ROP/RAC GTPase: an old new master regulator for plant signaling

Ying Gu[1], Zonghua Wang[2] and Zhenbiao Yang[1]

The ROP family of small GTPases has emerged as a versatile and pivotal regulator in plant signal transduction. Recent studies have implicated ROP signaling in diverse processes ranging from cytokseletal organization to hormone and stress responses. Acting as a switch early in signaling cascades, ROPs are also capable of orchestrating several downstream pathways to amplify a specific signal.

Addresses
[1] Department of Botany and Plant Sciences, University of California — Riverside, Riverside, California 92521, USA
[2] The School of Life Sciences, Fujian Agriculture and Forestry University, Fuzhou, PR China
e-mail: zheniao.yang@ucr.edu

Current Opinion in Plant Biology 2004, **7**:527–536

This review comes from a themed issue on
Cell signalling and gene regulation
Edited by Jen Sheen and Steven Kay

Available online 31st July 2004

1369-5266/$ – see front matter

DOI 10.1016/j.pbi.2004.07.006

Abbreviations

ABA	abscisic acid
ADF	actin depolymerization factor
CA	constitutively active
DN	dominant-negative
F-actin	filamentous actin
GAP	GTPase-activating protein
GDI	guanine nucleotide dissociation inhibitor
GEF	guanine nucleotide exchange factor
GFP	green fluorescent protein
HR	hypersensitive response
IAA	indole-3-acetic acid
PM	plasma membrane
RBOH	respiratory burst oxidase homolog
RIC	Rop-interactive CRIB-motif-containing
RNAi	RNA interference
ROS	reactive oxygen species

Introduction

As a binary switch cycling between an inactive GDP-bound and an active GTP-bound conformation, GTP-binding protein (also known as G protein or GTPase) provides a universal mechanism for controlling the transmission of extracellular signals to intracellular pathways in eukaryotes. Signaling G proteins are commonly associated with the plasma membrane (PM), endowing them with the capacity to signal directly from, or in close proximity to, PM-localized receptors. G proteins have been at the center stage of signal transduction research ever since the first demonstration of their role in signaling in 1980.

Two classes of signaling G proteins are known: classical heterotrimeric G proteins and monomeric Ras/Ras-like small GTPases (Table 1). Mammals possess numerous heterotrimeric G proteins — owing to the different combinations of the 20 Gα, 5 Gβ and 12 Gγ types of subunit — that regulate more than 30% of signaling pathways [1]. Pharmacological studies support a widespread role for heterotrimeric G proteins in plant signaling [1], but for the most part, this role has not been confirmed by molecular and genetic evidence. Strikingly, only one prototypical Gα, one Gβ and two Gγ subunits are present in *Arabidopsis* [1]. Analysis of Gα and Gβ knockouts shows that conical heterotrimeric G proteins have some roles in plant signaling, but their functions are not as widespread as those seen in animals [1,2].

In the Ras superfamily of small GTPases, only the Ras and Rho families have been clearly shown to transmit extracellular signals (Table 1). Plants do not possess a true Ras GTPase such as those that are pivotal signaling switches in animals and yeast [3,4]. Instead, they have a unique subfamily of Rho-family GTPases, called ROPs (Rho-related GTPase from plants) [4–6]. Rho-family GTPases control a wide range of cellular processes, most of which are linked to the regulation of the cytoskeleton, which probably reflects the conserved function of ancestral Rho GTPases [7]. The mammalian family of Rho GTPases consists of at least 14 members, including the best-characterized Rho, Rac and Cdc42 subfamilies [7]. Yeast possesses a single Cdc42 protein and a Rho subfamily [8], whereas ROP is the sole subfamily of Rho GTPase in plants [3,9,10]. Referred to as RACs in some literature [11–13,14•], ROPs share a common ancestor with Rho, Cdc42 and Rac, and fall into a distinct clade as the only Rho-like GTPases in plants [4,6,9]. *Arabidopsis* contains 11 ROPs or RACs [4,9,10], and comparable numbers of ROPs with similar subgroups are found in maize and rice [15].

It has been proposed that ROPs act as a predominant GTPase switch to control the transmission of extracellular signals in plants [3,9]. This hypothesis has been supported by the observation that ROPs are associated with receptor-like serine/threonine kinases [16], as well as by functional studies implicating ROPs in the regulation of many diverse processes in plants (reviewed in [3]).

In this review, we highlight recent advances in the studies of ROP-mediated pathways and ROP signaling

Table 1

Comparison of the G proteins of *Arabidopsis* and humans.

Type	General function	Number of genes in the genome	
		Arabidopsis	Human
Heterotrimeric G proteins			
α	Signaling	1	20
β	Signaling	1	5
γ	Signaling	2	12
Small GTPases			
Rab	Vesicle trafficking	57	60
Arf	Vesicle budding	21	45
Ran	Nuclear transport	4	1
Rho	Signaling	11	14
Ras	Signaling	0	7

mechanisms. Two major themes are emerging from these studies. First, ROPs act as master switches in the transmission of various extracellular and intracellular signals, as well as in the coordination of several downstream pathways controlled by a specific signal. Second, plants have invented novel modes of regulating ROPs and of transmitting ROP signals, while retaining some conserved mechanisms for the regulation of these GTPases.

Complexity of ROP functions: too many or too few ROPs?

Gain-of-function approaches using the overexpression of dominant mutant or wildtype ROP genes, together with loss-of-function approaches using T-DNA knockouts or RNA interference (RNAi), have revealed that ROPs have many physiological roles in *Arabidopsis* and other plant species. They are involved in the establishment of cell polarity in root-hair development, root-hair elongation, pollen-tube growth, cell-shape formation in various types of cell, responses to hormones such as abscisic acid (ABA) and auxin, responses to abiotic stresses such as oxygen deprivation, and disease resistance and disease susceptibility ([17••–19••,20•]; reviewed in [3]).

The functional diversity of ROPs is also reflected in the many cellular targets of ROP signaling, which include cytoskeletal organization and dynamics, the production of second messengers (such Ca^{2+} and reactive oxygen species), and the regulation of gene expression ([19••,21, 22••,23]; Y Gu *et al.*, unpublished; Y Fu *et al.*, unpublished). In addition, the function of individual ROPs can be complex. The eleven ROPs in *Arabidopsis* are divided into four phylogenetic groups: group I (ROP8), group II (ROP9–ROP11), group III (ROP7) and group IV (ROP1–ROP6). Evidence suggests that different groups have distinct functions [3]. Members of group IV share more than 86% amino acid identity and are expected to have redundant or overlapping functions. Indeed, at least two ROPs in this group are functionally redundant in the control of the morphogenesis of leaf pavement cells (Y Fu *et al.*, unpublished).

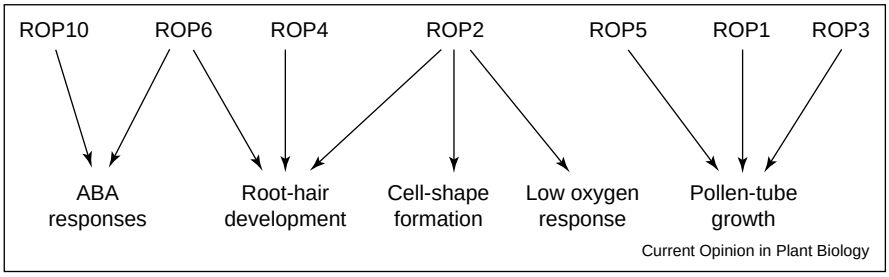
By contrast, an individual ROP can have several functions; that is, a single ROP can participate in the independent regulation of several unrelated processes by itself or with other functionally redundant or overlapping ROPs (Figure 1). For example, ROP2 has been implicated in the regulation of cell morphogenesis, root-hair initiation, root-hair elongation, ABA responses and responses to oxygen deprivation. But some ROPs seem to have a distinct function; for example, ROP10 is a specific negative regulator of ABA responses [18••]. Such complex functions for a specific family of signaling genes may be common among signaling protein families such as the calcium-dependent protein kinases (CDPKs), which explains the difficulties in functionally identifying these genes using forward genetics.

Are ROPs missing pieces of phytohormone signaling?

Some of the most significant advances in our understanding of ROP functions have been provided by studies showing that ROPs have a role in regulating plant responses to several major hormones, such as ABA and auxin. The first clue to the involvement of ROPs in hormone signaling came from the observation that transgenic *Arabidopsis* plants expressing constitutively active (CA) or dominant-negative (DN) mutants of ROP2 show altered responses to the exogenous application of ABA, indole-3-acetic acid (IAA) and brassinosteroids [24]. *35S::DN-ROP2* enhances the inhibition of seed dormancy by ABA in *Arabidopsis*, whereas *35S::CA-ROP2* has the opposite effect, suggesting that ABA responses are negatively regulated by ROPs. This notion has been further supported by a study from Chua's group [25] showing that *35S::CA-ROP6* and *35S::DN-ROP6*, respectively, inhibit and promote ABA induction of stomatal closure, and that ABA inactivates ROPs in a dose-dependent manner.

A role for ROPs in the negative regulation of ABA responses has been clearly demonstrated by studies using *rop10* knockout mutants. These mutants show enhancement in all aspects of ABA responses examined, including ABA regulation of stomatal movement, ABA inhibition of seed germination, the greening and root elongation of seedlings, and ABA-induced gene expression [18••]. *ROP10* is one of the few genes that are specifically involved in ABA responses. Furthermore, ROP10 is localized to the PM. These observations support the notion that ROP10 acts in an early step of ABA signaling, perhaps in proximity to a PM-localized ABA receptor. ABA seems to regulate ROP10 negatively in terms of both activity and gene expression. Transgenic expression of *CA-ROP10* reduces ABA sensitivity, whereas *ROP10* expression, as reported by a fusion construct with the

Figure 1



The functions of the ROP GTPase family are complex. Studies using gain-of-function and loss-of-function approaches have shown that the function of an individual ROP can be redundant or overlapping, distinct and/or multiple.

β-glucuronidase (GUS) promoter, is markedly suppressed by exogenous ABA [18••].

An interesting recent study from Cheung's group [17••] suggests that ROPs are involved in auxin signaling. First, *35S::NtRAC1* and *35S::CA-NtRAC1* stimulate auxin-responsive gene expression, whereas *35S:DN-NtRAC1*, *35S::NtRAC1-RNAi*, *35S::AtGDI* and *35S::AtGAP* suppress auxin-induced gene expression both in transgenic plants and in a protoplast transient expression system. Second, transgenic *35S::CA-NtRAC1* and *35S::NtRAC1-RNAi* tobacco plants show phenotypes that are consistent with a defect in specific aspects of auxin action. Notably, Cheung's group [17••] found that exogenous auxin applied at a concentration as low as 100 nM stimulates an increase in the GTP-bound form of NtRACs, and this stimulation occurs as early as 5 min after auxin treatment. They suggest that the ability of the *Arabidopsis* ROPs to stimulate auxin-responsive gene expression is correlated with the localization of ROPs to the PM.

Moreover, Cheung and co-workers [17••] reported that CA-NtRAC1 shows enhanced localization to the PM as compared with wildtype NtRAC1, whereas DN-NtRAC1 shows reduced PM localization. On the basis of these observations, they proposed that ROPs are involved in early auxin signaling [17••], similar to the role of ROP10 in ABA signaling [18••]. Thus, ROPs might transmit signals from a PM-localized auxin receptor. Auxin signaling is known to activate the degradation of IAA proteins, which act as transcriptional repressors of auxin-induced gene expression. It is possible that ROP signaling may lead to degradation of IAA. The use of *rop* knockout mutants in conjunction with various auxin-signaling mutants in *Arabidopsis* will definitively determine the role of ROPs in auxin signaling.

The findings about ROP participation in hormone signaling are of considerable significance because signaling pathways for ABA and auxin, particularly their early signaling steps, are less understood than those for the other major phytohormones, gibberellic acid, ethylene and cytokinin. ROPs could well provide a stepping stone for us to embark on the elucidation of the early signaling steps for ABA and auxin. In this respect, the identification of ROP-interacting proteins that are involved in ABA and auxin signaling will be extremely important.

Overlapping roles of ROPs in pollen-tube growth?

Regulation of pollen-tube growth is the first and the best-characterized ROP signaling system [3]. The function of ROP GTPases was initially investigated in pollen tubes because pea *ROP1*, the first member of the ROP gene family cloned from plants [5], is preferentially expressed in mature pollen and pollen tubes and is localized to the apical region of the pollen-tube PM [26,27]. Pollen tubes extend by growth at the tip (at up to 1 cm/h) over a long distance (>10 cm) as their tips are guided toward the ovule for sperm delivery. A significant amount of data indicates that ROPs localized at the tip control polarized tip growth in pollen tubes [12,23,26–28].

In *Arabidopsis*, three group IV ROPs (ROP1, ROP3 and ROP5, which are most closely related to pea ROP1), all group II ROPs (ROP9, ROP10 and ROP11), and the group I ROP (ROP8) are expressed in pollen [6,28]. Sharing essentially 100% similarity, ROP1, ROP3 and ROP5 have evolved from recent duplications and thus are expected to be functionally redundant in pollen tubes [10,28]. These ROPs are activated at the tip to promote the elongation of the pollen tube ([28]; Y Gu *et al.*, unpublished). The function of other ROPs in pollen seems to be distinct from those of ROP1, ROP3 and ROP5 ([28]; Y Gu *et al.*, unpublished).

The overexpression of ROP1 and ROP5 has been found to induce depolarized growth (i.e. increased radial expansion or tip swelling), probably because of their ectopic localization and activation at the subapical region [12,23]. The abnormal tobacco pollen-tube phenotypes caused by transient overexpression of green fluorescent protein (GFP)-tagged ROP8/AtRac9, ROP9/AtRac7 and ROP11/AtRac10 differ from those induced by overexpressing

GFP::ROP1 or GFP::ROP5 [29]. Furthermore, unlike overexpression of ROP1, transient overexpression of ROP8, ROP9 and ROP11 in tobacco pollen tubes does not alter the localization of GFP-tagged RIC1, an effector protein that binds to active GTP-bound ROP1 [28]. Although overexpression of ROP10 causes a ROP1-like phenotype, overexpression of a DN-ROP10 does not interfere with ROP1 signaling in pollen tubes (Y Gu *et al.*, unpublished).

These observations raise an interesting question: what is the function of ROP8–ROP11 in pollen and/or pollen tubes? It is possible that some of these ROPs participate in sensing signals from the pistil during the guidance of pollen tubes toward the ovule or during the last stage of pollen function (i.e. the release of sperm from the pollen tube). An *in vivo* function of ROPs is strongly supported by the recent observation that maize *rop2* knockouts do not show detectable defects in cultured pollen-tube growth but have a reduced pollen transmission rate [30•].

Role of ROPs in disease resistance

The involvement of ROPs in the regulation of defense responses has recently become a hot topic (reviewed in [31]). Pioneering work from Shimomato's group [11,32,33••] shows that a rice ROP, OsRac1, has an important role in signaling resistance (*R*)-gene-mediated defense responses against both a bacterial pathogen (*Xanthomonas oryzae*, which causes blight disease) and a fungal pathogen (*Magnaporthe grisea*, which causes rice blast disease).

In addition, ROPs are required for elicitor-induced defense responses in several species [34–37]. These defense responses are commonly expressed as a form of localized programmed cell death, which is known as the 'hypersensitive response' (HR). The HR results from an 'oxidative burst'; that is, the rapid production of reactive oxygen species (ROS) [38]. The oxidative burst in the HR requires the activation of NADPH oxidase, which in turn catalyzes the production of hydrogen peroxide (H_2O_2) [38]. Indeed, evidence indicates that ROPs activate the production of H_2O_2 through the gp91 NADPH oxidase, known as respiratory burst oxidase homolog (RBOH). Similarly, the mammalian ROP counterpart, Rac1, activates the neutrophil respiratory burst NADPH oxidase [34,36–38]. In addition, OsRac1 also suppresses the expression of genes encoding metallothioneins that scavenge ROS [39•]. Thus, ROPs seem to have a dual role in the activation of defense responses: activating the production of ROS and suppressing their removal [39•].

An interesting twist in ROP regulation of defense responses has been uncovered by two recent studies suggesting that a barley ROP (HvRacB) is important for susceptibility to, instead of resistance against, invasion by the powdery mildew pathogen *Blumeria graminis* f. sp. *hordei* [20•,40]. RNAi of HvRacB was found to enhance resistance, whereas expression of CA-HvRacB increased susceptibility. *B. graminis* f. sp. *hordei* is a biotrophic pathogen whose successful invasion is dependent on nutritional support from live host cells, thus, this pathogen must avoid triggering HR responses. Hence, the ROP-mediated oxidative burst is not an important defense mechanism against these pathogens.

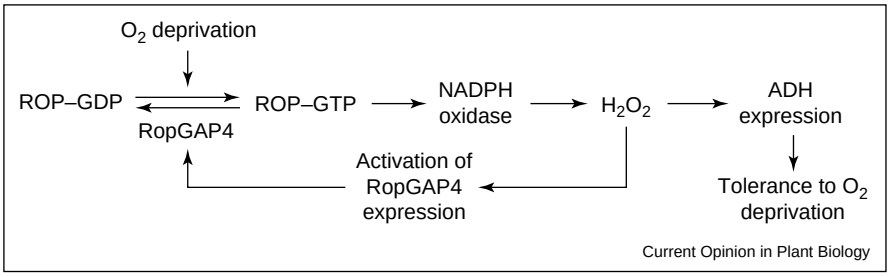
How do ROPs mediate the susceptibility of barley to *B. graminis* f. sp. *hordei*? One possible explanation is that the penetration of haustorium into host cells requires invagination of the PM of the host cell. This process is analogous to localized cell growth (in the reverse direction to the localized outgrowth that occurs, for example, during root-hair formation and lobe formation in pavement cells), which is known to require ROP signaling (see below). Alternatively, ROPs might regulate the secretion of a factor that is important for establishing invasion. Regardless of the mechanism, these results further support the notion that ROP signaling is a versatile signaling switch in the regulation of defense responses, as it is in other processes.

ROP functions as a rheostat in abiotic stress responses

The activation of H_2O_2 production by ROPs has been implicated in other signaling pathways, including those that regulate tolerance to oxygen deprivation and the tip growth of root hairs ([19••,41,42•,43]; M Jones *et al.*, unpublished). In these systems, ROP-dependent H_2O_2 acts as a second messenger to either induce the expression of genes that are crucial for tolerance against oxygen deprivation (e.g. the gene encoding alcohol dehydrogenase) or activate the generation of intracellular Ca^{2+} signals that are important for cell growth [19••,41]. In this case, H_2O_2 levels need to be tightly controlled because high concentrations of H_2O_2 are cytotoxic but a threshold of H_2O_2 is required to induce sufficient downstream responses. This contrasts with HR-type defense responses, in which oxidative-burst-induced localized cell death is an important mechanism for fencing off pathogens from uninfected tissues.

A recent study by Bailey-Serres and colleagues [19••] revealed an elegant mechanism that underlies ROP-mediated H_2O_2 homeostasis and is crucial for *Arabidopsis* tolerance of hypoxia stress (Figure 2). Oxygen deprivation turns on ROPs, which activate H_2O_2 production, most probably through a gp91 NADPH oxidase. H_2O_2 then acts as a second messenger to activate the expression of not only the gene encoding alcohol dehydrogenase but also the gene encoding RopGAP4, a member of the RopGAP family that deactivates ROPs by promoting hydrolysis of GTP [44]. Thus, ROP activation of RopGAP4 expression functions as a negative feedback loop to terminate ROP signaling. This feedback mechanism

Figure 2



A negative feedback loop generates a ROP rheostat to regulate hydrogen peroxide (H_2O_2) homeostasis. Analysis of the effect of a *ropgap4* null mutation on hypoxia regulation of ROP activity, H_2O_2 production, RopGAP4 expression, alcohol dehydrogenase expression and plant tolerance to oxygen deprivation reveals that a RopGAP4-dependent negative feedback loop is essential for *Arabidopsis* tolerance of hypoxic stress [19••].

maintains a threshold non-toxic level of H_2O_2 that activates responses required for low oxygen tolerance. Consequently, either inhibition of ROP signaling by expression of DN-ROP2 or superactivation of ROP signaling owing to loss-of-function of RopGAP4 reduces tolerance of hypoxia stress [19••].

This study shows that ROP acts as a rheostat rather than as a binary switch in hypoxia signaling [19••]. It is anticipated that a similar ROP rheostat mechanism may operate in other ROP signaling pathways in which ROP-mediated H_2O_2 is a crucial second messenger, such as in the control of other abiotic stresses and root-hair elongation. Given the emerging roles of NADPH-oxidase-dependent ROS in plant signaling [41,45], the ROP–NADPH oxidase–ROS signaling module is expected to be an important component underlying the functional diversity of ROP signaling.

ROP signaling to the actin cytoskeleton and polar cell growth

Most of the attention paid to ROP signaling has focused on its role in regulating polar cell growth, a fundamental process that is essential for shape formation in different cells, for root-hair development and for pollen-tube elongation. It is clear that ROPs are key regulators of polar cell growth in various cell systems (reviewed in [3,46]). This aspect of ROP functions follows the general theme of the conserved function of Rho GTPases in all eukaryotic cells. In yeast, Rho GTPases such as Cdc42 are also central regulators of cell polarity and cell morphogenesis [8].

Members of the Rho family, including Cdc42, Rac and Rho, also have pivotal roles in signaling pathways for cell morphogenesis, cell migration and growth guidance in animals [47]. Thus, Rho GTPase signaling provides a unifying mechanism for polar cell growth and cell migration across the plant and animal kingdoms. This link is particularly intriguing given the remarkable differences in the physical requirements for polar cell expansion or migration between plant and animal cells. Polar cell expansion and cell-shape formation in plants is thought to rely on differential wall extensibility and turgor-driven cell expansion, whereas morphogenesis and movement of animal cells are dependent on cytoskeleton-supported protrusion of the membrane. It is well established that members of the Rho family regulate the assembly and organization of the actin cytoskeleton and hence control cell polarity, cell morphogenesis and cell migration in yeast and animal systems [8,47].

The role of ROPs in regulating the actin cytoskeleton is a common thread that links ROPs to polar cell growth in different cell systems ([14•,21,22••,42•]). Specifically, ROPs promote the assembly of a fine and dynamic type of cortical actin microfilaments that are localized to the polar site of cell growth. These microfilaments are analogous to the Cdc42-mediated dynamic cortical actin patches that are localized to the site of growth in yeast or to the Cdc42/Rac-mediated fine actin meshwork that is localized to the leading edge in animal cells. Thus, ROP regulation of actin organization and polar growth reflects a conserved function of Rho GTPases that was probably the first function that the Rho family GTPase ancestor possessed. Notably, specific features of the biochemical mechanisms mediated by ROP-regulated cortical filamentous actin (F-actin) assembly seem to differ from those of Cdc42/Rac-dependent mechanisms in yeast and animal cells.

ROP effector proteins: reinventing wheels for relaying Rho GTPase signals

Activated GTPases transduce signals to downstream interacting proteins called 'effectors', which in turn regulate specific signaling targets such as the actin cytoskeleton. ROP signaling targets such as actin cytoskeleton dynamics and organization and the production of H_2O_2 are conserved in plants, and so one might expect that the ROP effectors that regulate these cellular processes would be also conserved. Surprisingly, no homologs of yeast and animal Rho effectors have been found in plants [3]. Instead, a class of novel plant proteins, known as

ROP-interactive CRIB-motif-containing (RIC) proteins, have been shown to be ROP effectors [48].

Several RICs act as ROP effectors that regulate the organization and dynamics of the cytoskeleton (Y Gu *et al.*, unpublished; Y Fu *et al.*, unpublished). Although it remains to be elucidated, the mechanism by which RICs regulate actin organization seems to differ from that of the Cdc42/Rac-mediated regulation of Arp2/3-dependent actin assembly [47]. There is no evidence to indicate that ROPs activate actin assembly through the Arp2/3 actin-nucleating complex [49].

As discussed above, overwhelming evidence indicates that ROPs promote H_2O_2 production through a PM-localized gp91 NADPH oxidase. In mammalian cells, the ROP counterpart, Rac1, regulates NADPH oxidase by interacting with its regulatory subunit, p47. Because this regulatory subunit is missing in plants, the regulation of NADPH oxidase by ROPs must involve a novel mechanism. Elucidation of this mechanism should be of great interest because of the importance of both ROPs and H_2O_2 in signal transduction in plants.

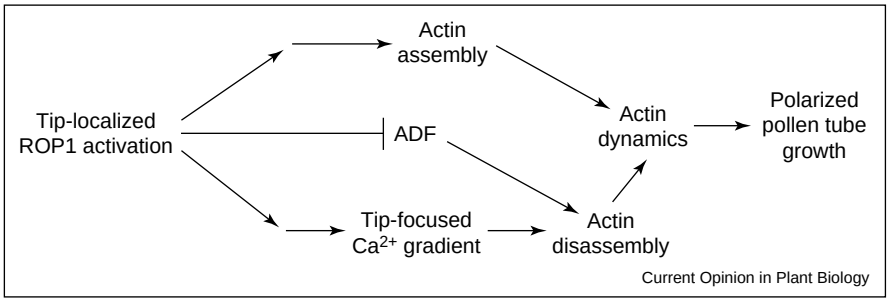
ROP signaling coordinates many downstream pathways

To generate a large output or major physiological responses, a signal or an early signaling event must regulate several downstream pathways. How early signaling events coordinate multiple branch pathways are not well understood. In animals, an activated autophosphorylated receptor tyrosine kinase simultaneously interacts with many proteins to regulate downstream targets. An intracellular signaling kinase can also phosphorylate multiple substrates to propagate upstream signals. For example, the SOS3-mediated SOS2 kinase has been shown to regulate various targets involved in salt tolerance in *Arabidopsis* (see [50] and references therein). Genetic, cell biological and biochemical studies have shown that ROP GTPases interact coordinately with downstream pathways through their regulation of multiple effector proteins (Y Gu *et al.*, unpublished).

Earlier work by our group suggested that ROPs regulate both the formation of a tip-focused Ca^{2+} gradient and actin dynamics, both of which are essential cellular activities for pollen-tube growth [21,23]. Our more recent work has shown that two distinct RICs are ROP1 effectors that promote the assembly of dynamic tip F-actin and modulate the formation of tip-focused Ca^{2+} gradients, respectively (Y Gu *et al.*, unpublished). The tip-localized Ca^{2+} maximum in turn promotes actin depolymerization. Coordination of the two downstream pathways is required for the dynamics of tip F-actin, which are achieved through a treadmilling mechanism (i.e. assembly at the plus end accompanied by disassembly at the minus end of actin polymer). Notably, a recent study by Cheung's group [14•] provides evidence that the tobacco NtRAC1 (an apparent ortholog of ROP1) regulates actin organization through the inactivation of actin depolymerization factor (ADF). Although the NtRAC1 effector for inactivating ADF is unknown, these observations show that ROP1 signaling coordinates at least two interacting pathways in its control of actin dynamics and tip growth in pollen tubes (Figure 3).

The formation of interlocking, jigsaw-puzzle-shaped pavement cells in the leaf epidermis requires the coordination of lobe outgrowth and localized suppression of radial cell expansion in the neck or indented region of pavement cells [22••]. This coordination is controlled by ROP signaling through the spatiotemporal regulation of two types of cytoskeleton: diffuse cortical actin microfilaments localized to the tip of expanding lobes, and cortical microtubules that are transversely oriented along the neck [22••]. ROP2 controls the morphogenesis of pavement cells through at least two functionally distinct RICs that regulate the organization of cortical

Figure 3



A model of the ROP1-mediated orchestration of multiple interacting downstream pathways. ROP1 is locally activated at the apical region of the plasma membrane (PM) and, in turn activates two downstream pathways that promote actin assembly and disassembly, respectively, leading to the dynamics of apical F-actin and tip growth [21,23,27,28,48]. ROP-mediated regulation of apical actin dynamics may also involve the inactivation of actin depolymerization factor (ADF) [14•], but it is not clear whether or not the ADF pathway is regulated by RICs.

microtubules and microfilaments, respectively (Y Fu *et al*., unpublished).

Other ROP-mediated processes probably involve the orchestration of multiple downstream pathways. For example, the regulation of root-hair tip growth by ROPs seems to involve F-actin, H_2O_2, tip-focused Ca^{2+} gradients, and microtubule organization ([41,42•,43]; M Jones *et al*., unpublished). ROP-mediated defense signaling may also coordinately activate the RBOH-dependent oxidative burst, inhibition of ROS scavenging (see above) and other defense mechanisms. *Arabidopsis* possesses 11 RICs, 9 gp91 NADPH oxidases (RBOHs) and probably many other unidentified potential ROP effectors. If one imagines that a ROP GTPase coordinately regulates a combination of these potential effector proteins, the opportunity for ROPs to exert functional diversity is enormous.

PM targeting of ROP GTPases

To transmit extracellular signals immediately from PM-localized receptors, intracellular signaling proteins need to be associated with the PM. Indeed, most ROPs examined are associated with the PM, although localization to the endomembrane has been also observed (reviewed in [3]). Mechanisms for targeting ROPs to specific cellular locations have been investigated in recent years. Three patterns of subcellular distribution have been described for PM-associated ROPs: first, exclusive distribution to the whole PM; second, distribution to both the PM and the nucleus; and third, dynamic distribution to specific domains of the PM and the cytosol.

Group IV ROPs (ROP1–ROP6) tend to show the third localization pattern. Like most yeast and animal Rho GTPases, these ROPs have a carboxy-terminal CAAL motif (where C is cysteine, A is an aliphatic amino acid, and L is leucine) for geranylgeranylation, a posttranslational modification that facilitates membrane targeting. It is known that membrane-localized prenylated Rho GTPases can be cycled to the cytosol by the action of guanine nucleotide dissociation inhibitors (GDIs), which interact only with prenylated and not with non-prenylated GTPases [51]. Thus, interaction with GDIs may provide a key mechanism for the localization of these ROPs to the PM in a spatiotemporally dynamic manner.

ROP9 and ROP10, which belong to group II, are exclusively are localized to the PM, as are their homologs in maize [18••,52,53•]. Both ROP9 and ROP10 contain a CAAX motif (where X is any amino acid except for phenylalanine or leucine) in their carboxyl terminus, which is a potential target for another type of prenylation modification. The PM localization of ROP9 or ROP10 is reduced but not abolished in *era1-2*, a protein farnesyltransferase knockout mutant that lacks farnesylation activity ([18••]; Z Zheng, Z Yang, unpublished), suggesting that a farnesylation-independent mechanism is sufficient for their localization to the PM, as shown for maize ROP7 [18••,52]. Indeed, ROP10/AtRac7 has been shown to be palmitoylated *in vitro* at one or two cysteine residues proximal to the CAAX motif [53•]. A palmitoylation inhibitor, bromopalmitate, inhibits the localization of both ROP9 and ROP10 to the PM, although the specificity of this inhibitor is unknown [18••,53•]. A prenylation-independent mechanism for PM targeting is further supported by the partial PM localization of a ROP11 variant that lacks the carboxy-terminal prenylation motif; this variant is distributed both at the PM and in the nucleus [53•]. ROP11 contains a cysteine residue and may be palmitoylated.

Taken together, these results suggest that both prenylation and palmitoylation are required for the localization of ROPs exclusively to the PM. Notably, palmitoylation coupled with prenylation enables small GTPases to localize exclusively to the PM in mammalian cells, but either modification alone is insufficient for PM localization. These observations imply that a unique mechanism underlies the targeting of apparently palmitoylated ROP GTPases to the PM in plant cells. Future studies should investigate whether this unique mechanism is mediated by a PM-localized protein factor or by specific lipid components of the PM in plant cells.

Upstream regulators of ROPs

At least four classes of Rho GTPase-interactors are known to regulate Rho-family GTPases: activators or guanine nucleotide exchange factors (GEFs,), deactivators or GAPase-activating proteins (GAPs), GDIs and scaffolding proteins. Rho scaffolding proteins, such as BEM1, are essential for the localization of Cdc42 to a specific PM domain in yeast [54], but are poorly characterized in animals and plants. GDIs regulate Rho GTPases both by sequestering them in the cytosol and by suppressing their activation by GEFs [51]. Humans and *Arabidopsis* possess four and three RhoGDIs, respectively [4,55]. It is unclear whether scaffolding and GDIs are regulated by extracellular signals; but both GEFs and GAPs are known to transmit extracellular signals for Rho GTPase regulation, providing another potential mechanism for regulating the functional diversity and specificity of Rho GTPases [7,47].

There is a marked difference between animals and plants in terms of RhoGEFs and RhoGAPs. Humans possess 80 RhoGAPs [47], whereas *Arabidopsis* has nine RhoGAP homologs, including six CRIB-motif-containing RopGAPs [44] and three pleckstrin homology (PH)-domain-containing GAPs (V Vernoud, J Hwang, Z Yang, unpublished). Knockout of RopGAP4 in *Arabidopsis* apparently affects only hypoxia signaling [19••], suggesting that RopGAPs can indeed mediate the functional specificity of ROP signaling. Rho is regulated by more

than 70 GEFs in mammals, but only one potential RhoGEF homolog is present in plants [47,56,57•,58]. Most mammalian and yeast RhoGEFs contain a Dbl (diffuse B-cell lymphoma) homology (DH) domain with GEF activity [47]. The *Arabidopsis* genome apparently does not encode a DH-domain containing protein [4].

Recently identified unconventional GEFs have shed new light on the regulation of Rho GTPases [59]. The bacterial pathogen *Salmonella typhimurium* expresses SopE, a cytotoxic protein that has Rac/Cdc42 exchange activity but lacks a DH domain [59]. Furthermore, a new family of mammalian GEFs containing a docker domain with GTP-loading activity, represented by the Dock180 protein, has been identified [56,58]. The *Arabidopsis* genome encodes a single homolog of Dock180, named SPIKE1 after the unbranched trichome phenotype in the *spk1-1* knockout mutant [57•]. Pavement cells in *spk1-1* show defects in lobe formation, similar to the *rop* loss-of-function phenotype ([22••], Y Fu *et al.*, unpublished data), supporting the potential GEF function of SPK1. The *spk1-1* mutant does not show many of the phenotypes caused by *rop* knockouts or by overexpression of *DN-rop*, such as defects in pollen-tube growth, root-hair development or morphogenesis, or in the hormone responses described above.

These observations raise the exciting and important possibility that there are novel RhoGEFs and/or mechanisms for the activation of ROPs in plants. Significantly, ROP GTPases have been shown to associate with receptor-like serine/threonine kinases [16,60]. With more than 600 such kinases in *Arabidopsis*, the prospect of their functional link to ROPs is exciting.

Conclusions

Considerable progress has been made in the past two years in our understanding of the functions of ROP GTPases in plants. ROP GTPases have emerged as versatile and powerful regulators of many important processes, such as hormone responses, cytoskeletal organization, root-hair development, cell morphogenesis, and defense and abiotic responses. Importantly, these advances have helped to shed light on the poorly understood signaling mechanisms behind the regulation of these processes.

A major challenge in analysis of ROP genes is the difficulty of unraveling the effects seen in loss-of-function mutants, owing to the functional complexity (i.e. redundant and multiple functions for any given ROP) of these GTPases. Meeting this challenge requires the elucidation of the ROP interacting partners that provide the functional specificity and diversity of ROPs. Significantly, progress is being made in identifying and functionally characterizing such ROP interactors (e.g. RICs and RopGAPs).

Clearly, more of these functional partners need to be isolated and investigated to uncover the multiple regulatory layers in each ROP-dependent pathway, including the initial signal perception, the subsequent targeting of a specific ROP at the cellular and subcellular levels, and the various amplifications of the signal through different ROP effectors. We anticipate that the next few years will bring many more groundbreaking findings in this exciting field of ROP GTPase signaling.

Acknowledgements

Work in ZY's laboratory is supported by grants from the National Science Foundation, the Department of Energy and the US Department of Agriculture. ZW is partly supported by grants from the Fujian Natural Science Foundation (B0210018) and the National Science Foundation of China (30070030).

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Assmann SM: **Heterotrimeric and unconventional GTP binding proteins in plant cell signaling**. *Plant Cell* 2002, **14**:S355-S373.
2. Ullah H, Chen J-G, Temple B, Boyes DC, Alonso JM, Davis KR, Ecker JR, Jones AM: **The β-subunit of the *Arabidopsis* G protein negatively regulates auxin-induced cell division and affects multiple developmental processes**. *Plant Cell* 2003, **15**:393-409.
3. Yang Z: **Small GTPases: versatile signaling switches in plants**. *Plant Cell* 2002, **14**:S375-S388.
4. Vernoud V, Horton AC, Yang Z, Nielsen E: **Analysis of the small GTPase gene superfamily of *Arabidopsis thaliana***. *Plant Physiol* 2003, **131**:1191-1208.
5. Yang Z, Watson JC: **Molecular cloning and characterization of Rho, a Ras-related small GTP-binding protein from the garden pea**. *Proc Natl Acad Sci USA* 1993, **90**:8732-8736.
6. Li H, Wu G, Ware D, Davis KR, Yang Z: ***Arabidopsis* Rho-related GTPases: differential gene expression in pollen and polar localization in fission yeast**. *Plant Physiol* 1998, **118**:407-417.
7. Burridge K, Wennerberg K: **Rho and Rac take center stage**. *Cell* 2004, **116**:167-179.
8. Johnson DI: **Cdc42: an essential Rho-type GTPase controlling eukaryotic cell polarity**. *Microbiol Mol Biol Rev* 1999, **63**:54-105.
9. Zheng Z-L, Yang Z: **The Rop GTPase: an emerging signaling switch in plants**. *Plant Mol Biol* 2000, **44**:1-9.
10. Winge P, Brembu T, Kristensen R, Bones AM: **Genetic structure and evolution of RAC-GTPases in *Arabidopsis thaliana***. *Genetics* 2000, **156**:1959-1971.
11. Kawasaki T, Henmi K, Ono E, Hatakeyama S, Iwano M, Satoh H, Shimamoto K: **The small GTP-binding protein Rac is a regulator of cell death in plants**. *Proc Natl Acad Sci USA* 1999, **96**:10922-10926.
12. Kost B, Lemichez E, Spielhofer P, Hong Y, Tolias K, Carpenter C, Chua N-H: **Rac homologues and compartmentalized phosphatidylinositol 4,5-bisphosphate act in a common pathway to regulate polar pollen tube growth**. *J Cell Biol* 1999, **145**:317-330.
13. Winge P, Brembu T, Bones AM: **Cloning and characterization of *rac*-like cDNAs from *Arabidopsis thaliana***. *Plant Mol Biol* 1997, **35**:483-495.
14. • Chen CY, Cheung AY, Wu HM: **Actin-depolymerizing factor mediates Rac/Rop GTPase-regulated pollen tube growth**. *Plant Cell* 2003, **15**:237-249.

This interesting work provides cell biological and biochemical evidence implicating ADF as a Rop/Rac signaling target in its control of polarized pollen-tube growth.

15. Christensen TM, Vejlupkova Z, Sharma YK, Arthur KM, Spatafora JW, Albright CA, Meeley RB, Duvick JP, Quatrano RS, Fowler JE: **Conserved subgroups and developmental regulation in the monocot rop gene family**. *Plant Physiol* 2003, **133**:1791-1808.

16. Trotochaud AE, Hao T, Wu G, Yang Z, Clark SE: **The CLAVATA1 receptor-like kinase requires CLAVATA3 for its assembly into a signaling complex that includes KAPP and a Rho-related protein**. *Plant Cell* 1999, **11**:393-405.

17. •• Tao LZ, Cheung AY, Wu HM: **Plant Rac-like GTPases are activated by auxin and mediate auxin-responsive gene expression**. *Plant Cell* 2002, **14**:2745-2760.

An important and extensive study suggesting that ROP/RAC participates in auxin signaling. The authors show that tobacco ROP/RAC proteins are rapidly activated by auxin in an dosage-dependent manner. Furthermore, downregulation of ROP/RAC inhibits the expression of auxin-responsive genes, whereas constitutive activation of ROP/RAC activates the expression of these genes.

18. •• Zheng Z-L, Nafisi M, Tam A, Li H, Crowell DN, Chary SN, Schroeder JI, Shen J, Yang Z: **Plasma membrane-associated ROP10 small GTPase is a specific negative regulator of abscisic acid responses in *Arabidopsis***. *Plant Cell* 2002, **14**:2787-2797.

Using knockout mutants and transgenic expression of dominant mutants, the authors show that ROP10 negatively regulates various ABA responses and that localization to the PM is required for its function. The authors propose that ROP10 might be involved in an early step of ABA signaling mediated by a PM-localized receptor.

19. •• Baxter-Burrell A, Yang Z, Springer PS, Bailey-Serres J: **RopGAP4-dependent Rop GTPase rheostat control of *Arabidopsis* oxygen deprivation tolerance**. *Science* 2002, **296**:2026-2028.

This is a highly significant paper showing that hypoxia activation of ROP signaling in response to ROS production is controlled by a ROS- and RopGAP4-mediated negative feedback loop. It is elegantly shown that this loop generates a ROP rheostat and modulates ROS homeostasis, enabling *Arabidopsis* plants to tolerate oxygen deprivation.

20. • Schultheiss H, Dechert C, Kogel KH, Huckelhoven R: **Functional analysis of barley RAC/ROP G-protein family members in susceptibility to the powdery mildew fungus**. *Plant J* 2003, **36**:589-601.

Together with an earlier paper [40] from the same group, this paper provides evidence that ROPs promote the invasion of barley leaves by the biotrophic fungal pathogen powdery mildew. This role of ROPs contrasts with their positive role in promoting host resistance against pathogens by eliciting HR responses (see [11,32]).

21. Fu Y, Wu G, Yang Z: **Rop GTPase-dependent dynamics of tip-localized F-actin controls tip growth in pollen tubes**. *J Cell Biol* 2001, **152**:1019-1032.

22. •• Fu Y, Li H, Yang Z: **The ROP2 GTPase controls the formation of cortical fine F-actin and the early phase of directional cell expansion during *Arabidopsis* organogenesis**. *Plant Cell* 2002, **14**:777-794.

A thorough analysis of the effect of transgenic *35S*::*CA-rop2* and *35S*::*DN-rop2* on the spatiotemporal control of cell expansion and the cytoskeleton during organogenesis. This work demonstrates that ROP2 has a general role in the control of cell-shape formation in developing tissues by promoting the formation of diffuse cortical F-actin and suppressing cortical microtubules.

23. Li H, Lin Y, Heath RM, Zhu MX, Yang Z: **Control of pollen tube tip growth by a Rop GTPase-dependent pathway that leads to the tip-localized calcium influx**. *Plant Cell* 1999, **11**:1731-1742.

24. Li H, Shen J, Zheng Z, Lin Y, Yang Z: **The Rop GTPase switch controls multiple developmental processes in *Arabidopsis***. *Plant Physiol* 2001, **126**:670-684.

25. Lemichez E, Wu Y, Sanchez JP, Mettouchi A, Mathur J, Chua NH: **Inactivation of AtRac1 by abscisic acid is essential for stomatal closure**. *Genes Dev* 2001, **15**:1808-1816.

26. Lin Y, Wang Y, Zhu J, Yang Z: **Localization of a Rho GTPase implies a role in tip growth and movement of the generative cell in pollen tubes**. *Plant Cell* 1996, **8**:293-303.

27. Lin Y, Yang Z: **Inhibition of pollen tube elongation by microinjected anti-Rop1Ps antibodies suggests a crucial role for Rho-type GTPases in the control of tip growth**. *Plant Cell* 1997, **9**:1647-1659.

28. Gu Y, Vernoud V, Fu Y, Yang Z: **ROP GTPase regulation of pollen tube growth through the dynamics of tip-localized F-actin**. *J Exp Bot* 2003, **54**:93-101.

29. Cheung AY, Chen CY, Tao LZ, Andreyeva T, Twell D, Wu HM: **Regulation of pollen tube growth by Rac-like GTPases**. *J Exp Bot* 2003, **54**:73-81.

30. • Arthur KM, Vejlupkova Z, Meeley RB, Fowler JE: **Maize ROP2 GTPase provides a competitive advantage to the male gametophyte**. *Genetics* 2003, **165**:2137-2151.

This elegant study uses knockout mutants to show that the ROP2, although not important for *in vitro* pollen-tube growth, is required for proper function of the male gametophyte, possibly in the signaling that guides the pollen tube.

31. Agrawal GK, Iwahashi H, Rakwal R: **Small GTPase 'Rop': molecular switch for plant defense responses**. *FEBS Lett* 2003, **546**:173-180.

32. Ono E, Wong HL, Kawasaki T, Hasegawa M, Kodama O, Shimamoto K: **Essential role of the small GTPase Rac in disease resistance of rice**. *Proc Natl Acad Sci USA* 2001, **98**:759-764.

33. •• Suharsono U, Fujisawa Y, Kawasaki T, Iwasaki Y, Satoh H, Shimamoto K: **The heterotrimeric G protein alpha subunit acts upstream of the small GTPase Rac in disease resistance of rice**. *Proc Natl Acad Sci USA* 2002, **99**:13307-13312.

The authors demonstrate that reduced disease resistance in a rice Gα knockout is suppressed by transgenic, constitutively active OsRac1, providing evidence that heterotrimeric G proteins may function upstream of ROP/RAC. Analogous regulation of Rho GTPases by heterotrimeric G proteins has been shown in animal cells.

34. Morel J, Fromentin J, Blein JP, Simon-Plas F, Elmayan T: **Rac regulation of NtrbohD, the oxidase responsible for the oxidative burst in elicited tobacco cell**. *Plant J* 2004, **37**:282-293.

35. Schiene K, Puhler A, Niehaus K: **Transgenic tobacco plants that express an antisense construct derived from a *Medicago sativa* cDNA encoding a Rac-related small GTP-binding protein fail to develop necrotic lesions upon elicitor infiltration**. *Mol Gen Genet* 2000, **263**:761-770.

36. Park J, Choi HJ, Lee S, Lee T, Yang Z, Lee Y: **Rac-related GTP-binding protein in elicitor-induced reactive oxygen generation by suspension-cultured soybean cells**. *Plant Physiol* 2000, **124**:725-732.

37. Park J, Gu Y, Lee Y, Yang Z: **Phosphatidic acid induces leaf cell death in *Arabidopsis* by activating the Rho-related small G protein GTPase-mediated pathway of reactive oxygen species generation**. *Plant Physiol* 2004, **134**:129-136.

38. Torres MA, Dangl JL, Jones JD: ***Arabidopsis* gp91phox homologues AtrbohD and AtrbohF are required for accumulation of reactive oxygen intermediates in the plant defense response**. *Proc Natl Acad Sci USA* 2002, **99**:517-522.

39. • Wong HL, Sakamoto T, Kawasaki T, Umemura K, Shimamoto K: **Downregulation of metallothionein, a reactive oxygen scavenger, by GTPase OsRac1 in rice**. *Plant Physiol* 2004, in press.

This paper presents data suggesting that OsRac1 signals suppression of the expression of a ROS scavenger gene — a process that may be important for the sustained accumulation of ROS in defense responses.

40. Schultheiss H, Dechert C, Kogel KH, Huckelhoven R: **A small GTP-binding host protein is required for entry of powdery mildew fungus into epidermal cells of barley**. *Plant Physiol* 2002, **128**:1447-1454.

41. Foreman J, Demidchik V, Bothwell JH, Mylona P, Miedema H, Torres MA, Linstead P, Costa S, Brownlee C, Jones JD *et al.*: **Reactive oxygen species produced by NADPH oxidase regulate plant cell growth**. *Nature* 2003, **422**:442-446.

42. • Jones MA, Shen J-J, Fu Y, Li L, Yang Z, Grierson CS: **The *Arabidopsis* Rop2 GTPase is a positive regulator of**

both root-hair initiation and tip growth. *Plant Cell* 2002, **14**:763-776.
This report shows that overexpression of *ROP2* alters the development of root hairs at different stages, including bulge formation, initiation of tip growth and polarity of tip growth.

43. Molendijk AJ, Bischoff F, Rajendrakumar CSV, Friml J, Braun M, Gilroy S, Palme K: ***Arabidopsis thaliana*** **Rop GTPase are localized to tips of root hairs and control polar growth**. *EMBO J* 2001, **20**:2779-2788.

44. Wu G, Li H, Yang Z: ***Arabidopsis*** **RopGAPs are a novel family of Rho GTPase-activating proteins that require the Cdc42/Rac-interactive binding motif for Rop-specific GTPase stimulation**. *Plant Physiol* 2000, **124**:1625-1636.

45. Kwak JM, Mori IC, Pei ZM, Leonhardt N, Torres MA, Dangl JL, Bloom RE, Bodde S, Jones JD, Schroeder JI: **NADPH oxidase *AtrbohD* and *AtrbohF* genes function in ROS-dependent ABA signaling in *Arabidopsis***. *EMBO J* 2003, **22**:2623-2633.

46. Fu F, Yang Z: **Rop GTPase: a master switch of cell polarity development in plants**. *Trends Plant Sci* 2001, **6**:545-547.

47. Etienne-Manneville S, Hall A: **Rho GTPases in cell biology**. *Nature* 2002, **420**:629-635.

48. Wu G, Gu Y, Li S, Yang Z: **A genome-wide analysis of *Arabidopsis* Rop-interactive CRIB motif-containing proteins that act as Rop GTPase targets**. *Plant Cell* 2001, **13**:2841-2856.

49. Li S, Blanchoin L, Yang Z, Lord EM: **The putative *Arabidopsis* arp2/3 complex controls leaf cell morphogenesis**. *Plant Physiol* 2003, **132**:2034-2044.

50. Cheng NH, Pittman JK, Zhu JK, Hirschi KD: **The protein kinase SOS2 activates the *Arabidopsis* H^+/Ca^{2+} antiporter CAX1 to integrate calcium transport and salt tolerance**. *J Biol Chem* 2004, **279**:2922-2926.

51. Faure J, Dagher MC: **Interactions between Rho GTPases and Rho GDP dissociation inhibitor (Rho-GDI)**. *Biochimie* 2001, **83**:409-414.

52. Ivanchenko M, Vejlupkova Z, Quatrano RS, Fowler JE: **Maize ROP7 GTPase contains a unique, CaaX box-independent plasma membrane targeting signal**. *Plant J* 2000, **24**:79-90.

53. Lavy M, Bracha-Drori K, Sternberg H, Yalovsky S: **A cell-specific,**
• **prenylation-independent mechanism regulates targeting of type II RACs**. *Plant Cell* 2002, **14**:2431-2450.
A series of biochemical and cell-biological analyses provides strong evidence that a prenylation-independent mechanism, probably palmitoylation, is sufficient for ROP targeting to membranes, consistent with an earlier report [52].

54. Irazoqui JE, Gladfelter AS, Lew DJ: **Scaffold-mediated symmetry breaking by Cdc42p**. *Nat Cell Biol* 2003, **5**:1062-1070.

55. Bischoff F, Vahlkamp L, Molendijk A, Palme K: **Localization of AtROP4 and AtROP6 and interaction with the guanine nucleotide dissociation inhibitor AtRhoGDI from *Arabidopsis***. *Plant Mol Biol* 2000, **42**:515-530.

56. Brugnera E, Haney L, Grimsley C, Lu M, Walk SF, Tosello-Trampont AC, Macara IG, Madhani H, Fink GR, Ravichandran KS: **Unconventional Rac-GEF activity is mediated through the Dock180-ELMO complex**. *Nat Cell Biol* 2002, **4**:574-582.

57. Qiu JL, Jilk R, Marks MD, Szymanski DB: **The *Arabidopsis***
• ***SPIKE1*** **gene is required for normal cell shape control and tissue development**. *Plant Cell* 2002, **14**:101-118.
In this paper, the *SPK1* gene is cloned on the basis of a mutant that shows defects in the formation of trichome branches and pavement-cell morphogenesis. The potential connection of SPK1 to ROP signaling is revealed when the mammalian Docker domain family of proteins, which show homology to SPK1, is found to represent a novel class of Cdc42/Rac GEFs.

58. Meller N, Irani-Tehrani M, Kiosses WB, Del Pozo MA, Schwartz MA: **Zizimin1, a novel Cdc42 activator, reveals a new GEF domain for Rho proteins**. *Nat Cell Biol* 2002, **4**:639-647.

59. Erickson JW, Cerione RA: **Structural elements, mechanism, and evolutionary convergence of Rho protein-guanine nucleotide exchange factor complexes**. *Biochemistry* 2004, **43**:837-842.

60. Wengier D, Valsecchi I, Cabanas ML, Tang WH, McCormick S, Muschietti J: **The receptor kinases LePRK1 and LePRK2 associate in pollen and when expressed in yeast, but dissociate in the presence of style extract**. *Proc Natl Acad Sci USA* 2003, **100**:6860-6865.