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REVIEW

# Small GTP-binding Proteins and their Functions in Plants

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**Abstract** Small GTP-binding proteins exist in eukaryotes from yeast to animals to plants and constitute a superfamily whose members function as molecular switches that cycle between "active" and "inactive" states. They regulate a wide variety of cell functions such as signal transduction, cell proliferation, cytoskeletal organization, intracellular membrane trafficking, and gene expression. In yeast and animals, this superfamily is structurally classified into at least five families: the Ras, Rho, Rab, Arf/Sar1, and Ran families. However, plants contain Rab, Rho, Arf, and Ran homologs, but no Ras. Small GTP-binding proteins have become an intensively studied group of regulators not only in yeast and animals but also in plants in recent years. In this article we briefly review the class and structure of small GTP-binding proteins. Their working modes and functions in animals and yeast are listed, and the functions of individual members of these families in plants are discussed, with the emphasis on the recently revealed plant-specific roles of these proteins, including their cross-talk with plant hormones and other signals, regulation of organogenesis (leaf, root, and embryo), polar growth, cell division, and involvement in various stress and defense responses.

**Keywords** Small GTP-binding proteins · Plant-specific functions

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## Introduction

Small GTP-binding proteins are monomeric G proteins with molecular masses of 20–40 kDa, which is different than the heterotrimeric G proteins. The first small GTP-binding protein was identified from a sarcoma virus as the oncogene. Two oncogenes, *v*-Ha-Ras and *v*-Ki-Ras, were found by comparing the genomic organizations of Kirsten and Harvey sarcoma viruses (Shih and others 1978). Their homologous genes, named Hs-Ras and Ki-Ras, were then identified in humans (Der and others 1982; Parada and others 1982). These Ras proteins were shown to be related to the heterotrimeric G proteins such as Gs, Gi, and elongation factor for protein synthesis (Shih and others 1980). Currently, more than 100 small GTP-binding proteins have been identified in eukaryotes from yeast to human, and they comprise a superfamily (Bourne and others 1990; Jiang and Ramachandran 2006). Members of this class of proteins are among the largest families of signaling proteins in eukaryotic cells. Their importance in cellular signaling processes is underscored by their conservation throughout the evolution of eukaryotic organisms from yeast to humans to plants. Small GTP-binding proteins are involved in regulation of diverse eukaryotic cellular processes such as cell proliferation, cytoskeletal assembly and organization, and intracellular membrane trafficking (Boguski and McCormick 1993; Jaffe and Hall 2005). Many upstream regulators and downstream effectors of small GTP-binding proteins have been identified, and modes of their actions have gradually been elucidated in yeast and animals (Vojtek and Der 1998; Corbett KD and Alber T 2001; Vlahou and Rivero 2006). In contrast, there are still limited data concerning the small GTP-binding proteins in plants. The completion of the *Arabidopsis* genomic sequence enabled the identification of a total of 93

small GTP-binding proteins in *Arabidopsis* by *in silico* analysis (Vernoud and others 2003). Several reports have demonstrated the similarity and the difference of small GTP-binding proteins between plants and animals. In this brief review, functions of small GTP-binding proteins and their modes of action in plants are discussed and compared with similar data from yeast and animals.

## Class and Structure of Small GTP-binding Proteins

The members of small GTP-binding proteins are structurally and functionally classified into at least five families: the Ras, Rho, Rab, Arf/Sar1, and Ran families (Kahn and others 1992; Jiang and Ramachandran 2006). Sequence annotation against complete genomic sequence in the yeast *Saccharomyces cerevisiae* has revealed that there are 4 Ras family members, 6 Rho family members, 11 Rab family members, 7 Arf/Sar1 family members, and 2 Ran family members (Garcia-Ranea and Valencia 1998). Humans have 34 Ras family members, 23 Rho family members, 70 Rab family members, 46 Arf/Sar1 family members, and 1 Ran family member (Jiang and Ramachandran 2006). Ninety-three and 111 small GTP-binding proteins have been identified in *Arabidopsis* and rice, respectively. These small GTP-binding proteins are classified within four of the five small GTP-binding protein families: *Arabidopsis* with 57 Rab, 21 Arf, 11 Rho, and 4 Ran, and rice with 47 Rab, 43 Arf, 17 Rho, and 4 Ran. Interestingly, *Arabidopsis* and rice do not contain any Ras family members based on phylogenetic analysis, perhaps reflecting unique mechanisms for control of cell signaling during development in plants (Vernoud and others 2003; Jiang and Ramachandran 2006).

A comparison of the amino acid sequences of all small GTP-binding proteins has revealed that they are conserved in primary structures and are 30–55% homologous to each other. The secondary and three-dimensional structures of small GTP-binding proteins are also highly conserved. Like other G proteins, all small GTP-binding proteins have consensus amino acid sequences responsible for specific interaction with GDP/GTP and for GTPase activity, which hydrolyzes bound GTP to GDP and Pi (Bourne and others 1991; Takai and others 2001). There are four guanine nucleotide-binding domains in the small GTP-binding protein sequence designated as GI-GIV, which are scattered in the different regions instead of combined together. However, these four binding domains are spatially located close together at the bend in the peptide by two highly flexible regions: the switch I and the switch II (Geyer and Wittinghofer 1997; Vetter and Wittinghofer 2001). Besides, there is an effector domain between GI and GII that is responsible for binding with effector proteins (Figure 1).

The major variations between the primary structures of the different small GTP-binding proteins are located in the N-terminus and the C-terminus. Small GTP-binding proteins belonging to Ras, Rho, and Rab have sequences at their C-termini that undergo post-translational modifications with lipids such as farnesyl, geranylgeranyl, palmitoyl, and methyl moieties, and proteolysis (Glomset and Farnsworth 1994; Magee and others 1992). This C-terminal motif for prenylation is designated as the P domain. The P domains of Ras, Rho, and Rab are classified into several groups: (1) **Cys-A-A-X** (A, aliphatic acid; X, any amino acid): The Cys-A-A-X structure is further subclassified into two groups: one has an additional Cys residue upstream of the Cys residue in the Cys-A-A-X structure, and the other has a polybasic region. Some Ras proteins belong to the Cys-A-A-X structure; these proteins are first farnesylated at the Cys residue followed by the proteolytic removal of the A-A-X portion and the carboxylmethylation of the exposed Cys residue (Fujiyama and Tamanoi 1990). (2) **Cys-A-A-Leu/Phe**: Some Ras proteins like Rap1 have this kind of structure; these proteins are first geranylgeranylated followed by the same modifications as that mentioned above (Kawata and others 1990). (3) **Cys-X-Cys**: Some Rab proteins with this structure are geranylgeranylated at both Cys residues, and the C-terminal Cys residue is carboxylmethylated (Farnsworth and others 1991). (4) **Cys-Cys**: Some Rab proteins with this structure are geranylgeranylated at both Cys residues, but the C-terminal Cys residue is not carboxylmethylated (Smeland and others 1994). Arf is myristoylated at the N-terminus, but Ran does not have such sequences to direct post-translational modifications. In plants, Rab proteins have the Cys-Cys-A-X structure and other small GTP-binding proteins just have similar post-translational modifications as those in animals (Yang 2002). Further lipid modifications of these small GTP-binding proteins are necessary for their binding to membranes and regulators and for their activation of downstream effectors as described below (Takai and others 2001).

## Functions of Small GTP-binding Proteins

Small GTP-binding proteins have two interconvertible forms: the GDP-bound inactive form and the GTP-bound active form (Bourne and others 1990). Upon stimulation by an upstream signal, the dissociation of GDP from the GDP-bound inactive form is followed by the binding of GTP and the formation of the GTP-bound active form, which leads to the conformational change of the downstream effector-binding region so that this region interacts with the downstream effector. This interaction causes the functional change of the downstream effector. The GTP-bound form

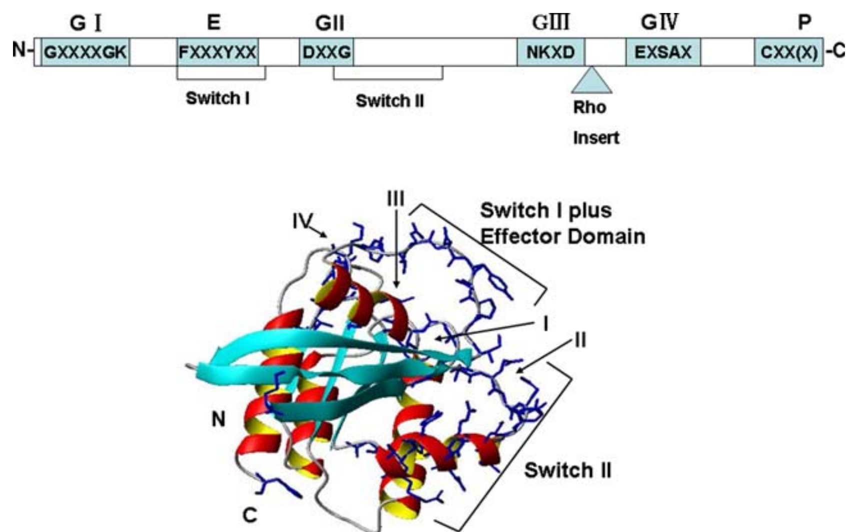


**Fig. 1** Structure of small GTP-binding proteins. All small GTP-binding proteins contain four conserved domains for guanine nucleotide binding and GTPase activities (GI-GIV) and an effector domain (E). The Rho insert (8-12 amino acids) is found only in Rho. Rab, Ras, and Rho also contain a C-terminal motif for prenylation (P). Arf is myristoylated at the N-terminus, but Ran has no known modification. Two switch regions (Switches I and II) make four guanine nucleotide-binding domains spatially close together. *Upper* The conserved domains and consensus amino acids of small GTP-binding proteins. *Lower* The three-dimensional structure of small GTP-binding proteins. Letters for amino acids: A, Ala; C, Cys; D, Asp; E, Glu; G, Gly; K, Lys; N, Asn; S, Ser; X, any amino acid

is converted by the action of the intrinsic GTPase activity to the GDP-bound form, which then releases the bound downstream effector. In this way one cycle of activation and inactivation is achieved and small GTP-binding proteins serve as molecular switches that transduct an upstream signal to a downstream effector during this process (Figure 2). Furthermore, this regulatory cycle of GTP binding and hydrolysis is controlled overall through the actions of two classes of regulatory proteins. Guanine nucleotide exchange factors (GEFs) catalyze the release of bound GDP, resulting in the formation of the GTP-bound active small GTP-binding proteins. GTPase-activating proteins (GAPs) stimulate the intrinsically low GTP hydrolytic activity of the small GTP-binding proteins, resulting in their conversion to the inactive GDP state (Figure 2). In addition, most small GTP-binding proteins cycle between membrane and cytosol. There is another class of regulatory proteins, called guanine nucleotide dissociation inhibitors (GDIs), that negatively regulate the activity of Rho and Rab proteins. GDIs sequester Rho and Rab access to GEFs and GAPs, preventing the dissociation of GDP and interactions with regulatory and effector molecules. GDIs also regulate membrane-to-cytosol cycling of Rho and Rab (Seabra and Wasmeier 2004; DerMardirossian and Bokoch 2005). These action modes of regulation are apparently conserved in all organisms, including plants. However, different forms of small GTP-binding proteins plus various regulatory and effector proteins still exhibit various specific functions; this has become a heavily investigated topic.

### In Animals and Yeast

There have been extensive reports on functions of small GTP-binding proteins in animals and yeast. Interested readers can refer to some recent articles and comprehensive review papers for more detailed information (Dasso 2002; Donovan and others 2002; Dovas and Couchman 2005; Dvorsky and Ahmadian 2004; Field 2005; Jaffe and Hall 2005; Jiang and Ramachandran 2006; Lal and others 2005;

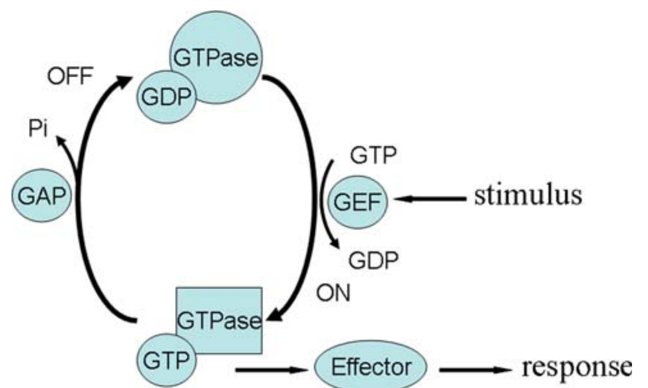


**Fig. 2** The action mode of small GTP-binding proteins. Small GTP-binding proteins (GTPase) cycle between an inactive GDP-bound form and an active GTP-bound form. This is catalyzed by a guanine nucleotide exchange factor (GEF) that displaces the GDP, allowing GTP to bind with a stimulus input. Inactivation is regulated by a GTPase-activating protein (GAP) that stimulates hydrolysis of GTP on the GTPase with signal termination. Active GTP-bound GTPase is competent to bind directly to effector proteins that propagate the response

Meller and others 2005; Nepomuceno-Silva and others 2004; Nie and others 2003; Pozzi and others 2006; Randazzo and Hirsch 2004; Takai and others 2001; Vlahou and Rivero 2006). Here we briefly list the important functions for each class of small GTP-binding protein.

### *Ras*

The extensive studies in *Caenorhabditis elegans, Drosophila*, and mammalian cells have demonstrated that the Ras family consists of several types of proteins, including R-Ras, Rap, Ral, Rheb, Rin, and Rit (Reuther and Der 2000; Jiang and Ramachandran 2006). They interact with similar downstream effectors, including phosphatidylinositol 3-kinase (PI3-K), Ral guanine nucleotide dissociation stimulator (RalGDS), Raf, $p120^{GAP}$, Tiam, Af6, Nore1, PLC$\varepsilon$, and PKC$\zeta$ (Rajalingam and others 2007). However, each of these Ras family members mediates highly divergent cellular processes (Campbell and others 1998; Bondeva and others 2002). Ras proteins can directly bind to and activate Raf protein kinase which then induces gene expression through the mitogen-activated protein (MAP) kinase cascade in response to various extracellular signaling molecules (Dent and others 1992; MacDonald and others 1993; Van Aelst and others 1993). The main functions of Ras are involved in the regulation of cell proliferation, differentiation, morphology, and apoptosis through gene expression (Feramisco and others 1984; Hagag and others 1986; Whitman and Melton 1992; Kauffmann-Zeh and others 1997; Pozzi and others 2006). Another important feature of Ras proteins is their link with cancer development because the mutations of Ras protein genes and their regulators cause human cancers (Basu and others 1992; Janssen and others 2005 and references therein). The localization of Ras proteins in the plasma membrane is crucial for their functions. It is generally believed that Ras proteins first associate with the endoplasmic reticulum and then with the Golgi apparatus, finally to be transported by exocytic vehicles. The association with endoplasmic reticulum requires the C-terminal Cys-A-A-X structure and farnsylation of Ras proteins (see the section "Class and Structure of Small GTP-Binding Proteins") (Choy and others 1999). However, the detailed mechanism of how Ras proteins get to the plasma membrane has not been fully understood.

### *Rab*

Rab proteins form the largest branch of the small GTP-binding protein superfamily; in *Homo sapiens* there are 70 Rab, *Drosophila melanogaster* 29, and *Arabidopsis thaliana* 57 (Pereira-Leal and Seabra 2001; Lal and others 2005; Saito-Nakano and others 2005; Ackers and others 2005; Jiang and Ramachandran 2006). Phylogenetic analysis reveals a clear phylogeny of function as opposed to a phylogeny of species in Rab proteins. In other words, Rab proteins with similar functions in different organisms always cosegregate. According to analysis from different organisms, the Rab superfamily can be divided into nine subfamilies, although this number may increase as new isoforms are identified.

A large body of evidence has accumulated in support of a role for Rab in intracellular vesicle trafficking from the endoplasmic reticulum to the Golgi apparatus, then to the plasma membrane (Stenmark and Olkkonen 2001; Zerial and McBride 2001). Macromolecules that are absorbed from the plasma membrane are transported inward to endosomes and lysosomes by vesicles. Some cell-surface proteins, including receptors, transit through a recycling endosome and are recycled back to the plasma membrane. These exocytosis, endocytosis, and recycling processes are performed by intracellular vesicle trafficking. There are at least four principal events in intracellular vesicle transport: (1) budding of a vesicle from the donor membrane, (2) targeting of the vesicle to the acceptor membrane, (3) docking of the vesicle to the acceptor membrane, and (4) fusion of the vesicle with the acceptor membrane. Most Rab proteins regulate the targeting/docking/fusion processes and some of them regulate the budding process, which is mainly regulated by Arf/Sar1 proteins (see section on Arf below). Besides, some Rab proteins, like Rab3A, play a key regulatory role in $Ca^{2+}$-dependent exocytosis, which is essential for newly synthesized secretory proteins translocated into the endoplasmic reticulum and then transported to the plasma membrane via the Golgi apparatus by vesicles (Geppert and others 1997). Moreover, some Rab proteins are linked with the microtubule cytoskeleton. For example, Rab6 protein associates with highly dynamic tubular structures that move along microtubules from the Golgi apparatus to the cell periphery (Echard and others 1998). The wide range of effectors/regulators and the different locations of Rab proteins suggest that Rab regulates variable cellular processes (Seabra and Wasmeier 2004). In addition, Rab proteins are also involved in intracellular signaling events with membrane traffic (Miaczynska and others 2004).

### *Rho*

Rho proteins also constitute a large small-GTP-binding protein superfamily. In humans, 23 Rho family members have been identified; these have been divided into eight subfamilies: RhoA (A, B, C isoforms), Rac (1, 2, 3

isoforms), Cdc42, Rnd (1, 2, 3 isoforms), RhoBTB, Miro, TC10, and TTF, of which RhoA, Rac, and Cdc42 are considered the “classical” members and have been the most extensively characterized (Aspenstrom and others 2004; Wennerberg and Der 2004; Jaffe and Hall 2005). However, the understanding of the Rho family structure nonetheless remains inconsistent, with some researchers dividing the Rho family into four subfamilies (Boureux and others 2007). *Saccharomyces cerevisiae* has five Rho proteins (Rho 1, 2, 3, 4, and Cdc42), whereas plants have a specific type of Rho called ROP (see section on Rho below).

In mammalian cells, it has now been demonstrated that Rho proteins primarily regulate cytoskeletal reorganization in response to extracellular signals. Reorganization of the actin cytoskeleton plays an important role in cellular functions such as cell shape change, cell motility, cell adhesion, and cytokinesis. The polymerization and depolymerization of cortical actin are tightly regulated in these processes. In most cases, this regulation of actin polymerization is directed by Rho proteins. For these subgroups, RhoA proteins regulate stress fiber formation, whereas Rac proteins regulate ruffling and lamellipodia formation, and Cdc42 regulates filopodium formation (Ridley and Hall 1992; Ridley and others 1992; Burridge and Wennerberg 2004; Schwartz 2004). Cdc42 and related Rho proteins play a central role in cell polarity (Etienne-Manneville 2004). Data have also shown that Rho proteins play additional roles in expression of other genes (Hill and others 1995; Westwick and others 1997) and in diverse cellular events such as cell growth (Olson and others 1995), membrane trafficking (Caron and Hall 1998), axon guidance, and extension. However, whether Rho proteins directly regulate these cellular events or indirectly regulate them through cytoskeletal reorganization and gene expression remains unresolved (Jaffe and Hall 2005). Recently, Rho signaling pathways were recognized as major regulators of cardiovascular functions (Rolf and others 2005; Loirand and others 2006), immunity (Bokoch 2005), and tumorigenesis (Sun and others 2007), which have very promising significance in application.

### *Arf*

Arf proteins (ADP ribosylation factors) were originally purified from rabbit liver and bovine brain membranes based on their ability to stimulate *in vitro* the cholera toxin-catalyzed ADP ribosylation of Gs. It has showed that Arf1 is localized to the Golgi apparatus in some cell lines and that Arf1 mutations cause secretion defects in *Saccharomyces cerevisiae* (Moss and Vaughan 1995). Six Arf proteins and some Arf-like proteins (Arl) have been identified in mammals, and three Arf proteins exist in *S. cerevisiae* (Moss and Vaughan 1998). In mammals, Arl proteins are 40–60% homologous with Arf proteins but cannot compliment Arf mutants in *S. cerevisiae*. The function of Arl is unclear but recent data suggest that Arl2 and Arl3 have related but distinct roles at centrosomes and in regulating microtubule-dependent processes in humans (Zhou and others 2006).

Arf proteins play crucial roles in membrane transport and actin remodeling in yeast and humans (Chavrier and Goud 1999; Randazzo and Hirsch 2004). It is now established that Arf proteins are involved in the budding of vesicles from the endoplasmic reticulum to the Golgi apparatus and the plasma membrane (Moss and Vaughan 1998; Schekman and Orci 1996; Donaldson and others 2005). Vesicle budding requires the assembly of specific proteins coating the cytoplasmic face of a donor membrane. The coat protein is thought to have at least two functions: it provides the mechanical force to pull the membrane into a bud and it helps to capture specific membrane receptors and their bound cargo molecules. Three classes of coated vesicles have been well-characterized to date: clathrin-, COP (coat protein) I-, and COPII-coated vesicles (Kartberg and others 2005). Arf proteins play crucial roles in the membrane recruitment of the COPI and COPII components. Arf proteins are also involved in the recruitment of clathrin adaptor proteins (AP) AP-1 and AP-3 components to the membranes. However, no Arf proteins have been reported to be involved in the clathrin/AP-2-coated vesicle formation from the cell surface (Lippincott-Schwartz and others 1998; Matheson and others 2006). Besides, proteins necessary for the targeting and docking processes, such as Rab proteins, must be incorporated into transport vesicles with Arf (Nebenführ 2002). However, the mechanism for this interaction between Arf and Rab is currently being investigated. Recently, Arf proteins were shown to be involved in endoplasmic reticulum to peroxisome vesicle transportation and peroxisome biogenesis (Lay and others 2006).

About 100 Arf protein sequences from animals, fungi, and plants cluster into five subgroups by phylogenetic analysis, including Arf, Arl, and Sar (Jiang and Ramachandran 2006). The functions of the Arf proteins in a metazoan model organism, *Caenorhabditis elegans*, were examined by use of RNA-mediated interference (RNAi). Of the six Arf family members examined in *C. elegans*, at least three were required for embryogenesis (Li and others 2004; Kahn and others 2006).

### *Ran*

Ran protein (Ras-related nuclear protein) was originally isolated based on its homology to Ras proteins (Drivsa and

others 1990). Different than other small GTP-binding proteins, there is only one Ran gene in many cell types (including human and *Schizosaccharomyces pombe*), although cells of other species (*S. cerevisiae*) contain two or more closely related Ran genes (Jiang and Ramachandran 2006). Furthermore, Ran proteins are not lipid modifications so they do not link with membranes. Except for GEF and GAP, which are in common with other families of Ras proteins, the GTP- or GDP-bound state of Ran proteins is also modulated by other interacting proteins such as Ran-binding protein (RanBP) and Mog1 (a guanine nucleotide release factor). Structural analysis indicates that Ran-RanBP-RanGAP forms a complex. RanBP is located away from the active site in this complex and does not influence the rate-limiting step of the GTP cleavage reaction but affects the dynamics of the Ran-RanGAP interaction (Vetter and others 1999; Seewald and others 2002, 2003). In animals and yeast, Ran GTP-binding proteins play a central role in controlling nucleocytoplasmic transport (Dasso 2002). Moreover, Ran proteins also have an essential role in microtubule organization during the M phase of the cell cycle, which controls nuclear processes throughout the cell mitotic cycle (Clarke and Zhang 2001).

Movement of macromolecules larger than 50–60 kDa, including proteins and RNAs, through the nuclear pore complexes (NPCs) is an active and selective process. The transport of these macromolecules requires at least three types of soluble proteins: transport receptors, adaptor molecules, and Ran with its binding proteins (Weis 2003). There are two types of nuclear transport receptors: import receptors, called importins, and export receptors, called exportins (Ullman and others 1997). Importins and exportins recognize nuclear location signals (NLS) and nuclear export signals (NES) in transport cargo, respectively, bind them, and transport bound cargos through the NPCs (Adam 1999). Transport receptors share a binding domain for RanGTP. During nuclear transport, the nucleotide-bound Ran acts as a switch to delineate the direction of movement, whereas the conversion between RanGTP and RanGDP may induce conformational changes of receptors so that they dissociate cargo (Weis 2003). The energy from GTP-Ran hydrolysis is not strictly required during this process. In animals, RNA picornaviruses encode factors that alter nuclear transport with the aim of suppressing synthesis of antiviral factors and promoting viral replication. Picornaviruses in the cardiovirus genus express a unique 67-aa Leader protein, known to alter the subcellular distribution of interferon (IFN) regulatory proteins targeted to the nucleus. Recent data have indicated that Leader protein binds directly to Ran and blocks nuclear export of new mRNAs. It is proposed that Leader proteins inhibit nucleocytoplasmic transport during infection by disrupting the RanGDP/GTP gradient. This inhibition triggers an efflux of nuclear proteins necessary for viral replication and causes IFN suppression (Porter and others 2006).

At the onset of mitosis in eukaryotes, the nuclear envelope and interphase microtubule array disassemble, and the duplicated centrosomes and nucleate microtubules form a bipolar spindle. Sister chromosomes balanced at the spindle equator segregate and move to opposite spindle poles during anaphase. Evidence has shown that Ran and Ran-binding molecules regulate aster formation and spindle assembly and stabilize kinetochore, formation of the nuclear envelope, and DNA replication (Arnaoutov and Dasso 2003; Blow 2003; Li and others 2003; Yamaguchi and Newport 2003; Roux and Burke 2006). In the absence of a nucleus, a natural gradient of GTP-Ran is produced that may be most concentrated at the chromosome surface. Ran and Ran-binding molecules will induce microtubule polymerization which offers a drive force for spindle assembly. Recently, a novel protein called MEL-28 has been identified; it shuttles between the nuclear pore complex and kinetochore and is essential for envelope assembly. HURP, as a novel target of Ran, is involved in the Ran-importin-regulated spindle assembly pathway (Galy and others 2006; Fernandez and Piano 2006; Sillje and others 2006; Roux and Burke 2006). In humans, the T-cell leukemia virus type-1 (HTLV-1) is an oncogenic retrovirus etiologically causal of adult T-cell leukemia. The virus encodes a Tax oncoprotein that functions in transcriptional regulation, cell cycle control, and transformation. It has been demonstrated that Tax directly binds Ran and Ran-binding protein-1, locates to centrosomes/spindle poles, and causes supernumerary centrosomes (Peloponese and others 2005). The essential actions of Ran on mitosis may lead to important new insights into the relationship between Ran and development of cancer (Sanderson and Clarke 2006).

## In Plants

There is accumulating evidence that analogous plant small GTP-binding proteins play important and diverse functional roles. In particular, the reports from *Arabidopsis* and rice indicated that basic structures and functions of small GTP-binding proteins were conserved in plants compared with their counterparts in yeast and animals. However, because the interaction of small GTP-binding proteins with various regulators and effectors generate functional diversity in different organisms, the new members and novel functions of the small GTP-binding proteins revealed in plants recently suggest that plants have the capacity to use small GTP-binding proteins as a key molecular switch for the modulation of many plant-specific signaling pathways.

*Rab*

Rab is the largest family of small GTP-binding proteins. Fifty-seven and 47 distinct Rab proteins are present in *Arabidopsis* and rice, respectively (Vernoud and others 2003; Jiang and Ramachandran 2006). The plant Rab family consists of eight subfamilies, designated as RabA to RabH, each with counterparts in yeast and animals (Rutherford and Moore 2002).

Functional studies of plant Rab homologs conform to their potential roles in vesicle trafficking (Kotzer and others 2004). During this process, different Rab proteins may act orderly. For example, RabE acts downstream of RabD (Zheng and others 2005), whereas RabD2 and RabB1 are required for transport between the endoplasmic reticulum and Golgi apparatus and for normal Golgi movement (Batoko and others 2000; Cheung and others 2002). Both AtRABF2a and AtRABF2b localize to transport cargo compartments. Another Rab protein, At-RABF1, appears to be novel and plant-specific. AtRABF1 lacks the traditional carboxy-terminal-CAAX motif for post-translational isoprenylation but is myristoylated at the amino terminus. This type of Rab is involved in post-Golgi trafficking events to the lytic vacuole in plant cells (Ueda and others 2001; Bolte and others 2004). An *Arabidopsis* RabF2 homolog, Rha1, localizes to the prevacuolar compartment and plays a critical role in the trafficking of soluble cargo from the prevacuolar compartment to the central vacuole (Sohn and others 2003; Lee and others 2004). At the same time, Rab effector protein is involved in vacuole trafficking. OsGAP1, a specific Rab-activating protein in rice, is essential for Golgi to plasma membrane (PM) and trans-Golgi network (TGN) to PM trafficking, respectively. Substitution of two invariant arginine residues (at positions 385 and 450, respectively) within the catalytic domain of OsGAP1 with alanine significantly inhibits its GAP activity. OsGAP1 localizes to the TGN or prevacuolar compartment (PVC). Wild-type OsGAP1 facilitates dissociation inhibitor 3 (OsGDI3)-catalyzed OsRabB1 recycling at an early stage, but the OsGAP1 ($R^{385}$A) and ($R^{450}$A) mutants do not. Moreover, mutation of the OsGAP1 inhibits the trafficking of some cargo proteins, including the PM-localizing $H^+$-ATPase, $Ca^{2+}$-ATPase8, and the central vacuole-localizing aleurain-like protein (AALP), which leads these proteins to accumulate at the Golgi apparatus. OsRabB1 overproduction relieved the inhibitory effect of the Os-GAP1 mutants on vesicular trafficking, whereas OsRabE1 had no such effect, which suggests that OsGAP1 facilitates vesicular trafficking from the TGN to the PM or central vacuole by both stimulating the OsRabB1 GTPase activity and increasing the recycling of OsRabB1 (Heo and others 2005).

Plant Rab proteins are involved in some plant-specific physiologic processes. Polar growth is important in plant development and this has been best characterized in two plant cell types: pollen tubes and root hairs. The accumulating data have shown that Rab and ROP (see the subsection "Rho" below) signalings are involved in polar growth. Rab proteins are involved in the apical and basolateral endocytosis for the polarized traffic, fusion of endocytic vesicles, and vesicular transport along microtubules, which is required for the establishment and maintenance of cell polarity (Cole and Fowler 2006; Campanoni and Blatt 2007). The tobacco pollen-predominant RabB1, NtRab2, functions in the secretory pathway between the endoplasmic reticulum and the Golgi in elongating pollen tubes (Cheung and others 2002). Tobacco pollen-expressed RabA2 is localized predominantly in the pollen tube tip that is almost exclusively occupied by transport vesicles. Altering RabA2 activity by expressing either a constitutive active or a dominant negative variant resulted in a reduced tube growth rate with curved pollen tubes which led to reduced male fertility, indicating that RabA2 activity is essential for tip-focused membrane trafficking and growth at the pollen tube apex (Graaf and others 2005). AtRabA4b was found to localize in the tips of growing root hairs, whereas AtRabF2b localized in leaf epidermal cells. These proteins may function in the vacuolar trafficking pathway in these tissues (Kotzer and others 2004; Preuss and others 2004). Further evidence showed that RabA4b acted on a phosphatidylinositol 4-OH kinase that polarized secretion of cell wall components in tip-growing root hair cells through $Ca^{2+}$ and PI-4,5$P_2$–dependent signals (Preuss and others 2006).

Rab may also be involved in plant hormone signal cross-talk. Expression of a tomato *RabA2* antisense RNA caused defects in the secretion of cell wall–degrading enzymes (pectinesterase and polygalacturonase) in ripening fruits which led the fruit to change color but failed to soften normally. There were other phenotypic effects in these plants, including reduced apical dominance, branched inflorescences, abnormal floral structure, ectopic shoots on the leaves, and reduced ethylene production (Lu and others 2001). The data from *Arabidopsis* and pea showed that ethylene rapidly upregulated RabE1 (Rab8 and Ara3) expression and its activity which indicated a role for Rab in ethylene signal transduction (Moshkov and others 2003a, b). Transgenic tobacco with RabA2 in either sense or antisense orientations showed distinct phenotypic changes with reduced apical dominance, dwarfism, and abnormal flower development. These abnormal phenotypes were associated with elevated cytokinin levels, stimulation of salicylic acid production in response to wounding, and increase resistance to tobacco mosaic virus infection. These data imply that cytokinins, salicylic acid, and Rab comprise

a complicated and finely tuned network in the defense signal-transduction system (Sano and others 1994; Sano and Ohashi 1995). A pea RabA2 has been shown to regulate light-mediated brassinosteroid biosynthesis. GTP-bound RabA2 directly binds the CPC p450 cytochrome. Transgenic plants with reduced RabA2 exhibited a dark-specific dwarfism, which was completely rescued by exogenous brassinolide. It suggests that RabA2 is a molecular mediator for the cross-talk between light and brassinosteroids in the etiolation process (Kang and others 2001). An *Arabidopsis* RabF2 homolog, Rha1, was found to localize on the plasma membrane and endosomes in root epidermal cells and root hairs. Auxin induced the expression of Rha1 and further affected its subcellular localization. Overexpression of Rha1 in the transgenic plants reduced the sensitivity to brefeldin A (BFA, the vesicle transport inhibitor) and N-1-naphthylphthalamic acid. It suggests that Rha1 is probably involved in auxin signal transduction (Qi and others 2005). Whether Rab proteins play any direct roles in the regulation of hormone accumulation or responses needs further investigation.

Tomato Api2, which is most similar to RabE1, is found to interact with avirulence protein AvrPto. RabE1 is involved in vesicular protein trafficking from the Golgi apparatus to the plasma membrane. Isolation of Api2 suggests the possibility that AvrPto contributes to disease by interfering with plant protein trafficking, possibly blocking the extracellular release of plant antimicrobial peptides (Bogdanove and Martin 2000). The expression of the RabF1 homolog gene was increased during early salt stress in *Mesembryanthemum crystallinum* (Bolte and others 2000). These data suggest the novel regulatory functions of Rab in disease-resistant and salt-stress responses.

### *Rho*

Rho proteins, which are involved in the regulation of the actin cytoskeleton organization and cell polarity development in eukaryotes, have three best characterized subfamilies, RhoA, Cdc42, and Rac, in yeast and animals. In plants, all small GTP-binding proteins that segregate with the Rho appear to be members of a unique subfamily. Because this subfamily has so far been identified only in plants, they have been designated as ROP proteins (for Rho-related proteins of plants) that apparently evolved from the ancestor RhoA, Rac, or Cdc42. ROP is distinct from other Rho proteins in several aspects. First, the effector domain (domain E in Figure 1) contains several amino acid residues unique to ROP, including $Ser^{28}$, $His^{29}$, and $Lys^{30}$. Second, the Rho insert region in ROP consists of 8–10 amino acid residues that share little homology with those (12 residues) in other Rho proteins. These unique features in effector domains suggest that plant ROP will have unique effectors from those in animals (Zheng and Yang 2000). There are 11 ROP proteins in *Arabidopsis*, 7 in rice, and at least 9 in maize (Gu and others 2004). The ROP subfamily is divided into four major subgroups according to phylogenetic analysis. In *Arabidopsis*, Group I contains ROP8 protein, Group II ROP9-11, Group III ROP7, and Group IV ROP1-6. All ROP proteins in Groups III and IV are putatively prenylated, whereas the Group I and Group II proteins are palmitoylated but not prenylated (Yang 2002; Christensen and others 2003).

Rho proteins play central roles in a wide range of cellular processes, many of which are associated with the actin cytoskeleton. Together with Ras, Rho proteins are *bona fide* signaling proteins known to transmit extracellular signals in yeast and animals (see Rho subsection in "In Animals and Yeast"). Mammalian and fungal Rho proteins regulate the cell polarity and influence cell morphogenesis (Settleman 2001). Plant ROP proteins have similar functions. Cell shape in plants is regulated by polar growth in one direction or diffused growth in different directions, and ROP proteins participate in the regulation of this process (Xu and Scheres 2005a; Nibau and others 2006). AtRop2 and AtRop4 localize to tips of elongating root hairs. Overexpressing *AtRop2* and *AtRop4* resulted in a strong root hair phenotype including isotropic growth or increased length in root hairs of *Arabidopsis*, whereas overexpressing *AtRop7* appeared to inhibit root hair tip growth, indicating that AtRop also controls tip growth during root hair development (Molendijk and others 2001; Jones and others 2002). At least four members of the *Arabidopsis* ROP family are expressed in pollen; these ROP proteins share similar biochemical activities and may integrate into the pollen cellular machinery regulating the polar tube growth process. The functional contribution by individual ROP to the pollen tube growth largely depends on their expression characteristics in pollen (Cheung and others 2003). In both root hairs and pollen tubes, the regulation of ROP is dependent on modulating the formation of both the dynamic fine tip F-actin and a tip-focused cytosolic calcium gradient (Li and others 1999; Fu and others 2001; Chen and others 2003). Pollen apical endo/exocytosis is also regulated by the $Ca^{2+}$ gradient and the activity of ROP (Camacho and Malho 2003). Further evidence indicated that AtRop1 activated two counteracting pathways involving the direct targets of tip-localized Rop1 to RIC3 or RIC4. RIC is named for ROP interactive with Cdc42/Rac-interactive binding motif (CRIB)–containing proteins. RICs are novel proteins as the specific effectors with ROP. Eleven RIC proteins have been identified from *Arabidopsis* and these proteins are classified into five groups that share little sequence homology outside of the conserved ROP-

interactive domain (Wu and others 2001). In Rop1-enhanced pollen tube growth, RIC4 promotes F-actin assembly, whereas RIC3 activates $Ca^{2+}$ signaling that leads to F-actin disassembly. Overproduction or depletion of either RIC4 or RIC3 caused tip growth defects that were rescued by overproduction or depletion of RIC3 or RIC4, respectively. Thus, AtRop1 controls actin dynamics and tip growth through checks and balances between the two pathways. The dual and antagonistic roles of AtRop1 may provide a unifying mechanism to modulate various processes dependent on actin dynamics in plant cells (Gu and others 2005). In contrast, RIC10 promotes pollen tube elongation but does not affect pollen tube growth polarity, suggesting that RIC10 may participate in a Rop1-independent pathway probably controlled by a different ROP (Wu and others 2001). Moreover, RIC4 and AtRop1 show an oscillatory pattern in tip membrane localization which correlates with the periodicity of pollen tube growth. The oscillatory activation of AtROP1 with RIC4-promoted F-actin assembly is ahead of growth and the RIC3-regulated accumulation of $Ca^{2+}$ in the pollen tip. These data suggest that temporal and spatial regulation of ROP activation is an important aspect of their signaling activity in these tip-growing cells (Gu and others 2005; Hwang and others 2005).

ROP also regulates formation of cell shape by controlling assembly of dynamic cortical F-actin in cells that do not undergo tip-based growth, such as epidermal cells. In the early phase of leaf differentiation, cell expansion occurs in both longitudinal and radial or lateral directions, mediated by AtRop2 with a mechanism known to control tip growth. In the late phase, expansion occurs only in the longitudinal direction and is not affected by AtRop2. This indicates that ROP uses a similar mechanism of control tip growth to modulate cell expansion in differentiating tissues (Fu and others 2002). ROP also affects the actin cytoskeleton by interacting with the SCAR complex that activates the actin nucleating protein complex ARP2/3, which is most evident in epidermal cells (Deeks and Hussey 2005; Smith and Oppenheimer 2005). PIROGI, a component of the *Arabidopsis* SCAR complex, interacts with AtRop2 as a direct effector (Basu and others 2004; Uhrig and others 2007). Locally activated AtRop2 activates RIC4 which then promotes the assembly of cortical actin microfilaments required for localized outgrowth. Meanwhile, AtRop2 inactivates RIC1 which then locally inhibits outgrowth. Thus, outgrowth-promoting ROP2 and outgrowth-inhibiting RIC1 pathways antagonize each other. Therefore, plants have an elaborate ROP-regulated mechanism whereby two downstream targets, RIC4 and RIC1, are activated and inactivated to regulate actin and microtubule-based pathways, respectively, which then achieve localized outgrowth that gives rise to the jigsaw puzzle pattern observed on the mature leaf epidermis (Fu and others 2005).

The Rac proteins of mammals control production of reactive oxygen species (ROS) by directly regulating the activity of plasma membrane-associated NADPH oxidase complexes (Ridley 1995). In *Arabidopsis*, oxygen deprivation rapidly and transiently activates ROP, resulting in $H_2O_2$ accumulation and alcohol dehydrogenase gene expression (Baxter-Burrell and others 2002). ROP-dependent $H_2O_2$ production is blocked by treatment with DPI, an inhibitor of the plasma membrane NADPH oxidase. Similarly, the transgenic *Arabidopsis* leaves with a constitutively active *rop2* mutant exhibited earlier cell death and higher levels of ROS than wild type, whereas those expressing a dominant-negative *rop2* mutant exhibited later cell death and lower ROS. Phosphatidic acid (PA) modulates an additional factor required for the active ROP-mediated ROS generation pathway (Park and others 2004). ROP also participates in the regulation of ROS production in soybean cells via activation of an enzyme complex similar to the NADPH oxidase of phagocytes in animal systems (Park and others 2000). Loss of ROP GAP function elevates alcohol dehydrogenase gene (ADH) expression in response to oxygen deprivation but decreases tolerance to hypoxia stress in *Arabidopsis* as a result of superactivation to ROP signaling. NADPH oxidase is necessary for induction of both ADH and ROP GAP expression. Tolerance of oxygen deprivation requires ROP activation and RopGAP-dependent negative feedback regulation. This suggests that ROP acts as a molecular rheostat, sensing oxygen deprivation in the environment like flooding (Baxter-Burrell and others 2002).

$H_2O_2$ has emerged as an important second messenger in plant signaling. Therefore, ROP regulation of $H_2O_2$ production proves to be a very important signaling module in plants, such as cell death and various stress and defense responses (Agrawal and others 2003). Rice ROP protein (OsRac1) has been shown to have a general role in disease resistance. The constitutive expression of active *OsRac1* caused HR-like responses and greatly reduced disease lesions against rice blast fungus and bacterial blight, further enhanced production of a phytoalexin, and altered expression of defense-related genes. The expression of the dominant-negative *OsRac1* suppressed elicitor-induced ROS production in transgenic cell cultures, and suppressed the HR induced by the avirulent fungus in plants (Ono and others 2001). Further evidence showed that the heterotrimeric G protein functioned upstream of OsRac1 in the early steps of disease resistance signaling of rice (Suharsono and others 2002). Mitogen-activated protein kinase cascades were also involved in this process because silencing of *OsRac1* by RNA interference or loss-of-function mutation of the heterotrimeric G-protein-subunit

gene resulted in a strong reduction of the OsMAPK6 protein levels. Furthermore, the OsMAPK6 protein was closely associated with the active form of OsRac1, not with inactive forms of OsRac1 (Lieberherr and others 2005). In another report, the expression of a metallothionein gene (*OsMT2b*) was found to be synergically downregulated by *OsRac1* and rice blast-derived elicitors. Transgenic plants overexpressing *OsMT2b* reduced elicitor-induced hydrogen peroxide production and increased susceptibility to bacterial blight and blast fungus. These results indicate that OsMT2b is an ROS scavenger and its expression is downregulated by OsRac1. Therefore, OsRac1 plays a dual role as an inducer of ROS production and a suppressor of ROS scavenging (Wong and others 2004). Constitutively expressed *RacB* in barley led to more susceptibility to *Blumeria graminis*, and at the same time increased water loss and enhanced transpiration associated with reduced responsiveness to ABA in regard to transpiration. RacB actions toward fungal attack were involved in receptor-like MLO protein and actin reorganization. Hence, RacB might be a common signaling element in response to both biotic and abiotic stresses (Opalski and others 2005; Schultheiss and others 2005). The rice *OsRacB* gene was strongly expressed in leaf sheath but downregulated by wounding, jasmonic acid, and blast pathogens. Transgenic rice overexpressing *OsRacB* showed increased symptom development in response to rice blast pathogens. It suggests that OsRacB functions as a potential regulator for a basal disease resistance pathway (Jung and others 2006). Systematic proteomics analysis of proteins whose expression levels were altered by OsRac1 and/or sphingolipid elicitor (SE) treatment revealed a total of 271 proteins, of which 100 proteins were upregulated by SE; 87 were also induced by active *OsRac1*. Results suggest that OsRac1 is able to induce many proteins in various signaling and metabolic pathways and plays a predominant role in the defense response in cultured rice cells (Fujiwara and others 2006).

ROP proteins are also involved in signal-transduction pathways mediated by plant hormones such as ABA, auxin, and brassinolide. ABA promotion of seed dormancy was enhanced and inhibited, respectively, by the constitutively active (*CA-Atrop2*) and the dominant-negative *rop2* mutants (*DN-Atrop2*) in *Arabidopsis* (Li and others 2001). Similarly, expression of *CA-Atrop6* in *Arabidopsis* inhibited ABA-induced stomatal closure in wild-type plants, whereas expression of *DN-Atrop6* caused stomatal closure in both the wild-type and the ABA-insensitive mutant *abi1-1* in the absence of exogenous ABA. This suggests that ABA inactivates one or more ROP proteins, which apparently act downstream of the ABI1 protein phosphatase, leading to stomatal closure (Lemichez and others 2001). Rop10 of *Arabidopsis* is also involved in ABA signaling. A null *rop10* mutant exhibited enhanced responses to ABA in seed germination, root elongation, and stomatal closure while inducing the expression of the transcription factor MYB2, which can be partially suppressed by *abi2*. Consistently, transgenic expression of a constitutively active form of Rop10 reduced ABA inhibition of seed germination. Further evidence suggests that Rop10 is a plasma membrane-localized signaling molecule that is specifically involved in the negative regulation of ABA signaling (Zheng and others 2002). Transcriptome analysis has been conducted to dissect Rop10 actions with ABA signaling. A particular subset of 21 genes was identified that was not altered by 1 μM ABA in the wild type but only activated in *rop10-1* of *Arabidopsis*. This indicates that Rop10 gates the expression of genes that are specific to low concentrations of ABA. Furthermore, almost all of these 21 genes are known to be highly induced by various biotic and abiotic stresses. It indicates that Rop10 negatively regulates ABA responses by specifically and differentially modulating genes such as protein kinases and zinc-finger family proteins. Consequently, Rop10 enhances the sensitivity of seed germination to salt stress inhibition (Xin and others 2005).

Besides being associated with ABA actions, the transgenic plants with CA-rop2 or DN-rop2 exhibit many morphologic phenotypes that resemble auxin and brassinolide overproduction or brassinolide-deficient or auxin-resistant mutant phenotypes, respectively. *CArop2* expression enhanced exogenous brassinolide-induced hypocotyl elongation of light-grown seedlings and increased lateral root formation induced by exogenous IAA, whereas *DN-rop2* expression inhibited hypocotyl elongation and root formation (Li and others 2001). The relationship among ROP, auxin, and brassinolide is still unclear. However, evidence shows that the auxin-activated ROP gene and ROP in turn stimulate auxin-responsive gene expression. Overexpressing a wild-type tobacco ROP gene, *NtRac1*, and its constitutively active mutant activated auxin-responsive gene expression. On the other hand, overexpressing the dominant-negative *NtRac1* gene, or reducing the endogenous *NtRac1* level, suppressed auxin-induced gene expression, suggesting that ROP proteins function in mediating the auxin signal to downstream responsive genes (Tao and others 2002). Furthermore, evidence has shown that ROP proteins are involved in auxin signaling (Tao and others 2005). Rop6 is activated by auxin, and it in turn stimulates auxin-responsive gene expression. At the same time, increasing ROP signaling activity promotes Aux/IAA degradation, whereas downregulating that activity results in the reduction of auxin-accelerated Aux/IAA proteolysis. Furthermore, Rop6 has been shown to be a regulator for ubiquitin/26S proteasome-mediated proteolysis. This process mediates the recruitment of nucleoplasmic Aux/IAAs into proteolytically active nuclear protein bodies of the $SCF^{TIR1}$ and COP9

signalosome components, in which TIR1 has been identified as an auxin receptor (Dharmasiri and others 2005; Kepinski and Leyser 2005). These data indicate that ROP proteins are regulators for ubiquitin/26S proteasome-mediated proteolysis and consolidate their role in auxin signaling (Tao and others 2005).

As more and more ROP proteins are identified, ROP regulation of other developmental processes has also been revealed. Besides the hormone actions, AtRop2 also induces other developmental phenotypes, including defects in embryo development, phyllotaxis, and pedicle orientation in *Arabidopsis* (Zheng and Yang 2000; Li and others 2001). Rop2 modulates NADPH oxidase activity which then regulates root hair growth through ROS production (Jones and others 2007). The *AtRop7* promoter directed a highly specific xylem-specific expression in the root, hypocotyl, stem, and leaves. Leaf epidermal cells of transgenic plants overexpressing constitutively active *AtRop7* exhibited highly impaired lobe formation, suggesting a role for AtRop7 in the secondary cell wall development of xylem vessels (Brembu and others 2005). Preferential and asymmetrical accumulation of *ZeRac2* mRNA has also been observed in differentiating xylem cells of *Zinnia elegans*, suggesting a general role in regulation of xylem development (Nakanomyo and others 2002). Rop2 from maize has an important role in the male gametophyte (Arthur and others 2003). It suggests that each group of ROP has a unique function. For example, group II tends to participate in stress responses, including ABA responses and $H_2O_2$ production, whereas group IV seems predominantly to regulate cell expansion through actin organization. Furthermore, different members within each group may regulate distinct processes in different cell types and by distinct mechanisms, which apparently depend on their expression patterns and the interactions with their functional partners (Christensen and others 2003).

ROP proteins also interact with various upstream regulators and downstream effectors, and many of these interactors are novel or unique to plants. Conversion of Rho from GDP- to GTP-bound form is catalyzed by guanine nucleotide exchange factors (GEFs). Rho GEFs in animals fall into two structurally distinct classes containing DH and DOCKER catalytic domains. Analysis of the RopGEF family of 14 members in *Arabidopsis* revealed that they shared a single conserved activity domain, designated PRONE (plant-specific ROP nucleotide exchanger; some authors also named DUF315 as plant-specific domain of unknown function 315) (Berken and others 2005). *In vitro* GEF assays showed that PRONE but not the full-length version of RopGEF1 had high GEF activity toward AtRop1. RopGEF1 was involved in ROP regulation through an autoinhibitory mechanism because *RopGEF1* overexpression in pollen tubes produced growth depolarization, as did a constitutively active *AtRop1* mutant, whereas the dominant-negative mutant of *AtRop1* suppressed the *RopGEF1* overexpression phenotype, probably by trapping RopGEF1. It suggests that AtRopGEF1 activates AtRop1 in control of polar growth of pollen tubes (Berken and others 2005; Gu and others 2006). The tip growth of root hairs is restricted to a small area at the surface of the hair-forming cell (trichoblast). A ROP GDP dissociation inhibitor (RopGDI) was shown to spatially restrict the sites of growth to a single point on the trichoblast, which is related to ROP-regulating ROS production and further modulates root hair growth (Carol and others 2005). The evidence showed that RopGDI2 and RopGAP1 restricted Rop4 activity in tobacco pollen tube. It has been proposed that inactivation of Rop4 by the subapically localized RopGAP1, together with dynamic relocalization of inactivated Rop5 to tip by RopGDI2, leads to spatial restriction of Rop4 to pollen tube apices, which subsequently promotes polar growth (Klahre and Kost 2006; Klahre and others 2006). RICs as specific effectors for ROP control various ROP-dependent signaling pathways in plants (Wu and others 2001; Nibau and others 2006). Recently, it was shown that the effector of OsRac1 is cinnamoyl-CoA reductase 1 (OsCCR1), an enzyme involved in lignin biosynthesis. OsRac1 was shown to bind OsCCR1 in a GTP-dependent manner in an *in vitro* interaction and two-hybrid experiments. Moreover, the interaction of OsCCR1 with OsRac1 led to the enzymatic activation of OsCCR1 *in vitro*. Transgenic cells expressing the *OsRac1* gene accumulated lignin through enhanced CCR activity and increased ROS production. Thus, it is likely that OsRac1 controls lignin synthesis through regulation of both NADPH oxidase and OsCCR1 activities during defense responses in rice (Kawasaki and others 2006). Therefore, the diversity of ROP interactors may contribute to functional versatility. Hence, future studies on ROP effectors are expected to reveal additional ROP functions and their mechanisms.

### *Arf*

Arf is involved in endocytic vesicle trafficking in plants. Inhibition of Golgi export by expression of a dominant-negative *Arf1* mutant decreased α-amylase secretion and simultaneously induced the secretion of the vacuolar protein phytepsin to the culture medium in tobacco. Increased secretion of the vacuolar protein was not observed after incubation with BFA. The differential effects to induce the secretion of one cargo molecule while inhibiting the secretion of another is dependent on GTP hydrolysis by Arf1p and is not caused by a general inhibition of Golgi-derived COPI vesicle traffic (Pimpl and others 2003).

Vesicle trafficking is essential for the generation of cell division asymmetries, which are central to cell differentiation and multicellular development. The essential functions of Arf in cell polarity have also been demonstrated in plants. Single loss-of-function mutants in six virtually identical *Arf1* genes revealed no obvious developmental phenotypes in *Arabidopsis*. However, apical-basal polarity of epidermal cells, reflected by the position of root hair outgrowth, was affected when *Arf1* mutants were expressed at early stages of cell differentiation. This suggests that the Rop2 and PIN2 proteins are targets of Arf1 actions during root hair tip growth and localization (Xu and Scheres 2005b). Except for cell polarity, Arf also affects cell size and cell expansion. The expression of the six highly similar putative Arfs was suppressed by antisense RNA in *Arabidopsis*. Antisense plants were severely stunted because the cell division rate and final cell size were reduced, which could directly reflect the changes in the rate of delivery of materials to expand the plasma membrane and to construct and plasticize cell walls, or reduced secretion of enzymes degrading the matrix polysaccharides. However, no gross changes in targeting or compartmentalization were seen in antisense plants and changes in cell wall composition were limited to increases in some noncellulosic polysaccharides and a relatively small decrease in cellulose. Therefore, Arf regulation of vesicle trafficking will directly affect delivery of proteins to intracellular and extracellular compartments, cellulose synthase to the plasma membrane, and noncellulosic polysaccharides to the cell wall, and will finally affect cell division and expansion (Gebbie and others 2005).

In plants, mutation of the *TITAN5* gene, which corresponds to AtArl2, resulted in dramatic alterations of mitosis and cell cycle control during seed development, which led to the presence of an abnormal endosperm with giant polyploidy with an enlarged nucleoli, whereas embryo development was accompanied by significant cell enlargement (McElver and others 2000; Tzafrir and others 2002). This suggests that Arf may play a role in membrane trafficking steps necessary for proper cell plate deposition during cytokinesis in developing seeds (McElver and others 2000). Interestingly, some Arf genes are also required for embryogenesis in *Caenorhabditis elegans* (Li and others 2004), suggesting that this may be a basic role for Arf proteins in multicellular organism.

Arf-regulating proteins also play important roles in regulation of plant development. *Arabidopsis* has two classes of ArfGEF, Gea/GBF/GNOM and Sec7/BIG, and two classes of ArfGAP, small BFA-sensitive GAPs and large GAPs. The mutant ArfGAP gene seedlings developed isotropically expanded, short and branched root hairs, with slow pollen tube elongation in *Arabidopsis*. This ArfGAP gene is specifically expressed in roots, pollen grains, and tubes; therefore, it is designated as ROOT AND POLLEN ARFGAP (RPA). RPA is localized at the Golgi complexes via its 79 C-terminal amino acids. It suggests that RPA plays a role in root hair and pollen tube growth, most likely through the regulation of *Arabidopsis* Arf1 and Arf1-like protein activity (Song and others 2006).

Evidence has shown that Arf and its regulating proteins are involved in auxin polar transportation and subsequently control plant development. A mutant named SCARFACE (sfc) has been identified with largely fragmented leaf veins in *Arabidopsis*. SFC encodes an ARFGAP which is required for normal intracellular transport of PIN1 (auxin efflux carriers) (Sieburth and others 2006). The constitutively expressed rice ArfGAP (*OsAGAP*) gene in transgenic *Arabidopsis* showed reduced apical dominance, shorter primary roots, increasing numbers of longer adventitious roots, and sharply increased free indoleacetic acid (IAA) levels. Many of these phenotypes can be observed in wild-type plants treated with exogenous IAA (Zhuang and others 2005). Overexpression of *OsAGAP* in transgenic rice impaired polar auxin transport which then interfered with both primary and lateral root development. The lateral root development could be rescued by the membrane-permeable auxin NAA, but not by IAA or 2,4-D, which require influx facilitators to enter the cells. Vesicle trafficking and localization of auxin-influx carriers have been changed without affecting auxin influx facilitators in transgenic rice. These results suggest that OsAGAP may be involved in the mediation of plant root development by regulating the auxin influx pathway (Zhuang and others 2006). GNOM as an ArfGEF coordinates polar localization with PIN1 during asymmetric embryo development (Steinmann and others 1999). Further evidence has shown that GNOM mediates the polarized distribution of PIN1 in *Arabidopsis* cells by residing on the endosomes. In transgenic plants with a BFA-resistant version of GNOM, PIN1 localization and auxin transport were no longer sensitive to BFA, whereas trafficking of other proteins was still affected by the drug. This demonstrates that GNOM is required for the recycling of auxin transport components which will result in polar auxin transport and establish cell polarity in roots (Geldner and others 2003). GNOM together with the auxin influx carrier AUX and the positive membrane regulator of the ethylene response pathway EIN2 coordinates cell polarity within the plane of a single tissue layer (planar polarity). Auxin overproduction in roots enhances planar ROP and hair polarity over long and short distances. Hence, auxin may provide vectorial information for planar polarity that requires combinatorial AUX1, EIN2, and GNOM activity upstream of ROP positioning (Fischer and others 2006).

*Ran*

Ran is involved in nucleocytoplasmic transport, the formation of spindle asters, and the reassembly of the nuclear envelope in mitotic cells. Because of the small number of Ran genes in different organisms, for example, humans and *Arabidopsis* contain only 1 and 4 Ran, respectively, it is generally believed that plant Ran proteins have similar roles as animal and yeast proteins. Overexpression of plant Ran proteins suppressed cell cycle defects in mutant fission yeast, indicating that they functioned similarly to their yeast homologs (Ach and Gruissem 1994; Wang and others 2004). AtRan1 interacts with AtXPO1, an *Arabidopsis* Exportin-1 homolog, and AtRanBP1a. RanGAP proteins from alfalfa and *Arabidopsis* were complementations of the yeast RanGAP mutant *rna1*; their localization pattern was consistent with the regulation of nuclear transport during interphase (Pay and others 2002). Besides targeting the nuclear envelope, nuclear import of Ran relies on a small RanGDP-binding protein and Nuclear Transport Factor 2 (NTF2). Three NTF2 proteins from *Arabidopsis* can functionally replace the essential *NTF2* gene of yeast, which suggests that nuclear import of Ran is also conserved in plants (Zhao and others 2006).

However, there are some plant-specific Ran proteins. AtRan4 from *Arabidopsis* is more divergent with the other AtRan sequences; in particular, it lacks the effector-binding domain for interaction with RanGAP (Haizel and others 1997). AtRan4 is annotated as "salt stress-inducible small GTP-binding protein Ran1-like protein" which may have distinct functions in *Arabidopsis*. Overexpression of *TaRAN1*, the Ran homologous gene from wheat, in transgenic *Arabidopsis* and rice increased the proportion of cells in the $G_2$ phase of the cell cycle, which resulted in an elevated mitotic index and prolonged life cycle. Increased primordial tissue, reduced numbers of lateral roots, and stimulated hypersensitivity to exogenous auxin were also observed in the transgenic plants. The results suggest that Ran is involved in the regulation of mitotic progress, either in the shoot apical meristem or in the root meristem zone in plants, where auxin signaling is also involved in these processes (Wang and others 2006). Methyl CpG-binding proteins (MBD) are considered to play critical roles in epigenetic control of gene expression by recognizing and interacting with 5-methylcytosine. A recent report showed that AtMBD5 from *Arabidopsis* interacted with AtRan3, suggesting that AtMBD5 becomes localized to the vicinity of chromosomes with the aid of AtRAN3 and may play an important role in maintenance of chromatin structures during plant cell division (Yano and others 2006).

Because there is only a limited number of Ran proteins in different organisms, they are likely to play key roles in Ran-regulated processes. Ran is not associated with membranes. Instead, the spatial sequestering of its accessory proteins, the RanGAP and the nucleotide exchange factor RCC1, appears to define the local concentration of Ran. *Arabidopsis* RanGAP1 (AtRanGAP1) lacks the SUMOylated C-terminal domain of vertebrate RanGAP but contains a plant-specific N-terminal domain (WPP domain, named by the highly conserved 16-amino-acid consensus sequence containing Trp-Pro-Pro), which is necessary and sufficient for targeting the nuclear envelope in interphase. Interestingly, the WPP domain is highly conserved among plant RanGAPs and the small, plant-specific nuclear envelope-associated protein MAF1 (small, soluble, serine/threonine-rich protein that is associated to MFP1, the matrix attachment region-binding filament–like protein) (Rose and Meier 2001). It is assumed that the binding of MAF1 to MFP1 blocks the binding of RanGAP. This interaction is regulated during the cell cycle and temporarily regulates RanGAP association with the nuclear vesicles and thereby prevents premature nuclear envelope assembly (Meier 2000). AtRanGAP1 has a uniquely mitotic trafficking pattern that is different from vertebrate RanGAP, which includes targeting to the outward-growing rim of the cell plate. Point mutations in conserved residues of the WPP domain abolished targeting to the nuclear rim and the cell plate. These results indicate that plant and animal RanGAP-targeting domains are kingdom-specific and may undergo different migration patterns during cell division (Jeong and others 2005). Furthermore, two *Arabidopsis MAF1* homologs, WPP1 and WPP2, are specifically associated with the nuclear envelope in undifferentiated cells of the root tip. RNA interference-based suppression of the WPP caused shorter primary roots, a reduced number of lateral roots, and reduced mitotic activity of the root meristem (Patel and others 2004). RCC1 is the Ran nucleotide exchange factor with a seven-bladed propeller that mediates various cellular events. Interestingly, mutation of RCC1 domains (*uvr8*) in *Arabidopsis* led to hypersensitivity to UV-B and reduced the UV-B-mediated induction of flavonoids and blocked chalcone synthase mRNA and protein induction, suggesting that UVR8 acts in a UV-B signal-transduction pathway leading to induction of flavonoid biosynthesis. The UVR8 protein contains five RCC1 repeats but does not possess nuclear localization sequences conserved in animal and yeast RCC1 homologs (Kliebenstein and others 2002).

Ran-binding protein (RanBP), the major effector of Ran, also has important roles. We have extensively analyzed the RanBP sequences, particularly from plants. Phylogenetic reconstruction of 33 RanBPs from 26 species of organisms revealed that RanBPs from plants, animals, and fungi clustered as distinct, remarkably unique groups in the different kingdoms. Structural analysis revealed that all RanBPs were highly conserved in the middle region of

their amino acid sequence, which included the Ran-binding domain and the three conserved motifs that have essential roles in binding with Ran protein. However, the N-terminus and C-terminus exhibited very low similarity between the different RanBPs. The highly diversified structure of RanBPs suggests that this kind of protein may have different actions in the different classes of organisms (Tian and others 2006). AtRanBP1c inhibited the EDTA-induced release of GTP from Ran and served as a coactivator of RanGAP *in vitro*. Neither the N- nor the C-terminus of AtRanBP1c was necessary for the binding of Ran protein, but both were needed for the cytosolic localization of AtRanBP1c (Kim and Roux 2003). Antisense expression of AtRanBP1c enhanced primary root growth, suppressed lateral root growth, and rendered transgenic roots hypersensitive to auxin, suggesting that it plays a key role in the nuclear delivery of proteins that suppress auxin action and regulate mitotic progress in root tips (Kim and others 2001). Upregulation or downregulation of wheat RanBP led to imbalance of protein biosynthesis in tobacco, suggesting a crucial role of RanBP in maintaining the orderly nucleocytoplasmic transport (our unpublished data).

The overview of plant small GTP-binding protein functions is listed in Figure 3. As the field progresses, new actions will be supplemented in the future.

## Perspectives

There have been numerous reports on small GTP-binding proteins in yeast and animals. Although the actions and functions of this superfamily protein are evident, there are still many issues that need to be addressed. For example, the mechanisms of when and how small GTP-binding proteins are activated by GEF and inactivated by GAP may lead to roles for small GTP-binding proteins as biotimers rather than as molecular switches. The mechanisms of temporal and spatial activation and inactivation of these small GTP-binding proteins may be related to their biological regulation. These efforts will clarify a working model for small GTP-binding proteins. For example, extensive reports have revealed a common molecular model that underlies Ran proteins (Joseph 2006). Compared with that in yeast and animals, the study of small GTP-binding proteins in plants is still in its infant stage, although over the last few years rapid progress has been made in our understanding of intracellular signaling pathways mediated by these proteins. Present data suggest that plant small GTP-binding proteins share many conserved features with those in yeast and animals, from protein structures, to the effectors, to the regulation networks. Yet, many plant-specific features have also been revealed. For example, plants lack Ras proteins, which may be due to the absence of the tyrosine kinase receptor, and ROP, as a plant-specific class of Rho proteins, has emerged as an important molecular switch in plant cellular signaling. In future works, similarities and differences in small GTP-binding proteins between plants and other organisms should be emphasized. Some special points should be highlighted. First, the interaction of different classes of small GTP-binding proteins in the same process is very important. For example, Arf and ROP are both involved in polar growth of the pollen tube and root hairs. Therefore, it is interesting to know if they act together or separately in these processes. Second, what is the relationship between the plant hormone signal and small GTP-binding protein regulation? Generally, we expect that plant hormones are the special signal molecules for plants, whereas small GTP-binding proteins are ubiquitous signal molecules that lie downstream in plant hormones. The receptors of plant hormones may link these two kinds of signals. Interestingly, it is clear that many plant hormone receptors are conserved proteins that exist in other organisms. For example, cytokinin receptors have been demonstrated as two-component regulators that are common in protokaryotes (Ding and Ma 2006). This hints that these links are present, although solid evidence is required. Finally, a comprehensive study using biochemical, proteomic, cell biological, and genetic approaches in a model system like *Arabidopsis* and rice is needed to determine the complex

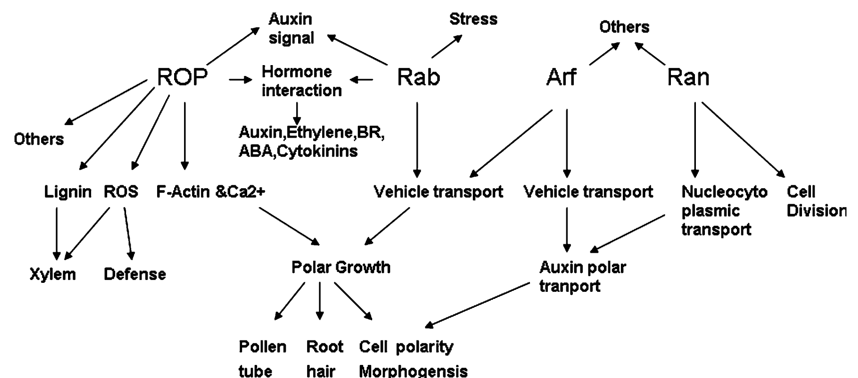


**Fig. 3** Schematic overview of functions of small GTP-binding proteins in plants

signaling network of this superfamily. At the same time, investigations of small GTP-binding protein actions in specific plant species are also important in determining their actions in the unique physiological processes in plants and to fully understand the functional diversity and specificity of these regulating and signaling proteins.

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