processes including cell polarity establishment, cell growth, morphogenesis, actin dynamics, $H_2O_2$ generation, hormone responses, and probably many other cellular processes in plants. Evidence suggests that plants have developed unique molecular mechanisms to control this universal molecular switch through novel GTPase-activating proteins and potentially through a predominant class of plant receptor-like serine/threonine kinases. Furthermore, the mechanism by which Rop regulates specific processes may also be distinct from that for other GTPases. These advances have raised the exciting possibility that the elucidation of Rop GTPase signaling may lead to the establishment of a new paradigm for G protein-dependent signal transduction in plants.

## Introduction

GTP-binding proteins are pivotal molecular switches in eukaryotic signal transduction that cycle from a GTP-bound 'on' to a GDP-bound 'off' state. Two major classes of signaling G proteins are known: heterotrimeric G proteins and the Ras superfamily of monomeric small GTPases. The Ras superfamily is composed of five familes (RAS, RHO, RAB/YPT, ARF, and RAN); however, only the RAS and RHO GTPases are considered *bona fide* signaling proteins, whereas RAB/YPT, ARF, and RAN are directly involved in the regulation of vesicular or nuclear trafficking. In mammals, >30% of signaling pathways are controlled by heterotrimeric G proteins, which are formed from the heterotrimeric combinations of 20 different $\alpha$, 5 $\beta$ and 7 $\gamma$ subunits (Sternweis, 1996). Many more signaling pathways in animals involve the RAS family (three members in mammals) and the RHO family (composed of Rho, Rac and Cdc42 subfamilies and several orphan members). In contrast, no RAS and only two G$\alpha$ and one G$\beta$ homologues have been identified in plants (Ma *et al.*, 1990; Ma, 1994; Weiss *et al*., 1994; Lee and Assmann, 1999). It is not yet known whether specific signaling pathways are controlled by heterotrimeric G proteins in plants. Loss-of-function mutants for G$\alpha$ display only reduced stem elongation and seed sizes in rice (Ashikari *et al*., 1999). These observations raise an intriguing question: do G proteins not play a major role in plant signal transduction, or alternatively, do plants use a different type of GTP-binding proteins as a predominant molecular switch? This is a particularly important question, considering that plants have few G protein-coupled seven-transmembrane receptors and no receptor tyrosine kinases, both of which are ubiquitous in animals. Plants, on the other hand, contain a huge family of receptor-like serine/threonine kinases (RLKs). Nonetheless, a plant-specific class of small GTPases, termed Rop, is emerging as an important switch in plant signal transduction.

**Rop is a plant-specific subfamily of RHO GTPases**

Since the cloning of the first plant cDNA encoding a RHO-related GTPase (Rho1Ps) from pea in 1993 (Yang and Watson, 1993), a large number of Rho1Ps-like GTPases have been identified from various plants including mosses and higher plants (36 available in the database) (Yang and Watson, 1993; Delmer *et al*., 1995; Winge *et al*., 1997; Li *et al*., 1998). All but one of the 36 genes fall into a unique RHO subfamily termed Rop (RHO-related proteins from plants) (Li *et al.*, 1998). Eleven *Arabidopsis* Rop genes have been identified to date (Li *et al*., 1998). Rops share >70% amino acid identity with each other and 45–64% identity with other members of the RHO family. Due to a slightly higher overall homology with the Rac subfamily of RHO GTPases, plant RHO-related GTPases have often been named Racs in the literature (Delmer *et al*., 1995; Winge *et al*., 1997; Kawasaki *et al*., 1999; Kost *et al*., 1999; Potikha *et al*., 1999). However, phylogenetic analysis of RHO GTPases from three representative species, yeast, man and *Arabidopsis* (Figure 1), and sequence comparison between *Arabidopsis* Rop and human Rho, Rac and Cdc42 (Figure 2) clearly suggests that Rop is distinct from the three subfamilies of RHO GTPases from animals. Importantly, Rop is specific to plants, and plants apparently do not possess Cdc42, Rac, and Rho.

Rop is distinct from other RHO GTPases in several aspects (Figure 2). First, the highly conserved effector domain (domain II) contains several amino acid residues unique to Rop (Figure 2). Second, the RHO insert region (domain V) in Rop consists of 9–10 amino acid residues that share little homology with those (12 residues) in other RHO GTPases. This RHO insert region also interacts with RHO effectors (Mackay and Hall, 1998). These unique features in effector domains are consistent with the observation that plants apparently possess few homologues of animal RHO effectors (see below). All Rops contain two putative serine/threonine phosphorylation sites, SYR and SSK (with the exception of Rop6At, which has SAK as Rho) (Figure 2). In addition, there are two other putative phosphorylation sites unique to specific Rop subgroups: SNK for Arac7, Arac8 and Arac10, and SKK for Rop1At, Rop3At, Rop5At, and Rop6At. These potential phosphorylation sites might be targets of RLKs, which have been suggested to associate with Rop (Trotochaud *et al*., 1999).

Rop can be further divided into at least four groups according to both overall sequence similarity and the variable region (domain VII) at the C-terminus (Li et al., 1998). Most Rops contain the geranylgeranylation motif CAAL (C, cysteine; L, leucine; A, aliphatic amino acids), the target for geranylgeranyl transferase II (GGTase II). The isoprenyl moiety covalently attached to the cysteine residue probably allows Rop to be anchored to membranes (Lin *et al*., 1996; Kost *et al*., 1999; Li *et al*., 1999; Rodriguez-Concepcion *et al*., 1999). In addition, most Rops (including groups III and IV) contain a CAAL-proximal polybasic region that presumably mediates the localization of Rop to specific membrane systems. Interestingly, the apparent founding Rop members (group II) contain either the farnesylation motif CAAX or the geranylgeranylation motif CXX for GGTase II. These Rops contain some extra amino acid residues in the variable region due to the presence of an additional exon right next to the CAAX box. This is quite unique to the plant Rop, because intron numbers and positions are normally conserved among different small GTPases. Interestingly, the cysteine residues in the CAAX of maize Rop1 and Rop6 (group II) are not important for plasma membrane localization, suggesting either that these proteins are not isoprenylated or that isoprenylation is not critical for their membrane targeting (John Fowler, personal communication). In contrast, two internal cysteine residues in the variable region (domain VII) of these Rops have been shown to be critical for Plasma Membrane (PM) localization (John Fowler, personal communication). Corresponding cysteine residues are also present in other members of this group. This suggests the existence of a novel PM targeting signal for this group of Rop GTPases (John Fowler, personal communication).

Given the plant specificity and the large size of the gene family, one might speculate that Rop may serve as a ubiquitous switch in plant signal transduction analogous to heterotrimeric G proteins and RAS in animals. The large gene family and possible functional redundancy among family members present a potential problem for determining the function of Rop in plants, because loss-of-function mutations for a given Rop gene most likely do not have a phenotype. This appears to be the case because no Rop genes have been identified through forward genetic approaches. However, the unique regulatory feature of GTPases, i.e., cycling between GDP- and GTP-bound forms, allows the generation of gain-of-function point mutations. These mutations have been extremely useful for functional studies of RHO and RAS GTPases in animals and yeast and have recently been proven to be

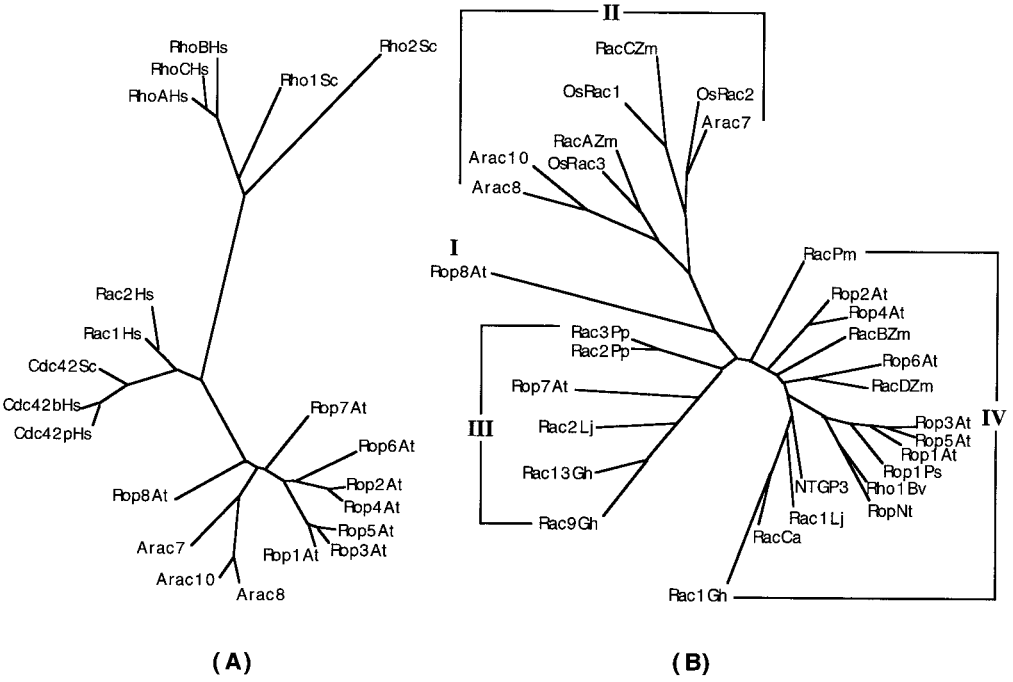


*Figure 1.* Phylogenetic analysis of Rop GTPases. A. A phylogenetic tree of RHO-family GTPases from the three representative species, yeast (Sc), man (Hs) and *Arabidopsis* (At or Arac) showing that Rop is a subfamily distinct from other RHO subfamilies including Rho, Rac and Cdc42. B. A phylogenetic tree of plant Rop GTPases. This analysis indicates that Rops can be further divided into four groups: I, II, III, and IV. Note that only one member (Rop8At) is found in the distinct group I. Abbreviations for species are *Arabidopsis thaliana* (At or Arac), *Beta vulgaris* (Bv), *Cicer arietinum* (Ca), *Gossypium hirsutum* (Gh), *Homo sapiens* (Hs), *Lotus japonicus* (Lj), *Nicotiana tabacum* (Nt), *Oryza sativa* (Os), *Physcomitrella patens* (Pp), *Picea mariana* (Pm), *Pisum sativum* (Ps), *Saccharomyces cerevisiae* (Sc) and *Zea mays* (Zm). NTGP3, identified as a geranylgeranylated protein 3 in Nt.

useful for understanding the function of Rop in plants as well (Kawasaki *et al*., 1999; Kost *et al*., 1999a; Li *et al*., 1999; Potikha *et al*., 1999). Recent studies using this approach complemented with other techniques, such as injection of neutralizing antibody and overexpression of sense and antisense genes, have implicated Rop in many pathways important for development and environmental responses in plants.

## Rop signaling to tip growth in pollen tubes

The role for Rop in signaling is best studied in pollen tubes. Pollen tube growth represents an extreme case of polarized growth, termed tip growth, during which Golgi vesicles fuse with a defined site of the plasma membrane, leading the PM and cell wall extension and the formation of a cylindrical tube. Thus a mechanism is required to restrict the site of exocytosis to the tip and the rate of exocytosis during pollen tube growth (Yang, 1998). Recent studies suggest that a Rop-dependent pathway couples the control of growth sites with the rate of growth (Li *et al*., 1999). First, it was shown that Rop1Ps preferentially accumulated in pea pollen and is localized to the apex of the pollen tube PM using an antibody raised against Rop1Ps (Lin *et al*., 1996). Anti-Rop1Ps antibodies injected into pea pollen tubes blocked pea pollen tube growth, suggesting that Rop is essential for tip growth (Lin and Yang, 1997). This was further confirmed using dominant negative (DN) mutants of *Arabidopsis* Rops (Li *et al*., 1999). In *Arabidopsis*, at least three Rop genes are expressed in pollen; *Rop1At* is pollen-specific whereas *Rop3At* and *Rop5At/At-Rac2* are also expressed in vegetative tissues (Li *et al*., 1998; Kost *et al*., 1999a). Pollen-specific expression of DN-rop1At or DN-rop5At/At-rac2 in transgenic *Arabidopsis* or transiently transformed tobacco pollen, respectively, inhibited pollen tube growth (Kost *et al*., 1999a; Li *et al*., 1999). Antisense *rop1At* expression only weakly inhibited pollen tube growth presumably due to a partial suppression of pollen Rop gene expression (Li *et al*., 1999). These results clearly demonstrate that one or more Rops control the rate

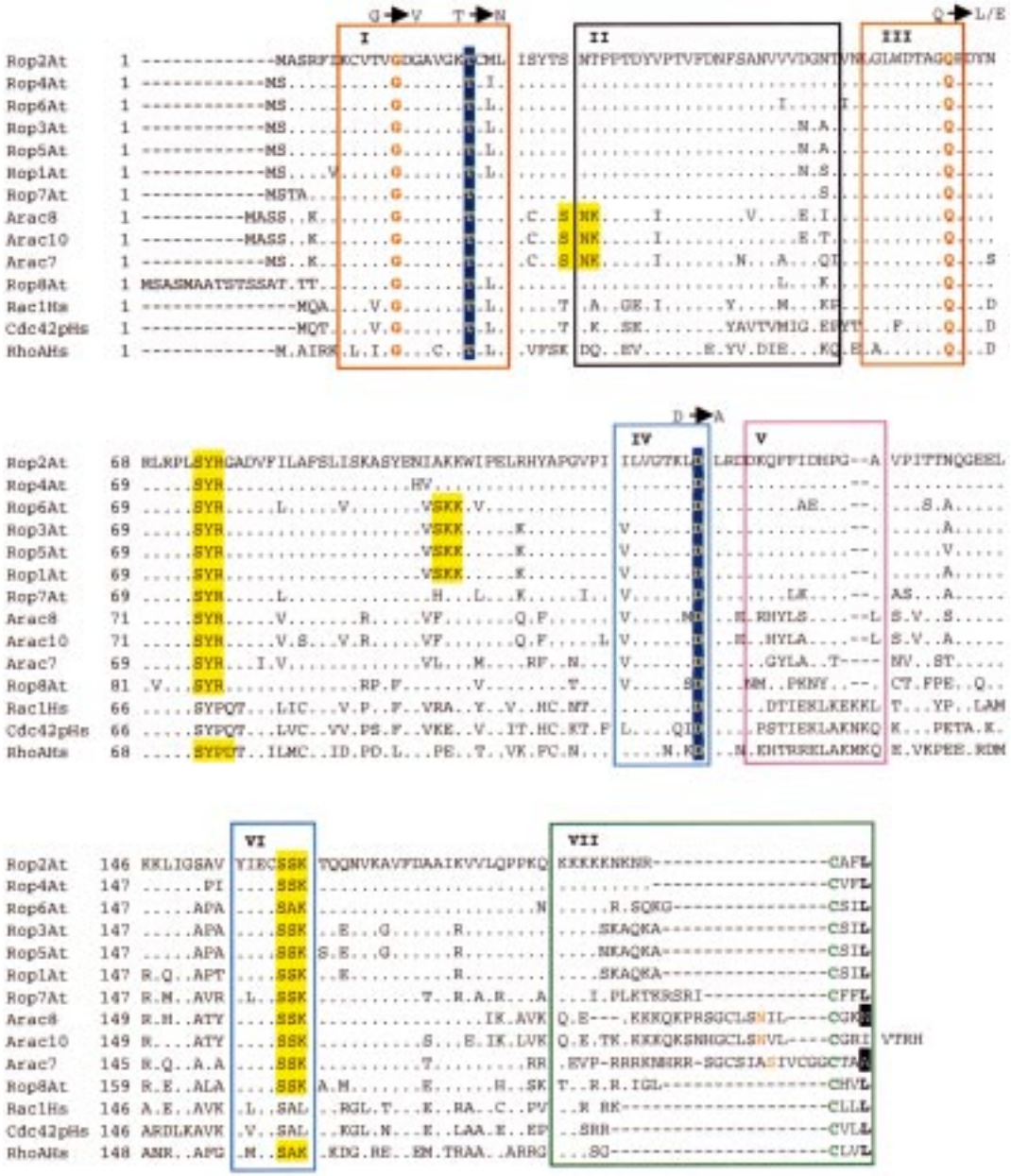


*Figure 2.* Comparison of RHO-family GTPase sequences from *Arabidopsis* (At or Arac) and man (Hs). The known constitutively active (red) and dominant negative (yellow) mutations are indicated. Distinct functional domains: GTPase domains (I and III), GDP/GTP-binding domains (IV and VI), effector domain (II), RHO insert region (V), and membrane localization domain (VII). In the VII domain, residue C (green) is conserved in all GTPases, and the gerenylgerenylation motif CAAL is present in most Rop GTPases except for Arac7, 8 and 10, in which the isoprenylation motif is not present (Arac10) or is the CAAX (the terminal X can be any amino acids except for L) farnesylation motif. For this unique group, the C-terminal fragment of peptide starting from the residue N/S (red) is encoded by an additional exon that is not found in other Rop members. Four motifs (SNK, SYR/SYPD, SKK, and SSK/SAK) shown in yellow are putative serine/theronine-dependent phosphorylation sites as predicted by the ProSite program. Distinct groups of RHO GTPases show some variations in these phosphorylation sites (see text).

of pollen tube growth. Because *Rop1At* transcripts are much more abundant than *Rop3At* and *Rop5At* (Li *et al*., 1999), Rop1At likely plays a major role in the control of pollen tube growth. Loss-of-function mutants are necessary to determine whether these Rops are functionally redundant or distinct in pollen tubes.

Overexpression of wild-type (WT) *Rop1At* in *Arabidopsis* pollen tubes induces depolarized growth, and the severity of depolarization is correlated with the level of WT-Rop1At proteins in pollen as well as the amount of Rop1At protein accumulated to the tip of pollen tubes (Li *et al*., 1999). Furthermore, expression of constitutively active rop1At (CA-rop1At) or CA-Rop5At/At-rac2 in pollen causes a complete loss of growth polarity, resulting in the bulbous tubes (Kost *et al*., 1999a; Li *et al*., 1999). These results suggest that both polar localization of Rop and the regulation of its activity at the tip region of PM are critical for defining the site of growth in pollen tubes. By coupling the temporal and spatial control of pollen tube growth, this Rop-dependent localized signaling, likely controlled by a localized tip-growth cue, assures that growth is restricted to the tip of pollen tubes (Zheng and Yang, 2000).

The Rop-dependent signaling pathway most likely controls localized exocytosis. This hypothesis is strongly supported by the result showing Rop acts in the same pathway as the mechanism that focuses $Ca^{2+}$ at the tip, which is thought to directly regulate exocytosis (Battey *et al*., 1999). Like tip-localized Rop, the tip-localized $Ca^{2+}$ flux and tip-focused $Ca^{2+}$ gradient are required for tip growth and control the site of tip growth in pollen tubes (Malhó *et al*., 1995; Malhó and Trewavas, 1996). Injected anti-Rop1Ps antibodies eliminated the tip-focused $Ca^{2+}$ gradient, and high extracellular $Ca^{2+}$ rescued *DN-rop1At*- and antisense *rop1At* gene-induced growth inhibition (Li *et al*., 1999). These results indicate that Rop acts upstream of the localized $Ca^{2+}$ activity in the activation of tip growth. Components connecting Rop and $Ca^{2+}$ are unknown, although phosphotidylinositol phosphate kinase appears to be a putative Rop effector linked to $Ca^{2+}$ signaling (see below).

**Rop provides a potential link between signaling to the actin cytoskeleton and cell morphogenesis**

The actin cytoskeleton has long been thought to play an important role in plant cell morphogenesis and cell growth (Kost *et al*., 1999b). This view has been strengthened by several recent studies in trichomes and pollen tubes (Gibbon *et al*., 1999; Kost *et al*., 1999a; Mathur *et al*., 1999; Szymanski *et al*., 1999). However, the mechanism that controls plant actin organization and dynamics is poorly understood. Because RHO-family GTPases are conserved regulators that link extracellular signals to the organization of the actin cytokeleton in yeast, *Dictyostelium*, and animals, it was speculated that Rop may also have a similar role in the regulation of the plant actin cytoskeleton. Indeed, alteration of F-actin in pollen tubes expressing CA-rop5At or DN-rop5At mutants has been reported (Kost *et al*., 1999a). The formation of extensive spiral cortical actin cables is associated with the expression of CA-rop5At, whereas DN-rop5At appears to cause reduced F-actin bundling in pollen tubes. However, it was suggested that these changes in actin organization are unlikely to account for the dramatic phenotypes induced by these mutants (Kost *et al*., 1999a). It remains to be determined whether the changes in F-actin are directly due to alteration of Rop signaling or indirectly from morphological changes induced by rop mutants.

Further support for the role of Rop in the regulation of actin organization in plant cells came from the studies of Rop2At in vegetative cells (Li and Yang, 2000). Expression of rop2At dominant mutants under the CaMV 35S promoter caused changes in cell morphology similar to that induced by rop1At mutants in pollen tubes. CA-rop2At induced isotropic cell expansion, whereas DN-rop2At inhibited cell expansion in *Arabidopsis* leaves (Fu, Li and Yang, unpublished data). However, changes in the actin cytoskeleton resulting from the expression of these mutants seem to be different from those observed in pollen tubes by Chua's group (Kost *et al*., 1999a). In CA-rop2At cells, actin bundles become subcortical, compared to cortical localization of these actin bundles in wild type cells. In DN-rop2At cells, however, the amount of fine cortical F-actin is drastically reduced, but thick actin cables do not seem to be affected. Although their significance in cell morphogenesis is not clear, these changes most likely are the direct effect caused by changes in Rop signaling activity. Rop signaling may be a general mechanism that controls actin-mediated plant cell morphogenesis.

**Rop in plant defense responses**

In mammalian cells, a multi-subunit plasma membrane NADPH oxidase is responsible for the gener-

ation of $H_2O_2$ in response to microbial stimuli. One key regulatory subunit is Rac2, which interacts with the p67phox regulatory subunit and is assembled into the catalytic subunit gp91phox together with the other regulatory subunit p47phox. Because of a crucial role for $H_2O_2$ in plant defense responses and programmed cell death and the existence of PM NADPH oxidase in plant cells, much attention has been devoted to the identification of a plant equivalent to the mammalian Rac2. It was shown that a Rac2-specific antibody detected a 21 kDa tomato protein that could be translocated to microsomal membranes in response to elicitor treatments (Xing *et al*., 1997). Because plants apparently do not have Rac orthologues, this Rac2 antibody-reactive protein, if a GTPase, most likely is a Rop. Indeed, recent studies show that constitutively active forms of the rice Rop OsRac1 constitutively activate $H_2O_2$ production (Kawasaki *et al*., 1999). CA-Osrac1 also induces spontaneous programmed cell death in rice leaves, whereas DN-Osrac1 inhibits $H_2O_2$ production and lesion formation induced by pathogen infection.

How does Rop regulate the production of $H_2O_2$? Rop could be a regulatory subunit of the NADPH oxidase like the mammalian Rac2 and/or could participate in a signaling pathway leading to the activation of NADPH oxidase. Expression of constitutively active mutants of human Rac1 in soybean cells enhanced $H_2O_2$ production induced by several different stress stimuli; however, unlike CA-Osrac1, this effect is not constitutive and thus is dependent upon the stimuli (Y. Lee, personal communication). These results imply that plants have a regulatory system analogous to the mammalian Rac-dependent regulation of NADPH oxidase. p91phox homologues are indeed present in plants, but plants apparently do not contain p67phox and p47phox homologues (Bolwell, 1999). It is possible that Rop could directly regulate NADPH oxidase in a p67phox-independent manner. Nonetheless, constitutive activation of $H_2O_2$ production by CA-Osrac1 suggests that Rop is involved in an additional or alternative signaling mechanism to regulate $H_2O_2$ production, likely in an early step of defense signaling pathways; for example, Rop could be regulated by receptor-like kinase R gene product (see below) or could regulate $Ca^{2+}$ influx (also an early step of defense signaling) as in pollen tubes.

**A potential role for Rop in the synthesis of secondary cell walls and vascular tissue differentiation**

The expression of the cotton Rop Rac13 is highly induced in cotton fiber cells during the transition from primary to secondary wall synthesis (Delmer *et al*., 1995). Interestingly, the timing of this transition also coincides with the production of $H_2O_2$, which has been shown to stimulate the synthesis of cellulose during cotton fiber differentiation (Potikha *et al*., 1999). Like CA-Osrac1, CA-rac13 also constitutively activates $H_2O_2$ production in tobacco or *Arabidopsis* cell cultures. These results imply that Rac13 may be involved in the differentiation of cotton fibers via $H_2O_2$. We found that CA-rop2At expression in *Arabidopsis* caused an increase in the size of vascular bundles in leaves and cotyledons, and preliminary analyses suggest that this is due to an increased number of tracheary elements (Fu, Li, and Yang, unpublished data). Further studies are needed to determine whether Rop2At promotes the differentiation of the xylem or the synthesis of secondary cell walls.

**Rop may act as a common molecular switch in many signaling pathways**

Apart from the functions described above, Rop is also implicated in other signaling pathways in plants as well. First, *in situ* immunolocalization of Rop in *Arabidopsis* with anti-Rop1Ps antibodies, which react with all *Arabidopsis* Rop isoforms, shows that Rop accumulates in all cell types (Lin and Yang, unpublished data). However, members of the Rop gene family display various spatial expression patterns (Winge *et al*., 1997; Li *et al*., 1998). Second, transgenic plants expressing CA-rop2At or DN-rop2At exhibit pleiotropic phenotypes including alterations in seed germination, leaf morphogenesis, apical dominance, hypocotyl and stem elongation, embryo development, root hair differentiation and development, and responses to phytohormones (such as ABA and brassinolides) and ozone (Li, Shen, Zheng and Yang, unpublished data). These phenotypes induced by dominant rop2At mutants most likely reflect the function of Rop2At and those of Rops that are closely related to Rop2At (e.g., members of Group IV Rop) that are expressed in vegetative tissues. However, Rops that are more distantly related to Rop2At (e.g., members of groups I and II) most likely have distinct functions not revealed by these

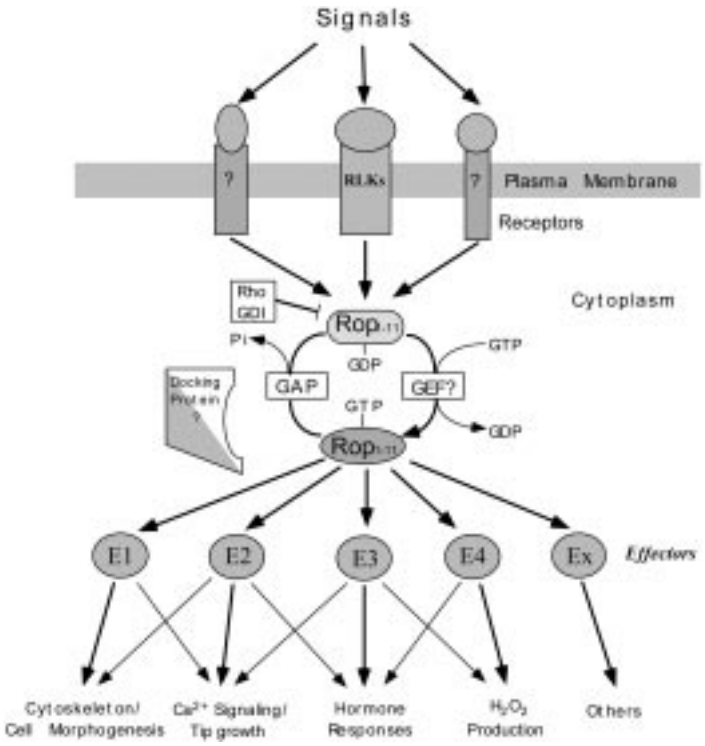


*Figure 3.* A general Rop signaling model. Rop may act as a common switch in signaling to many aspects of plant growth, development and defense responses. Rops relay extracellular signals perceived by receptor-like kinases (RLKs) or other unknown receptors through the active GTP-bound form of Rops. Docking proteins may be involved in pathway compartmentalization or determining functional specificity of each Rop. Guanine nucleotide exchange factor (GEF) for Rop has not been identified, and thus Rop activation may involve a novel mechanism. Each Rop or a group of Rops may have distinct effectors (E1, E2, E3, E4 and Ex), which in turn activate specific downstream signaling events.

mutant phenotypes. Third, yeast two-hybrid screens for Rop-interacting proteins have identified several genes encoding proteins related to receptor-like kinases, and Rop-like proteins have been shown to associate with the active CLAVATA1 RLK complex by co-immunoprecipitation using anti-CLV1 and anti-Rop antibodies (Trotochaud *et al*., 1999). Although which Rop is associated with the CLVATA1 complex remains to be determined, this finding is fascinating because the RLK family, with >100 paralogues in *Arabidopsis*, is a major class of transmembrane receptors in plants. Therefore, Rop may achieve its complex signaling through the interaction with RLK or other RLK-associated factors.

**Is the mechanism of Rop signaling different from that for the animal RHO family?**

Three types of RHO regulators are known in animals: GTPase-activating proteins (GAPs), guanine nucleotide exchange factors (GEFs), and guanine nucleotide dissociation inhibitors (GDIs). GEFs activate the GTPase switch by replacing GDP with GTP, whereas GAPs stimulate the intrinsic GTPase activity leading to the deactivation of the GTPase switch. GDIs prevent the activation process by removing GDP-bound RHO GTPases from membranes where GEFs are localized. Two RHO GDI homologues are present in the *Arabidopsis* genome but their functions have not been studied. RHO GAPs have been identified from *Arabidopsis* and *Lotus* by the yeast two-hybrid method (Borg *et al*., 1999; Wu, Li, and Yang, submitted). These GAPs preferentially stimulate the Rop subfamily of RHO GTPases and thus are named RopGAPs (Wu, Li, and Yang, submitted). All RopGAPs identified to date have a unique feature: the presence of an N-terminal Cdc42/Rac-interacting binding (CRIB) domain localized adjacent to the conserved GAP domain (Borg *et al*., 1999; Wu, Li, and Yang, submitted). In animals, the CRIB domain is only found in Cdc42/Rop effectors. Deletion and point-mutation analyses reveal a critical role for the CRIB domain in the regulation of RopGAP activity (Wu, Li, and Yang, submitted).

The ability for RopGAPs to be regulated is consistent with the *in vivo* function of *Arabidopsis* RopGAP (Wu, Li, and Yang, submitted). RopGAP overexpression under the pollen-specific LAT52 promoter caused pollen tube growth inhibition and a shift of the optimal extracellular $Ca^{2+}$ concentration from 2.0 mM to 0.5 mM. These results suggest that RopGAPs participate in the $Ca^{2+}$-dependent negative feedback regulation of Rop signaling as a negative regulator. This CRIB-dependent GAP regulation and a role for GAP in the negative feedback regulation of GTPase signaling are unique to Rop GTPase signaling in plants.

The activation of Rop signaling may also be distinct from other RHO GTPases. No RHO GEF homologues have been identified in plants, even though about 80% of *Arabidopsis* sequences have been completed. This raises an interesting possibility that plants have evolved a dramatically different mechanism to activate the GDP-bound Rop GTPases. This notion is consistent with our observation that Rop directly associates with RLKs as described above.

Little is known about Rop effectors, and the current plant databases contain few sequences homologous to typical RHO effectors found in animals and yeast. This suggests that the mode of action for Rop most likely is quite different from that for Cdc42 and Rac. Kost *et al*. (1999) found that phophotidylinositol monophosphate kinase (PIP-K) can associate with the recombinant Rop5At/At-Rac2, indicating that it is a putative Rop effector. Evidence supporting a role for PIPK in the control of pollen tube tip growth comes from two observations. First, phosphatidylinositol 4,5-bisphosphate (PIP2), the product of PIP-K, is localized to the tip of pollen tubes where Rop is presumably activated. Second, pollen tube growth is inhibited when the binding site of At-Rac2 for PIP-K is occupied by the fusion protein of GFP-pleckstrin homology domain. Although an *in vivo* interaction between Rop and PIP-K has yet to be determined, phospholipid kinases are known to serve as RHO effectors in animal systems. However, the potential regulation of PIP-K by Rop is unlike the action of the animal Rop relatives Rac/Cdc42, because Rac/Cdc42 activate PI3K, not PIPK; instead, PIPK is the target of Rho, a more distant relative of Rop (Aspenstrom, 1999). In both yeast and animal systems, each RHO GTPase regulates multiple targets to generate distinct effects (Aspenstrom, 1999). Given the multiplicity of Rop-dependent signaling pathways, Rop most likely interacts with multiple distinct effectors as well (Figure 3).

## Future prospects

Studies in the past few years have not only established a crucial role for Rop in signaling to cell polarity formation and cell morphogenesis, analogous to the roles of animal and yeast Cdc42, but have also provided evidence that Rop may serve as a common switch in plant signaling. The large Rop gene family and the multiplicity of Rop-dependent signaling pathways and Rop-interacting proteins contribute to the complexity of Rop signaling in plants and present a challenge for elucidating Rop-dependent signaling pathways. Many important questions regarding this emerging signaling switch await answers: (1) Which signaling pathways are controlled by the Rop gene family? (2) Does each Rop or Rop group participate in a distinct signaling pathway? (3) What are the signals and receptors that control the Rop switch, and how do they regulate this switch? (4) Does Rop act as a common switch to control RLK-mediated signaling pathways? (5) What are the direct targets of Rop? (6) Does each Rop control distinct targets and how? A multifaceted approach integrating genetic, genomic, bioinformatic, and biochemical methods is necessary for providing clear answers to these questions.

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