

Chapter 9

Small and Large G Proteins in Biotic and Abiotic Stress Responses in Plants

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Abstract The sessile nature of plants has enabled them to develop a higher level of plasticity in adaptation against the hostile environmental conditions such as biotic and abiotic stresses. The intricate molecular machineries in plants, consisting of signal sensors, signal transducers, and generation of response, are highly similar with animal's signal transduction components; however, a large number of pathways also show a high level of divergence. G proteins are a part of signal transduction components, which play pivotal role in regulating a large number of physiological and developmental processes in both plants and animals. G proteins or guanine nucleotide-binding proteins serve as molecular switches, existing in two forms: an active conformation, when bound to GTP, and an inactive conformation, when bound to GDP. In active conformation, G proteins interact with downstream effector molecules to initiate various cellular responses. Based on their structure, they have been divided into heterotrimeric and monomeric G proteins, also called large and small G proteins, respectively, where the former is composed of three subunits (α , β , and γ) and latter is composed of single subunit (α). Plant growth and development is partially regulated by the combined activity of large and small G proteins. Besides, identification of profuse number of stress-related genes interacting with monomeric and heterotrimeric G-protein complex in both *Arabidopsis* and rice suggests their involvement in modulation of abiotic stress responses. Further, based on the functional analysis of several G proteins (large and small) regulating cell growth, cell proliferation, seed germination, sugar sensing, and hormonal responses indicate these proteins are equally essential for plant development. Due to increased demand of food crops to sustain growing population in adverse environmental conditions, the role of G proteins and modulation of GTPase signaling has been studied in plants to produce genetically modified crop plants with increased stress tolerance and productivity.

Keywords Abiotic stress • Biotic stress • Plant G proteins • GTPase • Signal transduction

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9.1 Introduction

Plants being sessile have evolved an intricate network of molecular pathways to adapt to changes in their environment, including perception of signals by receptors, signal transduction via transducing molecules and proteins, and gene expression. The natural adaptation is a continuous process occurring by outbreeding over a span of several decades; however, to accelerate this process, plant biologists have adopted various approaches such as breeding and genetic manipulation of plants to generate resistant and tolerant varieties. With the global effort to sequence various plant genomes and availability of full genome sequence of model dicot like *Arabidopsis* (Arabidopsis Genome Initiative 2000) and model monocot crop like rice (Goff et al. 2002), a new field of functional genomics has emerged allowing analysis of the plant transcriptome, proteome, and metabolome employing techniques like epistasis analysis, microarrays, SAGE (serial analysis of gene expression), Y2H (yeast-two-hybrid), AP/MS (affinity purification and mass spectrometry), mutagenesis, and RNAi (RNA interference). Employing high-throughput technology, research in the past decades has identified key molecules acting in abiotic and biotic stress tolerance, including G proteins, also known as guanosine nucleotide-binding proteins. G family proteins are cytosolic proteins involved in transmitting a variety of extracellular signals into the cell through cell surface receptors to modulate cellular activities (Hepler and Gilman 1992). Their activity is regulated by certain cellular factors that control their ability to bind to GTP and hydrolyze it to GDP. G proteins when bound to GTP acquire an on or activated form, whereas GDP-bound form designates an inactivated or off state. Based on their structure, G proteins are mainly divided into two categories: monomeric small GTPases and heterotrimeric large G-protein complexes.

9.1.1 Heterotrimeric G Proteins

Heterotrimeric G protein (hereafter called G protein), also called large G protein, is a cytosolic protein and a complex containing three subunits including G-alpha ($G\alpha$), G-beta ($G\beta$), and G-gamma ($G\gamma$) (Jones and Assmann 2004; Ma 2001). In animals, G proteins are involved in transmitting extracellular signals, such as, hormones, neurotransmitters, chemokines, and light to intracellular signaling components to modulate gene expression, culminating into a response (Gilman 1987; Wettschureck and Offermanns 2005). Animal G protein is a multigene family encoded by 23 α , 6 β , and 12 γ genes (Hamm 1998; McCudden et al. 2005). Additionally, the G-protein signaling module is composed of G-protein-coupled receptors (GPCRs), containing seven-transmembrane α -helical proteins, acting upstream of G proteins and activating G proteins upon binding to a ligand. The human genome encodes for approximately 1,000 GPCRs, falling into six subgroups, comprising the largest receptor superfamily in metazoans (Kawasawa et al. 2003).

In comparison to mammals, plant G-protein family is relatively small; in *Arabidopsis*, $G\alpha$ is encoded by a single-copy gene, designated as *GPA1* (Ma et al. 1990), and single copy of $G\beta$, *AGB1* (Weiss et al. 1994), and three genes encoding the $G\gamma$ subunits have been isolated: *AGG1*, *AGG2*, and *AGG3* (Mason and Botella 2000, 2001; Chakravorty et al. 2011). The genome of crop plant rice contains one gene each encoding $G\alpha$ (*RGAI*) and $G\beta$ (*RGB1*) subunits and three genes encoding $G\gamma$ subunit (*RGG1*, *RGG2*, and *RGG3*) (Ishikawa et al. 1996; Kato et al. 2004). G-protein signaling components have also been identified in other plants: pea and soybean having two and four $G\alpha$ -subunits, respectively (Ashikari et al. 1999; Misra et al. 2007; Choudhury et al. 2011). The α -, β -, and γ -subunits of G proteins link extracellular signals to intracellular effector through GPCRs. Interestingly, direct homologs of metazoan GPCR (G-protein-coupled receptors) proteins are altogether absent in plants. However, recently in *Arabidopsis*, *GCR1* has been identified as a likely representative of GPCR-encoding gene because of its significant sequence similarity with them. The *Arabidopsis* *GCR1* is predicted heptahelical protein, a hallmark of bona fide GPCRs (Josefsson and Rask 1997; Pandey and Assmann 2004; Chen et al. 2004a, 2004b). Using bioinformatics tools, the *Arabidopsis* genome is predicted to encode for 394 7TMpRs (seven-transmembrane putative receptors) (Moriyama et al. 2006). An additional regulator of G-protein signaling (RGS) reminiscent to GPCRs has been identified in *Arabidopsis*; *AtRGS1* affects the intrinsic GTPase activity of $G\alpha$ -subunit, keeping it in inactivated conformation. It appears that the *Arabidopsis* genome contains only one member of the RGS family, *RGS1* (Chen et al. 2003). Additionally, 7 noncanonical, extra-large G (XLG) proteins were identified in plants, including 3 in *Arabidopsis* and 4 in rice (Lee and Assmann 1999). The physiological role of G proteins in plants was determined by the characterization of loss-of-function (lof) mutant and transgenic lines, in stomata opening/closure (Wang et al. 2001; Coursol et al. 2003; Chen et al. 2004a, 2004b), fungal defense (Llorente et al. 2005; Trusov et al. 2006; Trusov et al. 2009), oxidative stress (Joo et al. 2005; Booker et al. 2004), phytohormone signaling (gibberellic acid (GA)), and plant development (Ueguchi-Tanaka et al. 2000; Ullah et al. 2001; Ullah et al. 2002; Lease et al. 2001; Chen et al. 2006; Pandey et al. 2006). In mature leaves, G proteins transmit signals to molecules, including small GTPases, ion channels, and phospholipases, that are effectors in response to various biotic and abiotic stress conditions, including pathogen elicitation, ozone treatment, and water deficit.

9.1.1.1 Mechanism of Regulation of Heterotrimeric G Protein

Mechanism of mammalian G-protein activation involves binding of an activating ligand to its specific GPCR causing a change in its conformation, leading to the conversion of an inactive G protein to its active conformation. The GPCR acts as a guanine nucleotide exchange factor (GEF), causing $G\alpha$ to exchange GDP for GTP (Gilman 1987). As a result, $G\alpha$ -GTP separates from the $G\beta\gamma$ dimer, and both $G\alpha$ -GTP and the $G\beta\gamma$ dimer separate from the receptor and can individually activate downstream effector molecules. Subsequent to signal propagation, the GTP

that is bound to $G\alpha$ is hydrolyzed to GDP, thereby inactivating $G\alpha$ and allowing its reassociation with the $G\beta\gamma$ dimer to reform the inactive heterotrimeric G-protein complex.

In *Arabidopsis*, the mechanism of G-protein activation is different than in animals involving spontaneous disassociation of heterotrimer into $G\alpha$ s and $G\beta\gamma$ dimer, which allows $G\alpha$ to bind to GTP, and both the dissociated subunits separately activate downstream effectors (Urano et al. 2012). A 7TM-RGS protein, AtRGS1, promotes GTP hydrolysis of the $G\alpha$ s, and this GDP-bound form then interacts with $G\beta\gamma$ dimer and returns to the resting state (Fig. 9.1a) (Johnston et al. 2007; Jones et al. 2011). Here RGS proteins act as GTPase-activating proteins (GAPs) for $G\alpha$, attenuating signaling by promoting the return of the G protein to the resting state. Genetic evidence is consistent with D-glucose being the ligand that stops the AtRGS1 GAP activity and allows AtGPA1 to self-activate (Fig. 9.1a) (Chen et al. 2003; Johnston et al. 2007; Chen et al. 2006; Grigston et al. 2008).

9.1.1.2 Domain Structure of Heterotrimeric G Protein

G proteins contain highly conserved G domain/fold, shared by Ras superfamily proteins and translation elongation factors. G domain is composed of 6 β -sheets, 5 α -helices, and 5 regions/loops designated G1–G5, which play a pivotal role in GTP binding and hydrolysis. The organization of G domain from N-terminus is N- β 1-G1- α 1-G2/SW1- β 2- β 3/G3-SW2- α 2- β 4- α 3- β 5/G4- α 4- β 6-G5- α 5-C, where the G1 region or P-loop has a conserved GxxxxGK(S/T) motif (aka Walker A motif), required to contact α - and β -phosphate of GTP; the G2 region contains a conserved threonine residue for Mg^{2+} coordination important for GTP hydrolysis; G3 region (aka Walker B motif) is identified by sequence DxxG and the conserved aspartate (D) and glycine (G) bind Mg^{2+} and γ -GTP, respectively; G4 region has NKxD motif and the conserved aspartate contacts the guanine ring of GTP; and the G5 region is identified by E(A/C/S/T)(C/S)A(K/L) motif, which helps in guanine base recognition (Wittinghofer and Vetter 2011). Plant $G\alpha$ -subunit is around 35–46 kDa protein, characterized by functional motifs including myristoylation and S-acetylation sites (for membrane targeting), N- and C-terminal receptor binding sites, and three switch regions (SW) that sense nucleotide binding (Fig. 9.2). The N-terminal helical region, a small interface at switch I (SWI), and a large interface at switch II (SWII) bind $G\beta\gamma$ dimer (Li et al. 2009; Temple and Jones 2007), whereas the helical domain shelters the guanine nucleotide-binding pocket. The P-loop, G₄₅AGESGKS for NTP binding, the DxxGQ motif for GTP hydrolysis, and the NKxD motif for guanine recognition are conserved in plants (Temple and Jones 2007). The $G\beta$ s is 35–36 kDa protein, composed of an amino-terminal coiled-coil motif and a carboxyl-terminal WD40 (β -transducin) repeat domain (Fig. 9.2). The amino-terminus of $G\beta$ s forms the stable coiled-coil/hydrophobic interaction with the $G\gamma$ s (Li et al. 2009), and the WD40 repeat domain contains the effector- and $G\alpha$ s-binding surface. The effector-binding region on $G\beta$ s is normally masked by the GDP-bound form of $G\alpha$ s;

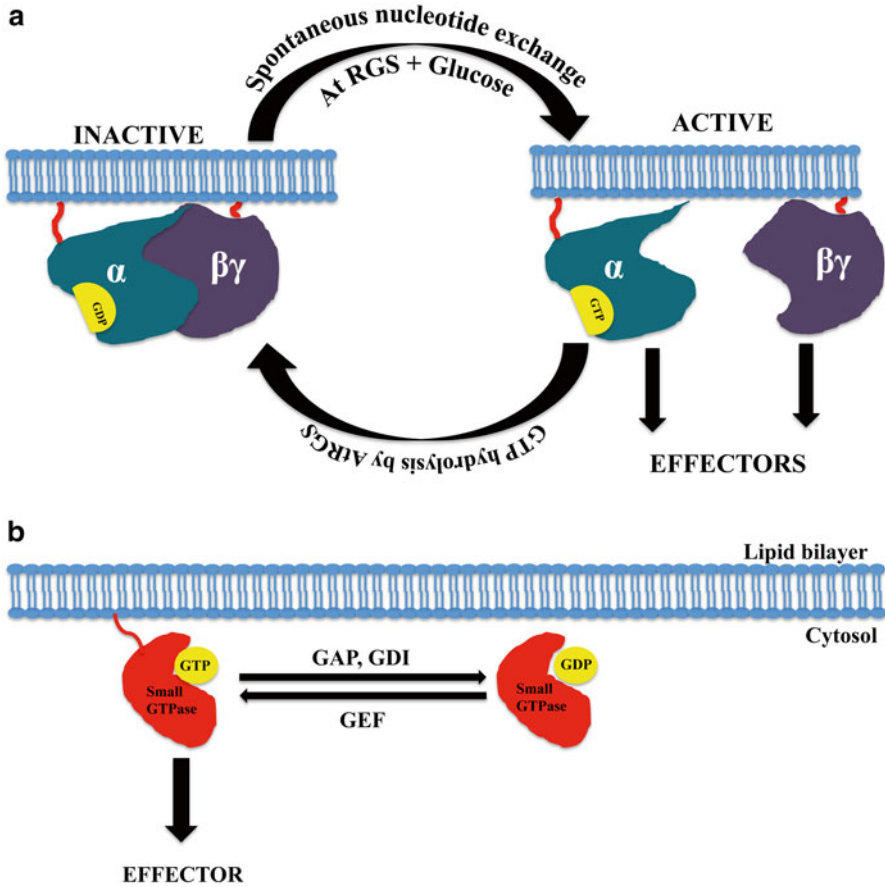


Fig. 9.1 Graphic depicting regulatory mechanism of heterotrimeric and monomeric G protein: (a) G proteins or large G proteins act as a molecular switch and regulate many cellular processes. The G protein is made up of three subunits: α , β , and γ . The $G\alpha_s$ binds to the guanosine nucleotide, GDP, in the inactive state and forms a complex with $G\beta\gamma$ dimer. Spontaneous disassociation of heterotrimer into $G\alpha_s$ and $G\beta\gamma$ allows $G\alpha_s$ to bind to GTP, and both the dissociated subunits separately activate downstream effector molecules. A 7TM-RGS (transmembrane-regulator of G-protein signaling) protein, AtRGS1, promotes GTP hydrolysis of the $G\alpha_s$, and this GDP-bound form then interacts with $G\beta\gamma$ dimer and comes back to the resting state. Here RGS proteins act as GTPase-activating proteins (GAPs) for $G\alpha_s$, attenuating signaling by hastening the return of the G protein to the resting state. D-glucose acts as the ligand that halts AtRGS1 GAP activity and allows AtGPA1 to self-activate. (b) Monomeric GTP-binding proteins or small GTPases are molecular switches that are “activated” by GTP and “inactivated” by the hydrolysis of GTP to GDP. Monomeric GTPase activity is determined by the ratio of GTP/GDP-bound form, regulated by three classes of proteins, including guanine nucleotide exchange factors (GEFs), GTPase-activating proteins (GAPs), and guanine nucleotide dissociation inhibitors (GDIs). Upon stimulation by an upstream signal, a GEF protein converts the GDP-bound inactive form into a GTP-bound active conformation, through GDP/GTP replacement. In the activated state through the effector-binding domain, it interacts with downstream effector proteins to transduce the signal. The small GTPases also have a weak intrinsic GTPase activity for GTP hydrolysis and require a GTPase-activating protein (GAP) for efficient deactivation. Additionally, most small GTPases cycle between membrane-associated and cytosolic states. And only membrane-bound GTPases can be activated by GEF; their removal by cytosolic GDI proteins negatively regulates GTPases

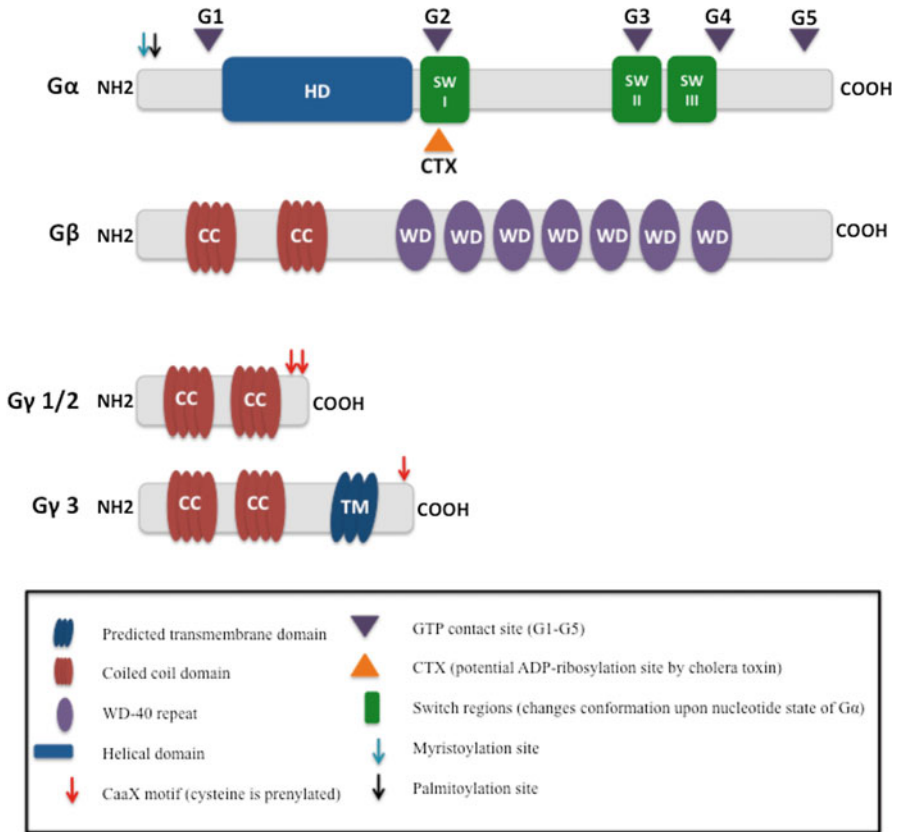


Fig. 9.2 Graphic representation of domain structure α , β , and γ subunits of plant heterotrimeric G protein: plant $G\alpha_s$ is around 35–46 kDa protein, characterized by functional motifs including myristoylation and S-acetylation sites (for membrane targeting), 5 G domains, N- and C-terminal receptor binding sites, and 3 switch regions (SW) that sense nucleotide binding. The N-terminal helical region, a small interface at switch I, and a large interface at switch II bind $G\beta\gamma$ dimer, whereas the helical domain (HD) shelters the guanine nucleotide-binding pocket. The P-loop, $G_{45}AGESGKS$ for NTP binding, the $D_{xx}GQ$ motif for GTP hydrolysis, and the $NK_{x}D$ motif for guanine recognition are conserved in plants. The $G\beta_s$ is 35–36 kDa protein, composed of an amino-terminal coiled-coil motif and a carboxyl-terminal WD40 repeat domain. The amino-terminus of $G\beta_s$ forms the stable coiled-coil/hydrophobic interaction with the $G\beta_s$, and the WD40 repeat domain contains the effector- and $G\alpha_s$ -binding surface. The effector-binding surface on $G\beta_s$ is normally masked by the GDP-bound form of $G\alpha_s$; therefore, $G\beta\gamma$ dimer is active only after dissociating from the $G\alpha_s$ -subunit. The $G\gamma_s$ is a small (less than 100 residue, 6–10 kDa) protein containing a coiled-coil region and a prenylation site at its carboxyl-terminus

therefore, $G\beta\gamma$ dimer is active only after dissociating from the α -subunit (Wettschureck and Offermanns 2005). The $G\gamma_s$ is a small (less than 100 residue, 6–10 kDa) protein containing a coiled-coil region and a prenylation site at its carboxyl-terminus (Fig. 9.2) (Gilman 1987), required for its membrane targeting (Wei et al. 2008).

9.1.2 Monomeric G Protein

Monomeric G proteins (21–30 kDa) are the small guanine nucleotide-binding proteins related to α -subunit of heterotrimeric G proteins, belonging to Ras (rat sarcoma oncoprotein) superfamily, named after human Ras genes, discovered as cellular homologs of the viral Ras oncogene (Parada et al. 1982).

The small GTPase superfamily in animals is divided into six distinct families, including Ras, Rho, Rab, Arf, Ran, and Miro (Kahn et al. 1992; Bischoff et al. 1999; Takai et al. 2001; Logan 2010). Members of each family are associated with specific biological function as Ras GTPases regulate cell proliferation; Arf (ADP-ribosylation factor) and Rab (Ras-like protein in brain) primarily regulate various steps in trafficking, where former is required for vesicle budding in the secretory system and latter controls transport and docking of specific vesicles to the plasma membrane. Ran (Ras-like nuclear protein) regulates trafficking of RNA and proteins across the nuclear membrane, and Miro (mitochondria Rho GTPase) GTPases regulate mitochondrial transport and morphology (Ridley et al. 1992; Nobes et al. 1998; Wennerberg et al. 2005; Reis et al. 2009).

In silico analysis identified 93 small GTPase superfamily members in *Arabidopsis*, classified into five small GTPase families: with 57 Rab GTPases, 21 Arf GTPases, 11 Rho GTPases, and 4 Ran GTPases. Interestingly, plant genomes do not encode homologs of Ras GTPases (Regad et al. 1993; Haizel et al. 1997; Vernoud et al. 2003). Furthermore, functional analysis of several members showed that they might regulate similar cellular processes in the plant cells as they do in animal cells. For example, Arf GTPases regulate vesicle trafficking between plasma membrane (PM) and cytoplasm (Geldner et al. 2001); the role of Ran GTPases is not known, but N-terminus of RanGAPs is homologous to nuclear matrix attachment proteins, providing new insight into their function in plant cells (Meier 2000). Rab GTPases comprise the largest family in *Arabidopsis*, functioning in vesicle trafficking (Batoko et al. 2000; Lu et al. 2001). Plants do not have true orthologs of Rho family GTPases, but have evolved a novel family of Rho-like proteins also known as Rho of plants (ROPs) (Zheng and Yang 2000; Shanmugam et al. 2013). Signaling proteins in plants play an important role in plant stress responses and pollen tube outgrowth (Kost et al. 1999; Li et al. 1999). In mammals 14 Rho family GTPases have been identified and divided into 7 subgroups (Nobes and Hall 1994; Aspenstrom 1999). In plants, two groups have been identified of which only one protein is Rho-like homolog while the rest are closely related to Rac (Newman et al. 1994; Yang and Watson 1993; Delmer et al. 1995; Winge et al. 1997).

9.1.2.1 Mechanism of Regulation of Monomeric G Protein

Small GTP-binding proteins are master molecular switches that are “activated” by GTP and “inactivated” by the hydrolysis of GTP to GDP, acting as global regulatory proteins in eukaryotic cells. In the cell, GTPase activity is determined by the ratio

of GTP-/GDP-bound form, regulated by three classes of proteins, including guanine nucleotide exchange factors (GEFs), GTPase-activating proteins (GAPs), and guanine nucleotide dissociation inhibitors (GDIs). Upon stimulation by an upstream signal, a GEF protein converts the GDP-bound inactive GTPases to a GTP-bound active conformation, through GDP/GTP exchange. Once in the activated state, small GTPases through their effector-binding domain interacts with downstream effector proteins to transduce the signaling response. The activated conformation possesses weak intrinsic GTPase activity for GTP hydrolysis, requiring a GTPase-activating protein (GAP) for efficient deactivation. Additionally, most of the small GTPases frequently cycle between plasma membrane and cytosolic compartments; only membrane-bound GTPases can be activated by GEF, and removal by cytosolic GDI proteins negatively regulates GTPases. This complex mode of regulation and their ability to interact with variety of upstream and downstream targets create functional diversity in GTPase activity (Fig. 9.1b).

In animals, most RhoGEFs possess a Dbl (diffuse B-cell lymphoma) homology (DH) domain and a pleckstrin homology (PH) domain. However, no proteins with DH/PH domains have been identified in plants so far. Instead, plant-specific Rop nucleotide exchanger (PRONE) domain constitutes a large family in plants (Berken et al. 2005; Gu et al. 2006).

9.1.2.2 Domain Structure of Small GTPases

Members of Ras superfamily share common structural features including guanine nucleotide-binding domains and an effector-binding domain (Zheng and Yang 2000; Takai et al. 2001). Plant small GTPases contain five G domains (G1-G5) of which the G5 domain shows high sequence divergence among the GTPases. The G domains are essential for binding guanosine nucleotide and the associated Mg^{2+} ion and for GTP hydrolysis. The G1 motif associates tightly with the α - and β -phosphate, G2 motif contacts the γ -phosphate, and the G3 motif helps in positioning the water molecule for GTP hydrolysis through highly conserved glutamine residue. Switch 1 and 2 regions bind to the effector or regulatory molecules. Rac/Rop GTPases have a Rho insert region, and all the GTPases have hypervariable region at the C-terminus facilitating localization (Fig 9.3).

9.2 Small GTPases in Biotic and Abiotic Plant Stress

9.2.1 *Rac/Rop Small GTPases in Biotic Stress*

In plants, Rac/Rop small GTPases participate in diverse signal transduction events including defense responses, root hair development, pollen tube growth, ROS generation, and hormone responses (Nibau et al. 2006; Yang and Fu 2007; Shanmugam et al. 2013). Plants respond to pathogen attack by various ways including the

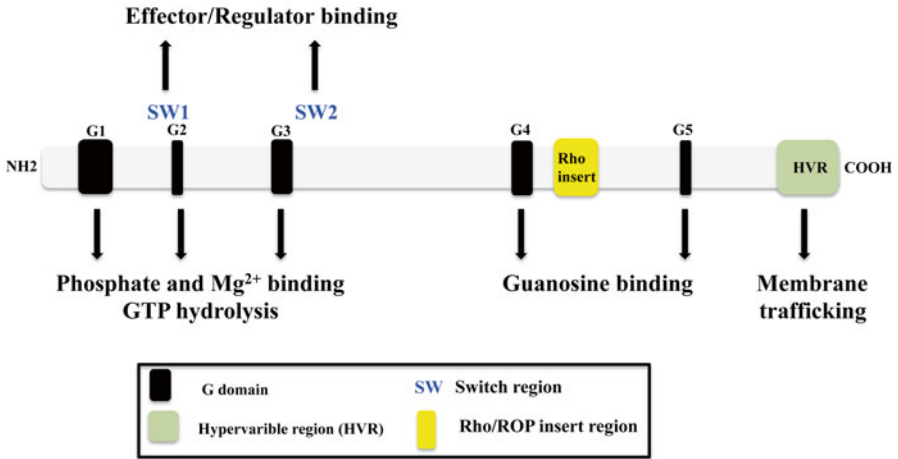


Fig. 9.3 Graphic representation of domain structure of monomeric G protein: plant small GTPases contain five G domains (G1-G5) of which the G5 domain has highly divergent sequence. The G domains are essential for binding guanosine nucleotide and associated Mg²⁺ ion and for GTP hydrolysis. The G1 motif associates tightly with the α- and β-phosphate, the G2 motif contacts the γ-phosphate, and the G3 motif helps in positioning the water molecule for GTP hydrolysis through highly conserved glutamine residue. Switch 1 and 2 (SW1 and SW2) regions bind to effector of regulators. Rac/Rop GTPases have a Rho insert region, and all the GTPases have hypervariable (HVR) region at the C-terminus for localization

activation of defense genes, formation of reactive oxygen species (ROS), synthesis of pathogenesis-related (PR) proteins, localized cell-wall reinforcement, and production of stress-related hormones and antimicrobial compounds. Moreover, plant defense system exists in two layers. The first layer is the PAMP (pathogen-associated molecular patterns)-triggered immunity (PTI) mediated by pattern recognition receptors (PRRs) (Dodds and Rathjen 2010), characterized by ion influx, mitogen-activated protein kinase (MAPK) activation and enhanced gene expression (Zipfel 2008). The second layer of defense is the effector-triggered immunity (ETI); it is in response to effector molecules secreted by pathogens, characterized by hypersensitive response (HR) including reactive oxygen species (ROS) production and programmed cell death (PCD) of infected tissue/cell (Jones and Dangl 2006).

ROS production is associated with PCD during resistance reaction to pathogens, and a plasma membrane NADPH oxidase (Rboh for respiratory burst oxidase homolog) similar to the neutrophil enzyme is suggested to be responsible for ROS production (Morel et al. 2004; Lamb and Dixon 1997; Hammond-Kosack and Jones 1996). In animals the oxidative burst produced by the NADPH oxidase in neutrophils is regulated by Rac2 (Babior 1999). Based on the assumption that the plant NADPH oxidase enzyme complex is similar to its mammalian counterpart, composed of six subunits including Rac (Bokoch 1994), the role of small GTPases was explored in plant defense response in ROS generation.

In rice, among seven Rac/Rop genes, *OsRac1* and *OsRacB* have been associated with defense response (Miki et al. 2005; Ono et al. 2001; Jung et al. 2006). The constitutively active (CA), containing a G19V mutation, and dominant-negative (DN), with T24N mutation, forms of *OsRac1*, when introduced into seed-originated calli of the wild-type cv. Kinmaze and a lesion-mimic mutant of rice plant, *sl* (Sekiguchi lesion), induced and blocked ROS production, respectively. This observation suggests that *OsRac1* mediates ROS production most likely by positive regulation of NADPH oxidase (Kawasaki et al. 1999). The role of NADPH oxidase in Rac-mediated ROS generation was supported by studies in tobacco, where Rac acts as a negative regulator of NADPH oxidase during ETI-triggered immune response. RT-PCR of tobacco cells upon elicitation with cryptogein, a fungal elicitor, exhibited repressed *NtRac5* mRNA levels, and tobacco cells with *CA-NtRac5* transgene showed decreased ROS production, by affecting the regulation of *NtrbohD*, a component of NADPH oxidase enzyme complex (Morel et al. 2004). *OsRac1* regulation of NADPH oxidase is further supported by biochemical studies where *OsRac1* interacts with the N-terminus of NADPH oxidase to regulate ROS production (Wong et al. 2007). Recent studies have also implicated PI3K as upstream regulator of *OsRac1* in recruiting *OsRac1* to PM to regulate NADPH oxidase activity and accelerate ROS production (Liu et al. 2012b). The role of Rac/Rop proteins in ROS production is conserved within plants and between plants and animals, pointing to existence of conserved signal transduction pathways in both the kingdoms, like maize Rac/Rop induces ROS production in mammalian cells (Hassanain et al. 2000). Similarly, human Rac was found to promote oxidative burst in soybean cell cultures (Park et al. 2000), and *CA-GhRac13* (*Gossypium hirsutum*) transgene increases ROS production in soybean and *Arabidopsis* cell cultures, probably serving as a signal for xylem differentiation (Potikha et al. 1999).

In search for molecules acting in the same signaling pathway as *OsRac1*, Pit, an NLR protein, was identified. *R* gene encodes proteins with a nucleotide-binding site and leucine-rich repeat region (NLR family), which act as intracellular receptors for recognition of pathogen effectors (also called avirulence [Avr] proteins). R-mediated disease resistance results in a host response culminating in a HR and ROS production. Pit, an NLR family protein interaction with *OsRac1* at the membrane, is supported by bimolecular fluorescence complementation (BiFC) assays using Vn- (N-terminus region of Venus) and Vc- (C-terminus region of Venus)tagged *OsRac1* (*Vn-CA-OsRac1*) and Pit (*PitWT-Vc*) in rice protoplasts. Moreover, Förster resonance energy transfer (FRET) analysis using Raichu (Ras and interacting protein chimeric unit) confirmed activation of *OsRac1* by Pit. Their role in HR and ROS production was confirmed by transient expression of autoactivated Pit, *PitD485V*, and *DN-OsRac1* in *N. benthamiana*, where *PitD485V* in the absence of pathogen induce HR and ROS production, whereas, upon co-expression with *DN-OsRac1*, ROS production was visibly reduced. Phenotypic analysis of *japonica* rice cultivar K59, carrying *Pit* resistance gene in which *OsRac1* was silenced by RNAi, showed enhanced fungal growth in RNAi lines, supporting the role of *Pit* acting upstream of *OsRac1* in conferring resistance to rice blast fungus (Kawano et al. 2010). Using FRET probe Raichu-*OsRac1* as an intracellular biosensor for monitoring the spatiotemporal

activation of *OsRac1* confirmed the induction of *OsRac1* in response to chitin elicitation. The chitin-elicited defense response involves chitin binding and forming a complex with OsCEBiP, a chitin-binding protein, and OsCERK1, a receptor-like kinase at the membrane. A PRONE GEF, *OsRacGEF1*, was shown to regulate *OsRac1* activity in chitin-elicited defense response. Phenotypic analysis of plants confirmed that OsRacGEF1 activation is required for chitin-induced immune response and resistance to rice blast fungus infection. OsRac1, OsRacGEF1, and OsCERK1 were shown to interact, supported by Y2H and in vitro binding assays, and the complex of OsRac1 and OsRacGEF1 was shown to localize to the PM by co-localization studies using OsRacGEF1-Venus and CFP-OsRac1-WT in rice protoplasts. Biochemical studies support the model where OsCERK1 phosphorylates OsRacGEF1 at the S549 residue, where the latter after activation regulates OsRac1. Based on these results, it can be hypothesized that OsCEBiP, OsCERK1, OsRacGEF1, and OsRac1 function as a “defensome” in chitin-triggered immunity (Akamatsu et al. 2013). These study indicate that *OsRac1* function in plant defense response is regulated by GEF proteins acting upstream of Rac/Rops. Another GEF implicated in regulating OsRac1 in chitin-triggered immune response and ROS production is the human SWAP70 homolog in rice, OsSWAP70A, with conventional DH domain, which interacts and exhibits GEF catalytic activity towards OsRac1. OsSWAP70A activity towards OsRac1 was analyzed in vitro, by measuring replacement of unlabeled GDP with fluorescently labeled *N*-methylanthraniloyl (mant)-GTP. Transient expression of *OsSWAP70A* and *OsRac1* together in *N. benthamiana* enhanced ROS production, whereas expressing OsSWAP70A alone had no effect. RNAi-mediated reduction of *OsSWAP70A* mRNA levels results in the suppression of chitin-induced defense response gene and ROS production, thereby indicating that *OsSWAP70A* positively regulates *OsRac1* (Yamaguchi et al. 2012). In another study, the *Arabidopsis* SWAP70 was shown to function in both PAMP- and ETI-triggered immune responses, supported by the phenotypic analysis of T-DNA insertion mutant of *AtSWAP70*, which were more susceptible to pathogen growth (Yamaguchi and Kawasaki 2012).

Several independent studies have identified signaling components acting downstream of OsRac1. Expression of *CA-OsRac1* transgene induces phytoalexin synthesis, a secondary metabolite with antimicrobial activity, and defense gene activation leading to resistance towards rice blast fungus (Ono et al. 2001; Fujiwara et al. 2006; Thao et al. 2007). Similarly, introduction of transgene, *CA-gOsRac1*, into rice, enhanced expression of *PAL1* and *PBZ1*, defense-related genes, and induced HR-response like PCD. Moreover, it also activates genes previously known to be induced by *Magnaporthe oryzae* and *Xanthomonas oryzae* pv. *oryzae* (Xoo) in cultured cells (Dang et al. 2013). OsRac1 has also been implicated in defense against the rice pathogen, the brown plant hopper (BPH), along with phytohormones, calcium, and MAPKs (Cheng et al. 2013).

Using proteomics, *OsCCR1* encoding *Oryza sativa* cinnamoyl-CoA reductase 1, an enzyme involved in lignin biosynthesis, was identified as a downstream effector of *OsRac1*. In the cell wall, lignin polymerization requires peroxidase activity using H₂O₂. The role of *OsCCR1* in plant defense was supported by the fact that

OsCCR1 expression level is enhanced by sphingolipid elicitor. *OsCCR1* and *OsRac1* form a complex, evidenced by Y2H and in vitro binding assay, where *OsRac1* activates *OsCCR1*. Transgenic cell cultures expressing *CA-OsRac1* accumulate lignin through enhanced *OsCCR1* activity and ROS production. Most likely, *OsRac1* regulates lignin synthesis by activating NADPH oxidase and *OsCCR1* in rice defense response (Kawasaki et al. 2006). *OsMT2b*, a rice metallothionein gene, was identified as a downstream effector of *OsRac1*. Metallothionein are involved in ROS scavenging and metal homeostasis. *OsMT2b* expression is downregulated synergically by rice blast-derived elicitor and *OsRac1*, supporting its function downstream of *OsRac1*. *OsMT2b* overexpressing cells showed reduced H₂O₂ production in contrast to homozygous *OsMT2b:Tos17* inserted mutant and *OsMT2b* RNAi-silenced transgenic cells. Most likely *OsRac1* functions by downregulating *OsMT2b* and activating NADPH oxidase in blast defense response (Wong et al. 2004)

Transcriptomics revealed the signaling components such as transcription factors acting downstream of *OsRac1*. Microarray analysis of *CA-OsRac1* transgenic plants exhibited enhanced levels of *RAI1* (Rac immunity 1), a transcription factor. In order to identify genes regulated by *RAI1*, microarray analysis of cells containing *RAI1-GR* (glucocorticoid receptor), a dexamethasone (DEX)-inducible construct was performed; induction with DEX-induced expression of *OsWRKY19* and *PAL1* (phenylalanine ammonia lyase) defense-related genes also upregulated in response to chitin and sphingolipids. Phenotypic analysis of *RAI1* T-DNA activation-tagged and RNAi transgenic lines, showing increased resistance to rice blast fungus infection, further supported the role of *RAI1* in plant defense response. Moreover, in vitro binding assay supports binding between *RAI1* and *MAPK3/6*, and BiFC assay using *RAI1-Vn* and *OsMAPK3/6-Vc* constructs in rice protoplasts supports in vivo interaction. qPCR of *OsMAPK3/6* overexpression (OE) transgenic lines showed enhanced expression of *PAL1* and *OsWRKY19*, supporting the model that *OsRac1* activates *RAI1* through *OsMAPK3/6* to regulate expression of defense-related genes (Kim et al. 2012). Another transcription factor regulated by *OsRac1* in chitin-triggered immunity is *OsRap2.6*. *OsRac1* has been shown to interact with *RACK1* (receptor for activated kinase C1). BiFC assay in rice protoplast with *Vn*- and *Vc*-tagged *OsRap2.6*, *RACK1*, and *OsMAPK3/6* showed these proteins interact and localize in nucleus and the cytoplasm. Moreover, *OsRap2.6*, *PAL1*, and *PBZ1* are all induced in response to chitin. Phenotypic analysis of *OsRap2.6* RNAi and OE lines showed that the former are highly susceptible, whereas the latter are more resistant to infection by rice blast fungus, *Magnaporthe oryzae* (Wamaitha et al. 2012), indicating *OsRap2.6* might act downstream of *OsRac1*, *RACK1*, and *MAPK3/6* in plant defense response.

In a study *OsRacB*, a close ortholog of barley *HvRacB* gene, transcript levels were downregulated in response to infection by rice blast fungus. Interestingly, transgenic plants overexpressing *OsRacB* showed increased symptom development. Moreover, *OsRacB* requires PM localization for initiating response evident from the *GFP:OsRacB*-transformed onion cells and *Arabidopsis* protoplasts (Jung et al. 2006). These results indicate that *OsRacB* functions as a negative regulator as opposed to *OsRac1* acting as positive regulator during rice blast fungus defense response.

To find out the role of the remaining Rop genes in defense responses, a tissue-specific expression pattern and subcellular localization of rice Rac/Rop genes were done using semiquantitative RT-PCR and transient expression system using GFP fusion proteins. Moreover, their role in disease resistance by testing single Rac/Rop RNAi plants against the rice blast fungus showed *OsRac2*, *OsRac6*, and *OsRac7* expressions at a very low level in leaf blades. Infection assays showed *OsRac1* as positive regulator of blast resistance, corroborating earlier results (Ono et al. 2001), whereas *OsRac4* and *OsRac5* act as negative regulators of blast resistance (Chen et al. 2010).

Three ROP proteins HvRACB, HvRAC1, and HvRAC3 in barley (*Hordeum vulgare*) are linked to both development and pathogen responses (Schultheiss et al. 2005; Pathuri et al. 2008; Hoefle et al. 2011). *HvRACB* functions as a common signaling component for both biotic and abiotic stresses, supported by phenotypic analysis of *CA-HvRACB* transgenic plants, exhibiting enhanced susceptibility to powdery mildew fungus (*Blumeria graminis* f. sp. *hordei* [*Bgh*]) and increased water loss in detached leaves due to reduced responsiveness to ABA, leading to failure in stomata closing (Schultheiss et al. 2005). In barley defense response, *HvRACB* acts as a negative regulator of disease resistance to *Bgh* (Schultheiss et al. 2002; Schultheiss et al. 2003). Knockdown of *HvRACB* expression by RNAi reduced susceptibility to powdery mildew by increasing resistance to penetration by *Bgh*, whereas the *CA-HvRACB* transgene enhanced susceptibility to *Bgh*. The mechanism of *HvRACB* action depends on the actin cytoskeleton disorganization and the mildew resistance locus (MLO). MLO encodes a seven-transmembrane protein and acts as negative regulator of cell death (Piffanelli et al. 2002; Schultheiss et al. 2002, 2003; Hoefle et al. 2011; Huesmann et al. 2011). HvMAGAP1 (microtubule-associated ROP GTPase-activating protein 1) was identified as a susceptibility factor in barley (Huesmann et al. 2011). Transient-induced gene silencing or OE of *HvMAGAP1* resulted in enhanced or reduced susceptibility to *Bgh*, respectively. Like *HvRACB*, *HvMAGAP1* also influences the polarity of cortical microtubules in interaction with *Bgh*, initiating MT disorganization to accommodate fungus haustoria. Moreover, interaction between HvMAGAP1 and HvRACB is required for the defense response, established in vitro, by Y2H and using FRET, in planta (Hoefle et al. 2011). The *Arabidopsis* homologs of *HvMAGAP1*, *AtROPGAP1*, and *AtROPGAP4* also function as plant defense. T-DNA insertion knockout enhanced susceptibility to the virulent powdery mildew fungus *Erysiphe cruciferarum*, indicating functional conservation of ROPGAPs in pathogen response in both monocots and dicots (Huesmann et al. 2011).

Adopting a proteomic approach, *HvRBK1* (*Hordeum vulgare* Rop-binding protein kinase1) was identified as a downstream effector of *HvRACB* and *HvRAC1* signaling pathways involved in MT reorganization in a Y2H assay. In in vitro assay, kinase activity of HvRBK1 was observed to be induced by CA-HvRACB or activated HvRAC1. Transient-induced gene silencing of barley *HvRBK1* supported penetration by *Bgh*, and transient knockdown influenced MT stability in barley epidermal cells; all these results support a model where HvRACB activates HvRBK1 to bring about cytoskeleton reorganization (Huesmann et al. 2012). It is evident

from research in both rice and barley that the mechanism of action of Rac/Rop protein function in plant defense is different, where in rice OsRac1 functions by activating enzymes involved in ROS generation and regulating gene expression; HvRACB primarily affects the MT organization in the infected cells by activating HvRKBK1.

Phosphatidic acid (PA), a secondary messenger, is implicated in numerous biotic and abiotic stresses including pathogen elicitation, wounding, freezing, hyperosmotic stress, and water deficit (Canonne et al. 2011). Studies have shown that PA mediates PCD and ROS generation in whole leaf and at single-cell level by activating AtROP2, which is shown to enhance ROS production in an in vitro assay. Although, epistasis analysis where in absence of exogenous PA, no spontaneous ROS production was observed in *CA-ROP2* transgenic plants; suggested activation of ROP2 alone is not sufficient to induce ROS generation (Park et al. 2004).

Mutations in plant signaling molecule salicylic acid (SA)-mediated pathways heighten disease susceptibility to pathogens, while induction of SA-dependent defense response reduces pathogen growth (Durrant and Dong 2004). SA-dependent signaling pathway known as systemic acquired resistance (SAR) induces expression of *PR* (pathogenesis-related) genes including phytoalexins and PR proteins. Microarray analysis of *DN-AtROP6* plants revealed a role for AtROP6 in SA-mediated defense response by causing major changes in gene expression associated with the constitutive SA-mediated plant defense responses. The total level of SA in *DN-AtROP6* transgenic plants resembled those of wild-type plants, inoculated with a virulent powdery mildew pathogen. The constitutive SA-associated response in *DN-AtROP-6* was suppressed in mutants defective in SA signaling (norepressor of PR genes1 (*npr1*)) or biosynthesis (salicylic acid induction deficient 2 (*sid2*)) (Poraty-Gavra et al. 2013), supporting Rac/Rops as negative regulators in SAR.

Similarly, in other plant species, the role of Rac/Rop GTPases in plant defense mechanism is conserved. Rac/Rop GTPases were shown to be involved in both PTI- and ETI-triggered immunity as their expression was found to be induced in pathogen-inoculated chickpea leaf and elicitor-treated cell culture (Ichinose et al. 1999); for example, in tobacco silencing of *NtRop* by expression of *Medicago*, *Ms-Rac1* antisense cDNA causes defects in defense response (Schiene et al. 2000).

9.2.2 *Rac/Rop GTPases in Abiotic Stress*

Stomata closure in aerial tissue is an important approach adopted by plants to respond to rapid water loss during drought, orchestrated by phytohormone ABA. ABA is also the key mediator of plant responses to other abiotic stresses including salinity and cold, and therefore is known as the stress hormone. The ABA signaling components include ABA receptors (PYL/PYR/RCAR family), a group of type 2C phosphatases (PP2C) (Park et al. 2009), three members of the SNF1-related protein kinase2 family (Fujii et al. 2009), and molecules regulating ABA

signaling including NADPH oxidase (Jiang and Zhang 2002; Zhang et al. 2009a, 2009b) and heterotrimeric G protein (Pandey and Assmann 2004).

Genetic and biochemical analysis in *Arabidopsis* points towards the involvement of ROP family GTPases in ABA signaling. Acting as negative regulator, expression of *CA-AtROP6* reduced ABA-mediated stomata closing (Lemichez et al. 2001). Zheng et al. in 2002 demonstrated *AtROP10* as negative regulator of ABA-mediated seed germination, stomata closing, and root elongation. A null *rop10* mutant exhibits enhanced responses to ABA in seed germination, root elongation, and stomata closure, whereas *CA-ROP10* reduces ABA inhibition of seed germination. Li and group in 2012 confirmed that *ROP11* also acts as a negative regulator of ABA-mediated stomata closure, seed germination, and drought response. Expressing *CA-ROP11* allele inhibiting stomata closure and *DN-ROP11* allele exhibited opposite effect. ABA primarily modulates the localization of ROP11, causing it to accumulate in the nucleus for transcriptional regulation. Moreover, phenotypic analysis of *rop10rop11* double mutant showed that they act in parallel pathways in ABA-mediated responses. Li et al. (2012a, 2012b), using luciferase complementation imaging (LCI), Y2H assay, and co-immunoprecipitation assay, showed that activated ROP11 binds ABI1 (ABA insensitive), a PP2C phosphatase, and allows the downstream SnRKs to be activated, which further activate TFs and upregulate stress-related genes like *RAD29*.

Insight into the mechanism of RacRop GTPase action was elucidated by studies providing a link between actin cytoskeleton remodeling in guard cells and ABA-induced stomata closure. Microscopic analysis of *WT/GFP-mTn* (GFP-mouse talin) and *WT/MAP4-GFP* (microtubule-associated protein 4-GFP), expressing GFP fusion proteins targeted to actin (Kost et al. 1998) and tubulin (Mathur and Chua 2000), respectively, supported ABA-mediated actin cytoskeleton reorganization during stomata closure. A role for ABA in actin cytoskeleton reorganization was further supported by studies done in *abi1-1/GFP-mTn* transgenic lines, where ABI1 is an upstream regulator of ABA signaling in stomata closure (Leung et al. 1994). *abi1-1* mutants display a wilted phenotype associated with impaired stomata closure. In *abi1-1/GFP-mTn* transgenic lines, both actin cytoskeleton and stomata closure remain unaffected (Meyer et al. 1994; Gosti et al. 1999). *AtRac1/AtROP3/ARAC3/Rop6AT* was isolated in a reverse genetic approach to identify genes acting in ABA signaling to induce stomata closure in *Arabidopsis* (Winge et al. 1997; Li et al. 1998; Lemichez et al. 2001). A role for *AtRac1/AtROP3* was confirmed by observing actin cytoskeleton organization in *AtRac1-T20N/GFP-mTn*, a DN Rac (Ridley et al. 1992), and *AtRac1-G15V/GFP-mTn*, a CA Rac (Diekmann et al. 1991), transgenic lines, where the former promoted actin cytoskeleton reorganization. Furthermore, *WT/AtRac1-T20N* transgenic lines blocked the ABA-induced stomata closure and restored stomata closure defects of *abi1-1/AtRac1-T20N* transgenic lines. In conclusion, *AtRac1* acts as a negative regulator linking the actin cytoskeleton remodeling and ABA signaling during drought response and water homeostasis in *Arabidopsis* (Lemichez et al. 2001).

Hypoxia/oxygen deficiency causes switch from mitochondrial respiration to anaerobic fermentation (Sauter 2000). Ethanol fermentation relies on activity of pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH); both the enzymes are activated upon hypoxia (Fukao et al. 2006). Importance of *ADH* and *PDC* (pyruvate decarboxylase) has been shown by mutant analysis, where *adh* and *pdh* mutants of maize and *Arabidopsis* rapidly succumb to hypoxia, confirming the importance of ethanol fermentation under oxygen stress (Ismond et al. 2003; Kürsteiner et al. 2003).

In animals, cellular hypoxia triggers H_2O_2 (ROS) production, leading to increased transcription of hypoxia-inducible factor 1 α (HIF1 α) via a Rho-GTP-dependent mechanism (Turcotte et al. 2003). In plants, hypoxia leads to H_2O_2 production by triggering the activity of ADH (Baxter-Burrell et al. 2002). In *Arabidopsis*, *ROPGAP4* was identified in an attempt to identify genes induced under hypoxia stress, using a *Ds-GUS* transposon gene trap (Baxter-Burrell et al. 2003). Loss-of-function *ropgap4* mutants exhibited increased *ADH* mRNA, indicating *ROPGAP4* negatively regulates *ADH*. Moreover, the DN form of *ROP2* (*35S:DN-Rop2*) did not show any increase in *ADH* mRNA, whereas the CA-*ROP2* (*35S:CA-Rop2*) lines were hypersensitive to oxygen deprivation, supporting *ROP2* as positive regulator in signaling during hypoxia. Even though H_2O_2 levels were found to increase significantly in *ropgap4* mutants under hypoxic conditions, no rise in H_2O_2 was detected in *35S:DN-Rop2* seedlings. These experimental results support the hypothesis, where *ROPGAP4* acts as a negative feedback regulator of Rop GTPases, which are involved in defense responses and H_2O_2 production required for ADH induction. A feedback loop is formed where H_2O_2 activates *RopGAP4*, which in turn inhibits *Rop2*. *Rop2* inactivation subsequently leads to reduction in hydrogen peroxide release (Baxter-Burrell et al. 2003).

Further, studies in tobacco revealed the role of Rac/Rops in salinity stress. *NtRop1* of tobacco when expressed in *Arabidopsis* conferred salt sensitivity. It was speculated that *NtRop1*-induced salt sensitivity is caused by increased H_2O_2 production, which acts as a second messenger molecule during salt stress (Cao et al. 2008), inducing PM H^+ -ATPase activity resulting in increased K^+ to Na^+ ratio (Zhang et al. 2007).

9.2.3 *Ran GTPases*

Plants respond to various biotic and abiotic stresses by modulating transcriptional activity of various stress-related genes or alternatively by regulating posttranscriptional nucleocytoplasmic trafficking of proteins and noncoding RNA molecules through Ran (Ras-related nuclear protein)-dependent karyopherin (importin β) protein (Mosammaparast and Pemberton 2004; Mazzucoteli et al. 2008; Chinnusamy et al. 2008). Ran GTPase activity is regulated by several proteins including GAPs, GEFs, and RanBP1. Ran in GDP-bound form, generated by action of RanGAP1, exists in the cytoplasm, whereas the GTP-bound form, generated by RCC1

(regulator of chromosome condensation 1), a GEF, exists in the nucleus. This asymmetric distribution of Ran GTPases is pivotal for their functional activities (Bischoff and Ponstingl 1991; Bischoff et al. 1994). Ran-binding protein 1 (RanBP1) acts as an accessory factor to increase RanGAP1-mediated nucleotide hydrolysis by inhibiting the GEF activity of RCC1, thereby promoting inactivated Ran (Bischoff et al. 1995). In plants, the identification of RanBP1 and RanGAP (Ach and Gruissem 1994; Haizel et al. 1997; Rose and Meier 2001; Pay et al. 2002) revealed their high sequence similarity and subcellular localization with animal Ran proteins, pointing to functional conservation in nucleocytoplasmic trafficking and mitotic process (Ach and Gruissem 1994; Haizel et al. 1997).

Ran GTPases are encoded by a family of 4 genes in *Arabidopsis* and 3 genes in rice (Haizel et al. 1997; Vernoud et al. 2003). Several independent studies have implicated Ran GTPases in plant development, for example, overexpression (OE) of wheat Ran, *TaRAN1*, in *Arabidopsis* and rice resulted in an elevated mitotic index and prolonged life cycle (Wang et al. 2006). Besides, Ran GTPases are also affected by oxidative stress, causing reduction in ATP levels, leading to decrease in Ran GTP levels, and their disordered distribution (Yasuda et al. 2006).

Virus-induced gene silencing (VIGS) of *NbRanBP1* in *N. benthamiana* exhibited stress responses, such as reduced mitochondrial membrane potential and excessive production of ROS (Cho et al. 2008). A number of studies in both *Arabidopsis* and rice using transgenic and RT-PCR approaches have shown involvement of Ran in phytohormone signaling and salt stress response (Miche et al. 2006). *OsRAN2* overexpression results in increased sensitivity to salt, osmotic stress, and ABA treatment, and RT-PCR analysis of transgenic lines showed reduced *OsRAN2* transcript levels in salt and osmotic stresses and ABA treatment, supporting their role as negative regulators in abiotic stress response (Zang et al. 2010). Furthermore, three ABA- or stress-responsive genes, *AtNCED3*, *PLC1*, and *AtMYB2*, encoding a key enzyme in ABA synthesis, a phospholipase C homolog, and a putative transcription factor, respectively, showed differentially induced expression in *OsRAN2* OE plants under salt and ABA treatment conditions (Liu and Zhu 1997; Xiong et al. 2002; Verslues et al. 2006). In order to find out the mechanism of *OsRAN2* action, *OsRAN2* and *Lc* (leaf color transcription factor) were co-expressed in tobacco epidermal cells, where *Lc* acts as a maize reporter gene for nuclear transport (Shieh et al. 1993), and it was observed that *OsRAN2* disturbed the nuclear transport of *Lc*, suggesting that the *OsRAN2* hypersensitivity phenotype could be due to its effect on nucleocytoplasmic trafficking of regulatory proteins such as transcription factors (Zang et al. 2010).

In *Oryza sativa*, *OsRAN2* was identified to confer resistance towards cold stress. The transcript level of *OsRAN2* increased several fold under cold stress condition. Insight into the mechanism of *OsRAN2* action came from the analysis of the mean mitotic index for *OsRAN2* OE and *OsRAN2* RNAi lines, which showed an enhanced and reduced mean mitotic index, suggesting its role in cell cycle to confer cold stress tolerance. Subcellular localization of *OsRAN2:GFP* revealed GFP fluorescence in nucleus at the spindle and tubulin during metaphase and anaphase pointing towards a function in maintaining cell division during cold stress. These results

were further corroborated by *OsRAN2* RNAi plants, exhibiting aberrant organization of spindles during mitosis and that *OsRAN2* helps in nucleocytoplasmic transport of tubulin and formation of intact nuclear envelope during cold conditions. In conclusion, *OsRAN2* regulates cold resistance in rice by maintaining cell division and by promoting accumulation of intranuclear tubulin at the end of mitosis and formation of intact nuclear envelope (Chen et al. 2011).

A recent report has shown that *OsRAN1* is ubiquitously expressed in rice and exhibit enhanced transcript levels upon cold and IAA treatment, where auxin is known for its effects on cell division. *OsRAN1* OE results in improved cold tolerance. It was found that at molecular level, it helps to maintain cell division and cell cycle progression and promote formation of intact nuclear envelope (Xu and Cai 2014). In conclusion, Ran GTPases confer cold stress tolerance by regulating the plant cell cycle; interestingly, cell division has been previously closely related to stress tolerance in plants (Kitsios and Doonan 2011).

9.2.4 RAB GTPases

RABs and SNAREs (soluble N-ethylmaleimide-sensitive (NSF) attachment protein receptors) are conserved regulatory molecules involved in membrane trafficking. RABs promote the tethering of transport vesicles/organelles to target membranes, followed by membrane fusion, which is executed by specific combinations of three glutamine-contributing SNAREs (Qa-, Qb-, and Qc-SNARE) and one arginine-contributing (R-)SNARE in a stable complex (Saito and Ueda 2009; Wickner and Schekman 2008).

Arabidopsis genome encodes 57 Rab GTPases (Pereira-Leal and Seabra 2001), grouped into 8 clades (RabA-RabH) (Segev 2001; Rutherford and Moore 2002; Vernoud et al. 2003). Among them, Rab5 (RabF) forms the largest clade (Stenmark et al. 1994), with three homologs encoded in *Arabidopsis* genome including *AtRabF1* (*Ara6*), *AtRabF2a* (*Rha1*), and *AtRabF2b* (*Ara7*) (Rutherford and Moore 2002; Vernoud et al. 2003). Co-expression of *ARA6-Venus* and *ARA7-GFP* under their native regulatory elements showed overlapping expression pattern in various endosomal population in *Arabidopsis*, confirming their role in endocytosis (Ebine et al. 2012).

Endocytosis, traditionally a constitutive housekeeping process, has recently been implicated in trafficking in stress responses in tobacco (Leyman et al. 1999). It has been shown that in yeast, oxidative stress inhibits vesicle trafficking between cytosol and PM, a phenotype partially restored by OE of *Arabidopsis* synaptobrevin homolog, providing tolerance to lethal concentrations of H₂O₂ (Levine et al. 2001).

Various Rab GTPases have been identified and implicated in biotic and abiotic stresses using SSH, differential display (DD), and northern blot techniques in different plant species. Phenotypic analysis of mutants and various proteomics approach helped to understand the mechanism of Rab GTPase action in conferring stress tolerance.

AtRabG3e (*AtRab7*) was isolated by DD approach for genes induced under abiotic and biotic stresses including salt, superoxide, SA, and necrogenic pathogens (virulent *P. syringae* bacteria or *B. cinerea* fungus) (Mazel et al. 2004). Overexpression of *AtRabG3e* in *Arabidopsis thaliana* leads to less sensitivity to salt and osmotic stress than WT plants. Analysis of general membrane endocytosis in *AtRabG3e* transgenic plants revealed accelerated uptake of membrane dye FM1-43, which could be due to faster vesicle trafficking at the end point, i.e., endosome-vacuole fusion, supporting the hypothesis that in the transgenic plants Na⁺ is transported into the vacuoles, reducing the overall cytosolic Na⁺ content. Moreover, transgenic plants exhibited reduced ROS content, due to decrease in the cytosolic Na⁺ content (Mazel et al. 2004). Another Rab5 member, *ara6*, lof confers hypersensitivity to salt stress, whereas OE transgenic plants showed elevated resistance to salinity stress and high-sorbitol stress (Ebine et al. 2012). Similarly, in rice root cells, ARA6 is secreted in the apoplast in response to salinity stress (Zhang et al. 2009a, 2009b).

PgRab7 of *Pennisetum glaucum*, isolated by subtractive hybridization (SSH) of treated plants, showed enhanced transcript level upon cold, dehydration, and NaCl and IAA treatment. The role of *PgRab7* in abiotic stress was confirmed by overexpression of *PgRab7* in heterologous system, enhancing NaCl and mannitol tolerance in transgenic tobacco plants (Agarwal et al. 2008). In a similar study, *Prosopis juliflora*, an abiotic stress-tolerant tree species of *Fabaceae*, was used for isolating genes functioning in abiotic stress tolerance. Northern analysis of leaf tissue revealed upregulation of *PjRAB7* during salt stress conditions. Moreover, expressing *PjRAB7* in heterologous system conferred salt tolerance to tobacco, probably by sequestering sodium in the vacuoles, suggesting functional conservation of Rab GTPase in different plant species (George and Parida 2011). Adopting differential display (DD) approach *Rab2* was identified in the desiccation-tolerant grass *Sporobolus stapfianus* in response to drought and rehydration in leaves. RT-PCR analysis showed *RAB2* transcript accumulation in response to decreased relative water content (RWC), whereas rehydration resulted in decreased levels. Furthermore, ABA was also found to function in this pathway as exogenous ABA slightly increased the *RAB2* transcript accumulation (O'Mahony and Oliver 1999). SmGTP related to *Rab2* gene family was identified in a salt stress SSH expression library of model grass species *Lolium temulentum*, exhibiting strong expression under salt and drought stress, a pattern similar to dehydration-specific gene such as delta 1-pyrroline-5-carboxylate synthetase (P5CS) and cold response gene *COR413* (Dombrowski et al. 2008).

The mechanism of Rab GTPase tolerance to biotic stress was shown in wheat, *TaRab7*, a Rab GTPase gene. It was isolated by reverse transcription technique from wheat cDNA library obtained from leaves infected with *Puccinia striiformis* f. sp. *tritici* (*Pst*), wheat stripe rust pathogen. It showed highest homology to *BdRab7*-like small GTPase from *Brachypodium distachyon*, *OsRab7* (*Oryza sativa*), *AtRabG3b* (*Arabidopsis*), and *HvRab7* (*Hordeum vulgare*). qRT-PCR analysis of *TaRab7* showed enhanced expression levels under both abiotic (salinity, drought, and low temperature) and biotic (*Pst* infection) stresses. Furthermore, knocking down

TaRab7 expression by virus-induced gene silencing (VIGS) enhanced the susceptibility of wheat cv. Suwon 11 towards an avirulent *Pst* race CYR23 (Liu et al. 2012a, 2012b). Previous studies have shown that Rab7 interacts with various downstream effectors (Zerial and McBride 2001) and plays a critical role in vesicular transport from early to late endosomes in the cytoplasm (Feng et al. 1995; Mukhopadhyay et al. 1997). In HeLa cells, Rab7 is critical for lysosomal aggregation and fusion in perinuclear region (Bucci et al. 2000). Transient subcellular localization assays in onion peel cells using *pCaMV35S:TaRab7-GFP* fusion construct revealed the cytoplasmic, perinuclear, and nuclear localization pattern, consistent with its role in regulating lysosome and phagosome to detoxify the toxic material secreted by pathogen into the host cells (Liu et al. 2012a, 2012b).

Studies in *Arabidopsis* have shown the involvement of auxins in stress response and tolerance. RABA (RAB11) group/clade contains 26 members, divided further into six subgroups (RABA1–RABA6). Cell biological and physiological analysis of RABA1 members implicated them in transport between trans-Golgi network and PM and their requirement during salt stress tolerance. Mutant and complementation analysis showed that the four RABs – RABA1a, RABA1b, RABA1c, and RABA1d – act redundantly in salt stress tolerance and are not involved in regulating intracellular distribution of sodium (Asaoka et al. 2012; Asaoka et al. 2013) and probably act by affecting the localization of PIN, auxin efflux transporters (Feraru et al. 2011), supported by the role of auxins in salt stress tolerance (Park et al. 2011). RABA1, subgroup of (RAB11) family members in *Arabidopsis*, shows a different mechanism for salt tolerance; it is involved in salinity stress by regulating the transport of sodium across the PM and accumulation in vacuoles as the quadruple mutants do not affect the sodium content or distribution (Asaoka et al. 2013).

In *Mangifera indica*, *MiRab5* is upregulated in response to cold, salinity, and PEG stress in addition to its involvement in seed germination (Liu et al. 2014). *Arabidopsis* CPRabA5e (where CP represents chloroplast) is a chloroplast-localized protein found to regulate oxidative stress signaling. The role of CPRabA5e was further validated by Y2H screen where 13 proteins previously predicted to be involved in stress and localized in thylakoids and plastoglobules were identified (Karim et al. 2014). However, the exact mechanism of *CPRab5e* activity is not known yet.

Proteome analysis of rice seedlings during drought stress and recovery upon rewatering revealed that 9 small GTP-binding proteins are highly expressed specifically under severe drought stress, while 3 out of 9 were induced in all stress conditions with highest expression in drought. Among these 9 stress-induced small GTP-binding proteins, 4 belong to Rab GTPases family, indicating Rab GTPases role in plant stress tolerance is conserved throughout the plant kingdom (Mirzaei et al. 2012).

9.2.5 ARF GTPases

High concentrations of salt arrest plant development and lead to plant cell death by disrupting ion and water homeostasis, inhibition of metabolism, and damage to membranes (Huh et al. 2002). Photosynthetic activity is suppressed by salt stress, and the reduction in photosynthetic activity can be accounted for by the decline in chlorophyll content (Al-Aghabary et al. 2004). Plants suffering from salt stress often display symptoms of oxidative damage as indicated by marked accumulation of ROS such as H_2O_2 (Hasegawa et al. 2000). MDA (malondialdehyde) is recognized parameter for lipid peroxidation, leading to increased permeability of membranes to various ions (Mittler 2002). Plants overcome salt stress by either transporting excess Na^+ into the vacuoles or by prohibiting its entry into plant cells, accomplished by various transporters (Apse et al. 1999; Zhang and Blumwald 2001; Rus et al. 2001) or by accumulating osmolytes.

ADP-ribosylation factor (Arf) GTPases are important regulators of membrane-trafficking pathways, initially identified due to their ability to stimulate the ADP-ribosyltransferase activity of cholera toxin A (Moss and Vaughan 1998). They are involved in generation of the three types of vesicle coat proteins (COPI, COPII, and clathrin) (Kirchhausen 2000). Plant genome encodes for Arf-like (Arl) GTPases with protein sequence highly similar to that of Arf GTPases in animals. Knockout of an Arl GTPase, *TITAN*, leads to dramatic alterations in mitosis and cell cycle control during seed development in *Arabidopsis* (McElver et al. 2000). Suppression subtractive hybridization (SSH) in *Medicago falcate*, tolerant to abiotic stresses, identified *MfARL1*, an Arl GTPase, which confers greater salt tolerance. Functional characterization of *MfARL1* in *Arabidopsis* confirmed its role in salt tolerance. The OE transgenic plants showed reduced accumulation of Na^+ and reduced expression levels of *AtHKT1*, a sodium transporter, which probably reduces the influx of Na^+ into the plant thereby conferring resistance to salt (Wang et al. 2013). Function of *AtHKT1* in sodium transport has been previously shown, where *AtHKT1* mutation suppress *sos3* mutant phenotypes. Analysis of ion contents in the *sos3hkt1* mutant demonstrated that *AtHKT1* is involved in mediating Na^+ influx into the plant cells (Rus et al. 2001). Moreover, the photosynthetic activity and chlorophyll content of the *MfARL1* OE transgenic lines were not affected by salt stress when compared to wild-type plants. The MDA and H_2O_2 content of transgenic plants was less, whereas the wild-type plants exhibited increased levels of H_2O_2 and MDA. Increased level of H_2O_2 in wild-type plants was due to decreased CAT (catalase) activity, whereas salt stress has no effect on CAT activity in the transgenic lines. In conclusion, *AtHKT1* provides greater salt tolerance by regulating a sodium transporter and CAT activity.

Similarly, *ARF1* of *Jatropha curcas* is induced in response to abiotic stresses, particularly upon treatment with PEG, which induces osmotic stress, and cold treatment (Qin et al. 2011).

9.2.6 *MIRO GTPases*

Miro GTPases function in mitochondrial trafficking and morphology in eukaryotes. The role for *AtCBG* (calcium-binding GTPase)/*AtMIRO2*, a Miro GTPase, has been explored in abiotic stress. Expression of *AtMIRO2* was upregulated in salt stress and ABA treatment suggesting its involvement in abiotic stress. Phenotypic analysis of *AtMIRO2* mutant confirmed their sensitivity towards both salt and ABA stress (Jayasekaran et al. 2006).

9.2.7 *Seed Plant (Spermatophyte)-Specific Small GTPase*

In plants, circadian clock regulates signaling pathways such as light, hormone, and stress (Covington et al. 2008). Plants respond to high salinity stress, by increasing cytosolic calcium, by activating the SOS1-mediated changes in ion homeostasis, and subsequently by generating secondary messengers such as phospholipids and ROS, which activate kinase cascade to regulate transcription of stress-inducible genes. These responses can be ABA dependent and independent and are linked to circadian clock (Sanchez et al. 2011). Kevei et al. in 2007 identified *LIP1* (*LIGHT INSENSITIVE PERIOD 1*), a small GTPase, in a genetic screen for novel circadian clock mutant in *Arabidopsis*. *lip1* mutant displayed increased sensitivity to salt stress, affecting morphogenesis and ploidy. qRT-PCR analysis confirmed that *LIP1* does not function in ABA-dependent or ABA-independent salt stress signaling pathways leading to expression of osmoprotectant genes like *RAD29A* (*RESPONSIVE TO DESICCATION 29A*), *RAD29B*, or *RAB18* (*RESPONSIVE TO ABA*) and ionic stress genes like *SOS2*, a Na⁺/H⁺ antiporter and activator of *SOS1*, respectively, though its function at the posttranslational level was not ruled out. Another possible explanation for salt stress sensitivity could be the role of *LIP1* in endoreplication, affecting the ploidy level (Terecskei et al. 2013). Previous studies have implicated endoreplication in seedling development upon exposure to stress including high temperature and drought (Engelen-Eigles et al. 2001). Cookson et al. in 2006 showed that increase in extent of endoreduplication reduced the negative impact of drought on the final leaf size.

9.3 Large G Proteins in Biotic and Abiotic Stress

9.3.1 *Biotic Stress*

Several independent studies using inhibitors and agonists of G proteins in different plant species supported the role of G proteins in defense signaling (Legendre et al. 1992; Beffa et al. 1995; Gelli et al. 1997). Particularly, changes in cytosolic calcium

concentrations, often observed in elicitor-treated plant cells, are regulated by G proteins (Aharon et al. 1998). Calcium then activates the downstream signaling pathway including calcium-activated kinases (Romeis et al. 2000), calmodulin (Heo et al. 1999), and NADPH oxidase (Sagi and Fluhr 2001).

Studies in rice have indicated the specificity of G-protein signaling in plant defense response. Rice *d1* mutants, lacking functional *RGA1* ($G\alpha_s$), showed reduced defense response to avirulent strains of bacterial blight (*Xanthomonas oryzae* pv. *Oryzae* [Xoo]) and rice blast fungus (*Magnaporthe grisea*) (Komatsu et al. 2004). A role supported by elevated *RGA1* mRNA levels upon rice blast infection and sphingolipid elicitor (SE) treatment in leaf and cultured cells. Further analysis supported G-protein function in ETI-triggered defense response, as there was suppression of ROS production and *PBZ1* expression by SE in the *d1* mutant cell cultures. Moreover, Rac signaling was placed genetically downstream of G proteins based on epistasis analysis using *CA-OsRac1*, where *OsRac1* restored the defense signaling, by promoting the *PR* gene expression and ROS production in *d1* mutant plants (Suharsono et al. 2002).

Similarly, *Arabidopsis* mutants of *gpa1* gene, encoding *GPA1*, have slightly increased resistance to several pathogens though the link between G proteins and plant defense is conclusively established through the *Arabidopsis* $G\beta\gamma$ dimer. Mutants deficient in *AGB1* ($G\beta_s$) are more susceptible to the fungal pathogens *Alternaria brassicicola*, *Botrytis cinerea*, *Fusarium oxysporum*, and *Plectosphaerella cucumerina* (Llorente et al. 2005; Trusov et al. 2009). Upon infection with *A. brassicicola* or treatment with methyl jasmonate (MeJA), where latter is produced during both biotic and abiotic stress response, *agb1* mutants exhibit delay in the induction of the MeJA-induced *PR* genes, *PDF1.2*, *OPR3*, and *PAD3* (Trusov et al. 2009), whereas expression of the SA-dependent *PRI* was increased after infection with *P. cucumerina* (Llorente et al. 2005). It was concluded that the *Gys* confer functional selectivity to $G\beta\gamma$ dimer. Among the three *Arabidopsis* *Gys*, *AGG1* has been implicated in plant defense response against *F. oxysporum* and *A. brassicicola* (Trusov et al. 2007), whereas *AGG3* has no defense-related response and the role of *AGG2* is not clear (Delgado-Cerezo et al. 2012). In conclusion, G protein plays an important role in both PTI- and ETI-triggered immune responses. Besides, the canonical G-protein subunits, one of three extra-large α -subunit, *XLG2* (Lee and Assmann 1999), is linked to plant defense. *xlg2* mutant shows enhanced susceptibility to *P. syringae* and reduced induction of the pathogenesis-related gene *PR2* indicating positive regulatory role in plant defense response. Microarray analysis revealed that, aside from *PR2*, other pathogen-inducible genes are downregulated in *xlg2* mutant in response to *P. syringae* infection, whereas overexpression of *XLG2* resulted in accumulation of aberrant transcripts for several defense-related genes. Biochemical analysis revealed that *XLG2* physically interacts with *AGB1* and that the interaction is restricted to infected tissues (Zhu et al. 2009).

G protein have been shown to be involved in elicitor-induced HR in plants by VIGS-induced silencing of the $G\alpha$, $G\beta1$, and $G\beta2$ subunits of *Nicotiana benthamiana* G-protein complex. The silenced plants when treated with bacterial and fungal elicitors showed impaired HR including stomata closure, nitric oxide (NO) produc-

tion, and ROS accumulation in guard cells. Besides, transcription of various plant defense-related genes was also impaired, including *NbrbohA* (NADPH oxidase), NOA1 (associated with NO), and NIA (responsible for NO production) (Zhang et al. 2012).

9.3.2 Abiotic Stress

Sessile nature of plants constantly exposes them to changing environmental conditions giving rise to oxidative stress. A mild oxidative stress induces antioxidant defenses, whereas severe stress leads to rapid necrosis or cell death. The induction and execution of PCD is regulated by various signaling molecules including ROS, jasmonic acid (JA), salicylic acid (SA), and ethylene (Lam et al. 1999). Ozone is a major oxidative stress and a major pollutant that damages crops, forests, and urban vegetation. The main symptoms of ozone damage are foliar lesions and reduction in stomata aperture mediated through ABA signaling (Mansfield 1998), and it shares signaling pathways and gene expression pattern with hypersensitive response (HR) (Tamaoki et al. 2003).

Arabidopsis plants with null mutations in the *gpa1* ($G\alpha s$) and *agp1* ($G\beta\gamma$ dimer) are less and more sensitive, respectively, to ozone damage than the wild-type plants. Analysis of *gpa1* mutant plants exhibited ozone-resistant phenotype, indicated by lack of leaf curling in response to ozone (Booker et al. 2004). At molecular level, it was shown that $G\alpha s$ activates membrane-bound NADPH oxidases, encoded by *AtrbohD* and *AtrbohF* genes, whereas the $G\beta\gamma$ dimer stimulates ROS production in chloroplast. The role of *gpa1* in activating NADPH oxidases was further supported by analysis of *atrbohD* and *atrbohF* double-mutant plants, which are more resistant to ozone than wild-type plants. Moreover, a link between G-protein and Rac signaling has been provided, where $G\alpha s$ acts through the Rop (Baxter-Burrell et al. 2002; Suharsono et al. 2002) and where Rop is activated by phosphatidic acid (PA), a product of phospholipase $D\alpha$ ($PLD\alpha$), which in turn is inhibited by $G\alpha s$. A direct interaction was shown between $PLD\alpha 1$ and $GPA1$, where binding inhibited $PLD\alpha 1$ activity, relieved upon GTP addition, suggesting in vivo G-protein activation leads to $PLD\alpha 1$ activation. PA upon activation and dissociation of $G\alpha s$ inhibits the activity of ABI-1 and ABI-2 (ABA insensitive), negatively regulating ABA signaling through stress-activated MAPK cascade (Zhang et al. 2004). *gpa1* mutant also exhibit reduced ozone sensitivity at single-guard-cell level, indicated by reduced ABA inhibition of guard cell inward K^+ channels and altered ABA promotion of slow anion currents (Wang et al. 2001). ABA is known to mediate its effects by acting through second messenger, S1P (sphingosine-1-phosphate) (Ng et al. 2001; Coursol et al. 2003) and through the effector molecule, PLCs (phosphatidylinositol-phospholipases) (Jacob et al. 1999; Zhang et al. 2004). Guard cells of *gpa1* mutants exhibit insensitivity to inhibition of stomata opening by S1P. Moreover, studies supported existence of two parallel pathways: S1P dependent and independent (Wang et al. 2001; Coursol et al. 2003).

The role for G proteins in ABA-mediated response is further validated by studies in *gcr1* mutants, exhibiting hypersensitivity to ABA and S1P in both stomata opening and promotion of stomata closure, indicating that *GCR1* acts as negative regulator of *GPA1*-mediated ABA responses in guard cells. A hypothesis further supported by higher levels of expression of drought- and ABA-regulated genes after exogenous ABA treatment and inhibited improved recovery following drought stress in *gcr1* mutant plants (Pandey and Assmann 2004). PLCs, the downstream effectors of ABA, have been shown to both genetically and physically interact with G-protein signaling component, indicated by studies done in BY2 tobacco cells overexpressing *GCR1*, where GCR1 activates DNA synthesis in PLC-dependent manner (Apone et al. 2003).

G proteins have been shown to function in closure of stomata aperture by production of H_2O_2 induced by ExtCaM (extracellular calmodulin) and NO (nitric oxide). G-protein involvement in H_2O_2 production is evident by analysis of *gpa1* mutants; ExtCaM-induced H_2O_2 production is impaired in *gpa1* mutants, indicated by impaired stomata closure. ExtCaM-mediated NO generation is regulated by GPA1, whereas GPA1 activation of NO production depends on H_2O_2 (Chen et al. 2004a, 2004b).

Recently, eATP (extracellular ATP) has been reported as a signaling molecule, existing in the apoplast of plant cells. In *gpa1* mutant plants, eATP-promoted stomata opening, cytoplasmic ROS generation, and Ca^{2+} and H^+ efflux are all suppressed, indicating G-protein involvement during eATP signaling (Hao et al. 2012). Proteomics has identified MAPK (mitogen-activated protein kinase) as a downstream effector molecule for G-protein-mediated signaling in response to ABA and MeJA (Methyl Jasmonate), supported by yeast two-hybrid assays and upregulation of PsG β and PsMAPK3 (*Pisum sativum* MAPK3), upon treatment with MeJA and ABA (Bhardwaj et al. 2011).

Besides, *Arabidopsis* and rice, the signaling pathways involving G protein during abiotic stress conditions are also conserved in other plants. Overexpression of *AGG3* under the control of CaMV35S promoter in *Camelina sativa* resulted in transgenic seeds with less sensitivity to ABA, osmotic, and salt stress during germination and post germination. In addition, stomata in *CaMV35:AGG3* plants are more sensitive to ABA, imparting better drought recovery (Roy Choudhary et al. 2004).

Some plants respond to hypoxia or oxygen deprivation like underwater logging by enhanced ethylene production (Cohen and Kende 1987; Wang and Arteca 1992; Zarembinski and Theologis 1993). Ethylene signaling regulates adaptation to hypoxic conditions by promoting morphological, anatomical, and metabolic adaptations (He et al. 1996a; He et al. 1996b; Steffens and Sauter 2009). *RGAL1*, the rice homolog of G α_s , functions downstream of ethylene and H_2O_2 under oxygen deprivation condition to induce cell death, indicated by reduced cell death in *rgal1* (*d1*) mutant plants, with reduced *D1* mRNA (Steffens and Sauter 2010). The cell death phenotype of *rgal1* mutant plants could not be restored by treatment with ethylene or H_2O_2 , indicating that G proteins act downstream of these signaling molecules. Microarray analysis revealed that the *D1* gene itself was not regulated in epidermal cells by ethylene or H_2O_2 , pointing to posttranscriptional regulation

of the D1-dependent G-protein activation (Suharsono et al. 2002; Steffens and Sauter 2009).

Studies in maize further support the role of G protein in hypoxia condition. Maize roots incubated with GTP γ S, an activator of G proteins, at normoxic conditions, resulted in cell death and aerenchyma formation. This could probably occur due to constitutively activated G proteins, as GTP γ S is nonhydrolyzed substrate for GTPases. Moreover, formation of aerenchyma was accompanied by an increase in cellulase activity, where cellulases contribute to the degradation of the cell wall and thus to the removal of the cell corpses. These results demonstrated a central role for G protein in hypoxia by activating cellulases (He et al. 1996a).

ABA, a plant stress hormone, is known to regulate adaptation of plants to environmental stresses including cold, drought, and salt by modulating the expression of stress-responsive genes and controlling the stomatal aperture to regulate water content (Spartz and Gray 2008). *BnGAI*, encoding *Brassica napus* G α s, is shown to be induced in response to ABA. *BnGAI* is upregulated by salinity and drought stress tolerance while downregulated by temperature stresses (Gao et al. 2010) supporting the role of *BnGAI* in ABA-mediated responses. Similarly, *Arabidopsis GPA1* acts as a regulator of transpiration efficiency (TE). *gpa1* mutant plants, despite having guard cells that are hyposensitive to ABA-induced inhibition of stomata opening, exhibit increased TE under ample water and drought stress conditions and when treated with exogenous ABA, most likely due to increased stomatal density. *GPA1* regulation of stomatal density was further supported by analysis of *gpa1* mutant plants, which showed reduced stomatal density (Nilson and Assmann 2010a). This supports the role of *GPA1* downstream of ABA signaling. *AtRGS1* was identified as one of the components of G-protein signaling during salt stress in *Arabidopsis*. Lof *AtRGS1* (*rgs1-2* mutant) in presence of salt showed better survival and low senescence as opposed to lof G β s (*agb1-2* mutant), which showed low survival and accelerated senescence under salt stress. Most likely in the presence of Na⁺, *AtRGS1* undergoes encosytosis due to increased glucose levels, thereby activating the G α s and releasing the heterotrimer. In conclusion G α s and G β s act antagonistically during salt stress affecting the survival and senescence process (Colaneri et al. 2014).

Asakura and Kurosaki (2007) demonstrated that the expression of G α s in carrot seedlings was transiently decreased by treatment with high concentration of NaCl and decreased by the exposure to high temperature, and no induction was seen at low temperature in carrot. In pea, the expression of G α s increases upon treatment with high concentration of NaCl and high temperature (Misra et al. 2007).

Functional genomics approach has shown conservation of G-protein signaling across plant species during biotic and abiotic stresses. Transcription profiling of rice *rga1* showed upregulation in the transcript level following NaCl, cold, and drought stresses. Where higher temperatures downregulate *RGAI* transcript levels, heavy metal(loid) stress exhibits upregulation. Similar, transcription profiling of rice *RGG1* and *RGG2* showed that their transcript levels are upregulated following

NaCl, cold, heat, and ABA treatments. However, in drought stress, only *RGG1* is upregulated. Promoter analysis of *RGG1* and *RGG2* revealed presence of stress-related *cis*-regulatory signature motifs, supporting their role in abiotic stress response (Yadav et al. 2012; Yadav et al. 2013). Similar study involving in silico characterization of *cis*-regulatory elements of *RGB1*, encoding the G β s of Indica rice, revealed the presence of stress-related *cis*-regulatory elements. Transcript profiling revealed enhanced *RGB1* transcript levels under KCl, cold, drought (dehydration), and micronutrient (Mn²⁺ and Zn²⁺) stresses. Moreover, nuclear localization of *RGB1* supports its possible interaction with transcription factors in regulating salt stress-responsive genes (Yadav et al. 2014).

9.4 Conclusions and Future Perspectives

Increased demand of high-yielding food crops and depleting natural resources has resulted in exponential growth in the number of genetically modified crops. To efficiently generate genetically engineered plants, use of high-throughput technologies has led to rapid identification and subsequent manipulation of target genes. Primarily, target genes are involved in signal transduction pathways, which either increase the yield or generate stress-tolerant crop plants. Using high-throughput techniques like proteomics, metabolomics, and phenomics along with functional genomics approach has identified several key signaling molecules in plant stress responses, including large and small G proteins. G proteins are excellent molecular switches in various cellular and biological processes and can serve as excellent targets for generating stress-tolerant crop plants. Monomeric GTPases have been shown to act as molecular switches in various abiotic and biotic stress conditions signaling downstream of heterotrimeric G proteins, elicitor molecules, phytohormones like ABA, ethylene, and others. In GTP-bound form, they undergo conformation change and activate downstream effector molecules like NADPH oxidase, MAPKs, SnRKs, metallothionein, and transcription factors, to generate response either by modulating enzyme activity or gene expression. Similarly, research has shown that heterotrimeric G proteins act in signaling pathways in response to pathogens, ROS, salt, and others. The ease of manipulation of pathways involving large and small G proteins by using constitutively active or dominant-negative forms has made them excellent targets to generate stress-tolerant high-yielding crop plants. Although research done in the past several decades has delineated many signaling pathways involved in both biotic and abiotic stresses as evident from Fig. 9.4, the picture is far from complete, and future efforts have to be focused on establishing the intricate network and cross talk between various networks to find a target pathway for creating genetically modified crop plants.

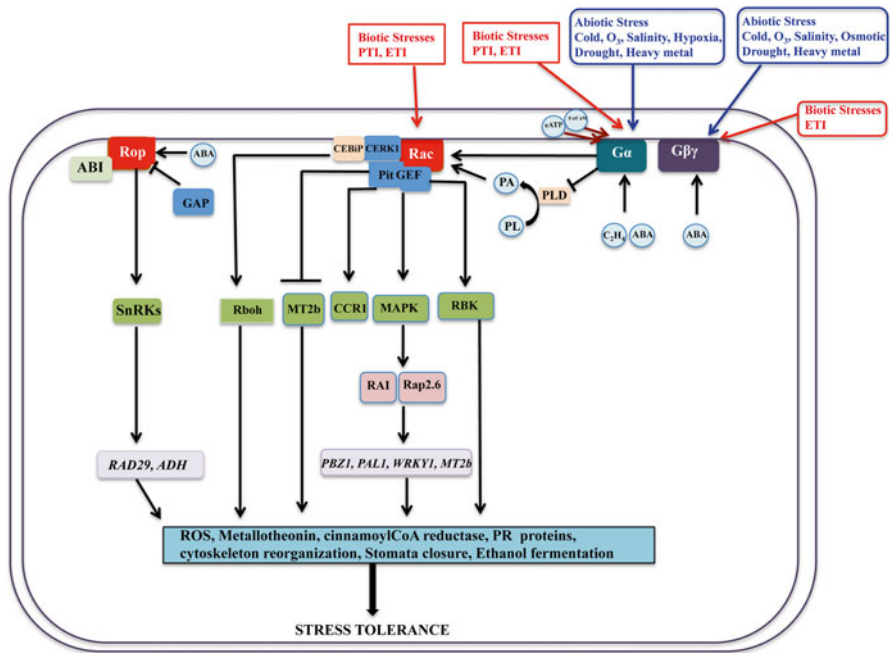


Fig. 9.4 Schematic depiction of various signaling pathways involving small and large G proteins during biotic and abiotic stress responses in plants: the G proteins and small GTPases are activated in response to various abiotic and biotic stimuli and second messenger molecules to activate several signaling pathways. The G-protein subunits activate downstream effectors when activated, and one of the effector molecules is Rac GTPase thus providing a link between G proteins and small GTPases. The Rac GTPase acts in plant defense response by forming a defensome at the membrane consisting CEBiP (chitin-binding protein), CERK (chitin-activated receptor kinase), and Pit (NLR protein). The Rac complex then activates various effector molecules including Rboh (NADPH oxidase), MT2b (metallothionein), CCR1 (cinnamoyl-CoA reductase1), MAPK (mitogen-activated kinase), and RBK (receptor-like kinase). Downstream of effector molecules is transcription factor such as RAI (Rac immunity) and Rap2.6, which regulate transcription of defense-related gene such as *PBZI*, *PALI*, *WRKY1*, and *MT2b*, leading to response and stress tolerance

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