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MINI-REVIEW

Small GTPases in plant biotic interactions

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ABSTRACT
The superfamily of small monomeric GTPases originated in a common ancestor of eukaryotic multicellular organisms and, since then, it has evolved independently in each lineage to cope with the environmental challenges imposed by their different life styles. Members of the small GTPase family function in the control of vesicle trafficking, cytoskeleton rearrangements and signaling during crucial biological processes, such as cell growth and responses to environmental cues. In this review, we discuss the emerging roles of these small GTPases in the pathogenic and symbiotic interactions established by plants with microorganisms present in their nearest environment, in which membrane trafficking is crucial along the different steps of the interaction, from recognition and signal transduction to nutrient exchange.

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Introduction

Plants respond to the environment in a different way than animals considering their sessile nature. This condition has shaped the evolution of plant genomes to develop genetic and epigenetic programs that integrate external stimuli with endogenous cues in a more versatile manner, leading to the occurrence of plant-specific processes. Thus, it is common to observe gene families that have expanded in plants as compared with other eukaryotic kingdoms, giving origin to sub- or neo-functionalization associated to plant-specific functions. The small GTPases superfamily is one of the most important gene families associated to vesicle trafficking, signaling, cytoskeleton rearrangements and nuclear transport, constituting a remarkable example of the evolution associated to kingdom-specific processes. The composition of this gene family in humans, yeast and plants has been previously covered.[1-3] Here, we will review the current knowledge of the functions that small GTPases exert in trafficking and cytoskeleton rearrangement during the interaction of plants with other organisms (Table 1). Other recent reviews have covered the more comprehensive topic of membrane trafficking associated to plant-microbe interactions.[4-5]

Plant small GTPases

Small monomeric GTPases are present in all studied eukaryotic organisms, from yeast to mammals and higher plants, being one of the most important gene families exerting regulatory functions in a myriad of cellular processes. First discovered by their role in cancer caused by viral infection,[6] they were subsequently characterized as modulators of signaling pathways, membrane trafficking, cytoskeleton rearrangements and nuclear transport. The superfamily of small GTPases (also referred to as the Ras superfamily) is divided into 5 families in yeast and animals; Rab, Ras, Arf, Rho and Ran; however, the Ras family has not been found in plants (Fig. 1A). Comparison of the genomes of yeast (*Saccharomyces cerevisae*) and more complex organisms suggests that the expansion of the small GTPase superfamily is associated to pluricellularity. Even though the number of Rab proteins encoded in the human genome is similar to the predicted number of gene members in *Arabidopsis thaliana*, phylogenetical analyses revealed asymmetric evolution of certain clades, as for example the branch of Rab11/Rab25 (referred to as RabA in plants), which has largely expanded in plants[1]: whereas this subfamily represents a small proportion of Rab proteins in human and yeast (5 and 18%, respectively), it accounts for 45% of the total of Rabs encoded in the genome of Arabidopsis, as well as other plant species.[3,7] The amino acid sequences of Rab and Arf family members are extremely well conserved across kingdoms, whereas members of the Rho/Rac family are more divergent between plants and animals, with members of the plant family (designated as ROPs for

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Table 1. List of small GTPases involved in plant interactions with microorganisms.

GTPase	Type	Organism	Accession number	References
Root nodule symbiosis				
Rab7p	RabG	*Glycine max*	Glyma.20G171600	16
Rab7p	RabG	*Vigna aconitifolia*	L14928	16
Rab7A1	RabG	*Medicago truncatula*	Medtr4g012940	36
Rab7A2	RabG	*Medicago truncatula*	Medtr4g069850	36
RabA2	RabA	*Phaseolus vulgaris*	Phvul.011G061100	21,24
Rab1p	RabD	*Glycine max*	Glyma.12G165300	16
ROP10	type-II ROP	*Medicago truncatula*	Medtr3g078260	22
ROP6	type-I ROP	*Lotus japonicus*	JF260911	23,34,35
ROP9	type-I ROP	*Medicago truncatula*	Medtr5g022600	19
Plant Defense against Pathogen				
ARF1	ARFA	*Nicotiana benthamiana*	DQ531849	42
ARFA1b	ARFA	*Hordeum vulgare*	AK252968	47
ARFA1c	ARFA	*Hordeum vulgare*	AJ508228	47
ARA7/RabF2b	RabF	*Arabidopsis thaliana*	At4g19640	37
RABA1b	RabA	*Arabidopsis thaliana*	At1g16920	38
RABA4c	RabA	*Arabidopsis thaliana*	At5g47960	38,45
RABA6a	RabA	*Arabidopsis thaliana*	At1g73640	38
RabE1d	RabE	*Arabidopsis thaliana*	At5g03520	40,41
RabG3b	RabG	*Arabidopsis thaliana*	At1g22740	43
YPT1-like	RabD	*Hordeum vulgare*	AK361131	46
Rac1	type-II ROP	*Oryza sativa*	LOC_Os01g12900	54,55
Rac4	type-II ROP	*Oryza sativa*	LOC_Os06g12790	54
Rac5	type-I ROP	*Oryza sativa*	LOC_Os02g58730	54
RACB	type-I ROP	*Hordeum vulgare*	AJ344223	50,51,53
ROP6	type-I ROP	*Arabidopsis thaliana*	At4g35020	52
ROP9	type-I ROP	*Medicago truncatula*	Medtr5g022600	19

Rho of Plants) displaying higher sequence identity with Rac proteins from animals.

Small GTPases act by switching between an active-state bound to GTP and an inactive GDP-associated form. In their active state, small GTPases interact with effector proteins. In addition, their subcellular localization is modified by covalent binding to lipid molecules that regulate their transient and reversible association to membranes (Fig. 1B). Rabs are modified by prenylation (primarily by geranyl-gernanylation), whereas Arfs are myristoylated,[8] changing their localization from cytosol to membranes. Rop proteins are also modified by prenylation, but in addition they can be constitutively or reversibly modified by S-acylation (by palmitic or stearic acids) as part of their regulatory mechanisms.[9-12] Based on the primary sequence of proteins, Rops have been classified into 2 types: type I Rops contain a CaaL box in the hypervariable C-terminal region and undergo geranylgeranylation, whereas type II Rops contain a GC-CG box that is S-acylated.[12-13] Members of the Ran family, which participate in the import/export across the nuclear pore, are not subjected to post-translational modification by lipids. Since they have not been associated to any of the biologic processes considered in this review, this family will not be further discussed.

Rab and Arf proteins in their active state -associated to GTP- participate in all stages of membrane trafficking: Arfs have been mainly associated to vesicle budding, whereas Rabs are necessary for cytoskeleton-guided transport and regulation of tethering and fusion of vesicles with target membranes (Fig. 1C). This vesicle movement connects cellular organelles with the extracellular/surrounding cell microenvironment through the different endocytic and exocytic pathways (Fig. 1D). The function of small GTPases of the Rab and Arf families as regulators of these membrane trafficking pathways is well conserved in eukaryotes. On the other hand, Rop proteins exert their function mainly by controlling cytoskeleton dynamics. Rop proteins control assembly and disassembling of F-actin modulating the action of proteins that shape the actin networks (Fig. 1E) and changes in the dynamic of cortical microtubules. In animal systems, these changes are crucial for ligand-activation of plasma membrane receptors and have been linked to the production of reactive oxygen species (ROS).[14–15]

Plant-microbe interactions: Small GTPases in a trans-kingdom world

Plants engage in different types of ecological relationships with surrounding microorganisms, from interactions that benefit both partners -symbiosis or mutualism- to pathogenic interactions, where fungi or bacteria infect and colonize their hosts. In both types of interactions, the role of small GTPases was established over 20 y ago[16-17] and since then, significant advances has been made to understand the mechanisms by which the small GTPases modulate endomembrane trafficking in processes such as changes in cell polarity or the

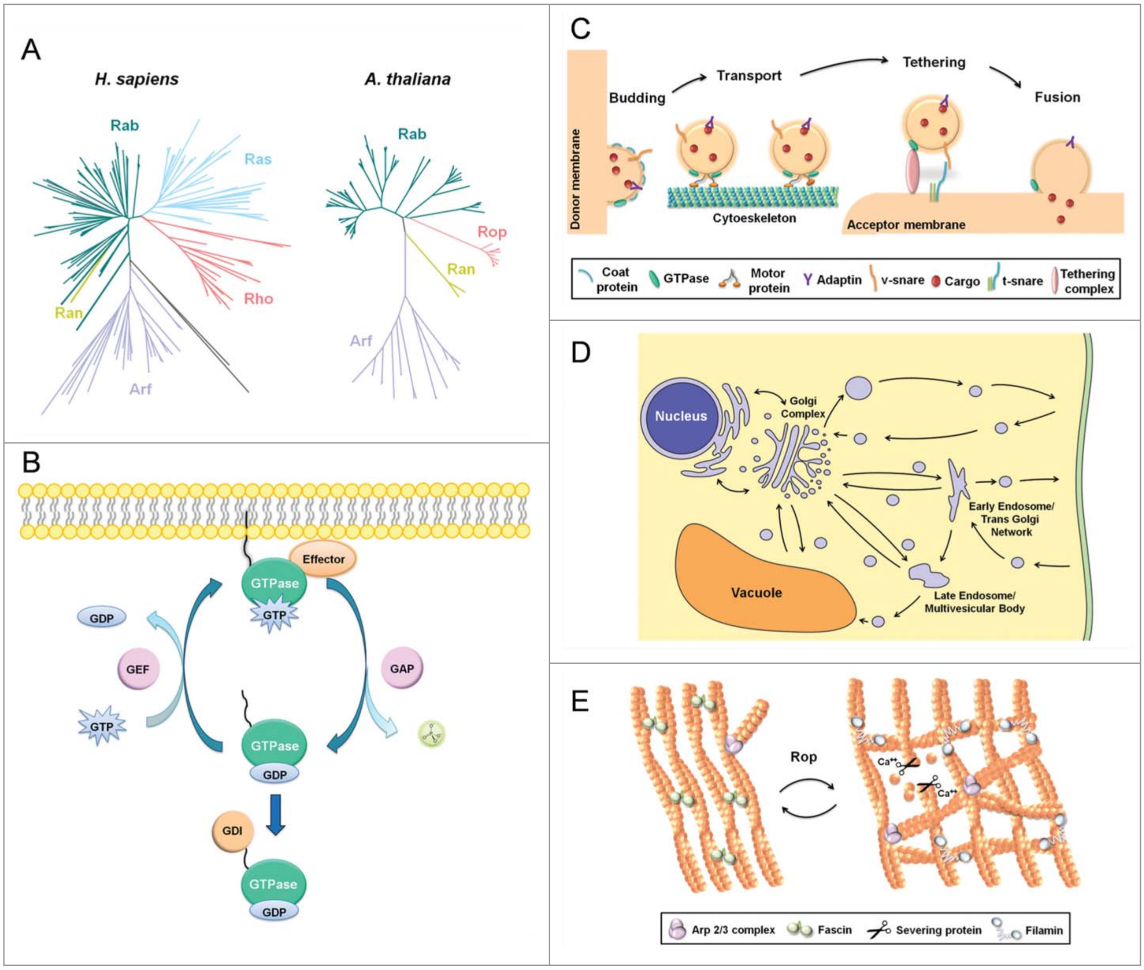

Figure 1. (A) Phylogenetic diagrams of the small GTPase superfamily from human (*Homo sapiens*) and *Arabidopsis thaliana*. The superfamily of small GTPases is divided into 5 and 4 families in animals and plants, respectively. Note that the Ras family is absent in plants. (B) Regulatory cycle of small GTPases. Small GTPases cycle between a GTP-bound active state and a GDP-bound inactive state. This activation is regulated by guanine nucleotide exchange factors (GEFs) that catalyze the exchange of GDP to GTP, GTPase activating proteins (GAPs) that increase the rate of GTP hydrolysis, and guanosine nucleotide dissociation inhibitors (GDIs) that inhibit the exchange of GDP to GTP, keeping a pool of inactive GTPases in the cytosol. Small GTPases associate to membranes (whit the exception of Ran GTPases) by lipid modifications. Membrane localization and binding to GTP are necessary for the interaction of small GTPases with specific effectors that activate downstream signaling pathways. (C) Vesicle trafficking controlled by Rab/Arf GTPases. Arf and Rab GTPases regulate distinct membrane trafficking steps: vesicle budding from donor membranes, vesicle transport facilitating vesicle motility along the cytoskeleton and tethering and fusion of vesicles and target membranes. (D) GTPases are involved in the endocytic, secretory and recycling pathways. GTPases are components of vesicle trafficking machinery that regulate traffic between intracellular compartments. (E) Rop GTPases control cytoskeleton rearrangements. The scheme shows how actin dynamics is regulated by proteins that control the association of actin fibers forming bundles or networks, branching and remodeling by severing proteins. These rearrangements are controlled by Rop GTPases and the action of calcium.

activation of molecular mechanisms triggered by microbial signals.

Symbiotic interactions between plants and microorganisms

One way in which plants cope with the limitation of macronutrients in the soil is by establishing endosymbiotic interactions with fungi and bacteria. The most extended association of this kind, called arbuscular mycorrhiza (AM), was the key for aquatic plants to colonize terrestrial lands. AM is established with glomeromycota fungi by approximately 90% of the plant species, facilitating solubilization and acquisition of phosphorus and nitrogen from the soil. Chitooligosaccharides produced by AM fungi are perceived by plasma membrane receptors, activating a genetically defined signaling pathway that results in the intracellular accommodation of AM fungi. Tree-shaped hyphal structures called arbuscules are formed, where a plant-derived membrane surrounds fungal hyphae and plays important functions in nutrient acquisition.[18] MtROP9 has been implicated in the colonization of *Glomus intraradices* in *Medicago truncatula* plants; knock-down of *MtROP9* by using an RNA interference (RNAi) strategy stimulates initial colonization of roots and progression of fungal hyphae through epidermal cell layers, most likely by a mechanism that reduces the generation of ROS.[19] Interestingly, knock-down of MtROP9 also stimulates the colonization by the pathogenic oomycete *Aphanomyces euteiches*, suggesting a dual role for this small GTPase in beneficial and detrimental biotic interactions.

Another ecologically and agronomically relevant mutualistic interaction, the root nodule symbiosis (RNS), is established between a limited number of plants concentrated in the legume family (with the exception of small trees from the genus *Parasponia*) and diazotrophic soil bacteria called rhizobia.[20] This interaction allows legume plants to obtain nitrogen in assimilable forms from the bacteria in exchange to photosynthetic products. The most expanded and sophisticated mode of infection requires a coordinated series of changes at molecular and morphological levels in the root hair to form a tubular structure, the infection thread (IT). The IT leads bacteria to the inner tissues of the root, where they will be internalized to form nitrogen-fixing structures called symbiosomes. The genetic program that controls the infection is exquisitely coordinated with an organogenic program that leads to the development of a root post-embryonic organ that will allocate the nitrogen-fixing bacteria, the nodule. Small GTPases are required for the development of functional nitrogen-fixing nodules at different stages of the symbiotic interaction (Fig. 2). At pre-infection stages, establishment of the interaction requires an actively growing root hair. This polar growth ceases temporarily to restart in a different direction, resulting in a curl at the root hair tip that entraps the attached rhizobia in a new structure called the infection pocket, from which the IT starts to form. Since all these processes involve local degradation and new synthesis of membrane and cell wall, it is not surprising that a group of small GTPases that act in the root hair polar growth are also required for the root hair deformation produced by rhizobia, or by the bacterial secreted molecule referred to as the Nod Factor, which triggers the molecular and physiological changes that initiate the infection process.[21-23] This signal molecule is recognized by receptors exposed in the plant plasma membrane and activates a signal transduction pathway that will turn on the genetic programs associated to root hair infection and nodule organogenesis. Evidences of the involvement of small GTPases on the rhizobial infection process were obtained in common bean, where it was shown that a Rab protein, PvRabA2, is required for the reorientation of the root hair polar growth to curl around the rhizobia microcolony and form the infection pocket.[21] Post-transcriptional silencing mediated by RNAi affected both root hair initiation and elongation, suggesting PvRabA2 is part of the vesicle trafficking at the site where the cell wall and the plasma membrane are degraded and newly synthesized to form root hairs and sustain the tip polar growth. PvRabA2 belongs to the RABA/Rab11 subgroup, which has been involved in the trafficking from the Golgi apparatus to the plasma membrane (the secretory pathway) or to the lytic central vacuole. A more recent report showed that a functional RabA2 is required also for IT formation and maintenance of IT integrity, since the constitutive expression of GTP- or GDP-locked versions of this protein led to the formation of abnormal ITs that burst in the root hair.[24] The IT is formed in infected trichoblasts by transport of membrane and cell wall material, which is mediated by vesicles that move through the cytoskeleton filaments. It has been suggested that phragmoplast-like material is transported to the place of IT formation,[20] which is consistent with the subcellular localization of PvRabA2 at Golgi stacks and the trans-Golgi network around the IT.[24] Supporting this hypothesis, it was shown that RabA2 from Arabidopsis participates in the synthesis of

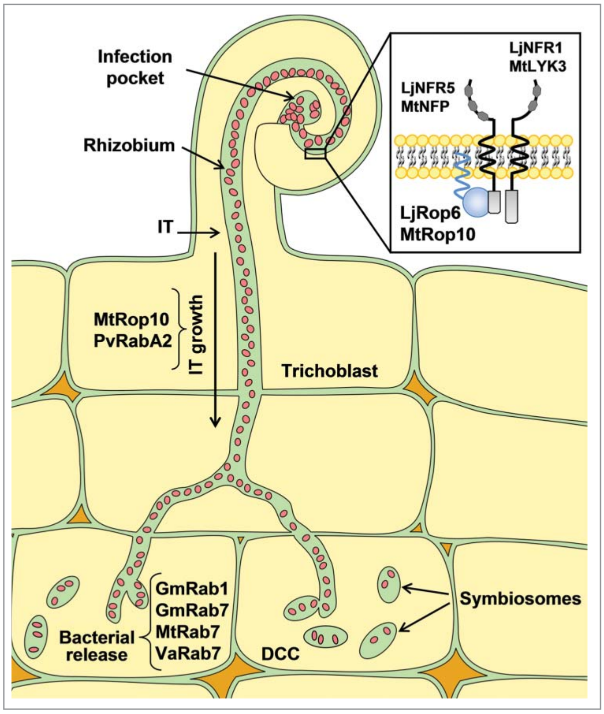

Figure 2. Functions of small GTPases in plant-symbiotic interactions. Small GTPases involved in early events of root-nodule symbiosis, including root hair curling and formation of the infection pocket, infection thread (IT) growth and formation of symbiosomes in the dividing cortical cells (DCC). The inset shows the direct interaction between the Nod Factor receptor (LjNFR5 or MtNFP) and Rop proteins (LjRop6 or MtRop10).

the cell plate before cytokinesis,[25] suggesting that particular components of the machinery used to form the IT would have been recruited from the secretory pathways that sustain root hair expansion and the one that connects Golgi with the cell plate.

Upon rhizobial infection, actin and microtubule organization is altered in root hairs, allowing IT formation and elongation.[26-28] Supporting the proposed roles of changes in the arrangements of cytoskeleton filaments during RNS, genetic evidences showed a link between mutations affecting microtubule and actin dynamics and the IT formation and bacterial release.[29-31] Considering the role of Rop proteins in cytoskeleton rearrangements, it is not surprising that a group of ROPs from *M. truncatula* and *L. japonicus* were identified as required for root hair polar growth and the morphological changes associated to the infection process. RNAi-mediated silencing of the type I LjRop6 did not affect IT formation, but their progression toward cortical cells.[23] This effect on IT elongation resulted in a reduction of the nodules formed in Rop6-RNAi roots as compared with controls, positioning LjRop6 as a positive modulator of the genetic programs associated to RNS. Another Rop that participates in both polar growth and early symbiotic responses was identified in *M. truncatula*.[22] MtRop10 is a type II Rop that in its active state (bound to GTP and localized at the plasma membrane) regulates root hair polar growth. Moreover, overexpression of MtRop10 or a constitutively active form (Rop10CA) interfered with root hair curling around the rhizobial microcolony, reducing the number of ITs and nodules formed. These evidences reveal that Rops I and II participate at very early stages of RNS, modulating physiological changes that allow bacterial penetration.

Interestingly, the absence of physiological changes in root hairs provoked by genetic alteration of small GTPases during rhizobia infection affected the molecular responses triggered by Nod Factor perception,[21-23] suggesting that vesicle trafficking and cytoskeleton rearrangements are necessary at very early stages for activation of symbiotic genetic programs. Taken together, all these studies that connect small GTPases with rhizobial infection also revealed a link between vesicle transport across the cytoskeleton and the construction of the IT, possibly by transporting enzymes, cell wall and membrane components associated to the reprogramming of root cells for symbiosis, such as a nodule pectate lyase and ENOD11, a cell wall repetitive proline-rich protein.[32–33]

In addition to the assigned role in cytoskeleton dynamics, ROP proteins exert their biological functions by participating in signal transduction pathways. Both MtROP10 and LjROP6 interact with Nod Factor receptors at the plasma membrane,[22-23,34] suggesting that they can act in the Nod Factor signaling pathway in a similar way that receptor-associated Ras GTPases act in animal systems, connecting the perception of rhizobia with the cytoskeleton changes required to initiate the infection process. LjROP6 also interacts with the heavy chain of clathrin (CHC1) in moving vesicles near the infection pocket and the growing IT.[35] Expression of a dominant negative version of CHC1 showed that the endocytosis mediated by clathrin is necessary for expression of early noduline genes and IT formation, further supporting the hypothesis that the Nod Factor receptor NFR5 is recycled to the plasma membrane upon rhizobial infection as part of the Nod Factor signaling pathway.[35]

Another critical step of infection in which vesicle trafficking plays a role is the release of bacteria from the IT, where a membrane derived from the plant surrounds the rhizobium to form the symbiosome. Even though this internalization has been frequently described as an endocitosis-like process, a detail study based on molecular markers have shown that the symbiosome membrane does not contain the early endosome marker Rab5, but acquires the late endosomal marker Rab7, which persists up to the senescence stage. Knock-down of Rab7 (Rab7 correspond to RabG3 in the nomenclature used for plants) using RNAi led to the arrest of symbiosome maturation, suggesting that Rab7 is required for the transition to nitrogen-fixing symbiosomes.[36] Similarly, GmRab1p (corresponding to a Rab D) and GmRab7p from soybean (*Glycine max*) and VaRab7p from *Vigna aconitifolia* act during symbiosome membrane maturation.[16] Although evidences of the importance of small GTPases at different stages of rhizobial infection have been obtained in the last years, our knowledge is still very fragmentary and much more research is needed to fully understand how membrane trafficking and cytoskeleton rearrangements have been adapted in legume plants to cope with the specific processes that allow the symbiotic nitrogen fixation.

Plant defense against pathogens

Plant defense is based on several layers of responses, from pre-formed physical and chemical barriers to inducible changes triggered by pathogen perception. Recognition of molecules derived from the pathogen (called pathogen-associated molecular patterns, or PAMPs) is mediated by cell-surface receptors (known as pattern recognition receptors, PRR), followed by signal transduction and activation of defense responses. The defense response triggered by PAMP recognition is referred to as PAMP-triggered immunity (PTI). Localization of PRRs and activation of some defense responses, such as the secretion of antimicrobial proteins, are mediated by the secretory pathway. This is the case of the FLAGELLIN SENSING2 Receptor (FLS2) from Arabidopsis, which recognizes flagellin 22, a peptide derived from the protein component of the bacterial flagellum. This receptor is internalized in a ligand-specific manner by an endocytic pathway that requires ARA7, a Rab of the Rab5/RABF2 subgroup, constituting a different pathway than the constitutive recycling of FLS2 (Fig. 3).[37] A subset of proteins of the Rab11/RABA group is involved in either the secretory pathway that positions the *de novo* synthesized FLS2 in the plasma membrane (RABA1b) or the ligand-specific endocytosis (RABA6a and RABA4c).[38] These studies position members of the Rab11/RABA group, which has expanded in the plant kingdom, as key regulators of the secretory and endocytic pathways that operate during symbiotic and pathogenic interactions. In this way, RABA proteins would modulate the number of receptors present in the plasma membrane and the fate of the receptor-ligand complexes by regulating the recycling and vacuolar routes.

Some particular pathogens have evolutionary acquired the capacity to interfere with PTI by the action of secreted molecules known as pathogen effectors or suppressors, leading to the effector-triggered susceptibility (ETS). One example is AvrPto, a protein produced by the tomato and Arabidopsis pathogenic bacteria *Pseudomonas syringae*. AvrPto is internalized into the plant cytoplasm by the type III secretion system and targets FLS2[39] and RabE GTPases.[40-41] Expression of a GTP-locked version of RABE1d (RABE1d-Q74L) resulted in an enhanced response to *P. syringae* and the reduction of disease symptoms.[41] Considering that RABE proteins are involved in trafficking from Golgi to the plasma membrane, it would be interesting to test the possible connection between the RABE1d-Q74L mode of action and the localization of plant receptors or secreted defense proteins.

Some plant varieties possess Resistance (R) proteins that recognize pathogen effectors and trigger a second layer of inducible defense, known as effector-triggered immunity (ETI). ARFA1 from *Nicotiana benthamiana* was implicated in an R-mediated interaction since virus-induced silencing of *ARFA1* produced an increase in the lesion size caused by the tobacco mosaic virus in plants that carry the N resistance gene.[42] Interestingly, NbARFA1 was identified by its capacity of triggering cell death in paraquat-treated *N. benthamiana* leaves in its GTP-associated active form, suggesting a link between the hypersensitive response (a form of programmed cell death

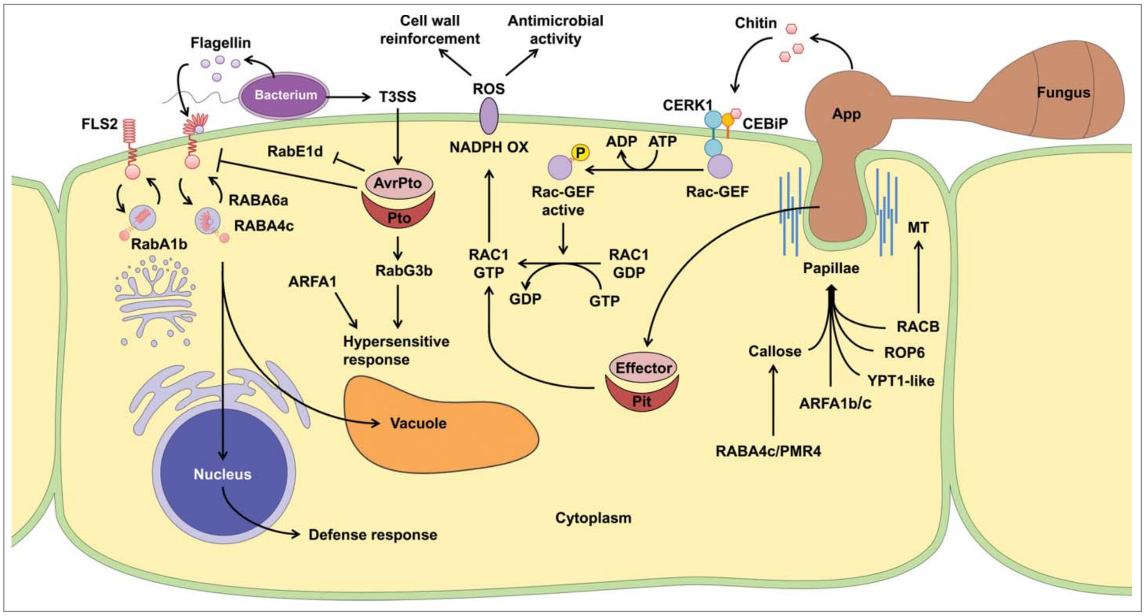

Figure 3. Functions of small GTPases during plant pathogen interactions. Several members of the RabA family participate in re-cycling of the flagellin receptor FLS2 in a ligand-dependent or -independent manner, thus promoting PAMP-triggered immunity (PTI). Bacterial effectors, such as AvrPto, are internalized by the type 3-secretion system (T3SS) and interfere with the signaling and action of Rab proteins. Perception of bacterial effectors by cytoplasmic receptors (such as Pto and Pit) leads to enhanced defense responses and the hypersensitive response associated to the Effector-triggered immunity (ETI), where proteins of the Rab and Arf families are involved. Different Rop and Arf GTPases stimulate the activity of plasma membrane NADPH oxidases (NADPH OX) and the production of reactive oxygen species (ROS). During fungal infection, Rop and Arf proteins alter microtubule (MT) dynamics around the infection site and promote the formation of papillae to interfere with fungal penetration. OsRac1 can modulate ROS production in a PTI response after chitin perception by the receptor complex CERK1-CEBiP or in an ETI response triggered by a cytoplasmic receptor, Pit, which recognizes an effector produced by the pathogen. App: appressorium.

frequently observed in ETI) and the vesicle trafficking mediated by ARF GTPases. Another small GTPase of the Rab family, RabG3b, was also associated to the programmed cell death during ETI.[43] Expression of a GTP-locked version of RabG3b caused cell death and accumulation of autophagic structures following infection with a *P. syringae* strain that triggers ETI in Arabidopsis. Taken together, these reports revealed an interesting link between small GTPases, autophagy and programmed cell death; however, the molecular mechanisms subjacent to this connection are poorly understood.

In addition to the role of RABA4c in FLS2 localization, this GTPase interacts with PMR4, a callose synthase involved in deposition of a (1,3)-β-glucan polymer that accumulates at the site of fungal penetration.[44-45] Enhanced accumulation of callose was correlated with penetration resistance to the causal agent of powdery mildew (*Golovinomyces cichoracearum*) in plants expressing the GTP-locked version of RABA4c.[45] Another Rab of the D group from barley (*Hordeum vulgare*), named HvYPT1-like, locates at the Golgi and concentrates around the site of non-successful penetration attempts by *Blumeria graminis*. Knock-down of *HvYPT1-like* enhanced the susceptibility to *B. graminis*, suggesting that Golgi transport mediated by this RabD is also implicated in barley defense against haustoria colonization.[46] Similarly, HvARFA1b and HvARFA1c from barley have been implicated in penetration resistance since they contribute to the synthesis of papillae in response to powdery mildew.[47] Papillae is formed by apposition of cell wall material (mainly callose, lignin and cell wall structural proteins) at the site of haustoria penetration and, in addition to ARFA1 proteins, it requires the action of ROR2, a membrane-located syntaxin that seems to be involved in the same secretory pathway. Besides some controversy, HvARFA1 appears to be located at the Golgi and trans-Golgi network.[24,48] Supporting the role of ARF proteins in callose deposition after pathogen attack, treatment with brefeldin A, an inhibitor of ARF-GEF proteins, prevents papillae formation and promotes penetration of the pathogen.[49] Furthermore, genetic studies showed that a mutation in one of the ARF-GEFs called GNOM resulted in the same phenotype observed in brefeldin A-treated plants.

Microtubule rearrangements are also critical to limit fungal penetration at the site of powdery mildew infection. A type I ROP from barley called HvRACB interacts with MAGAP1, a ROP-GAP that associates to microtubules at the sites of haustoria penetration to create a network that confines fungal growth.[50] Interestingly, HvRACB is also required for root hair polar growth, constituting another example of small GTPases that seems to have been recruited from the polar growth molecular machinery to participate in the interactions of plants with microorganisms.[50-51] In Arabidopsis, ROP6 is involved in powdery mildew resistance, but also affects several developmental processes as a consequence of its effect on cytoskeleton organization, further supporting the partial specificity of GTPases linked to pathogen-responses.[52-53] All these evidences illustrate the key roles played by small GTPases from different families in cytoskeleton rearrangements and vesicular trafficking that contributes to cell wall reinforcement activated in response to penetration attempts by pathogenic fungi (Fig. 3).

Different reports highlighted the role of the 7 members of the ROP family from rice in plant defense. Whereas expression of a GTP-locked version of the type II Rop OsRac1 resulted in cell death and enhanced resistance against *Magnaporthe grisea*, other members of the ROP family, namely OsRac4 and OsRac5, negatively regulate blast resistance.[54-55] OsRac1 modulates production of ROS,[55] a similar function that animal Rho proteins exert in pathogen defense. Several roles have been proposed for ROS in the context of plant defenses: the direct antimicrobial action against the pathogen at the infection site, as a second messenger in signal transduction pathways or producing changes in the cell wall by modifying the crosslinking among their components. OsRac1is activated by chitin (one of the best characterized PAMPs) perception mediated by OsCERK1, a receptor-like kinase that forms a complex with OsCEBiP, a chitin binding protein without an intracellular domain[56] (Fig. 3). In the presence of chitin, OsCERK1 interacts, phosphorylates and activates OsRacGEF.[57] OsRacGEF, as other GEF proteins, participates in the GDP by GTP exchange of small GTPases, switching OsRac1 to its active state, which in turns regulates ROS production by activation of the NADPH oxidase rbohB (for respiratory burst oxidase homolog).[55,58-59] In addition, OsRac1 activation results in the downregulation of OsMT2b, a ROS scavenger with antioxidant activity against superoxide and hydroxyl radicals, indicating that OsRac1 plays a dual role inducing ROS production and suppressing ROS scavenging during plant-pathogen interactions.[60] Interestingly, OsRac1 is also activated during ETI by recognition of an effector produced by the rice blast fungus mediated by a receptor called Pit, showing that the same Rop participates in both layers of defense.[61]

As above mentioned, the Rac1 GTPase MtROP9 is required for ROS production and defense response against *Oomyces* in *M. truncatula*.[19] This production of apoplastic ROS is mediated by the activation of a plasma membrane RBOH NADPH oxidase. Silencing of MtRop9 altered ROS production and *MtRBOH* gene expression, affecting both pathogenic and symbiotic interactions. Normal levels of ROS produced in response to microorganisms seem to be necessary to sustain the infection process by rhizobia and to control invasion by an oomycete pathogen.[19] These evidences support the idea that, besides their different nature, both types of interactions share molecular components of their signaling pathways. RBOH activation is a common response to the presence of PAMP in the apoplast. Perception of pathogen molecules stimulates ROS production through the activation of Rop-GEF proteins.[62] Understanding molecular aspects of the link between ROP proteins and ROS production will help to integrate the role of these small GTPases in different biological processes such as polar cell growth and interactions of plants with microbes. Additionally, this connection constitutes an interesting evolutionary link with animal systems, raising the possibility that this mechanism was already present in the common ancestor of plant and animals or, alternatively, the function of ROPs in the regulation of ROS production is a consequence of convergent evolution.

Taken together, clear roles for small GTPases in plant immunity have emerged in the last 2 decades. These roles are played in both layers of induced plant immunity, PTI and ETI, involving transport of receptors and defense components to the plasma membrane, recycling of receptors, cytoskeleton rearrangements and modulation of defense responses mediated by ROS and pathogen-related proteins. It is also clear that plant pathogens exploit membrane trafficking mediated by small GTPases to either interfere with defense responses triggered by the plant or directly favor their virulence strategy.

Our current vision and future challenges

The participation of plant monomeric GTPases in the interactions of plants with microorganisms has been largely documented in the past years. However, our current knowledge of the function of each member of the different subfamilies of small GTPases, their mechanisms of action and the downstream effectors is far from complete and more research is needed to answer key biological questions. To understand how this gene superfamily has been shaped during evolution, comparison of the families present in species of each lineage can help to understand how different variations have acquired new functions to solve biological problems associated to the ecological challenges imposed to different pluricellular organisms.

One particularly interesting aspect of plant small GTPases is related to similarities and differences between their mode of action during pathogenesis and symbiosis. Although the information is still limited, some common themes, such as the connection between ROP proteins and ROS production, revealed an exciting link between both types of interactions. Members of the Rop and Rab families have been shown to play important functions at different stages of the interaction between legumes and rhizobia. Also, a member of the Rop family has been implicated in AM symbiosis. Thus, the presence of common molecular components of signal transduction pathways (e.g. SYMRK, Symbiotic Receptor Kinase, and CCaMK, Calcium Calmoduline-dependent protein kinase) and transcription factors (e.g., NSP1 and NSP2) that act in both root nodule and AM symbiosis, also raise interesting question about the specificity of members of the small GTPase families in different types of interaction and their roles in signaling, the entrance of the symbiont and at the interface between both symbionts for nutrient exchange. Comparative genomics could help to establish if asymmetrical evolution has occurred in particular groups of plants during evolution associated to a particular interaction.

Another interesting aspect of small GTPases in biotic interactions is the integration of the different roles played by these proteins during early infection events in signal transduction, vesicle trafficking and regulation of cytoskeleton dynamics. An interesting link between 2 different types of GTPases was established on root hair polar growth. Plants co-expressing GDP- or GTP-locked versions of ArfA1 and a GTP-locked version of Rop2 revealed that the activity of ArfA1 is required for the correct localization and functioning of Rop2, suggesting that Rop proteins can be carried by vesicles whose traffic is regulated by Arf proteins.[63]

Membranes are at the center of the interaction between plants and microorganisms, participating in recognition, remobilization of cell components and nutrient exchange. The knowledge generated in the small GTPases field should be integrated in a broader context of the role of vesicle transport, cytoskeleton rearrangement and signaling in plant-specific biological processes.

Disclosure of potential conflicts of interest

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