# Regulatory and Cellular Functions of Plant RhoGAPs and RhoGDIs

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**Abstract** Rho GTPases play central roles in the regulation of essential cellular processes, such as directional expansion, motility, and division. RhoGEFs (Guanine Nucleotide Exchange Factors) have key functions in the stimulus-induced spatio-temporal control of Rho GTPase activity. RhoGAPs (GTPase activating proteins) and RhoGDIs (Guanine nucleotide dissociation inhibitors) have long been seen as less important regulators of Rho GTPase activity, with functions largely restricted to the constitutive attenuation of Rho signaling. Extended families of diverse RhoGAPs, as well as small families of structurally similar RhoGDIs, have been identified in yeast, animals, and plants. Recent research has established that members of these protein families play much more important and complex roles than previously anticipated in the regulation of Rho GTPase activity and cellular processes. Non-plant RhoGAPs and RhoGDIs were shown to be tightly regulated by upstream signaling, and the same is likely to be true for their plant homologs as well. The recent functional characterization of plant RhoGAPs and RhoGDIs has allowed exciting and universally important insights into the molecular mechanisms underlying the control of Rho GTPase activity by these proteins.

## 1 Introduction

Rho family small GTPases are important eukaryotic regulators of signaling lipid metabolism, ROS production, transcription, cytoskeletal dynamics, and membrane trafficking. Through the coordination of these processes, Rho GTPases play key roles in the control of cell motility, division, and growth. In plants, Rho signaling also regulates hormone and stress responses (Jaffe and Hall 2005; Nibau et al. 2006,

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see chapter "RAC/ROP GTPases in the Regulation of Polarity and Polar Cell Growth").

The diverse functions of Rho GTPases all depend on tight spatial and/or temporal control of their activity. In many cell types, spatially restricted Rho signaling orchestrates pronounced polarization (Etienne-Manneville and Hall 2002; Kost 2008). Most Rho GTPases are associated with the plasma membrane, often at specific sites of activation, depending on posttranslational prenylation and, at least in some cases, on activation-trigged acylation (Wennerberg and Der 2004; Yalovsky et al. 2008, see chapter "ROPs, Vesicle Trafficking and Lipid Modifications"). Rho GTPases stimulate downstream signaling when bound to GTP, and are inactive with respect to signaling in the GDP bound conformation after GTP hydrolysis (Fig. 1). RhoGEFs (guanine nucleotide exchange factors), a large and diverse family of typically membrane-associated factors, activate Rho GTPases by promoting GDP for GTP exchange and play a key role in the control of Rho signaling in response to upstream regulators (Fig. 1; Berken et al. 2005; Rossman et al. 2005; see chapter "Structure and Function of ROPs and Their GEFs").

RhoGAPs (GTPase activating proteins) and RhoGDIs (guanine nucleotide dissociation inhibitors) have long been seen as less important, in comparison to RhoGEFs, regulators of Rho activity, with functions largely restricted to constitutive signal attenuation. RhoGAPs can inactivate the signaling functions of Rho GTPases by increasing their low intrinsic GTPase activity, which promotes conversion to the GDP-bound conformation (Fig. 1; Tcherkezian and Lamarche-Vane 2007). RhoGDIs contain a hydrophobic binding pocket capable of accommodating the prenyl tail of Rho GTPases. These proteins can transfer Rho GTPases from the plasma membrane to the cytoplasm, where they are thought to form inactive heterodimers with them (Fig. 1; Hoffman et al. 2000). Activation-dependent acylation of Rho GTPase is likely to inhibit RhoGDI binding (Yalovsky et al. 2008, see chapter "ROPs, Vesicle Trafficking and Lipid Modifications"). Some Rho GTPases appear to interact with RhoGDIs, specifically, in the inactive GDP-bound form (Ueda et al. 1990; Klahre et al. 2006; see section "NtRhoGDI2: Maintenance of Polarized Rho GTPase Activation at the Tip of Tobacco Pollen Tubes), whereas others seem to bind to these proteins independently of their activation status (Nomanbhoy and Cerione 1996). Accordingly, RhoGDIs have been proposed to generally act as negative regulators of Rho signaling (Etienne-Manneville and Hall 2002), and to contribute to the control of the intracellular targeting of activated forms of some Rho GTPases (Del Pozo et al. 2002). Dissociation of Rho GTPase/RhoGDI complexes and subsequent reassociation of Rho GTPases with the plasma membrane, a prerequisite for RhoGEF-dependent activation, appear to be promoted by membrane-associated proteins or lipids acting as RhoGDFs (RhoGDI dissociation factors; Fig. 1; DerMardirossian and Bokoch 2005).

Recent work in non-plant systems has shown that upstream regulators control Rho signaling not only via RhoGEFs, but also by directly regulating RhoGAP activity and Rho GTPase/RhoGDI interaction. A number of signaling pathways that control essential cellular functions alter the activity of RhoGAPs based on different mechanisms including direct binding of lipid or protein factors, proteolytic

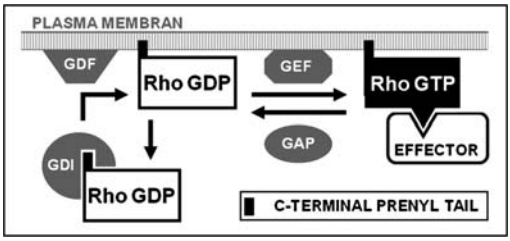


**Fig. 1** *Control of Rho GTPase activity by regulatory proteins*. Most Rho GTPases associate with the plasma membrane via posttranslationally attached C-terminal prenyl tails. They interact with effectors to activate downstream signaling when bound to GTP, and are inactive in the GDP-bound form. RhoGAPs (GTPase activating proteins) enhance the low intrinsic GTPase activity of Rho GTPases, and inactivate their signaling function. RhoGEFs (Guanine nucleotide exchange factors) activate Rho GTPases by promoting GDP for GTP exchange. RhoGDIs (Guanine nucleotide dissociation inhibitors) transfer GDP-bound Rho GTPases to the cytoplasm, where they form inactive heterodimers with them. They can also translocate GTP-bound Rho GTPases between different membrane domains. RhoGDFs (GDI displacement factors) destabilize Rho GTPase/RhoGDI complexes and promote reassociation of Rho GTPases with the plasma membrane, which is required for RhoGEF-mediated activation

degradation, and phosphorylation (Bernards and Settleman 2004; Tcherkezian and Lamarche-Vane 2007; Yoshida and Pellman 2008). Rho GTPase/RhoGDI interactions are also modulated by stimulus-induced phosphorylation either of Rho GTPases or of RhoGDIs by a range of different protein kinases (DerMardirossian and Bokoch 2005; DerMardirossian et al. 2006; Knezevic et al. 2007; Qiao et al. 2008). Much less is known about regulatory mechanisms controlling RhoGAP and RhoGDI activity in plants, although membrane association of a RhoGAP in tobacco pollen tubes was proposed to be modulated by phosphorylation-dependent interaction with a 14-3-3 protein (Klahre and Kost 2006; see section "NtRhoGAP1: Polarity Maintenance at the Tip of Tobacco Pollen Tubes").

Consistent with the regulation of RhoGAP and RhoGDI activity by elaborate signaling mechanisms, recent research has shown that members of these two protein families play much more important and complex roles in the control of Rho GTPase activity and essential cellular functions than previously anticipated (Bernards and Settleman 2004). Work in plants has been at the forefront of this exciting discovery. The purpose of this chapter is to discuss recent advances in our understanding of RhoGAP and RhoGDI functions in plants in the light of a comparison of the plant RhoGAP and RhoGDI families with those of non-plant organisms.

## 2 RhoGAP Protein Families

Interestingly, yeast and animal RhoGAP families generally appear to be two to three larger than the corresponding Rho GTPase families. In budding yeast (*Saccharomyces cerevisiae*), five Rho GTPases and eight RhoGAPs have been

characterized, with two additional predicted RhoGAP genes identified in the genome. Mammalian genomes encode 22 Rho GTPases and 59–70 predicted RhoGAPs, of which about half have been characterized. Fly (*Drosophila melanogaster*) and worm (*Caenorhabditits elegans*) genomes are predicted to contain 11 and 10 Rho GTPase genes, respectively, along with about 20 RhoGAP genes (Tcherkezian and Lamarche-Vane 2007).

All characterized and predicted RhoGAP proteins contain a homologous RhoGAP domain (Lamarche and Hall 1994) with a conserved arginine residue required for catalytic activity (Graham et al. 1999; Rittinger et al. 1997). Outside this domain, non-plant RhoGAPs are highly diverse and contain a bewildering variety of additional domains with many different confirmed or predicted functions, which include protein–protein interaction (e.g., SH3, IQ, RA, etc.), lipid/membrane binding (e.g., PH, C2, PX, etc.) and enzymatic activity (RhoGEF, S/T kinase, myosin motor, etc.) (Bernards and Settleman 2004; Tcherkezian and Lamarche-Vane 2007). Up to 11 different functional domains have been identified in individual non-plant RhoGAPs (Tcherkezian and Lamarche-Vane 2007). An important function of multidomain RhoGAPs was proposed to be the integration of different signaling pathways (Bernards and Settleman 2004; Tcherkezian and Lamarche-Vane 2007). Consistent with this hypothesis, the domain organization of complex RhoGAPs appears to be conserved between different organisms (Bernards 2003).

Despite an extensive discussion in the literature, the significance of the striking diversity within non-plant RhoGAP families has remained a bit of mystery. Although a few members of these families are differentially expressed in distinct cell types or tissues, and/or display RhoGAP activity selectively towards single Rho GTPases, most of them are ubiquitous and can attenuate a range of Rho signaling pathways (Tcherkezian and Lamarche-Vane 2007). Several proteins have been found to contain inactive RhoGAP domains, which bind to Rho GTPases without promoting GTP hydrolysis. These proteins modulate Rho signaling by acting as a scaffold for complex formation (Chiang et al. 2003). A plausible hypothesis suggests that each RhoGAP selectively regulates a single signaling pathway, which is embedded in a complex Rho signaling network, and controls a specific cellular function. Consistent with this idea, knock-out or knock-down of individual RhoGAPs can cause highly specific cellular or developmental defects in mammals and in flies (Tcherkezian and Lamarche-Vane 2007).

The situation in plants is considerably less complex. The *Arabidopsis* genome encodes 11 highly similar Rho GTPases referred to as AtROPs (Rho of plant; Vernoud et al. 2003), of which most have been characterized, at least to some extent (Gu et al. 2004; Nibau, et al. 2006; Yalovsky et al. 2008, see chapter "ROP Evolution and ROPs in Grasses"). Only nine *Arabidopsis* genes coding for predicted AtROPGAP proteins with a RhoGAP domain have been identified (Wu et al. 2000; Hwang et al. 2008). These proteins contain a single recognizable functional domain in addition to the RhoGAP domain, and can be divided into two subfamilies each consisting of structurally very similar proteins, which share a high degree of sequence identity (Fig. 2). One subfamily consists of six relatively small proteins (331–466 amino acids) called AtROPGAP1-6 (Wu et al. 2000), which all contain a

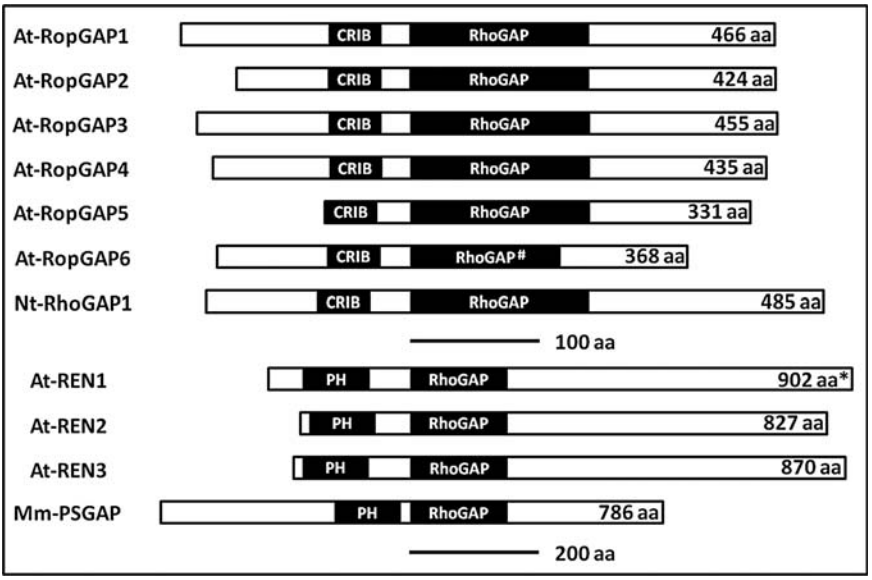


**Fig. 2** *Domain structure of AtROPGAPs and related proteins.* Structures of AtROPGAP1-6 (At5G22400, At4G03100, At2G46710, At3G11490, At1G08340, At2G27440), NtRhoGAP1 (DQ813657), AtREN1-3 (At4G24580, At5G12150, At5g19390), and MmPSGAP (AF297030) are shown drawn to scale (CRIB domain proteins > scale bar: 100 amino acids; PH domain proteins > scale bar: 200 amino acids). All structures are aligned such that the first amino acid of the RhoGAP domain is positioned on a vertical line. #AtROPGAP6 is missing a fragment of the conserved RhoGAP domain. *Displayed is the structure of AtREN1 according to TAIR (www.arabidopsis.org). Full-length cDNA sequencing by Hwang et al. (2008) has shown that the part of the protein between the RhoGAP domain and the C-terminus contains 18 additional amino acids

CRIB (CDC42/Rac interactive binding) domain adjacent to the N-terminal end of the RhoGAP domain (Fig. 2). The gene encoding AtROPGAP6 has been annotated as a pseudo gene, presumably because corresponding EST/cDNA sequences remain to be identified (http://www.arabidopsis.org/). This gene has a normal structure, contains an intact ORF and appears to be expressed according to the GeneVestigator database, although at somewhat lower levels as compared to other subfamily members (https://www.genevestigator.com). However, the predicted AtROPGAP6 protein is lacking a part of the conserved RhoGAP domain (Fig. 2). The second *Arabidopsis* RhoGAP subfamily consists of three larger (870–902 amino acids) proteins called AtREN1-3 (Hwang et al. 2008), which contain a PH domain near the N-terminus (Fig. 2). Rho GTPase and RhoGAP families similar to those of *Arabidopsis* also appear to be present in other plant species (Wu et al. 2000; Klahre and Kost 2006; Hwang et al. 2008). Members of both plant RhoGAP subfamilies display RhoGAP activity towards ROP GTPases in vitro and in vivo (Klahre and Kost 2006; Hwang et al. 2008). Plant RhoGAPs with CRIB domains were also shown to stimulate the GTPase activity of mammalian Rho GTPases in vitro (Wu et al. 2000; Klahre and Kost 2006; see section "NtRhoGAP1: Polarity Maintenance at the Tip of Tobacco Pollen Tubes").

PH domain containing RhoGAPs with similar structure as AtREN1-3 are also found in non-plant systems (Ren et al. 2001; Tcherkezian and Lamarche-Vane 2007; Fig. 2). Consistent with the ability of PH domains to bind to phospholipids, the PH domain of the mammalian RhoGAP PSGAP (Ren et al. 2001) has been shown to be essential for membrane association and correct intracellular targeting. The presence of a CRIB domain in the RhoGAPs of one of the plant subfamilies is more surprising, as such domains are not found in any of the non-plant RhoGAPs identified to date (Tcherkezian and Lamarche-Vane 2007). CRIB domains mediate the interaction of many plant and non-plant Rho effectors, specifically with activated forms of Rho GTPases (Pirone et al. 2001). Because RhoGAP domains on their own are sufficient for specific binding to activated Rho GTPases (Wu et al. 2000; Klahre and Kost 2006), the functions of the CRIB domain of plant RhoGAPs is not entirely clear. In any case, CRIB domains of plant RhoGAPs have been shown to modulate interactions of these proteins with Rho GTPases, as well as their RhoGAP activity (Wu et al. 2000; Klahre and Kost 2006; see section "NtRhoGAP1: Polarity Maintenance at the Tip of Tobacco Pollen Tubes").

GeneVestigator data supported by some experimental verification (Baxter-Burrell et al. 2002; Klahre and Kost 2006) suggest that plant RhoGAPs with a CRIB domain are constitutively expressed at very low levels, whereas genes coding for two of the three PH domain containing *Arabidopsis* ROPGAPs proteins (AtREN1+2, no expression data are available to date for AtREN3) seem to be differentially expressed at a much higher levels, similar to genes encoding AtROPs. One possible explanation for these observations, which clearly need further experimental confirmations, could be that CRIB domains confer a particularly high affinity for active ROP GTPases to plant RhoGAPs. Together with the catalytic nature of RhoGAP activity, this may allow RhoGAPs with CRIB domains to function at low expression levels.

Results of the recent functional characterization of members of both RhoGAP subfamilies found in plants (Baxter-Burrell et al. 2002; Klahre and Kost 2006; Hwang et al. 2008) are summarized in the following sections of this chapter. These results demonstrate that despite the comparably low complexity of these protein families, RhoGAPs play the key roles in the control of Rho GTPase activity and cellular processes also in plants. Furthermore, they have allowed exciting new insights into RhoGAP-dependent signaling mechanisms, which are likely to have important functions also in non-plant systems.

## *2.1 Plant RhoGAP SubFamily I: CRIB domain proteins*

### 2.1.1 AtROPGAP4: Regulation of Oxygen Deprivation Tolerance

To maintain energy-dependent metabolism under flooding-induced oxygen deprivation, plant roots activate ethanolic fermentation and induce alcohol dehydrogenase (ADH) expression. In *Arabidopsis* roots, oxygen deprivation induces increased

ADH expression via ROP activation, which stimulates NADPH oxidase-dependent production of reactive oxygen species (ROS) that act as second messengers. Rho GTPase-dependent stimulation of ROS production also has important functions in the control of tip growth in root hairs (Takeda et al. 2008; see section "AtROPGDI1: Maintenance of Cellular Polarity Required for Root Hair Initiation and Growth") and in pollen tubes (Potocky et al. 2007), as well as of pathogen defense in plants (Ono et al. 2001) and in animals (Bokoch 1994). Constitutive overexpression of dominant negative AtROP2 (DN-AtROP2) in *Arabidopsis* roots prevents ADH activity from increasing under low oxygen conditions, whereas ADH activity is enhanced under nonstress conditions in the presence of constitutively active forms of this protein (CA-AtROP2). After the transfer of wild-type *Arabidopsis* roots to oxygen deprived conditions, levels of activated GTP-bound ROP, which can be pulled down from extracts using a CRIB domain containing effector protein, increase for 12 h, and then start dropping. By contrast, ADH transcript levels and activity keep rising for at least 24 h.

A mutant in which AtROPGAP4 expression is disrupted displays increased levels of GTP-bound ROP, as well as slightly enhanced ADH expression and activity, under nonstress conditions. During the first 12 h of oxygen deprivation, levels of GTP-bound ROP increase and ADH expression and activity in mutant roots rise much steeper than in wild-type roots. However, in striking contrast to what happens in wild-type roots, within the next 12 h under low oxygen conditions, ADH expression and activity in mutant roots massively drop, whereas levels of GTP-bound ROP remain constant. Consistent with these unexpected observations, mutant roots display reduced resistance to oxygen deprivation, and are compromised in their ability to recover from this condition.

Within 12 h, oxygen deprivation also results in the accumulation of about three times higher ROS levels in mutant roots than in wild-type roots, presumably because of increased NADPH oxidase stimulation by ROP overactivation in the absence of AtROPGAP4. Oxidative stress caused by the accumulation of excessive amounts of ROS is likely to be responsible for the collapse of ADH activity in mutant roots after 12 h of oxygen deprivation, as well for reduced resistance to this condition.

On the basis of these observations, AtROPGAP4 appears to have an essential function in restraining ROP activation, and consequently NADPH oxidase stimulation, during oxygen deprivation. Interestingly, AtROPGAP4 transcription is induced by low oxygen conditions, CA-AtROP2 overexpression, and by treatments resulting in increased ROS levels. Furthermore, the stimulation of AtROPGAP4 transcription by oxygen deprivation can be blocked by DN-AtROP2 overexpression and by treatments preventing ROS accumulation. These observations strongly suggest negative feedback regulation of the ROP/NADPH oxidase/ROS signaling pathway in *Arabidopsis* roots via the stimulation of AtROPGAP4 expression. A delicate balance between ROP activation and AtROPGAP4 expression maintained by this feedback loop appears to be required for an effective response of *Arabidopsis* roots to oxygen deprivation. Consistent with this hypothesis, constitutive overexpression of CA-AtROP2, which carries a mutation that disrupts GTPase

activity even in the presence of RhoGAPs (Klahre and Kost 2006), also results in reduced resistance to low oxygen conditions.

### 2.1.2 NtRhoGAP1: Polarity Maintenance at the Tip of Tobacco Pollen Tubes

Vegetative pollen tubes cells rapidly elongate in a strictly polarized manner based on F-actin-dependent tip-directed targeted secretion of cell wall material (Hepler et al. 2001). Rho GTPases, such AtROP1 and the closely related tobacco NtRac5, accumulate at the plasma membrane selectively at the tip of growing pollen tubes (Lin et al. 1996; Kost et al. 1999, Klahre et al. 2006). Specific activation of these Rho GTPases at the pollen tube apex (Hwang et al. 2005) is thought to play a central role in the control of pollen tube tip growth through the coordination of F-actin dynamics and membrane trafficking (Yalovsky et al. 2008). Consistent with this view, overexpression of wild-type or CA Rho GTPases depolarizes pollen tube growth and causes massive tip ballooning, presumably as it results in an extension of the area of the apical plasma membrane-associated with Rho GTPase activity. By contrast, pollen tube elongation is strongly inhibited by the overexpression of DN Rho GTPases (Li et al. 1999; Kost et al. 1999; Klahre et al. 2006). These observations strongly suggest that tight spatial control of Rho GTPase activity is essential for the maintenance of the polarity of pollen tube tip growth.

AtROPGAP1 and its close tobacco homolog NtRhoGAP1 were identified in yeast-two hybrid screens using CA-AtROP1 and CA-NtRac5, respectively, as bait. Pull-down and yeast-two hybrid assays established that full-length AtROPGAP1 and NtRhoGAP1, as well as their isolated CRIB and RhoGAP domains, preferentially interact with active GTP-bound forms of their target Rho GTPases (AtROP1 and NtRac5, respectively). The CRIB domain of AtROPGAP1, but not the one of NtRhoGAP1, also displays affinity to the nucleotide-free transition state of its target Rho GTPase (Wu et al. 2000; Klahre and Kost 2006).

AtROPGAP1 and Nt-RhoGAP1 dramatically enhance the GTPase activity of their target Rho GTPases in vitro. Removal of the CRIB domain strongly reduces the in vitro RhoGAP activity of both proteins towards these targets. AtROPGAP1 and NtRhoGAP1 also show in vitro RhoGAP activity towards mammalian Cdc42 and Rac1, respectively, although in the case of AtROPGAP1, this activity is relatively weak. Surprisingly, removal of the CRIB domain enhances A-ROPGAP1 activity towards Cdc42. These observations are in agreement with the hypothesis that the function of the CRIB domains of plant RhoGAP is to modulate the strength of target Rho GTPase binding. RhoGAP activity may be reduced not only by decreased Rho GTPase affinity, but also by excessively strong target binding, which interferes with substrate turnover (Wu et al. 2000; Klahre and Kost 2006).

Consistent with the ability of AtROPGAP1 and NtRhoGAP1 to inactivate Rho signaling, overexpression of these proteins strongly inhibits pollen tube growth (Hwang et al. 2005; Klahre and Kost 2006). However, the intracellular distribution of N- and C-terminal NtRhoGAP1 YFP (Yellow Fluorescent Protein) fusion

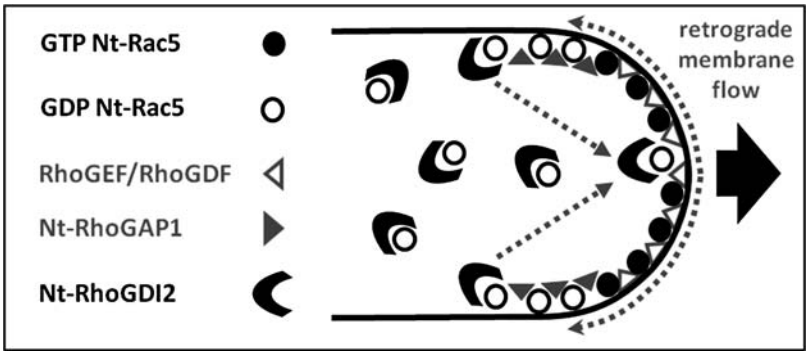


**Fig. 3** *Maintenance of apical NtRAC5 activity at the tip of tobacco pollen tubes*. This model of the molecular mechanisms responsible for the polarization of NtRAC5 activity at pollen tube tip predicts that the stimulation of the fusion of secretory vesicles with the apical plasma membrane by active NtRAC5 generates a constant retrograde flow of plasma membrane material, which laterally displaces this protein. NtRAC5 inactivation by NtRhoGAP1 at the flanks of the tip is required to prevent a depolarization of NtRAC5 activity. NtRhoGDI2 extracts inactive NtRAC5 from the plasma membrane at the flanks of the tip and escorts it through the cytoplasm back to apex. To complete NtRhoGDI2-mediated recycling at the apex, RhoGDFs (such as perhaps the membrane lipid $PIP_2$) promote NtRAC5 reassociation with the plasma membrane by destabilizing NtRAC5/NtRhoGDI2 complexes, and RhoGEFs subsequently reactivate NtRAC5 by promoting GDP for GTP exchange

proteins can be visualized in normally elongating tobacco pollen tubes based on low-level transient expression. Interestingly, both YFP fusion proteins associate with the plasma membrane at the flanks of the pollen tube tip, but not at the apex where active NtRAC5 presumably accumulates (Klahre and Kost 2006). These findings strongly suggest that NtRhoGAP1 plays a central role in the maintenance of the polarized distribution of NtRAC5 activity at the pollen tube tip, which controls directional cell expansion. The stimulation of the fusion of secretory vesicles with the apical plasma membrane by activated NtRAC5 presumably causes constant lateral displacement of this protein. The resulting depolarization of NtRAC5 activity and cell growth can be prevented by NtRhoGAP1-mediated stimulation of GTPase activity at the flanks of the tip. RhoGDI-mediated recycling of inactivated NtRAC5 from this location back to the apex appears to be required for the maintenance of apical NtRAC5 activity (see section "NtRhoGDI2: Maintenance of Polarized Rho GTPase Activation at the Tip of Tobacco Pollen Tubes"; Fig. 3).

A key function of NtRhoGAP1 in the spatial control of NtRAC5 signaling is supported by the observation that overexpression of a dominant negative (DN) mutant form of this protein in tobacco pollen tubes induces ballooning at the tip (Klahre and Kost 2006). DN-NtRhoGAP1 is missing an essential arginine residue in the catalytic domain and does not stimulate NtRAC5 GTPase activity in vitro. As expected, based on the fact that NtRhoGAP1 preferentially associates with GTP-bound NtRAC5 (see *above*), DN-NtRhoGAP1, which fails to promote GTP

hydrolysis, displays enhanced interaction with NtRac5 in yeast two-hybrid assays. When overexpressed as a YFP fusion protein, DN-NtRhoGAP1, particularly, strongly accumulates at plasma membrane of swollen pollen tube tips, which is consistent with increased NtRac5 activation (Klahre and Kost 2006). T-DNA insertions disrupting the expression of the *Arabidopsis* NtRhoGAP1 homolog AtROPGAP1 do not affect pollen tube growth (Klahre and Kost 2006; Hwang et al. 2008). GeneVestigator data indicate that at least four additional AtROPGAPs with a CRIB domain are expressed in *Arabidopsis* pollen tubes, two of them at higher levels. This suggests redundant functions of multiple CRIB domain AtROPGAPs in the control of Rho GTPase activity in pollen tubes, which underscores the importance of the role this protein family plays in the control of tip growth.

Essential functions in cell polarity establishment or maintenance of RhoGAPs, which are associated with distinct plasma membrane domains and confine Rho activity to others, have recently also been identified in animal cells (Simoes et al. 2006; Anderson et al. 2008) and were proposed to underlie bud formation in yeast (Knaus et al. 2007). To further advance our understanding of the spatial control of Rho signaling during cellular polarization, it is essential to investigate the molecular mechanisms responsible for RhoGAP targeting to specific membrane domains. The analysis of the intracellular distributions of truncated forms NtRhoGAP1 fused to YFP (Klahre et al. 2006) has established that a large C-terminal CRIB/RhoGAP-domain-containing fragment, as well as the CRIB domain alone, associates with the plasma membrane at the apex of normally elongating tobacco pollen tubes, where active Rho GTPases accumulate. Interestingly, a short (95 amino acid) N-terminal fragment, which is complementary to the C-terminal CRIB/RhoGAP domain containing fragment (see above) and displays an even cytoplasm distribution on its own, is required for the subapical association of full-length NtRhoGAP1 with the plasma membrane at the flanks of the pollen tube tip.

A yeast-two hybrid screen for proteins interacting with the N-terminal 95 amino acid NtRhoGAP1 fragment resulted in the identification of Nt14-3-3b-1 (Klahre et al. 2006), a member of a protein family implicated in the relocation of target proteins between cellular compartments (Aitken 2002). Nt14-3-3b-1 is specifically expressed at high levels in tobacco pollen and pollen tubes, has no effect on tobacco pollen tube growth when overexpressed on its own, and displays an even distribution throughout the cytoplasm of these cells when fused to YFP. However, Nt14-3-3b-1 coexpression strongly alleviates the inhibition of tobacco pollen tube growth induced by NtRhoGAP1 overexpression, and almost completely prevents the accumulation of NtRhoGAP1 YFP fusion proteins at the plasma membrane. NtRhoGAP1 contains a consensus motif predicted to confer phosphorylation-sensitive binding to 14-3-3 proteins. A point mutation mimicking the phosphorylated state of this consensus motif, which is predicted to enhance interaction with 14-3-3 proteins, reduces membrane association of NtRhoGAP1 YFP fusion proteins, whereas mutations preventing phosphorylation of this motif has the opposite effect (Klahre et al. 2006). Altogether, these observations establish an important function of Nt14-3-3b-1 in the control of NtRhoGAP1 targeting, although the exact molecular mechanism of this process remains to be determined. Recently, the mammalian

RhoGAP DLC1 was also shown to be inactivated by the interaction with a 14-3-3 protein (Scholz et al. 2009).

## *2.2 Plant RhoGAP Subfamily II: PH Domain Proteins*

### 2.2.1 AtREN1: Polarity Maintenance at the Tip of *Arabidopsis* Pollen Tubes

AtREN1 was identified in a screen for *Arabidopsis* mutants showing enhanced effects of low-level overexpression of a GFP AtROP1 fusion protein. The disruption of AtREN1 expression results in massive ballooning at the tip of pollen tubes expressing this fusion protein. In a wild-type background, the absence of AtREN1 expression has the same effect in a less severe form, and also causes precocious pollen germination and results in male sterility. AtREN1 overexpression under the control of the strong pollen-specific Lat52 promoter (Twell et al. 1991) restores normal fertility and in vitro pollen tube growth, whereas an AtREN1 GFP fusion protein complements the *ren-1* mutant phenotype only partially under the same conditions. Interestingly, overexpression of CA-AtROP1 causes similar defects in pollen tubes as the disruption of AtREN1 expression. These data show that AtREN1 is required for the maintenance of polar pollen tube growth, and indicate that it may participate in the restriction of Rho GTPase activity.

GeneVestigator data and RT-PCR suggest preferential expression of AtREN1 at high level in mature pollen. Full-length AtREN1 and an isolated N-terminal fragment of this protein containing the PH and RhoGAP domains selectively bind to activated AtROP1 in vitro. The in vitro GTPase activity of AtROP1 is strongly enhanced in the presence of full-length AtREN1, but not in the presence of mutant AtREN1 missing a conserved arginine residue in the catalytic domain. These observations establish that AtREN1 has RhoGAP activity towards AtROP1.

By contrast to the overexpression of AtROPGAP1 or NtRhoGAP1, which strongly inhibits pollen tube growth (see 2.1.1.2.), overexpression of AtREN1 on its own does not seem to affect this process (see *above*). However, AtREN1 cooverexpression suppresses the depolarization of tobacco pollen tubes induced by the overexpression of a GFP AtROP1 fusion protein, whereas cooverexpression of mutant AtREN1 without RhoGAP activity has the opposite effect, presumably because the mutant protein dominant negatively inhibits endogenous ROPGAPs. Swollen tips of *Atren1* mutant pollen tubes display enhanced plasma membrane association of ROP GTPases, and of the CRIB domain containing AtROP effector AtRIC4. All these data are consistent with a role of AtREN1 in the downregulation of pollen tube ROP GTPase activity.

Immunolabeling and GFP tagging show that AtREN1 accumulates in the cytoplasm at the pollen tube tip, where it extensively overlaps with the styryl dye FM4-64 and with a YFP AtRabA4D fusion protein, which are thought to be associated with subapical endocytic and/or post-Golgi secretory vesicles (Parton et al. 2001; Szumlanski and Nielsen 2009). The intracellular distribution of full-length AtREN1

was compared to that of an N-terminal fragment, which contains the PH and RhoGAP domains, specifically interacts with active AtROP1 (see above), and activates its GTPase activity, but lacks the C-terminus with two coiled-coil domains. This fragment does not accumulate at the pollen tube tip when expressed as a GFP fusion protein. It also fails to suppress the pollen tube phenotype of *Atren1* mutants when expressed as a free protein under the control of the Lat52 promoter. These results demonstrate (1) that the C-terminus is more important than the PH for the intracellular targeting of AtREN1, and (2) that the accumulation of this protein at the pollen tube tip is essential for its function.

Vesicle transport and dynamics at the pollen tube apex are thought to be highly sensitive to latrunculin B (LatB), which at low concentrations appears to specifically disrupt fine apical F-actin structures (Vidali et al. 2001), and to brefeldin A (BFA), which blocks secretion and causes endocytic and post-Golgi organelles to aggregate to "BFA compartments". These compartments have been shown to trap FM4-64 and other plasma membrane markers undergoing endocytic recycling (Nebenführ et al. 2002; Helling et al. 2006). Treatment with LatB or BFA reduces the rate of pollen tube growth and results in loss of tip-specific accumulation of an AtREN1-GFP fusion protein. Unlike FM4-64, this fusion protein does not accumulate in BFA compartments, suggesting that it may be associated with secretory, rather than with endocytic, vesicles. It will be interesting to test whether LatB and BFA have similar effects on the intracellular distribution of an AtRabA4D-YFP fusion protein, which is also thought to be associated with post-Golgi vesicles. In any case, the intracellular distribution of an AtREN1 GFP fusion protein is severely disrupted in mutant *Atraba4d* pollen tubes, which display depolarized growth resulting from defects in the targeted of secretory vesicles (Szumlanski and Nielsen 2009). Furthermore, the introduction of a weak *Atren1* allele into the *Atraba4d* background results in a synergistically enhanced phenotype.

Additional support for an import role of vesicle transport in the function of AtREN1 is provided by the observation that mutant *Atren1* pollen tubes with a phenotype partially complemented by the overexpression of an AtREN1-GFP fusion protein (see *above*) are hypersensitive to LatB and BFA. Treatment of such pollen tubes with these drugs at concentrations that affect wild-type pollen tube only weakly causes pronounced growth depolarization and tip ballooning. Cultured pollen tubes can show growth oscillations. A quantitative correlation analysis was performed (1) of the fluorescence emitted by an AtREN1-GFP fusion protein directly adjacent to the apical plasma membrane of oscillating pollen tubes, and (2) of the growth rate of these cells. Fluorescence levels were found to peak immediately before growth rate maxima both in *Arabidopsis* and in tobacco pollen tubes, although the AtREN1-GFP fusion protein did not display a strong accumulation near the tip of the latter. On the basis of the established phasal relationship between peak growth rate and maximal ROP GTPase activity at the apex of tobacco pollen tubes (Hwang et al. 2005), it was concluded that maximal ROP GTPase activity is followed first by a peak in GFP fluorescence near the apical plasma membrane, which may indicate delivery of secretory vesicles associated with GFP-tagged AtREN1, and then by a peak in growth rate.

Together, all these observation have led to the proposal that global inhibition of AtROP1 by AtREN1 is required for the maintenance of the polarity of pollen tube tip growth, and that delivery of secretory vesicles, with which AtREN1 is associated, to the plasma membrane is essential for the function of this protein. Conceivably, the maintenance of polarized Rho signaling during tip growth depends on two proteins with RhoGAP activity, one that associates subapically with the plasma membrane where it spatially restricts ROP GTPase activity to the apex (NtRhoGAP1, see section "NtRhoGAP1: Polarity Maintenance at the Tip of Tobacco Pollen Tubes"), and one that globally attenuates this activity after delivery to this site by secretory vesicle (AtREN1).

## 3 RhoGDI Protein Families

By contrast to the large families of diverse RhoGAPs found in eukaryotic organisms, in each of these organisms, only a few RhoGDIs have been identified, which share a high degree of sequence identity and are structurally very similar. The human genome appears to encode three RhoGDIs: HsRhoGDI, HsLy/D4GDI, and HsRhoGDIγ. HsRhoGDI is ubiquitously expressed, while the two other proteins are selectively present in a few cell types and tissues. While HsRhoGDI and HsLy/D4GDI, like most other RhoGDIs, are localized in the cytoplasm, HsRhoGDIγ is associated with vesicular membranes (DerMardirossian and Bokoch 2005). In budding yeast, a single cytoplasmic RhoGDI seems to be expressed (Masuda et al. 1994).

RhoGDIs are small proteins with two highly conserved functional domains (Fig. 4). A C-terminal immunoglobulin-like domain (Fig. 4; IG-like) contains a hydrophobic binding pocket, which can accommodate prenyl tails anchoring Rho GTPases in the plasma membrane. This domain is responsible for the ability of

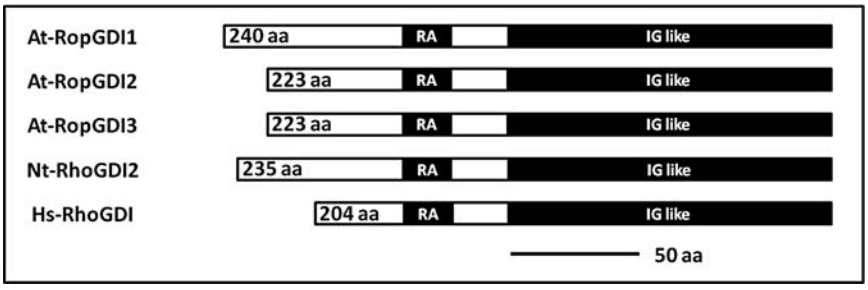


**Fig. 4** *Domain structure of AtROPGDIs and related proteins*. Structures of AtROPGDI 1-3 (At3g07880, At1g12070, At1g62450), NtRhoGDI2 (DQ416769), and HsRhoGDI (AAP35530) are shown drawn to scale (scale bar: 50 amino acids). All structures are aligned such that the first amino acid of the IG-like domain is positioned on a vertical line. *RA* regulatory arm, *IG-like* immunoglobulin like domain

RhoGDIs to transfer Rho GTPases from the plasma membrane to the cytoplasm. RhoGDIs typically seem to preferentially translocate inactive Rho GTPase to the cytoplasm (Ueda et al. 1990; Klahre et al. 2006), although some Rho GTPases have been found to interact equally well in the GDP and in the GTP bound with RhoGDIs (Nomanbhoy and Cerione 1996). Once a cytoplasmic Rho GTPase/RhoGDI heterodimer has been formed, the regulatory arm (Fig. 4; RA) located between the N-terminus and the IG-like domain of RhoGDIs interacts with Rho GTPase regions involved in guanine nucleotide, GEF, and effector binding. This interaction prevents GTP hydrolysis even in the presence of RhoGAP activity, GDP dissociation, GEF-mediated nucleotide exchange, and the activation of downstream signaling pathways. In agreement with the biological inertness of Rho GTPase/RhoGDI heterodimers, RhoGDIs are generally thought to act as negative regulators of Rho signaling (DerMardirossian and Bokoch 2005).

In a range of mammalian cell types, the molar RhoGDI amount is roughly equal to the combined molar amount of all major Rho GTPases (Michaelson et al. 2001). In some of these cell types, Rho GTPases are largely present in cytoplasmic heterodimers with RhoGDIs, whereas in others variable levels of free Rho GTPases are detected (Chuang et al. 1993; Fritz et al. 1994). Consistent with these observations, mammalian Rho GTPases differ widely in their affinities to RhoGDIs, suggesting that they may be subject to regulation by these proteins to a variable extent (DerMardirossian and Bokoch 2005).

RhoGDI overexpression has been shown to downregulate Rho signaling, and to interfere with Rho regulated process in many different cell types and organisms. However, disruption of the genes encoding the single RhoGDI identified in budding yeast (Masuda et al. 1994), or the ubiquitously expressed mouse homolog of HsRhoGDI (MmRhoGDI), causes surprisingly mild phenotypes. Because mice in which additional RhoGDI genes have been disrupted are not yet available, it is possible that stronger defects in the development of MmRhoGDI knock-out mice were masked by the upregulation of other RhoGDI genes (DerMardirossian and Bokoch 2005).

The *Arabidopsis* genome contains three genes encoding proteins with a high degree of sequence identity, which are very similar to characterized mammalian and yeast RhoGDIs (Fig. 4). These proteins are called AtROPGDI1-3 and, as their homologs identified in other plants species, contain short, highly divergent N-terminal extensions, which remain to be functionally characterized (Carol et al. 2005; Klahre et al. 2006). GeneVestigator data suggest that transcripts of all three AtROPGDI genes reach high levels in mature pollen, and are ubiquitously present at much lower levels in other organs throughout plant development. AtROPGDI1 transcripts seem to be the most abundant in all cell types and tissues. Interestingly, the GeneVestigater expression patterns displayed by all three AtROPGDI genes nicely overlap with that of the AtROP gene family as a whole. Also, the combined expression levels of all AtROPGDIs and AtROPs in each tissue and cell type seem to be similar. This suggests that, as in mammalian cells, roughly equal molar amounts of AtROPGDIs and AtROPs may be present in *Arabidopsis* cells. The recent functional characterization of AtROPGDI1, and of a tobacco pollen tube

homolog of this protein, has established that these proteins are not simply down-regulating ROP signaling, but are essential for the establishment and maintenance of spatially restricted ROP activity, which controls polarized cell expansion (Carol et al. 2005; Klahre et al. 2006). The results of these studies are summarized in the sections below.

## 3.1 *AtROPGDI1: Maintenance of Cellular Polarity Required for Root Hair Initiation and Growth*

Root hairs are single, uniaxial, highly elongated protrusions growing out from the basal end of root epidermal cells (trichoblasts). They elongate by tip growth much in the same way as pollen tubes (see section "NtRhoGAP1: Polarity Maintenance at the Tip of Tobacco Pollen Tubes"). AtROP2 and other AtROP GTPases accumulate at the trichoblast plasma membrane selectively at sites from which root hairs will emerge, and remain associated specifically with the apical plasma membrane of elongating root hairs. Root hair tip growth, like the expansion of pollen tubes, is depolarized upon expression of CA-AtROP GTPases, and is blocked by DN forms of these proteins (Jones et al. 2002; Molendijk et al. 2001). The stimulation of ROS production by NADPH oxidases appears to play a key role in the control of root hair elongation downstream of ROP GTPase activation, as it does in the regulation of other processes by ROP GTPases, including the response of *Arabidopsis* roots to oxygen deprivation (see section "AtROPGAP4: Regulation of Oxygen Deprivation Tolerance"). ROS accumulate in trichoblasts at sites of future root hair outgrowth, as well as at the apex of elongating root hairs, depending on the activity of the NADPH oxidase RHD2/AtrbohC. Trichoblast of *Arabidopsis* mutants defective in the gene coding for this protein form correctly positioned but highly stunted root hairs, which fail to accumulate ROS at the tip (Foreman et al. 2003).

By contrast, trichoblasts of the *Arabidopsis supercentipede1* (*scn1*) mutant initiate multiple (about three in average) root hairs at random positions, which remain very short and often split at the tip to form multiple growth sites. An AtROP2 GFP fusion protein displays strongly enhanced association with the plasma membrane of mutant trichoblasts, and is mislocalized to all sites of cell expansion, where ROS also rise to high levels. Interestingly, the *scn1* phenotype is caused by defects in the gene that encodes AtROPGDI1, which either disrupt gene expression, or result in the production of a mutant protein in which a highly conserved glutamate residue at position 181 is replaced by glycine. Consistent with an important function of this residue in Rho GTPase binding predicated based on modeling of an AtROP GTPase/AtROPGDI1 complex, recombinant mutant AtROPGDI1$^{Glu188Gly}$ displays a strongly reduced ability to pull-down ROP GTPases from cauliflower extracts (Carol et al. 2005).

These observations demonstrate that in trichoblasts, AtROPGDI1 is essential for the establishment and the maintenance of cellular polarity. Interaction of

AtROPGDI1 with AtROP GTPases, which spatially restricts ROP GTPase activity and RHD2/AtrbohC-mediated ROS production, is required for the determination of a single site of cell expansion during root hair formation.

## *3.2 NtRhoGDI2: Maintenance of Polarized Rho GTPase Activation at the Tip of Tobacco Pollen Tubes*

Like NtRhoGAP1, NtRhoGDI2 was identified in a yeast two-hybrid screen for proteins that interact with the Rho GTPase NtRac5, a key regulator of tobacco pollen tube tip growth (see section "NtRhoGAP1: Polarity Maintenance at the Tip of Tobacco Pollen Tubes"). NtRhoGDI2 is highly similar to *Arabidopsis* and mammalian RhoGDIs (Fig. 4). Northern analysis has shown that transcripts encoding this protein accumulate to high levels specifically in tobacco pollen and pollen tubes. In yeast two-hybrid assays, NtRhoGDI2 shows strong interaction with NtRAC5, weaker interaction with CA-NtRAC5, and no interaction with DN-NtRAC5. These observations suggest that NtRhoGDI2, like other RhoGDIs (see Section "RhoGDI Protein Families"), preferentially interacts with its target Rho GTPase in the inactive GDP bound form (Klahre et al. 2006). RhoGDIs generally do not interact well with DN forms of Rho GTPases, which are considered nucleotide-free because of their low affinity for both GDP and GTP (Strassheim et al. 2000). Interestingly, a point mutation preventing the prenylation of NtRAC5, which is expected to promote nuclear targeting required for the detection of yeast two-hybrid interactions, weakens the interaction of NtRAC5 with NtRhoGDI2 in yeast two-hybrid assays. This is in agreement with an important function of interactions between the prenyl tail of NtRAC5 and the hydrophobic pocket in the IG-like domain of NtRhoGDI2 in the binding of the two proteins to each other (Scheffzek et al. 2000).

Cell fractionation, as well as C- and N-terminal YFP tagging, has established that NtRhoGDI2 accumulates in the cytoplasm of tobacco pollen tubes. NtRhoGDI2 overexpression strongly inhibits the growth of these cells and effectively suppresses the accumulation of a coexpressed NtRAC5 YFP fusion protein at the apical plasma membrane. Cooverexpression of NtRhoGDI2 and NtRAC5 at different relative levels has shown that excess NtRhoGDI2 activity inhibits the elongation without inducing tip swelling, whereas excess NtRAC5 activity depolarizes the growth and results in ballooning at the tip. Interestingly, pollen tubes overexpressing NtRhoGDI2 and NtRAC5 at similar levels can grow normally, suggesting that the two proteins can neutralize each other. NtRhoGDI2 was also cooverexpressed at similar levels with YFP tagged or free CA-NtRAC5. In these experiments, plasma membrane accumulation of CA-NtRAC5 fused to YFP was only partially suppressed and growth depolarization by free CA-NtRAC5 was not inhibited at all. These results provide further evidence for the preferential interaction of NtRhoGDI2 with inactive GDP-bound NtRac5. Together, the observations summarized in this section are consistent with the view that NtRhoGDI2, like other

RhoGDIs (see section "RhoGDI Protein Families"), transfers GDP-bound NtRAC5 from the plasma membrane to the cytoplasm, where the two proteins form inactive heterodimers (Klahre et al. 2006).

Replacement of the arginine at position 69 by alanine specifically disrupts the ability of NtRAC5 to interact with NtRhoGDI2. The in vitro GTPase activity of NtRAC5 is not affected by this mutation. In yeast two-hybrid assays, $Ntrac5^{arg69ala}$ does not interact with NtRhoGDI2, but shows normal interaction with NtRhoGAP1 as well as with NtRAC5 effectors. Interestingly, an $Ntrac5^{arg69ala}$ YFP fusion protein accumulates strongly at the plasma membrane at the flanks of tobacco pollen tube tips, but is almost completely absent from the apex, where NtRAC5 accumulates (see section "NtRhoGAP1: Polarity Maintenance at the Tip of Tobacco Pollen Tubes"). YFP-tagged DN-Ntrac5, which does not interact with NtRhoGDI2 either, shows the same aberrant intracellular distribution. Furthermore, in contrast to NtRAC5, $Ntrac5^{arg69ala}$ fails to depolarize pollen tube growth when overexpressed and does not block the inhibition of this process by cooverexpressed NtRhoGDI2. Together, these results strongly suggest that interaction with NtRhoGDI2 is required for the accumulation of NtRAC5 at the pollen tube apex, as well as for the activation of this protein at this location (Klahre et al. 2006).

On the basis of the observations summarized above, NtRhoGDI2 has been proposed (1) to extract NtRac5 from the plasma membrane at the flanks of the pollen tube tip after its inactivation by NtRhoGAP1 (see section "NtRhoGAP1: Polarity Maintenance at the Tip of Tobacco Pollen Tubes"), and (2) to subsequently escort this protein in the GDP-bound form through the cytoplasm back to the apex (Fig. 3). RhoGDF activity, which destabilizes NtRAC5/NtRhoGDI2 complexes and promotes NtRAC5 reassociation with the plasma membrane, together with Rho-GEF activity, which promotes GDP for GTP exchange, is thought to reactivate NtRAC5 at the apex (Fig. 3). Consistent with this model, an *Arabidopsis* protein with RhoGEF activity accumulates at the plasma membrane at the tip of tobacco pollen tubes when expressed in these cells (Gu et al. 2006). The membrane lipid phosphatidylinositol 4,5-bisphosphate ($PIP_2$) also accumulates specifically at the apex of tobacco pollen tubes, is generated by a lipid kinase activity pulled down form extracts of these cells together with ROP GTPases (Kost et al. 1999), and has GDF activity in animal cells (Fauré et al. 1999). The stimulation of $PIP_2$ production by active NtRAC5, together with the promotion of NtRAC5 activation by $PIP_2$-mediated destabilization of NtRAC5/NtRhoGDI2 complexes, could potentially create a positive feedback loop that helps focusing NtRAC5 activity at the pollen tube apex (Klahre et al. 2006).

The results of the functional characterization of NtRhoGDI2 suggest that the primary role of this protein is not to downregulate NtRac5 signaling. Rather, NtRhoGDI2-mediated recycling from the flanks of the pollen tube tip to the apex appears to be required for NtRac5 activation specifically at this location (Kost 2008). Consistent with this view, the growth of tobacco pollen tubes containing constructs designed to silence NtRhoGDI2 expression is strongly reduced, but not depolarized (Fig. 5). AtROPGDI1 in *Arabidopsis* trichoblasts and root hairs (see section "AtROPGDI1: Maintenance of Cellular Polarity Required for Root Hair

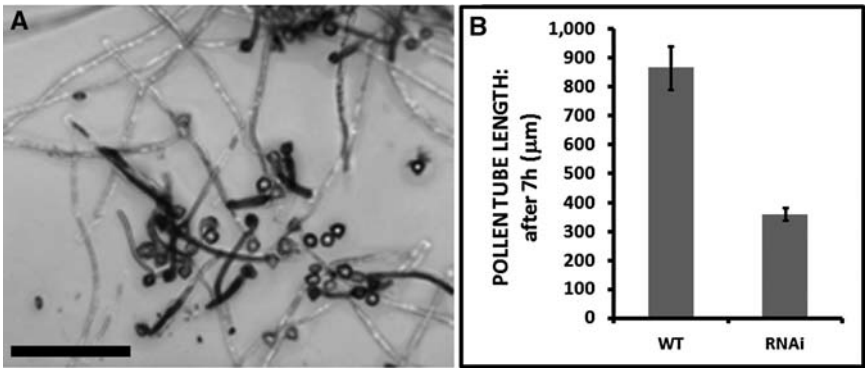


**Fig. 5** *Constructs designed to silence NtRhoGDI2 expression inhibit tobacco pollen tube growth without depolarizing this process*. Pollen was collected from heterozygous transgenic tobacco plants containing a single T-DNA inserts that confers pollen-specific expression of (1) an RNAi construct (Wesley et al. 2001) designed to downregulate NtRhoGDI2 transcript levels (Lat52 promoter; Twell et al. 1991), and (2) a GUS (β-glucuronidase) gene (AtProfilin4 promoter; Christensen et al. 1996). (**a**) Seven hours after pollen plating on solid culture medium (Read et al. 1993), a histochemical GUS assay was performed, which resulted in the selective blue (dark) staining of transgenic pollen tubes, whereas nontransgenic pollen tubes remained transparent. As expected for pollen produced by heterozygous transformants, 50% of the analyzed pollen tubes were transgenic and displayed GUS activity. These pollen tubes were clearly shorter that non-transformed pollen tubes, but did not display ballooning at the tip. (**b**) Statistical analysis of pollen tube length showed that within the first 7 h after germination the presence of an NtRhoGDI2 RNAi construct reduced pollen tube growth by roughly 50%. Essentially, the same results were obtained with 3 independent transgenic tobacco lines. Scale bar: 200 µm; error bars: 95% confidence interval

Initiation and Growth"), as well as non-plant RhoGDIs at least in some systems (Lin et al. 2003), are likely to function in a similar manner as NtRhoGDI2.

## 4 Conclusions

Recent research has established that RhoGAPs and RhoGDIs are not simply attenuating Rho GTPase signaling in a constitutive manner. Members of both protein families play important and highly complex roles in the control of Rho GTPase activity and of cellular processes in animals, yeast, and plants. The functions of non-plant RhoGAPs and RhoGDIs are tightly regulated by upstream signaling and there are indications in the literature that the same is also true for plant homologs of these proteins. The functional characterization of plant RhoGAPs and RhoGDIs during the past few years has allowed exciting insights in the molecular mechanism underlying the spatio-temporal control of Rho GTPase activity by these proteins, which are likely to be relevant also for non-plant systems.

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