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The Small GTPase Superfamily in Plants: A Conserved Regulatory Module with Novel Functions

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Abstract

Small GTP-binding proteins represent a highly conserved signaling module in eukaryotes that regulates diverse cellular processes such as signal transduction, cytoskeletal organization and cell polarity, cell proliferation and differentiation, intracellular membrane trafficking and transport vesicle formation, and nucleocytoplasmic transport. These proteins function as molecular switches that cycle between active and inactive states, and this cycle is linked to GTP binding and hydrolysis. In this review, the roles of the plant complement of small GTP-binding proteins in these cellular processes are described, as well as accessory proteins that control their activity, and current understanding of the functions of individual members of these families in plants—with a focus on the model organism *Arabidopsis*—is presented. Some potential novel roles of these GTPases in plants, relative to their established roles in yeast and/or animal systems, are also discussed.

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INTRODUCTION

At least a billion years of evolution has occurred since plants, fungi, and animals diverged into their distinct eukaryotic lineages (32). While it is unclear whether multicellularity arose independently in these different eukaryotic systems, this vast evolutionary time has allowed for the emergence of highly distinct mechanisms of development and structural organization in these phyla. As plants are sessile photosynthetic organisms, sensing and responding to the external environment are possibly as important to plant development as signaling among cells within the organism. Consequently, signaling and developmental pathways in plants are more strongly coupled to light and other environmental cues and are more heavily influenced by their environment than is typical in animals (102). It is therefore somewhat remarkable the degree to which aspects of specific molecular regulatory modules involved in cell signaling have remained conserved. At the same time, however, some of these conserved regulatory modules have been adapted to reflect the dramatically different lifestyles and organization distinguishing plants, animals, and fungi (**Table 1**).

Ras-LIKE SMALL GTPases: A CONSERVED REGULATORY MODULE BASED ON A GTP MOLECULAR SWITCH

One of these conserved molecular regulatory modules is the GTPase molecular switch (53). Early research describing the functions of the prokaryotic ribosomal elongation factor, EF-Tu (8, 83), and mammalian G proteins (114) established the hallmark by which these molecular switches function: the ability to undergo a guanine nucleotide–dependent conformational change. Within the diverse classes of regulatory proteins that utilize this conserved GTPase molecular switch,

Table 1 Novel and conserved functions of small GTPases in plants

GTPase family	Conserved functions	Novel plant roles
Ras GTPases	RLK signal transduction	Absent from plants
Ran GTPases	Nucleocytoplasmic transport	Plant-specific NE localization Roles in female gametogenesis
Rab GTPases	Endomembrane trafficking, vesicle tethering, membrane fusion fidelity	Non-canonical lipid modification of endocytic RabF1/ARA6 GTPases Elaboration of Rab11/RabA GTPases
Arf GTPases	Vesicle budding, coat protein recruitment	Arf1-like GNOM involvement in polar recycling to PM TPLATE adaptors essential for endocytosis from PM
Rho GTPases	RLK signal transduction, actin cytoskeleton dynamics, NADPH oxidase activation	Plant-specific Rop GTPase clade Plant-specific PRONE RopGEFs Cell expansion by regulation of cortical actin/MT dynamics

Abbreviations: GEF, guanine exchange factor; MT, microtubule; NE, nuclear envelope; PM, plasma membrane; PRONE, plant-specific Rop nucleotide exchanger; RLK, receptor-like kinase; Rop, Rho of plants.

several distinct superfamilies have been described: the small GTPases, heterotrimeric GTPases, and high-molecular-weight GTPases. Small GTPases, which are the subject of this review, are defined as monomeric guanine nucleotide-binding proteins with masses of 21 to 30 kDa, and they differ from heterotrimeric G proteins in terms of both the mechanisms by which they are regulated by upstream factors and those by which they activate downstream targets (153). High-molecular-weight GTPases [>70–80 kDa (165)] include the dynamins, as well as several other families of proteins with less well-characterized functions. An example of one such non-dynamin, high-molecular-weight GTPase, the *Arabidopsis* Root Hair Defective3 protein (RHD3), is a member of the atlastin family of GTPases, and appears to regulate endoplasmic reticulum (ER) membrane tubulation (18, 58).

All small GTPases belong to the Ras superfamily, named after the human *Ras* genes initially discovered as cellular homologs of the viral *ras* oncogene (119). In plants, small GTPases can be divided into four main groups: Ras-related nuclear (Ran) GTPases, Ras-related brain (Rab) GTPases, ADP-ribosylation factor (Arf) GTPases, and Rho of plant (Rop) GTPases. Notably, canonical Ras GTPases, after which this superfamily is named, are absent (167).

Small monomeric GTPases cycle in the cell between inactive GDP-bound and active GTP-bound states (**Figure 1**). In the active GTP-bound state, the GTPase protein can bind to effector proteins and regulate cellular processes. Interaction between the GTPase and its cognate effectors can regulate the activity of these downstream signaling components via a variety of methods, such as alteration of their conformation to allow binding of substrates, release from or induction of autoinhibitory states, or recruitment of these proteins to a specific subcellular location (e.g., a specific subcellular membrane or nucleoplasmic compartment). This GTPase cycle is tightly regulated by guanine exchange factors (GEFs), which promote exchange of GDP for GTP upon binding and act to turn on the protein, and GTPase-activating proteins (GAPs), which turn off the protein by providing essential amino acids to the GTPase domain that dramatically increase the rate at which bound GTP is hydrolyzed to GDP+Pi (112). Mutations in these highly conserved GTPase domains that disrupt this cycle of nucleotide exchange and hydrolysis can stabilize either the GTP or the GDP conformation, leading to proteins that are constitutively active (CA), constitutively inactive, or dominant negative (DN) inhibiting. In addition, in the Rab and Rho GTPase subfamilies, guanine nucleotide dissociation inhibitors perform a chaperone-like activity by removing inactive GDP-bound forms of these proteins from cellular membranes and sequestering and stabilizing these proteins in the cytosol by masking their posttranslational lipid modifications (112).

Endoplasmic reticulum (ER): an interconnected network of membrane-bounded elements and tubules that are widely distributed throughout the cytoplasm

Ras-related brain proteins (Rab GTPases): regulate aspects of vesicle trafficking between compartments of the endomembrane trafficking pathway

ADP-ribosylation factor proteins (Arf GTPases): a family of small GTPases that are recruited and associate with subcellular organelle membranes and assist in formation of transport vesicles

Guanine exchange factors (GEFs): proteins or protein domains that activate GTPase proteins by stimulating the exchange of guanine-diphosphate (GDP) for guanine-triphosphate (GTP)

Ran GTPases IN PLANTS AND THEIR FUNCTIONS IN NUCLEAR IMPORT/EXPORT

Ran GTPases are a conserved family of small signaling GTPases that, along with their regulatory factors RanGEFs and RanGAPs, are involved in nucleocytoplasmic transport of RNAs and proteins based on the presence of nuclear import or export signals (**Figure 1*b***) (3, 108). In addition

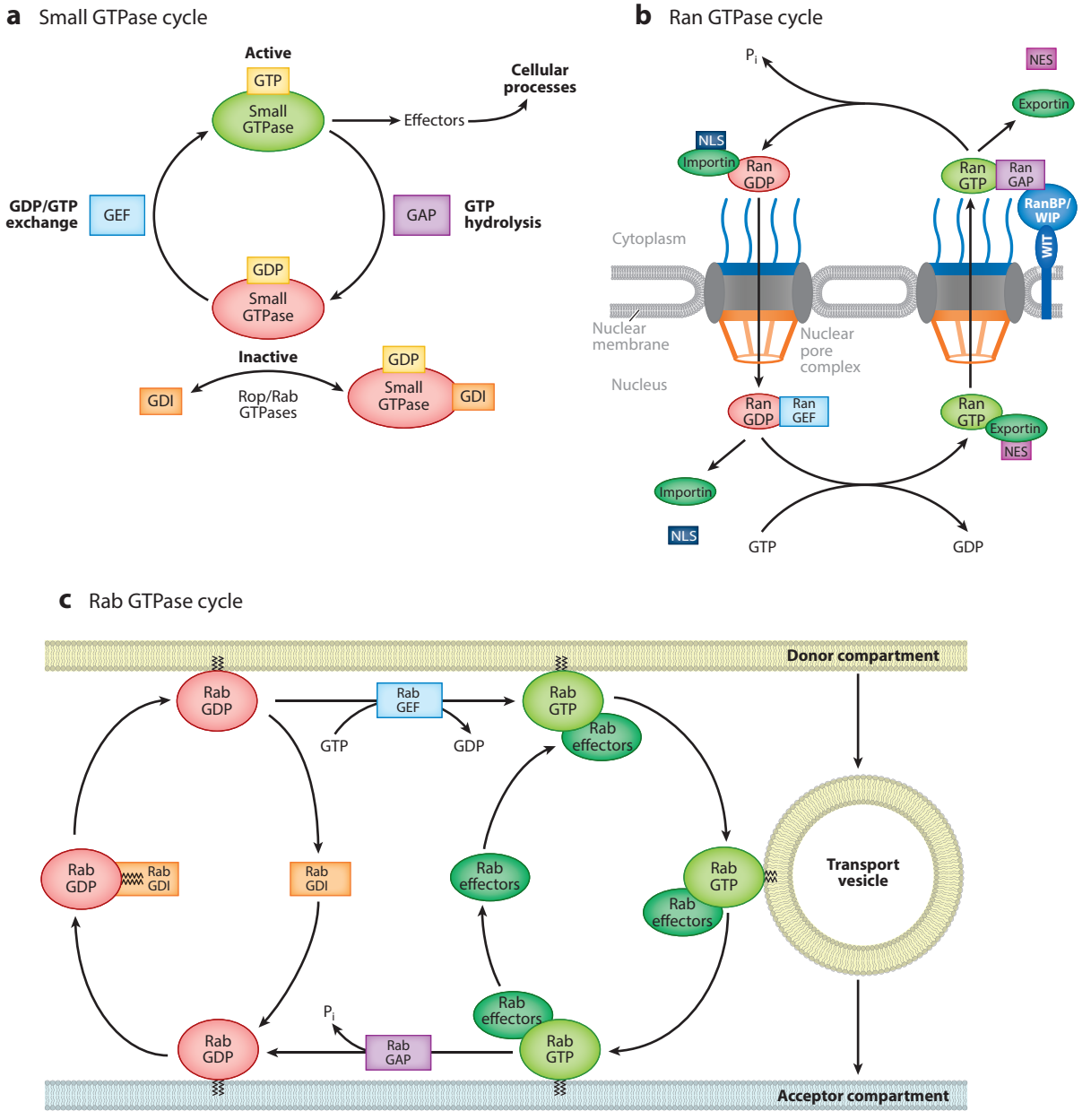

Figure 1

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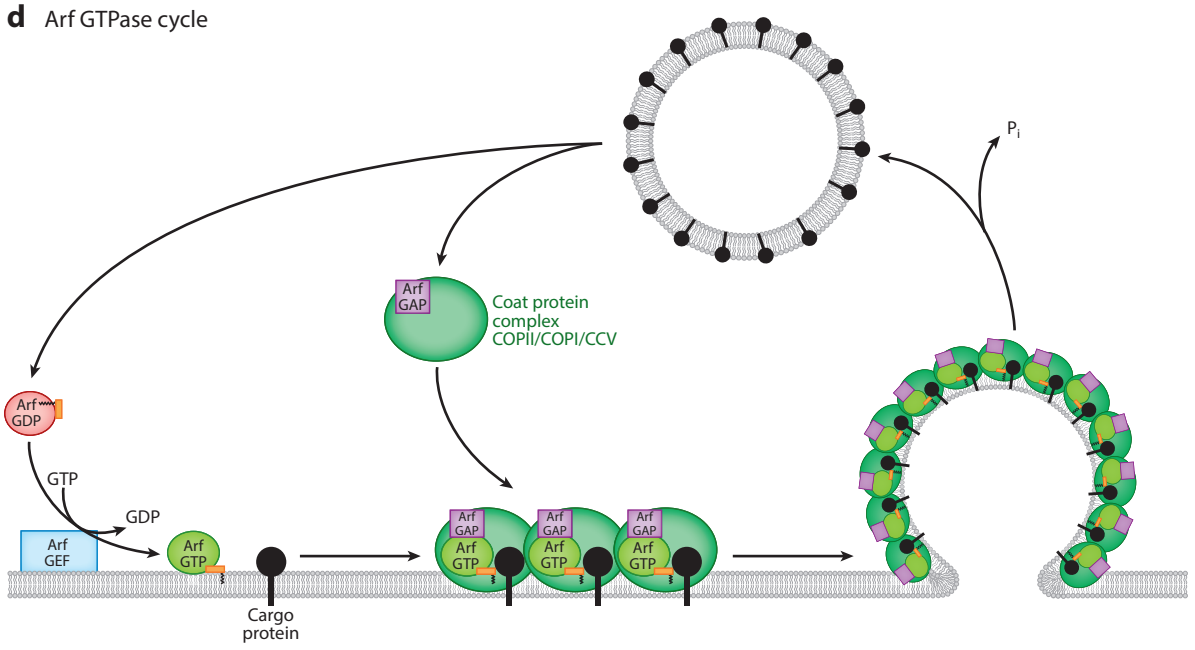

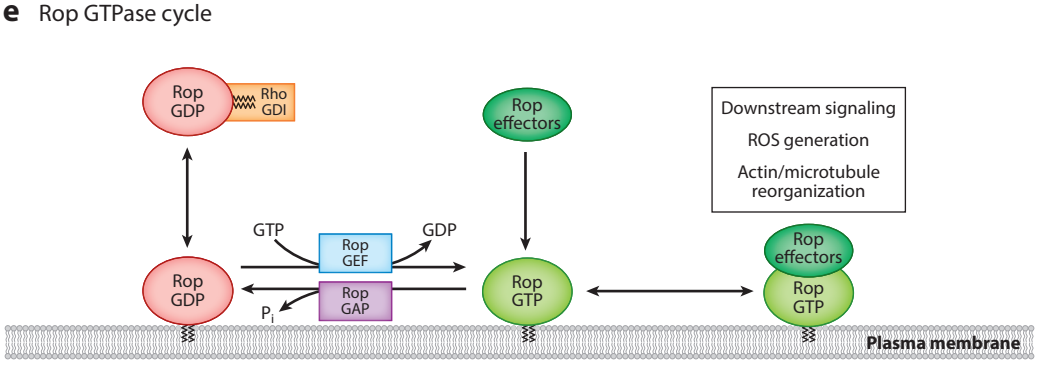

Figure 1

(*a*) Small GTPase regulatory module. Ras-related GTPases cycle between an active GTP-bound and an inactive GDP-bound state. GDP/GTP exchange factors (GEFs) activate these small GTPases by promoting release of GDP and binding to GTP, which induces conformational changes that stimulate their interaction with specific effectors to mediate cellular processes. Due to their low intrinsic GTPase activity, GTPase-activating proteins (GAPs) bind and stimulate the inherent GTPase activity of these small G proteins, accelerating the inactivation of their regulatory activity. In Rop and Rab GTPases, the cycle of GTP binding and hydrolysis is coupled to changes in their subcellular localization. GDP dissociation inhibitor (GDI) proteins bind to the GDP-bound protein, sequestering these G proteins in the cytosol by masking their C-terminal lipid moieties. Upon membrane binding, the GDI is released, and these GTPases are activated by GEFs. (*b*) The Ran GTPase cycle during nucleocytoplasmic trafficking. (*c*) The Rab GTPase cycle during membrane trafficking and vesicle fusion. (*d*) The Arf GTPase cycle during cargo recruitment and coat protein–mediated vesicle budding. (*e*) The Rop GTPase cycle during cell signaling and regulation of cytoskeletal dynamics.

to having roles in nucleocytoplasmic transport, Ran GTPases are involved in a number of mitotic processes, such as cell cycle progression, spindle assembly, chromosome segregation and decondensation, and nuclear envelope formation (23, 49, 61, 128, 140). Ran GTPases cycle between GTP- and GDP-bound states, which is linked to transport into or out of the nucleus (108). Unlike other small GTPases, Ran GTPases are not posttranslationally lipid modified, and they do not associate with cellular membranes (135). Instead Ran-GTP is found in the nucleoplasm because of an association between RanGEF activity and chromatin DNA, while Ran-GDP is favored in the cytoplasm because of the presence of RanGAP activity (65).

GTPase-activating proteins (GAPs): a family of regulatory proteins that function to inactivate GTPase proteins by stimulating the hydrolysis of GTP to GDP

Ras-related nuclear proteins (Ran GTPases): a family of Ras-related GTPases that regulate transport of proteins and RNA between the cytoplasm and nucleus during interphase and regulate aspects of mitosis

Nuclear localization signals: specific amino acid motifs present on the surface of proteins that are recognized by elements of the Ran GTPase regulated transport machinery and allow these proteins to be targeted to the interior of the nucleus

Nuclear export signals: specific amino acid motifs present on the surface of proteins that allow elements of the Ran GTPase regulated transport machinery to bind and export these proteins out of the nucleus into the cytoplasmic compartment

Conserved Nucleocytoplasmic Transport Functions

In eukaryotic cells, import and export of proteins and RNAs are linked to the Ran GTPase cycle through a number of soluble receptor proteins that interact with cargo molecules. In the case of nuclear import, cytosolic proteins contain nuclear localization signals that associate with soluble import receptor proteins (importin α and importin β) and Ran-GDP (reviewed in 25). Upon entry into the nucleoplasm, these complexes dissociate as a result of conversion of Ran-GDP to Ran-GTP by nuclear-localized RanGEF action. During nuclear export, specific members of these importin α and importin β family members recognize protein cargoes with nuclear export signals, forming complexes with Ran-GTP (instead of Ran-GDP) that dissociate upon conversion of Ran-GTP to Ran-GDP through the action of cytoplasmically localized RanGAP activity. *Arabidopsis* has 8 putative importin α genes and 17 putative importin β genes, several of which function similarly to their yeast and animal counterparts (reviewed in 103) (**Table 2**). Perhaps reflecting the important roles microRNAs play in plant development, two proteins involved in nuclear export of RNA species have been identified in classical genetic screens. The *PAUSED* mutant, which affects plant development pleiotropically, is affected in an Exportin t-like protein, which is required in transfer RNA export (62, 88), while HASTY mutants, which are defective in an Exportin 5–like protein required for double-stranded RNA export, show accelerated juvenile-adult transitions (13, 120).

Novel Roles During Plant Gametogenesis and Cell Division

Arabidopsis contains four Ran GTPase isoforms, AtRAN1–AtRAN4 (**Table 2**). AtRAN1–AtRAN3 are soluble proteins, detected in both the cytoplasm and the nucleus, and are ubiquitously expressed in all organs during development (51). Plant Ran GTPases from a number of plant species rescue mutants of the *Saccharomyces pombe* Ran GTPase gene (1, 51, 105), suggesting similar roles in nucleocytoplasmic trafficking for these GTPases in plants (**Figure 1*b***). Overexpression of wheat Ran1 in rice and *Arabidopsis* led to meristem defects (169), while overexpression of rice Ran2 in rice and *Arabidopsis* caused the plants to become hypersensitive to salinity and osmotic stress (179). More recent studies in which endogenous AtRAN1 expression levels were altered revealed female gametophyte–specific defects associated with endosperm development, specifically with the timing of cellularization events associated with this tissue (94).

While Regulator of Chromosome Condensation 1 (RCC1)-like RanGEFs have not yet been identified in plants, orthologous RabGAPs and RabBPs have been identified (104) (**Table 2**). In all three eukaryotic lineages, RanGAP proteins share two conserved domains, a central leucine-rich repeat domain (LRR) that binds Ran GTPases (50) and a more C-terminal acidic domain. The budding yeast RanGAP, Rna1p, is a cytosolic protein, but in plants and animals RanGAP-RanBP complexes associate with nuclear pore complexes (**Figure 1*b***) and cytoplasmic nuclear envelope membranes (69, 73, 74, 100). In animals, RanGAP protein localization depends upon interaction between a SUMOylated C-terminal domain and RanBP2/Nup358 (96, 99), which is absent in

Table 2 Ran GTPases and their interacting proteins

Gene name	AGI number	Other names	Localization/expression/function	References
Ran GTPases				
AtRAN1	At5g20010	RAN-1	Ubiquitous expression, roles in seed and endosperm development	94
AtRAN2	At5g20020	NA	Same as AtRAN1	51
AtRAN3	At5g55190	NA	Same as AtRAN1	51
AtRAN4	At5g55080	NA	ND	NA
Ran GAPs				
AtRANGAP1	At3g63130	RG1	Associates with nuclear envelope membranes	121
AtRANGAP2	At5g19320	RG2	Associates with nuclear envelope membranes	121
Ran effectors				
AtIMPA-1	At3g06720	AtKAP	Interacts with multiple plant nuclear localization signals	60
AtIMPA-2	At4g16143	NA	NA	NA
AtIMPA-3	At4g02150	AtMOS6	*modifier of snc1 6* (MOS6), required for plant immunity to pathogens	118
AtIMPA-4	At1g09270	NA	Nuclear import receptor for *Agrobaterium tumefaciens* VirD2 and VirE2	10
AtIMPA-5	At5g49310	NA	NA	NA
AtIMPA-6	At1g02690	NA	NA	NA
AtIMPA-7	At3g05720	NA	NA	NA
AtIMPA-8	At5g52000	NA	NA	NA
AtIMB1	At5g53480	AtKPMB1	Sensitive to ABA, roles in drought stress	95
AtIMB2	At2g16950	AtTRN1	Interacts with ARGONAUTE1 (AGO1), roles in miRNA loading	27
AtIMB3	At5g19820	EMB2734, AtKETCH1	Nuclear import receptor for Hyponastic Leaves 1 (HYL1), roles in miRNA processing	181
AtIMB4	At4g27640	NA	Nuclear import receptor for GRF-interacting factors (GIFs), roles in ovule development	93
AtIMB5	At1g26170	NA	NA	NA
AtKA120	At3g08960	NA	NA	NA
AtSAD2	At2g31660	AtEMA1	Sensitive to ABA, defective trichome activity, enhanced miRNA activity	168
AtPLANTKAP	At3g17340	NA	NA	NA
AtXPO1A	At5g17020	AtHIT2, AtCRM1	Sensitive to heat and oxidative stress	172
AtXPO1B	At3g03110	AtCRM1B	Gametophyte defects in *xpo1a/xpo1b* double mutants	11
AtXPO2	At2g46520	NA	NA	NA
AtXPO4	At3g04490	NA	NA	NA
AtXPO5	At3g05040	AtHASTY	Involved in timing of shoot maturation, miRNA transport	120
AtXPO7	At5g06120	NA	NA	NA
AtXPOT	At1g72560	AtPAUSED	Delayed leaf formation, inflorescence defects	120
AtTNPO3	At1g12930	NA	NA	NA
AtMOS14	At5g62600	NA	*modifier of snc1 14* (MOS14), impaired plant immunity	173

(*Continued*)

Table 2 (*Continued*)

Gene name	AGI number	Other names	Localization/expression/function	References
RanGAP-interacting proteins				
AtWIP1	At4g26455	NA	WPP-domain interacting protein, recruits RanGAPs to outer nuclear envelope membranes	174
AtWIP2	At5g56210	NA	WPP-domain interacting protein, recruits RanGAPs to outer nuclear envelope membranes	166
AtWIP3	At3g13360	NA	WPP-domain interacting protein, recruits RanGAPs to outer nuclear envelope membranes	42
AtWIT1	At5g11390	NA	WIP-interacting tail-anchored protein, participates in recruiting RanGAPs to outer nuclear envelope membranes	182
AtWIT2	At1g68910	NA	WIP-interacting tail-anchored protein, participates in recruiting RanGAPs to outer nuclear envelope membranes	182

Abbreviations: ABA, abscisic acid; NA, not available.

plants. Instead, each of the two *Arabidopsis* RanGAPs, RG1 and RG2 (121), contains a unique N-terminal domain called the WPP domain with highly conserved tryptophan-proline-proline motifs. These WPP domains interact with a small family of coiled-coil proteins, the WIPs (WPP-domain interacting proteins), which associate with WITs (WPP-domain interacting tail-anchored proteins), and both are required to recruit plant RanGAP proteins to nuclear envelope membranes (134, 174, 182) (**Table 2**). Interestingly, RG2 also appears to play important roles in plant innate immunity through interactions with several different (Coiled-Coil-Nucleotide Binding-Leucine-Rich Repeat) CC-NB-LRR resistance proteins, although the RG2 role in immunity does not appear to be strictly dependent upon its GAP activity (156).

In animals, RanGAP proteins play important roles during mitosis, where they selectively localize to kinetochores and the spindle midzones and, along with the RanGEF RCC1, participate in regulating spindle pole dynamics and chromosome segregation (24, 74). Interestingly, two recent studies have highlighted important roles for plant RanGAPs in cell division in plants as well, although they appear to reflect plant-specific aspects associated with cell division. In *Arabidopsis*, RG1 is recruited to the preprophase band (PPB), which forms through the rearrangement of plant microtubule (MT) arrays prior to entry into mitosis (175). This localization is maintained at this peripheral zone, even after disassembly of the PPB, and during later mitotic stages is recruited to the leading edges of the phragmoplast and newly forming cell plate, where it is required for proper positioning of the cell division plane (69, 121, 174). While RG1 and RG2 are redundant, lethal phenotypes of *RG1 RG2* double mutants occur early during female gametophyte development and appear to be linked to defects in cellularization of the endosperm (15, 132).

Transport vesicle: small, membrane-bounded compartments with specifically sorted cargo that are pinched off of one of the membrane compartments of the endomembrane trafficking pathway

Rab GTPases ORGANIZE THE PLANT ENDOMEMBRANE NETWORK

Rab GTPases constitute the largest small GTPase family of regulatory molecules. Soon after their discovery, their specific subcellular localizations and accumulation of transport vesicle intermediates in mutants led to the hypothesis that Rab GTPases, in conjunction with SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor) proteins, regulated specificity for membrane fusion events (reviewed in 149). Plants also maintain a large Rab GTPase family, with 57 members identified in *Arabidopsis* that organize into eight distinct

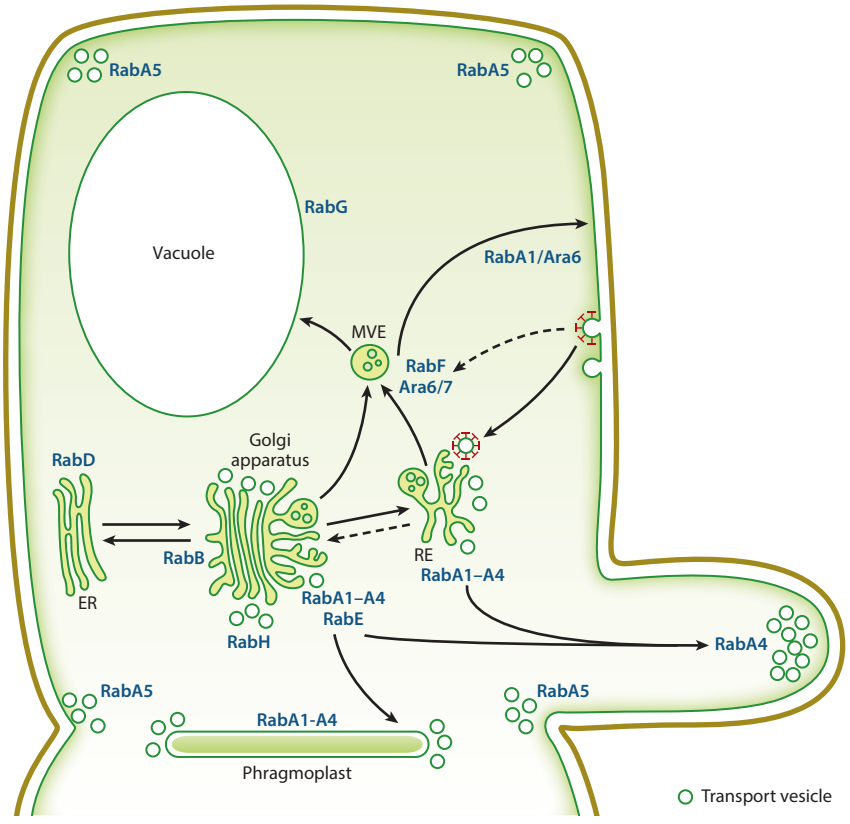

Figure 2

A schematic illustration of Rab GTPase–regulated pathways in plant cells. Phragmoplast and polarized outgrowth are depicted as examples of cellular structures that require high levels of polarized secretion for their formation. Abbreviations: ER, endoplasmic reticulum; MVE, multivesicular endosome/prevacuolar compartment; RE, recycling endosome.

clades (RabA–RabH) on the basis of their sequence similarity to one another and to yeast and animal Rab GTPases (reviewed in 136, 167) (**Supplemental Table 1**). In most cases, sequence conservation among yeast, animal, and plant Rab GTPases has correlated with subcellular localization to analogous membrane compartments in the endomembrane trafficking systems of these eukaryotic lineages. Although to date knockout mutations of plant Rab GTPases have not been broadly useful in determining function, likely because of functional redundancy, the use of functional fluorescent fusions and expression of CA and DN versions of these Rab GTPases have proved useful in assigning subcellular localization and function to plant Rab GTPases (**Figure 2*a***).

Supplemental Material >

Golgi apparatus: a series of flattened, membrane-bounded compartments, called cisternae, which are assembled by fusion of ER-derived transport vesicles

Plant Rab GTPases Involved in Trafficking from the Endoplasmic Reticulum to the Golgi Apparatus

In plants, trafficking between the ER and Golgi apparatus appears to be regulated by Rab1/RabD and Rab2/RabB GTPases (reviewed in 111). In *Arabidopsis*, the Rab1/RabD family contains five

members (**Supplemental Table 1**), of which one, Rab1b/RabD2a, localizes to ER and Golgi compartments; expression of a *DN-Rab1b RabD2a* mutant resulted in accumulation of secreted and Golgi-targeted cargo proteins, consistent with a role in ER–to–Golgi trafficking (6). Further investigation of the function of additional members of the RabD family in *Arabidopsis* suggested that RabD1 and members of the RabD2 subclades provided overlapping but functionally distinct activities during ER–to–Golgi trafficking (124). While *Saccharomyces cerevisiae* cells do not appear to contain members of the Rab2/RabB GTPases (142), the Rab2 GTPase family appears to be involved in intra–Golgi apparatus and retrograde trafficking from the Golgi apparatus to the ER in mammals (157). In *Arabidopsis* and other angiosperms, the Rab2/RabB family is relatively small, with two to four members (**Supplemental Table 1**). The *Arabidopsis Rab2A/RabB1b* gene was initially shown to be expressed at high levels in pollen grains and seedlings; subsequently, expression of GFP-NtRab2 was shown to localize to Golgi compartments in tobacco pollen (20, 107). An intriguing development is the recent identification of mammalian Rab18 GTPases in lipid droplet formation (97, 117). In plants, the family of Rab GTPases most analogous to the Rab18 GTPase-like family is the RabC family (**Supplemental Table 1**), of which RabC2a appears to associate with peroxisomes and assist in recruitment of myosin motors (26, 55). While no direct connection between RabC function and lipid droplet formation has been established in plants, it is interesting that in mammalian cells Rab18 has also been reported to be associated with peroxisomal membranes, which often are closely associated with lipid droplets in eukaryotic systems (45).

***Trans*-Golgi network (TGN):** a highly dynamic association of interconnected tubules and vesicles that forms as fully modified cargo is sorted and packaged into transport vesicles as it leaves the Golgi apparatus for delivery to subsequent compartments of the endomembrane trafficking pathway

Supplemental Material >

Plant Rab GTPases Involved in Trafficking from the Golgi Apparatus to the Plasma Membrane

In plants, as in animals and yeast, members of the Rab11/RabA GTPase family localize to and regulate trafficking of both secretory and endocytic cargo through *trans*-Golgi network (TGN) compartments (7, 19, 22, 63, 68, 125, 163). However, while in animal cells Rab11-labeled compartments are typically pericentriolar-localized membranes associated primarily with polarized recycling of endocytic cargo (163), the plant RabA4b GTPase family associates with both Golgi apparatus–associated TGN and freely distributed TGN compartments (78, 141, 159). These RabA GTPase–labeled membranes accumulate at sites of polarized secretion in the tips of growing root hairs and pollen tubes in *Arabidopsis* and tobacco as well as at the leading edges of newly forming cell plates during cell division. These membranes also accumulate endocytic tracers, highlighting the dual nature of these compartments in secretion and endocytosis (22, 86, 125, 152). Interestingly, while animals and yeast maintain relatively small families of these Rab11/RabA-like GTPases (122), the RabA GTPases are greatly expanded in plants, with 26 members in *Arabidopsis* (**Supplemental Table 1**), and similarly large families in rice, maize, and *Medicago truncatula* (14, 167). Several recent observations suggest that this large family may indeed reflect complex regulation of post-Golgi membrane trafficking in plants. Distinct RabA GTPases colocalized with the defense-related flagellin sensing receptor 2 at different stages during secretion, endocytosis, and recycling (21). Fluorescent RabA1e and RabA2a colocalized to cell plates during cytokinesis but displayed differing levels of resistance to Endosidin7 inhibitor treatment, suggesting that their targeting and trafficking functions may involve distinct cellular components (29). Finally, RabA5c GTPases localized to novel membrane compartments that define the geometric edges of plant cells. Loss of RabA5 function resulted in defects in plant cell shape maintenance arising from the inability to generate stable cell corners during cytokinesis (81).

In yeast and animals, the Rab8/Sec4 GTPases regulate polarized secretion of proteins from the Golgi apparatus to the plasma membrane (PM), such as during the budding process in *S. cerevisiae* (48, 139), during basolateral membrane trafficking in mammalian epithelial cells (59),

and during formation of primary cilia in vertebrates (109). In *Arabidopsis*, Rab8/Sec4 GTPases are organized into the RabE group (**Supplemental Table 1**), which contains five closely related members (136, 167). A fluorescent fusion protein of RabE1d localizes to both Golgi compartments and the PM, and expression of a DN-mutant RabE1d impairs delivery of secretory cargo from the Golgi apparatus to the PM, diverting this cargo to vacuolar compartments (2, 183). In tomato, interaction between the RabE GTPase and the bacterial pathogen effector avrPto from *Pseudomonas* sp. in an *R* gene–dependent manner raised the possibility that members of the RabE GTPases may be targeted by these bacterial pathogens during subversion of host defenses (12). Downregulation of *Arabidopsis* RabE1d by cosuppression resulted in severe leaf morphology defects, and overexpression of a *CA RabE1d* mutant conferred resistance to infection with *Pseudomonas syringae*, highlighting important roles for RabE GTPases both during plant growth and development and during establishment of plant defense responses (146). More recently, RabE GTPases were shown to interact with the Stomatal Cytokinesis Defective (SCD) complex, a multiprotein complex that, in turn, interacts with exocyst components, which together facilitate aspects of secretion and endocytosis during cytokinesis (101).

Early endosomes: the initial membrane bounded organelles that newly internalized transport vesicles from the plasma membrane fuse with within the cytoplasm

Rab6/RabH GTPases also appear to regulate trafficking between Golgi and PM locations, although the precise functions remain less well characterized than those of the Rab11/RabA and Rab8/RabE trafficking pathways. In animals, Rab6 GTPases localize mostly to the Golgi apparatus and regulate aspects associated with vesicle budding from the Golgi apparatus (106, 147) as retrograde trafficking to the ER (170), trafficking from endosomes to the Golgi apparatus (164), or post-Golgi trafficking of enveloped viruses to the PM (72). In *S. cerevisiae*, the Rab6 homolog, Ypt6, regulates retrograde trafficking from the endosome to the Golgi apparatus and appears to participate along with the Arf-like GTPase, Arl1, in vesicle budding/recycling events associated with macroautophagy (150). In *Arabidopsis*, the Rab6/RabH isoforms RabH1b and RabH1c localize to the plant Golgi apparatus (71), and more recently RabH1b has been implicated in formation of Golgi apparatus–derived vesicles from the periphery of Golgi cisternae, and may regulate trafficking of cellulose synthase proteins between the Golgi apparatus and the PM (56, 70) (**Supplemental Table 1**).

Plant Rab GTPases Involved in Endocytosis and Vacuolar Sorting

Endocytic membrane trafficking pathways include those involving the retrieval of lipids, extracellular macromolecules, and integral membrane proteins from the PM to internal membrane compartments for sorting and recycling back to the PM, or redirection to lysosomal/vacuolar compartments. In addition, many newly synthesized proteins are sorted in endosomal compartments en route to their final destinations in the vacuole or PM. In animals and yeast, Rab5/Ypt51 GTPases regulate membrane trafficking between PM-derived transport vesicles and early endosomal compartments, where initial cargo sorting occurs between recycling and degradation pathways (reviewed in 85, 116). While in animal cells newly internalized endocytic cargo first reaches tubulovesicular, Rab5-positive early endosomes, in yeast and plants endocytic cargo first appears either in Golgi apparatus–associated membranes (in *S. cerevisiae*) or in a subset of freely distributed TGN compartments, called TGN/early endosomal (EE) compartments (in plants) (22, 30). In *Arabidopsis* there are three Rab5/Ypt51 homologs (**Supplemental Table 1**): two canonical Rab GTPases, RabF2a/RHA1 and RabF2b/ARA7, and a unique, plant-specific Rab-like GTPase, RabF1/ARA6, which lacks normal C-terminal lipid modification and instead is lipid modified at the N terminus (161). The presence of this plant-specific RabF1/ARA6 GTPase appears well conserved in the plant lineage but is absent from yeast and animals (35). All three RabF GTPases localize to populations of multivesicular endosomes (MVEs) that partially overlap and

Supplemental Material >

can be labeled with the endocytosis tracer FM 4-64 (67, 154, 160). While all three RabF GTPases are thought to mediate trafficking to late endosomes and vacuoles (16, 82, 137, 145), the plant-specific RabF1/ARA6 GTPase also supports MVE-mediated recycling of cargo to the PM, and it appears to play particularly important roles during plant responses to environmental cues, such as salinity and gravitropic responses, as well as flowering (35, 37).

Mammalian RAB7 is known to regulate membrane fusion at late endosomes, and its yeast counterpart, Ypt7, mediates the fusion of vacuoles. The *Arabidopsis* genome encodes eight putative RAB7/Ypt7-like Rab GTPases, organized into the RabG subgroup (**Supplemental Table 1**), and seven of these localize to the vacuolar membrane (137). While many of these RabG family members appear to be functionally redundant, *vacuoleless1* mutants, in which a subunit of a putative downstream effector complex of RabG GTPases was mutated, exhibits embryonic lethality (133), suggesting important RabG functions in vacuolar biogenesis and plant development.

Supplemental Material >

Plant Rab Interacting Proteins: Conserved GEFs and Lipid Kinase Effectors

Rab GTPases cycle between an inactive GDP-bound form located in the cytosol, where they form complexes with Rab-GDP dissociation inhibitors, and an active GTP-bound form that promotes membrane trafficking–associated functions once they are localized to the appropriate endomembrane compartment (**Figure 1*c***). In order to become active on the membrane, they must first interact with a complement of RabGEFs that activate these Rab GTPases by stimulating GDP/GTP exchange. In yeast and animals, distinct RabGEFs that act on different Rab GTPase groups have been identified. These include the Transport Protein Particle I/II (TRAPPI/II) complexes, which are Rab1/Rab11 GEFs; the Ribosome Control 1-Reduced Growth Phenotype 1 (RIC1-RGP1) Rab6 GEF; the SEC2 Rab8 GEF; VPS9 domain proteins, which are Rab5 GEFs; the Monensin Sensitivity 1-Calcium Caffeine Zinc Sensitivity 1 (MON1-CCZ1) family of proteins, which are Rab7 GEFs; and the Differentially Expressed in Neoplastic vs. Normal (DENN) domain–containing family of proteins that activate diverse Rab GTPases in animals but are absent in yeast (reviewed in 84). Plants have maintained most of these RabGEF complexes (**Supplemental Table 1**), and a number of plant proteins containing these RabGEF domains are required for Rab GTPase–mediated membrane trafficking in plants. *Arabidopsis* contains two sequences with high sequence similarity to VPS9, termed VPS9A and VPS9B, which activate the two canonical *Arabidopsis* Rab5-like GTPases, RabF2a/RHA1 and RabF2b/ARA7, as well as the plant-specific RabF1/ARA6 (47, 151). Of these two RabGEFs, VPS9A appears to provide most of the activity, and it plays important roles in RabF-mediated membrane trafficking events involved in endosomal sorting and trafficking of cargo to plant vacuolar compartments in *Arabidopsis* (16, 47, 64), as well as important roles in plant innate immunity to fungal pathogens (113).

In yeast and animals, Rab5/Ypt51-positive early endosomal compartments mature into Rab7/Ypt7-positive late endosomes as recycling cargo is progressively sorted away from lysosomal/vacuolar targeted cargo. MON1-CCZ1 RabGEF complexes link Rab7 recruitment to this endosomal maturation process and are initially recruited to early endosomes by direct interaction between MON1, GTP-bound Rab5/Ypt51, and Rab5/Ypt51-generated phosphoinositide 3-phosphate (PI3P) on these membranes. MON1 simultaneously sequesters Rab5/Ypt51 from VPS9 RabGEFs and stimulates Rab7/Ypt7 recruitment and activation through CCZ1-mediated RabGEF action (reviewed in 39). Plants appear to have maintained this Rab5-to-Rab7 endosomal maturation module as MON1-CCZ1 complexes are present and act as RabGEFs for RabG GTPases in plants, and regulate MVE-to-vacuolar trafficking (28, 36, 144).

Other conserved RabGEF complex components, previously identified in animals and yeast, also appear to function in RabGEF complexes in plants. The SCD complex, containing the DENN

domain protein, is thought to be the GEF for RabE (101), and a LOT (loss of TGN) protein may act as a GEF for RabH (70). Additionally, plants contain essential TRAPPII complex components, which are GEFs for RabA GTPases (66, 76, 127).

Once Rab GTPases are recruited to their specific membrane compartment and activated by their cognate RabGEF, they regulate membrane fusion events as well as other aspects of organellar dynamics through recruitment of specific Rab effector proteins. While the specific Rab effector complements vary between the types of subcellular organelles and in different eukaryotic lineages, two generally conserved classes of Rab effectors that are recruited by Rab GTPases to cellular membranes appear to be phosphoinositide kinases and membrane tethering components. In yeast and animals, Rab5/Ypt51 recruit phosphoinositide 3-OH kinases to endocytic membranes (85), resulting in specific enrichment of PI3P in endosomal membranes. This process recruits additional proteins with PI3P interaction domains, such as FYVE fingers and PX domains. Endosomal PI3P enrichment is conserved in plants, as GFP-FYVE markers accumulate on RabF endosomes in plants, and interference with PI3P production, either in *pi3k* mutants or by treatment with inhibitors, disrupts vacuolar trafficking (145). The recently characterized RabF2a/F2b interacting effector protein EREX binds PI3P through a PX domain, and knockout mutants display defective trafficking of seed storage proteins (138). Two phosphoinositide 4-OH (PI4P) kinases, PI4Kβ1 and PI4Kβ2, are RabA4b effectors, and PI4P generation/turnover is important in RabA-mediated polar secretion during tip growth in root hairs and pollen tubes (126). Additionally, PI4P 5-kinase 2 was identified as a RabE effector protein (17). This kinase utilizes PI4P as a substrate to produce phosphatidylinositol 4,5-bisphosphate, which accumulates in polarized PMs in tip-growing cells and at the leading edges of newly forming cell plates during cytokinesis, two processes linked with RabE GTPase–mediated trafficking (2, 146).

A highly interesting development is the identification of plant Rab GTPases, and components recruited by these Rab GTPases, as targets for inactivation by plant pathogens. Trafficking of antimicrobial compounds by RabA1a is inhibited by its direct interaction with a RxLR (arginine-*X*-leucine-arginine) virulence effector from the oomycete pathogen *Phytophthora brassicae*, and secreted effectors from several bacterial and fungal pathogens have been found to bind plant secretory components (158). Similarly, mutation of membrane trafficking components often either compromises or enhances plant pathogen resistance (recently reviewed in 38). One of the features of plant membrane trafficking is the striking enlargement of gene families associated with post-Golgi membrane trafficking when compared with animal and yeast lineages. This expansion includes the RabA GTPases, PM-localized SNAREs, and Exo70 subunits of the exocyst complex (77, 167). While some of this diversification undoubtedly reflects the elaboration of plant-specific membrane trafficking pathways, it may also highlight the ongoing evolution of plant host-pathogen interactions.

SAR/Arf GTPases: COAT RECRUITMENT DURING FORMATION OF TRANSPORT VESICLES IN PLANTS

The formation of transport vesicles on donor membranes begins with assembly of coat protein complexes, mediated by SAR/Arf GTPases. Sar1/Arf1 proteins are characterized by the presence of an N-terminal, amphipathic α-helix that is also *N*-myristoylated in the Arf GTPases, but not in Sar1 GTPase. When bound to GDP, Sar1/Arf1 proteins are cytosolic and inactive, with the amphipathic α-helix sequestered in a hydrophobic pocket. Upon GDP/GTP exchange by either the Sar1GEF, Sec12, or one of a number of distinct ArfGEFs, structural changes occur that expose the amphipathic α-helix, which then inserts into its target membrane and initiates recruitment of transport vesicle coat proteins (**Figure 1*d***). *Arabidopsis* contains 3 Sar1 GTPases and 12 Arf

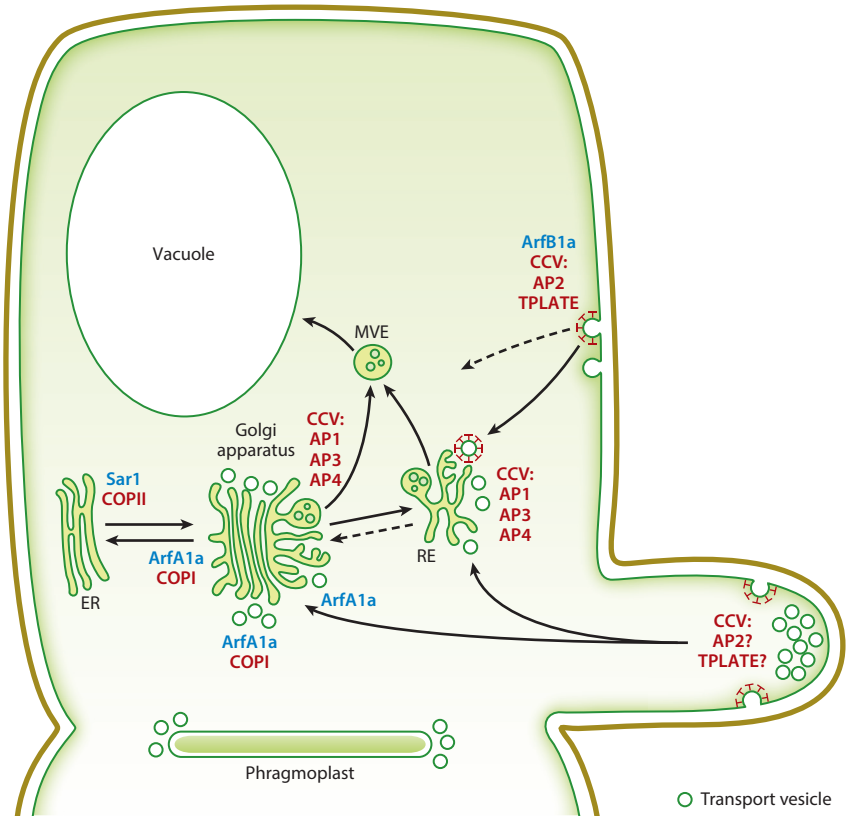

Figure 3

A schematic illustration of Sar/Arf GTPase *(blue)* and adaptor/coat protein (*red*) regulated pathways in plant cells. Abbreviations: CCV, clathrin-coated vesicle; COPI, coatomer protein complex I; COPII, coatomer protein complex II; ER, endoplasmic reticulum; MVE, multivesicular endosome/prevacuolar compartment; RE, recycling endosome.

Supplemental Material >

GTPases (**Supplemental Table 2**) that correspond to the mammalian Arf1, a class I Arf GTPase (75, 167). These plant Arf GTPases segregate into four distinct clades (ArfA–ArfD), with ArfA1a localizing to plant Golgi compartments while ArfB1a associates with PMs (98). ArfA1 family members are implicated in a variety of trafficking steps, including trafficking from the ER to the Golgi apparatus, endocytosis and recycling, and vacuolar trafficking (recently reviewed in 143).

Sar1 is present only on ER membranes, where it organizes assembly of coatomer protein complex II–coated vesicles at ER exit sites, while coatomer protein complex I–coated vesicles assemble at Golgi compartments and are probably recruited by Arf GTPases (123, 129). A number of distinct clathrin-coated vesicle (CCV) classes are formed on the PM and TGN/EE compartments in plants (131). Because the triskelion outer coat complexes that typify CCVs do not directly interact with cargo, their formation on distinct cellular membranes and packaging of distinct cargoes are regulated by Arf GTPase recruitment of a series of conserved adaptor protein complexes (AP1–AP4) and the TPLATE complex (**Figure 3**). In *Arabidopsis*, AP1 adaptor recruitment to the TGN requires the BIG1–BIG4 family of ArfGEFs, forming CCVs that contain cargo destined for delivery to vacuoles, the PM, and newly forming cell plate membranes during cytokinesis (143). How AP1-CCV transport vesicles containing different cargos are

delivered to these different compartments is presently unknown, although differential recruitment or activation of RabGEF complexes on these transport vesicles is an attractive possibility. In animals, AP2 adaptors are responsible for most CCV-mediated endocytosis; however, mutations that eliminate plant AP2 adaptor components remain viable, although endocytosis of some PM-localized proteins are altered, and these plants display varying developmental and infertility defects (4, 31, 80, 176). Instead, the TPLATE complex plays a major role in CCV-mediated endocytosis, and mutations of TPLATE components are gametophyte lethal (44, 180). Yeast and animal AP3 adaptors function in trafficking to vacuolar compartments. However, mutants of plant AP3 components do not display significant defects in growth and development (41, 171), perhaps due to redundancy with other AP complexes that function at the TGN, such as AP1 or AP4, each of which interacts with the vacuolar sorting receptor (43).

Rho of plant proteins (Rop GTPases): a plant-specific family of small GTPases that regulate aspects of cellular signaling pathways and cytoskeletal dynamics

While ArfA1-class GTPases regulate transport vesicle formation on a number of distinct subcellular organelles in plants, the spatiotemporal regulation of these small GTPases is thought to be controlled through interaction with plant ArfGEF and ArfGAP proteins (**Supplemental Table 2**). *Arabidopsis* contains eight ArfGEFs, organized into two main families (167). The first consists of GNOM and GNOM-like 1 and 2 (GNL1 and GNL2), and the second consists of BIG1–BIG5 (143). GNOM is essential for polarized endocytic recycling of PIN1 auxin transporter proteins to polarized basal PM domains in root tissues (46). GNOM, along with GNL1, also regulates coatomer protein complex I–mediated Golgi apparatus–to–ER retrograde trafficking (129). BIG1–BIG4 act redundantly at the TGN where they function to recruit AP1 adaptor protein complexes to these membranes (130). BIG5 also regulates TGN/EE trafficking steps and is a target for inactivation by bacterial effectors during *Pseudomonas* infection (115). Of the 15 ArfGAP proteins (called AGDs) identified in *Arabidopsis*, AGD1 regulates aspects of polarized membrane trafficking in root hairs and localizes to PMs via interaction with PI4P (178). AGD3/VAN3 (also known as SCARFACE) also binds to PI4P but localizes to the TGN and is required for vascular development (110). AGD5/NEV4 (also called MTV4), a plant-specific ArfGAP, localizes to TGN, and its mutation delays floral organ abscission (91, 148). AGD7–AGD10 are thought to localize to the Golgi apparatus and regulate ArfA1a during coatomer protein complex I–mediated trafficking, functioning in Golgi apparatus–to–ER retrograde trafficking, intra–Golgi apparatus trafficking, or both (reviewed in 143). Interestingly, mutation of the plant-specific AGD12 displayed root and shoot gravitropism defects, and AGD12 may act in conjunction with EHB1, a structural isoform lacking the ArfGAP domain, to regulate Ca^{2+}-dependent regulation of these processes (34).

Supplemental Material >

Rop GTPases: SIGNALING GTPases IN PLANTS

In yeast and animals, small GTPase involvement in signal transduction across the PM as well as its integration and propagation in cytosolic protein kinase cascades, ultimately resulting in changes in gene transcription in the nucleus, are provided by the Ras and Rho/Rac GTPase families (52). While Ras GTPase–mediated signaling generally controls aspects of cell proliferation and differentiation, Rho/Rac GTPases also regulate cytoskeletal dynamics and cellular polarity (reviewed in 57). In plants, the Ras GTPase is absent, and both receptor-mediated signaling and regulation of cytoskeletal dynamics and cell polarity are provided by Rop GTPases (167) (**Supplemental Table 3**). Receptor-mediated signaling modules that involve Rop GTPase–mediated signaling involve a wide variety of plant processes, including cell polarity, cell shape, hormone responses, and pathogen defense. For the sake of brevity, this section focuses on a few recent developments in our understanding of plant-specific wrinkles in Rop GTPase–regulated processes and directs readers to a number of excellent recent reviews covering these topics (40, 90).

Novel Mechanisms of Rop GTPase Activation

Like Rab GTPases, Rop GTPases are posttranslationally modified at their carboxyl terminus and cycle between cytosolic localized inactive (GDP-bound) and membrane-bound active (GTP-bound) forms in a process mediated by their association with specific Rop GDP-dissociation inhibitor proteins (**Figure 1*e***). Similar to other small GTPases, Rop GTPases require interaction with RopGEFs in order to facilitate activation by GDP/GTP exchange. However, early attempts to identify RopGEFs in plant genomic sequences failed to identify sequences with classical RhoGEF, Dbl-homology catalytic domains (167). Instead, a novel plant-specific class of RopGEFs, the plant-specific Rop nucleotide exchanger (PRONE) family of proteins, was demonstrated to activate plant Rop GTPases (9). *Arabidopsis* contains 14 PRONE domain–containing RopGEFs (**Supplemental Table 3**), which interact with a number of plant receptor-like kinases (RLKs) linked with Rop GTPase signaling (33, 79). A second class of RopGEFs is defined by SPIKE1 (SPK1), which shares limited sequence homology with DOCK/CZH RhoGEFs, initially characterized in mammals. It is found in complex with SCAR/WAVE complexes, which in conjunction with activated Rop GTPases associate with the Arp2/3 complex, initiating actin polymerization and branched actin networks (5, 162).

Supplemental Material >

Spatial Coordination of RopGEF Activation and RopGAP Recruitment Controls Plant Cell Shape

In plant cells, two cytoskeletal networks, filamentous actin (F-actin) and cortical MT arrays, play important roles in the regulation of a number of cellular functions important for cell growth and changes in cell morphology. These include cytoplasmic streaming and vesicle transport, which depend on dynamic F-actin (87). The placement and organization of cortical MT arrays also guide and orient PM-localized cellulose synthase complexes as they synthesize and deposit cellulose microfibrils (89), which are the primary load-bearing components of the plant cell wall and whose deposition affects the future shape of plant cells as they expand. Localized RLK activation from extracellular signals that promote cell growth, such as LURE peptides in tip-growing pollen tubes (155) or RALF peptides in cells undergoing diffuse growth (54), results in localized recruitment and activation of Rop GTPases in these regions. In tip-growing cells, Rop-mediated actin polymerization may assist in the recruitment and fusion of vesicles carrying new cell wall material. In diffusely expanding cells, depletion or reorientation of cortical MT arrays is likely mediated by activated Rop GTPases through the recruitment of the Rop effector RIC1, which in turn activates Katanin1-mediated MT severing (92). Loss or reorientation of cortical MT arrays likely alters the density or orientation of new cellulose microfibril deposition by PM cellulose synthase complexes, which in turn affects cell expansion characteristics. In what is perhaps an extreme example of this type of interplay between Rop-mediated activation of localized actin polymerization and depletion of cortical MTs, SPK1/SCAR/WAVE accumulation at the apex of developing trichomes was correlated with a MT-depleted zone, and the boundaries of this domain appeared to be surrounded and restricted by a ring of cortical MTs (177).

CONCLUSIONS

In the approximately one billion years since plant, animal, and fungal lineages diverged from a common eukaryotic ancestor, these three types of organisms have had ample time to diversify and evolve novel lifestyles and developmental mechanisms. Nevertheless, it is clear that at least one core signaling module, the GTPase molecular switch, whose presence preceded the evolutionary divergence of these three kingdoms, has been maintained and continues to be broadly utilized to regulate cellular mechanisms in present-day eukaryotes. Over the quarter-century since the first

plant small GTPases were identified and characterized, our understanding of the roles and organization of plant small GTPases has greatly improved. Plants have maintained several distinct small GTPase families: the Ran, Rab, Arf, and Rho/Rac GTPases. Each of these classes displays core functions in plants that are conserved in fungal and animal lineages. Plant Ran GTPases regulate nucleocytoplasmic trafficking; Rab GTPases specify distinct endomembrane compartments and regulate vesicle fusion between them; Arf GTPases stimulate transport vesicle formation and cargo selection through the recruitment of coat protein complexes; and Rop GTPases control aspects of plant cellular signaling, cell polarity, and cytoskeletal dynamics. However, as our understanding of the functions of these small GTPases has improved, it has become abundantly clear that many of these core functions have been supplemented with new, plant-specific functions. Future studies are likely to reveal additional examples of how plants have evolved novel uses for these conserved regulatory modules during their growth and development.

SUMMARY POINTS

1. Plant Ran GTPases maintain conserved nucleocytoplasmic transport functions, but have evolved novel mechanisms to target RanGAP proteins to nuclear envelope membranes. Ran GTPases appear to perform novel functions during female gametogenesis, particularly in the cellularization process during endosperm development.
2. The subcellular localization of plant Rab GTPases on subcellular compartments of the endomembrane trafficking pathway reflects its evolutionary conservation. Specific differences in the organization of these endomembrane compartments between plants are reflected in expansion of RabA GTPases and the appearance of a noncanonical endocytic Rab GTPase, RabF1/ARA6.
3. Sar/Arf GTPases in plants have conserved roles in vesicle coat protein recruitment, but appear to have swapped the major requirement for adaptor protein 2 (AP2) components during clathrin coat–mediated endocytosis at the plasma membrane (PM) with the TPLATE adaptor complex.
4. Lacking obvious Ras GTPase family members, plants instead utilize the Rop GTPases to mediate receptor-like kinase (RLK) signal transduction. Plants have also evolved a novel family of RopGEF proteins that are recruited to many of these RLKs during activation of Rop GTPase signaling cascades.
5. Many of the plant GTPase families of regulatory proteins appear to be targets for inactivation or modulation by invading plant pathogens. In several cases, plant membrane trafficking components involved in post-Golgi trafficking events display large gene families when compared with their yeast and animal counterparts. Whether this discrepancy reflects increased regulation or complexity of these pathways or the evolution of host-pathogen interactions will be an area of great interest in the future.

FUTURE ISSUES

1. What is the nature of the plant RAN GTPases GDP/GTP exchange (RanGEF) activity? Canonical RCC1-like sequences have not yet been identified in plants. Given the central role of these DNA-associated RanGEF activities in both animals and yeast, it is somewhat surprising that similar sequences have not yet been identified in plants.

2. Do plant Rab GTPases regulate vesicle tethering and fusion as observed for both yeast and animals? In both, Rab GTPases regulate vesicle fusion events by (*a*) recruiting coiled-coil tethering proteins that assist in stabilizing vesicle-target membrane associations, and (*b*) recruiting and modulating the activity of Sec1-like proteins that regulate v-t SNARE pairing.
3. To what extent are Rab GTPase-specific effector protein families conserved or divergent from yeast and animals? While the core Rab GTPase roles in regulation of membrane fusion events is likely conserved, additional Rab effector protein roles in cargo selection, attachment and/or movement of subcellular compartments on cytoskeletal elements, and signal transduction have been described in animals. Whether similar diversification of Rab-specific effector protein functions in plants remains largely unexamined.
4. While plant SAR1/ARF GTPases appear to have maintained roles in vesicle coat protein recruitment, what roles do plant ARL GTPases play in plant development? In animals, some ARL GTPases have been implicated in packaging and trafficking of specific cargo subsets; do plant ARL GTPases also display similar diversified roles for specific subsets of cargo proteins?

DISCLOSURE STATEMENT

The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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