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Heterotrimeric G-Protein Signaling in Plants: Conserved and Novel Mechanisms

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Abstract

Heterotrimeric GTP-binding proteins are key regulators of a multitude of signaling pathways in all eukaryotes. Although the core G-protein components and their basic biochemistries are broadly conserved throughout evolution, the regulatory mechanisms of G proteins seem to have been rewired in plants to meet specific needs. These proteins are currently the focus of intense research in plants due to their involvement in many agronomically important traits, such as seed yield, organ size regulation, biotic and abiotic stress responses, symbiosis, and nitrogen use efficiency. The availability of massive sequence information from a variety of plant species, extensive biochemical data generated over decades, and impressive genetic resources for plant G proteins have made it possible to examine their role, unique properties, and novel regulation. This review focuses on some recent advances in our understanding of the mechanistic details of this critical signaling pathway to enable the precise manipulation and generation of plants to meet future needs.

Contents

1. INTRODUCTION

G protein: a guanine nucleotide–binding protein complex that switches between active and inactive forms depending on the receptor activation

G protein–coupled receptor (GPCR): a seven transmembrane–containing, plasma membrane–localized protein that perceives changes in its surrounding environment and transfers this information intracellularly to elicit a response

Guanine nucleotide exchange factor (GEF): a protein (e.g., G protein–coupled receptor) that facilitates activation of G proteins by promoting the release of GDP from Gα protein

All living cells must constantly respond to environmental and endogenous cues and adjust their growth and development. Perception of these cues and the resulting response require the coordinated action of multiple, distinct sensory modules. One such module is the heterotrimeric G-protein complex present in all eukaryotes. The protein complex was originally described as the transducer component of the signal transduction core, which could couple the sensor or discriminator present at the plasma membrane with the secondary messengers or amplifiers inside the cells for fast and specific response to a signal. Decades of elegant studies identified the transducer function to be guanine nucleotide–dependent: hence the term G proteins (27, 89).

1.1. The Classical Metazoan G-Protein Cycle

The term heterotrimeric G proteins (G proteins, hereafter) refers to a core protein complex comprised of one Gα, one Gβ, and one Gγ subunit, which swap between active and inactive forms depending on which guanine nucleotide is bound to Gα (**Figure 1**). When GDP-bound, Gα remains associated with the Gβ and Gγ proteins in a trimeric complex, representing the inactive or resting phase of signal transduction. Upon activation, the GDP on Gα is exchanged for GTP, and the complex dissociates to release GTP-bound Gα and a nondissociable Gβγ dimer. Each of these entities interacts independently with a multitude of downstream effectors to transduce the signal, representing the active stage of signaling. The Gα protein also has GTPase activity, which causes hydrolysis of bound GTP to GDP. This hydrolysis generates GDP-bound Gα, which reassociates with the Gβγ dimer to form the inactive heterotrimer, ready to be activated again (72, 78, 104). This recurring swapping of active versus inactive stages of the protein complex results in extremely efficient and specific signal–response coupling. The cyclic nature of G-protein signaling requires precise, synchronized activation (i.e., GDP-to-GTP exchange) and deactivation (i.e., GTPase activity). GDP-to-GTP exchange is typically facilitated by G protein–coupled receptors (GPCRs) which act as guanine nucleotide exchange factors (GEFs). The inherent rate of GTP hydrolysis by the Gα proteins is relatively slow and is usually

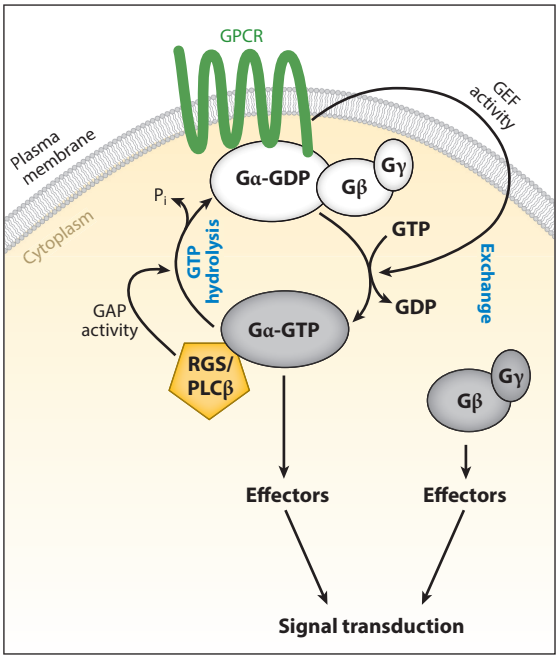

Figure 1

Conventional G-protein signaling mechanism. The heterotrimeric G proteins comprised of Gα, Gβ, and Gγ subunits toggle between the inactive and active forms depending on the GDP-bound versus GTP-bound state of the Gα protein. The GDP-to-GTP exchange reaction of Gα is facilitated by GPCR, whereas the slow inherent GTPase activity of Gα protein is aided by GAPs, such as RGS or PLCβ. When active, the G-protein subunits interact with downstream effectors to transduce the signal. Abbreviations: GAP, GTPase activity–accelerating protein; GEF, guanine nucleotide exchange factor; GPCR, G protein–coupled receptor; PLCβ, phospholipase Cβ; RGS, Regulator of G-protein signaling.

aided by the GTPase activity–accelerating proteins (GAPs), such as the regulator of G-protein signaling (RGS) protein or specific phospholipases (72, 78, 104).

The core G-protein components and their basic properties are conserved across phyla. Despite limited sequence similarities, Gα proteins from yeast, algae, human, or plants can bind and hydrolyze GTP and associate with or dissociate from the Gβγ dimers depending, respectively, on whether Gα has bound to GDP or GTP. A Gβ protein from any species can interact strongly with Gγ proteins, and an RGS protein can accelerate the rate of GTP hydrolysis by binding to a Gα protein regardless of the species (32). However, in addition to what we have learned from the well-explored mammalian and fungal systems, study of G proteins and the signaling pathways regulated by them in plants has yielded some surprising variations in the components and regulatory modes of the complex.

GTPase activity–accelerating protein (GAP): a protein (e.g., regulator of G-protein signaling) that accelerates GTP hydrolysis by Gα proteins and thus helps to regenerate inactive G-protein complexes

Phospholipase: member of a diverse group of plasma membrane–localized enzymes that hydrolyzes phospholipids and has roles in signaling and development in all organisms

1.2. Plant Heterotrimeric G Proteins

The study of plant G proteins initially focused on reference organisms, such as *Arabidopsis* and rice. Each of these species has a relatively limited number of canonical G-protein components (one Gα, one Gβ, and a few Gγ subunits) in contrast to the large repertoire of G-protein subunits in metazoans (5, 43, 87). However, physiological and genetic analyses uncovered the roles of G proteins in almost every aspect of plant growth and development. In *Arabidopsis*, where the most

Extra-large G protein (XLG): a plant-specific Gα protein that has a large N-terminal extension

extensive data exist, G proteins are involved in processes as fundamental and diverse as control of cell division and cell expansion; regulation of ion channel activities; modulation of responses to almost all plant hormones; response to bacterial, fungal, and viral pathogens; and response to various environmental variables, such as light, drought, and salinity (16–18, 21, 40, 58, 64, 71, 74, 82, 83, 86, 132, 140, 145, 147). Studies in additional plant species, such as rice, maize, soybean, pea, and *Camelina sativa*, have suggested that G proteins affect the real agronomic potential of plants by controlling plant architecture, nitrogen and water use efficiency, seed size and number, and nitrogen fixation (9, 10, 47, 51, 93, 100, 106–108, 123, 129, 134–136).

Even though diverse roles have been identified for G proteins in plants, the mechanistic details of their signaling pathways are only beginning to be explored. Earlier studies in *Arabidopsis* implied that G proteins are not essential because plants lacking one or more G-protein subunits are able to complete their life cycles. This led to the hypothesis that the role of G proteins is primarily to modulate overall plant plasticity by regulating multiple aspects of growth and development (4, 109). However, recent research in grasses has changed this view because some G-protein components are indeed essential for survival. Rice and maize plants lacking canonical *Gα* genes are dwarf (a phenotype not shared by dicot plants lacking Gα) (9, 26, 77, 120, 123, 126), but plants lacking the *Gβ* gene survive only to the early seedling stage (126, 128). Rice and many other monocots also lack an obvious *RGS* homolog in their genome, even though RGS is required for the G protein–dependent signaling in *Arabidopsis* and soybean (23, 32, 42, 95, 102). More importantly, a GPCR with GEF activity has not yet been identified in plants, so the activation mechanism of plant G proteins remains somewhat elusive.

During the past two decades, plant G-protein research has progressed from establishing that plants possess functional G proteins to identifying similarities with and differences from the conventional mammalian model. In the current phase, it has become clear that plants use the same G-protein components that exist in metazoan systems but wire them differently. This rewiring includes new components working together with the core G proteins, novel regulators, and effectors as well as unique signaling and regulatory mechanisms. Recent research has redefined multiple aspects of G-protein signaling, including novel G-protein components and their mechanistic details. This is the focus of the following sections.

1.3. Novel G-Protein Core Components in Plants

The first plant G proteins were identified through homology-based searches using mammalian G-protein sequences. The plant Gα, Gβ, and Gγ proteins exhibit limited sequence identities with their mammalian counterparts but possess all of the required domains for proper localization, interaction, and activity. However, certain novel plant-specific components have also been identified. These show notable variations compared with the canonical G-protein subunits, but they have been confirmed to constitute the core of heterotrimeric G proteins.

1.3.1. Extra-large G proteins. The extra-large G proteins (XLGs) are a larger form of canonical Gα proteins. Plant XLGs are encoded by genes distinct from those encoding Gα, unlike certain mammalian extra-large Gα proteins (e.g., XLG_{olf}), which result from alternative splicing of a Gα transcript (14, 54, 126). All higher plants possess multiple genes encoding XLGs. The C-terminal region of XLGs is similar to that of a canonical Gα protein, with almost complete conservation of the G1, G2, and G4 domains, a partially conserved G3 domain, and no conservation of the G5 domain. Consequently, the proteins can bind GTP, but their GTPase activity remains debatable. The unique feature of these proteins is the presence of a long N-terminal extension of 300 to 500 amino acids, which does not show any recognizable features except for a nuclear localization or

export signal. XLGs have been detected both at the plasma membrane and in the nuclei of plant cells (13, 61, 126).

XLGs work both redundantly and independently of canonical Gα proteins in plants. In *Arabidopsis*, the three *XLG* genes (*XLG1*, *XLG2*, and *XLG3*) largely work redundantly. Plants lacking all three *XLG* genes (*xlg1.xlg2.xlg3* triple mutants) exhibited altered abscisic acid sensitivity, sugar sensitivity, defense response, and root waving—phenotypes similar to those of the *Arabidopsis Gβ* mutant (*agb1*)—providing indirect evidence for XLG involvement in canonical G protein–regulated processes (21, 83). Recent studies have conclusively established that they can replace Gα in the core of the G-protein complex in plants, leading to distinct signaling regulation (13, 126, 147). XLGs physically interact with Gβγ proteins. Exquisite genetic and biochemical studies have elucidated the roles of XLGs, especially XLG2 and XLG3, as parts of the heterotrimeric G-protein complex involved during antibacterial defense responses in *Arabidopsis*. A complex comprised of XLG2/3, AGB1, and AGG1/2 interacts with the *Arabidopsis* immune receptor complex flagellin sensing 2 (FLS2)/Brassinosteroid-insensitive 1–associated receptor kinase 1 (BAK1) to control signal output during defense response (58, 71, 131). Plants lacking *XLG* genes are compromised in pathogen-associated molecular pattern–triggered oxidative burst, disease resistance, and programmed cell death (58, 71, 147).

In maize, where the phenotypes of plants lacking G proteins are more severe, a clear role of XLGs working with the canonical G proteins has emerged. The maize Gα protein CT2 functionally interacts with the CLAVATA (CLV) receptors to control shoot apical meristem (SAM) development (9, 41). *ct2* mutants have a larger SAM and dwarf stature. The three XLGs of maize also regulate this pathway. Although knocking out any single *XLG* gene did not cause any phenotypic differences from the wild-type plants, knocking out any two *XLG* genes in the *ct2* mutant background significantly enhanced the *ct2* phenotypes, resulting in severely dwarf plants and larger SAMs (41, 135). The complete loss of all three *XLG* genes in maize is seedling lethal, a phenotype not observed in *Arabidopsis*, where *xlg* triple mutants grow and develop normally except under specific conditions (21, 132, 135). The lethality of maize *xlg* triple mutants is identical to the phenotype of maize or rice *Gβ* mutants and suggests that XLGs and Gβ proteins are indispensable for plant survival, potentially by affecting immune signaling (135).

Another remarkable example of XLG-mediated G-protein signaling exists in the moss *Physcomitrella patens* (34). This species is unique because it has lost its canonical Gα protein but possesses an XLG along with the Gβ and Gγ subunits of the canonical protein complex. Genetic analysis of *P. patens* plants lacking the sole *XLG* gene or one of the *Gβ* genes reveals identical phenotypes. The mutant plants grow more slowly during the vegetative phase and form shorter gametophytes with fewer leaves, as compared with the wild-type moss. Moreover, they are unable to form sporophytes, which represent the only diploid stage of the moss life cycle. These moss mutant phenotypes can be fully complemented by introducing *Arabidopsis XLG2* or the *AGB1* (*Gβ*) genes, confirming that it is indeed a G protein–regulated process (34).

Interestingly, some of the functions of XLGs are independent of the canonical Gα proteins, as has been clearly demonstrated in maize. While SAM development and dwarf plant phenotypes were regulated by both XLGs and CT2, ear fasciation, which is caused by altered inflorescence meristem size, is regulated by CT2 but not by XLGs (41, 135).

1.3.2. Plant-specific Gγ proteins. Canonical Gγ proteins, present in all eukaryotes, are 100–120 amino acids in length and have a coiled-coil domain in the middle with a conserved DPLL motif and a few additional amino acids, which are required for the interaction of Gγ and Gβ proteins (92, 110, 113). The C-terminal region of these proteins ends in a prenylation motif CXXL (where X is any aliphatic amino acid), which ensures their proper plasma membrane targeting.

Shoot apical meristem (SAM): a group of undifferentiated, meristematic cells at the shoot apex that can give rise to all above-ground plant parts

***Physcomitrella patens*:** a reference bryophyte (nonvascular plant) with a unique life cycle that is important for the study of plant evolution

With the discovery of two additional types of Gγ proteins in plants, these canonical proteins have been classified as type I (or group A) proteins. Type II (or group B) represents proteins that are similar to the type I Gγ proteins except for their C-terminal region, which does not end in a prenylation motif (92, 113). However, the lack of prenylation motif in these proteins does not seem to affect their localization (144), and the proteins are able to work with the Gβ proteins during regulation of defense signaling pathways in tomato (106). This is different from the mammalian systems, where disruption of the prenylation motif in a Gγ protein results in altered function and severe phenotypes (20, 68).

Another novel plant-specific variant group of Gγ proteins is represented by AGG3 in *Arabidopsis*; DEP1, GS3, and GCA2 in rice; and Gγ 8–10 in soybean (92, 113). These proteins are categorized as type III (or group C) Gγ and exhibit a unique, modular structure. The N-terminal region of these proteins is similar in size and sequence to the type I and II Gγ proteins and connected to the C terminus by a putative transmembrane (TM) domain (16, 22). The C-terminal region is expanded in plants that have more than one copy of this gene. For example, the three type III Gγ proteins in soybean have highly similar N-terminal regions, but their C-terminal regions differ in length (92). This C-terminal region is extremely rich in the amino acid cysteine (Cys), which can account for up to 35% of total amino acids in this region (92, 113). This unique Cys-rich region has predicted segments showing some similarity to tumor necrosis/nerve growth factor receptor, multiple repeats of the von Willebrand factor type C modules, and a Sprouty domain—all of which are thought to be involved in large protein complex formation (92). The C-terminal region is also predicted to be extracellular (16, 22). The proteins have been hypothesized to act as a receptosome based on the distinctive domains and predicted extracellular localization (10), although both the identity of proteins with which they might interact and the signals they might perceive are not known. The type III Gγ proteins are key determinants of multiple agronomic traits in plants, including seed size and number, panicle erectness and branching, nitrogen use efficiency (NUE), and abiotic stress responses (22, 39, 47, 51, 57, 70, 100, 129, 134).

1.3.3. Plant regulator of G-protein signaling proteins. RGS proteins are key GAPs of Gα proteins in all organisms. Plant RGS proteins are unique due to the presence of two distinct domains, which were identified in AtRGS1, the first RGS GAP cloned and characterized in plants (18). All plant RGS proteins identified to date are highly similar to AtRGS1. The C-terminal region of these proteins has the conserved RGS domain that is similar to the ones found in all other eukaryotes. This region is connected to an N-terminal region, which has a seven transmembrane (7TM) domain topology reminiscent of metazoan GPCRs (32). Intriguingly, mammalian proteins containing an RGS domain possess a variety of domain associations, but never possess a 7TM domain, although the genomes of many basal eukaryotes encode 7TM-containing RGS proteins (1). The 7TM domain anchors the plant RGS proteins to the plasma membrane and, therefore, places them in close proximity to the G-protein complex. AtRGS1 has also been proposed to be a sensor for D-glucose under certain conditions (88, 127).

1.3.4. Plant-specific phospholipases. Phospholipases of the phospholipase Cβ (PLCβ) family constitute a key regulatory part of the G-protein cycle in mammalian systems. PLCβ interacts with and accelerates the GTPase activity of mammalian Gα proteins, similar to the function of RGS proteins. Although these two proteins bind to different regions of a Gα protein, they change its conformation to increase the rate of GTP hydrolysis (90, 91).

Conventional PLCβ homologs are not found in plants, but another class of phospholipases, phospholipase Dα1 (PLDα1), has been shown to interact with and regulate the activity of the

Gα protein in *Arabidopsis*. Both biochemical data and genetic data confirm the role of this unique enzyme in regulating the plant G-protein cycle in combination with the RGS protein (97, 146). Plants have a large family of phospholipases. If many of them, not just PLDα1, turn out to be G-protein regulators—which is likely given their involvement with G protein–regulated processes (48, 75, 93)—they would provide considerable flexibility to the regulation of the G-protein cycle.

Receptor-like kinase (RLK): a plasma membrane–localized protein that has an extracellular receptor domain and an intracellular protein kinase domain connected by a single transmembrane

1.3.5. Receptor-like kinases. There is mounting evidence that plant G proteins interact with the plant-specific receptor-like kinases (RLKs). Multiple genetic screens have identified specific RLKs that function in G protein–dependent pathways. A classic example is the identification of the *Arabidopsis* protein ERECTA as a Gβ suppressor (65). Additional experiments on plant–microbe interactions have also suggested the involvement of RLKs in G-protein signaling (2, 64, 116). This is tantalizing because *RLK*s represent one of the largest gene families in plants (approximately 600 genes in *Arabidopsis*) and are involved in sensing a variety of environmental, chemical, and developmental cues (28). **Table 1** lists the potential functions of receptor-like proteins (RLPs) known to interact with G proteins in plants. The roles of specific RLKs are discussed in more detail later in this review.

1.4. Missing G-Protein Components

Although G-protein core complexes in plants contain novel, unconventional proteins, they also lack a number of proteins known to be central to the G-protein cycle in mammals. These include enzymes such as adenylyl cyclases, regulator proteins such as β arrestins, GPCR kinases, and GEF activity–possessing GPCRs. The absence of these proteins reiterates the fact that G-protein networks are wired differently in plants and metazoan systems. Future research directed toward the

Table 1 Receptor-like proteins known to function with G proteins in plants

Receptor/receptor-like protein	Biological processes or pathways	Species	Reference(s)
GCR1	ABA signaling, early seedling development, stress responses, flavonoid biosynthesis	*Arabidopsis*	12, 82, 133
GTG1/GTG2	ABA signaling	*Arabidopsis*	84
RGS1	D-glucose sensing and signaling	*Arabidopsis*	62, 127
MLO2	Immunity	*Arabidopsis*	66
ER	Response to necrotrophic fungus	*Arabidopsis*	65
CLV/FEA2	SAM development, stem cell proliferation	Maize, *Arabidopsis*	9, 40, 41, 135
NFR1/NFR5	Nodule formation	Soybean	95
FER	Stomatal movement and salinity response	*Arabidopsis*	141, 142
CERK1, FLS2, EFR, BAK1, BIR1	Immunity	*Arabidopsis*	2, 64, 116, 118
FLS2/BIK1	Immunity	*Arabidopsis*	58, 59, 118, 131, 138
ZAR1	Zygote asymmetric cell division and daughter cell fate	*Arabidopsis*	140
BRI1/BAK1	Sugar-responsive growth and development	*Arabidopsis*	86

Abbreviations: ABA, abscisic acid; BAK1, Brassinosteroid-insensitive 1–associated receptor kinase 1; BIK1, *Botrytis*-induced kinase 1; BIR1, Brassinosteroid-insensitive 1–associated receptor kinase 1–interacting receptor 1; BRI1, Brassinosteroid-insensitive 1; CERK, Chitin elicitor receptor kinase 1; CLV, CLAVATA; EFR, Elongation factor Tu receptor; ER, ERECTA; FEA2, Fasciated ear 2; FER, FERONIA; FLS2, Flagellin sensing 2; GCR1, G protein–coupled receptor 1; GTG, G protein–coupled receptor type G protein; MLO2, Mildew locus O2; NFR, Nod factor receptor; RGS1, Regulator of G-protein signaling 1; SAM, shoot apical meristem; ZAR1, Zygotic arrest 1.

discovery of novel components and their signaling mechanisms will certainly uncover multiple new facets of this signaling module.

1.5. G-Protein Interactors and Effectors

After receptor-mediated activation, the G-protein subunits interact with various cytosolic effectors to transduce the signal. However, no well-characterized bona fide effector of plant G proteins is known with the mechanistic details of how it transduces the signal, except in a handful of instances. Potential G-protein interactors and effectors are listed in **Table 2**. The extant data are from various targeted or large-scale protein–protein interaction screens, which identify multiple classes of proteins, many of which could be effectors (15, 25, 49, 50, 55, 61, 76).

2. THE EXPANSION OF G-PROTEIN COMPONENTS IN PLANTS

One obvious difference noted early on between the plant and mammalian G proteins was in the numbers of each of the subunits. Humans have 23 Gα proteins, which are grouped into five different families depending on their biochemical characteristics, and together with the 5 Gβ and 12 Gγ proteins, they can form a multitude of different heterotrimeric combinations. In contrast, plants, such as *Arabidopsis* and rice, were reported to have only single *Gα* and *Gβ* genes. This had led to the hypothesis that plant G-protein networks are extremely limited and only a few heterotrimeric combinations can exist, depending entirely on the multiplicity of Gγ proteins (121, 124). However, the discovery of additional novel components that can constitute the core of the heterotrimer suggests that diversity of the plant proteins is also high. Moreover, the availability of massive sequence information confirms that the duplicated G-protein components are retained in plant genomes after recent whole-genome duplication events, predicting an expansion of and diversity in this signaling module that are vastly greater than what was expected just a few years back. For example, the allotetraploid soybean genome encodes 4 canonical and 12 extra-large Gα proteins, 4 Gβ proteins and 14 Gγ proteins (8, 79, 92, 102; S. Roy Choudhury & S. Pandey, unpublished data). Similar expansion of G-protein components has been seen in other plant species with recent genome duplications, such as wheat, *Brassica rapa*, and *C. sativa* (3, 79, 92; S. Pandey, unpublished data). Additionally, even though the multiplicity of G proteins in these species has arisen due to genome duplications such that the proteins are highly similar to each other in sequence and in biochemical properties, the limited amino acid substitutions that do exist provide for specific response regulation, as was reported for soybean Gα (94, 99). Because more than 70% of plant species are polyploid, this diversity should be considered a norm.

The second important development has been the identification of G-protein signaling components in the entire plant lineage, from green algae to higher angiosperms. The reported absence of sequence homologs of G-protein genes in some of the earliest sequenced green algae, such as *Chlamydomonas reinhardtii*, *Volvox carteri*, and *Chlorella vulgaris*, had led to the proposal that the algal lineages have lost G-protein genes. Even early land plants such as moss have only a few of the components, ostensibly as evolutionary leftovers. However, research during the past five years has confirmed that the complete repertoire of heterotrimeric G proteins is present in green algae, such as *Chara braunii* and other charophycean algae, and the proteins exhibit biochemical properties similar to the ones present in higher plants (33, 35). Furthermore, the missing Gα function in the moss *P. patens* is substituted by the XLG (34). A recent report also describes a possible sequence homolog of a Gα protein in *C. reinhardtii*, which is involved in regulating heat stress response (53). These observations reaffirm that G protein–mediated signaling operates in the entire plant lineage and is likely as prevalent and diverse as in metazoans.

Table 2 Proteins that interact with different components of heterotrimeric G proteins[a]

G-protein subunit	Interacting protein	Pathway/phenotype	Species	Reference(s)
Arabidopsis Gα (GPA1)	AtPirin 1	Interacts with a Pirin protein during early seedling growth and development	*Arabidopsis*	52
GPA1	Abscisic acid (ABA)-insensitive 1 (ABI1) and phospholipase Dα1 (PLDα1)	Interacts with ABI1 phosphatase and PLDα1 during ABA-dependent stomatal opening and closure	*Arabidopsis*	74
GPA1	Thylakoid formation 1 (THF1)	Interacts with THF1 in sugar signaling pathway	*Arabidopsis*	38
GPA1	Prephenate dehydratase 1 (PD1)	Interacts with PD1 during blue light–induced phenylalanine production	*Arabidopsis*	133
GPA1/*Arabidopsis* Gβ1 (AGB1)	PLDα1	Gα and Gβ both interact with PLDα1; PLDα1 also interacts with Regulator of G-protein signaling 1 (RGS1), and both proteins regulate the other's biochemical activity; PLDα1 and RGS1 also regulate GPA1's GTPase activity	*Arabidopsis*	97, 98, 146
GPA1/AGB1	Mitogen-activated protein (MAP) kinase and Receptor of activated protein C kinase 1 (RACK1)	G proteins interact with MAP kinases and RACK1 in activation of multiple defense and development pathways	*Arabidopsis*	19, 73, 105, 143
Rice Gα protein1 (RGA1)	Taihu Dwarf1 (TUD1)	Interacts with a ubiquitin ligase TUD1 to regulate brassinosteroid (BR) signaling	Rice	37
Pisum sativum Gα1 (PsGα1)	Phospholipase Cδ (PLCδ)	Interacts with PLCδ to regulate stress responses	Pea	75, 119
Triticum aestivum Gα (TaGa1)	Phosphoinositide phospholipase C1 (PI-PLC1)	Interacts with the calcium-binding protein Clo3 and a PI-PLC1	Wheat	48
Extra-large G protein (XLG)	Related to vernalization 1 (RTV1)	XLG2 interacts with RTV1 to control vernalization and flowering	*Arabidopsis*	36
XLG	Plant U-box protein 2 (PUB2) and PUB4	XLGs interact with E3 ligases PUB4 and PUB2 and function in cytokinin-dependent developmental processes	*Arabidopsis*	132
AGB1	N-MYC downregulated-like (NDL)	Interacts with NDL proteins to regulate auxin transport and root architecture	*Arabidopsis*	76
AGB1	Aci-reductone dioxygenase 1 (ARD1)	ARD1 acts as an effector of AGB1	*Arabidopsis*	25
AGB1	Bri-EMS suppressor 1 (BES1)	Interacts with BES1 to regulate BR signaling and cell elongation	*Arabidopsis*	145

(*Continued*)

Table 2 (*Continued*)

G-protein subunit	Interacting protein	Pathway/phenotype	Species	Reference(s)
AGB1	VirE2-interacting protein (VIP1)	Interacts with a bZIP protein VIP1	*Arabidopsis*	115
AGB1	Adaptor protein 3 μ (AP-3 μ)	Interacts with AP-3 μ to regulate ABA-dependent germination and postgermination plant growth	*Arabidopsis*	45
AGB1	Nonphototropic hypocotyl 3 (NPH3)	Interacts with a NPH3 to regulate phototropism	*Arabidopsis*	46
AGB1	B-box domain protein 2 (BBX2)	Interacts with BBX2 transcriptional activator to promote hypocotyl elongation	*Arabidopsis*	137
Camelina sativa Gβ (CsaGβ)	Patatin-like phospholipase III δ (pPLAIIIδ)	Gβ interacts with pPLAIIIδ to control cell and organ shape by affecting lipid metabolic pathways	*Camelina*	93

[a]The interactions between G proteins and receptors and the results of large-scale screens for protein–protein interactions are not included.

3. MECHANISTIC DETAILS OF G-PROTEIN SIGNALING IN PLANTS

In the mammalian paradigm, G-protein signaling begins with the signal perception at a GPCR, which activates the Gα protein by its GEF activity, i.e., it facilitates the exchange of GDP for GTP (**Figure 1**). This causes dissociation of the heterotrimer into active GTP-bound Gα and inseparable Gβγ. The proteins return to their trimeric conformation by the GTPase activity of Gα proteins, which is accelerated by GAPs, such as RGS (72, 90). Because many of the classic signaling components are missing in plants and because the plant Gα proteins have unique biochemical properties (42, 125), alterations to this broad outline are expected. Tremendous progress has been made in recent years to address both the activation mechanisms and the deactivation mechanisms of plant G-protein signaling, especially in the context of receptors that couple to plant G proteins.

3.1. Activation of the G-Protein Signaling Cycle in Plants

Canonical GPCRs have two defining features: (*a*) They are 7TM domain–containing proteins that interact with the G proteins, and (*b*) they possess GEF activity (104). Analysis of plant genomes, especially *Arabidopsis* and rice, identified many proteins with 7TM topology, reminiscent of GPCRs (30, 31, 66, 82, 84). Many of these proteins also interact with Gα, thereby fulfilling one of the two requirements for being a GPCR. Additionally, *Arabidopsis* GCR1 (and its rice homolog), which shares significant sequence similarity with the *Dictyostelium discoideum* GPCR CAR1, also acts with Gα in a subset of genetic pathways to regulate physiological responses (12, 82, 133, 139). Similarly, some variant proteins, such as GTG1 and GTG2, show similarities with GPCRs and interact with and function in the same genetic pathways as Gα, suggesting that these might couple specific signals of the G-protein cycle (84). However, none of these proteins exhibits GEF activity, the second and probably more important requirement for being a classical GPCR. Therefore, activation of plant G proteins represents one of the biggest mysteries in the field today, with three potential scenarios to account for it.

In the first scenario, canonical GPCRs possessing GEF activity exist in plants but have not yet been identified due to the lack of the exquisite biochemical assays available for the metazoan systems. Proteins with 7TM topology are notoriously difficult to purify in active form; proteins such as adenylyl cyclases, which provide a good readout of receptor activation in mammalian

systems, are absent in plants; and fast, millisecond-duration, cell-based assays for plant G proteins, receptors, and effectors are not well established. In this context, it is notable that at least some canonical plant Gα proteins can be activated by a classic GPCR system in a heterologous system. Complementation of the yeast *gpa1* mutant with a subset of soybean Gα proteins resulted in complete restoration of all growth and mating phenotypes (101). These pathways are controlled by a classical GPCR-mediated signal perception and transduction in yeast.

The second scenario is proposed based on the in vitro biochemical properties of the *Arabidopsis* Gα protein GPA1. Recombinant GPA1 has an extremely high on-rate of GTP binding coupled with very slow GTPase activity (an order of magnitude slower than the slowest mammalian Gα protein). These observations have led to the hypothesis that plant Gα proteins are self-activated, i.e., that they inherently exist in the GTP-bound, active form. This eliminates the requirement of a GPCR for their activation (25, 42, 125). If this is the case, then deactivation of the G-protein cycle in plants is the key mechanism to control signaling. Although some genetic and physiological data support this hypothesis, it is not known whether the hypothesis applies to all G proteins in all plant species. Biochemical characterization of XLGs is extremely limited, and, even though they can bind GTP, the rates of GTP binding or GTP hydrolysis are not known. Their interaction with the RGS proteins also remains to be unequivocally established.

The third and most plausible scenario is that proteins other than classical 7TM GPCRs fulfill this role in plants. Plant genomes code for a large family of single TM domain–containing RLKs and RLPs, which are known to perceive a variety of signals. There is growing evidence that plant G proteins are coupled with and are regulated by RLKs and RLPs during specific signal–response coupling (2, 9, 19, 58, 59, 64, 71, 85, 86, 95, 118, 138, 140, 142).

There are three distinct signaling pathways where some mechanistic details of the role of RLKs in influencing the G-protein cycle have been deciphered. Possibly the best characterized of these is the regulation of immune signaling pathways by G proteins in *Arabidopsis*, where an almost complete model of the activation of G proteins has been uncovered.

3.1.1. Regulation of G-protein signaling during immune responses in *Arabidopsis*. *Arabidopsis* G proteins interact with multiple immune response and defense-related RLKs and RLPs both at the biochemical/physical level and in functional/genetic pathways. These include chitin elicitor receptor kinase 1 (CERK1), BAK1, BAK1-interacting receptor 1 (BIR1), FLS2, and *Botrytis*-induced kinase 1 (BIK1) (2, 19, 58–60, 64, 71, 96, 111, 130, 138). The activation mechanism of G proteins has been demonstrated via FLS2, a key immune receptor that perceives bacterial flagellin (or its derived epitope, flg22). FLS2 interacts with additional RLKs, such as BAK1, and receptor-like cytoplasmic kinases (RLCKs), such as BIK1, to transduce the immune signal via a phosphorylation-based mechanism (63, 67). The activity and stability of BIK1 (and its homologs) have been shown to be central to the regulation of these responses (44, 56, 58, 59, 130, 131). The FLS2/BIK1 immune receptor complex also interacts with the *Arabidopsis* G-protein complex, including both canonical and extra-large Gα, Gβγ, and the RGS1 protein (58–60, 131).

As per the current model of the plant G-protein cycle (**Figure 2*a***), in the resting state, the heterotrimer composed of XLG2 (or XLG3 or Gα), Gβ, and Gγ1 or Gγ2 associates with FLS2 and BIK1. RGS1 also forms a part of this complex as it interacts with both FLS2 and Gα and maintains the G-protein complex in its inactive state via its GAP activity. Upon activation by flg22 binding, FLS2 forms an active complex with BAK1, its co-receptor, and activates BIK1 (or its homologs) (63, 67). Active BIK1 phosphorylates RGS1, which results in its dissociation from the FLS2 receptor complex. Another parallel mechanism suggests that, upon activation by flg22, BAK1 is activated, which can phosphorylate RGS1. Phosphorylated RGS1 undergoes endocytosis, causing its dissociation from the FLS2 complex (116–118). Removal of RGS1 from the complex

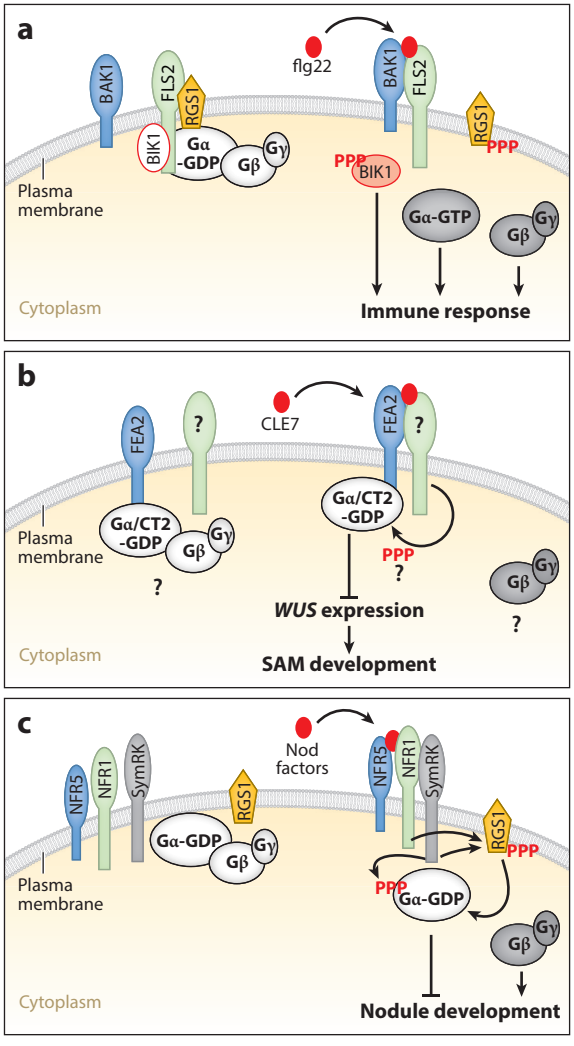

Figure 2

Mechanistic details of the G-protein cycle in plants. Regulation during (*a*) immune response in *Arabidopsis*, (*b*) SAM development in maize, and (*c*) nodule development in soybean. A receptor-like kinase–mediated regulation of the G-protein cycle seems to be more prevalent in plants. Abbreviations: BAK1, Brassinosteroid-insensitive 1–associated receptor kinase 1; BIK1, *Botrytis*-induced kinase 1; CLE7, CLAVATA3/Embryo surrounding region–related 7; CT2, Compact plant 2; FEA2, Fasciated ear 2; flg22, Flagellin 22; FLS2, Flagellin sensing 2; Nod factors, Nodulation factors; NFR, Nod factor receptor; RGS1, Regulator of G-protein signaling 1; SAM, shoot apical meristem; SymRK, Symbiosis receptor kinase; *WUS*, *WUSCHEL*.

releases Gα, which then spontaneously becomes GTP-bound and consequently dissociates from the Gβγ dimer (58, 59, 131). In this active form, the freed proteins interact with the downstream effectors to transduce the signal (**Figure 2*a***). This mechanism demonstrates for the first time that the RGS1-dependent GDP-bound versus GTP-bound forms of a plant Gα protein can determine the off or on states of signaling, similar to what is known for the mammalian G proteins. However, in contrast to the mammalian systems where activation is via the GEF activity of a

GPCR, in plants the activation is via derepression of a constitutively active Gα. Furthermore, the specificity of response regulation during immune signaling is achieved by specific subunit usage. Stomatal immunity is regulated by the activation of canonical Gα, GPA1, whereas the mesophyll immunity is regulated by the activation of XLG2/3 (59). Because BIK1 and its homologs are downstream components of many RLKs and because G proteins interact with a variety of RLKs and affect BIK1 stability, this may be a highly sophisticated, widespread mechanism involved in the regulation of many responses in plants (131, 138). Overall, this model explains the potential activation/deactivation by RLKs of G proteins in a guanine nucleotide–dependent manner in the context of the unusual biochemistry of plant G proteins.

Nodulation: the process of organogenesis (nodule formation) during leguminous plant and rhizobia symbiosis to fix atmospheric nitrogen in plant roots

3.1.2. Regulation of G-protein signaling during shoot apical meristem development in maize. A second example of the role of RLKs in regulating G-protein signaling is during SAM development in maize (**Figure 2*b***). SAM development in plants is controlled by a feedback loop between the CLV and WUSCHEL (WUS) signaling pathways (103). The CLV signaling module in *Arabidopsis* consists of an RLK, CLV1, an RLP, CLV2, and the small signaling peptide CLV3. A genetic screen in maize identified CT2 as an interactor of CLV2 (maize FEA2). Knocking down either *CT2* or *Fea2* resulted in increased SAM size. Using synthetic CLV3 peptide, it was shown that CT2 transmits the CLV-dependent signal to control shoot stem cell proliferation (9). Furthermore, the GTP-binding and exchange activities of CT2 are required for proper function. A constitutively active version of CT2, $CT2^{CA}$ (which exhibits no GTPase activity), when introduced in the *ct2* mutant background, acts as its weak allele (135). The XLGs of maize are also involved in the regulation of SAM size, both redundantly and independently of CT2. Even though the *xlg* triple mutants of maize are seedling lethal, the young seedlings showed normal SAM development; however, knocking down any two of the three maize *XLG* genes in the *ct2* background resulted in significantly larger SAM size, suggesting partial redundancy (135, 136). Incidentally, in *Arabidopsis*, the Gβ protein AGB1 is also involved in regulation of meristem size by its interaction with another CLV1-like receptor, RPK2 (40). These data confirm that specific RLKs are functionally linked with plant G proteins for the regulation of critical developmental programs. However, a clear activation mechanism of G proteins through these receptors is still lacking.

3.1.3. Regulation of G-protein signaling during nodule formation in soybean. A third example of the regulation of the G-protein cycle by an RLK was demonstrated during nodulation in soybean (**Figure 2*c***). Nodule formation is an important signaling and developmental event, which is rigorously controlled at multiple levels. Genetic analysis demonstrated that the soybean Gα proteins are negative regulators of nodule formation, whereas the Gβγ or RGS proteins are positive regulators (94). Nodulation signaling begins with the perception of rhizobial nodulation factors (Nod factors) by the Nod factor receptors 1 (NFR1s), a class of lysine motif (LysM)-containing RLKs (11, 69). NFR1 proteins (two in soybean) interact with the Gα and RGS proteins of soybean and can phosphorylate the latter. Phosphorylation promotes the GAP activity of RGS proteins, which helps maintain the Gα proteins in their inactive state and thereby allows for nodule formation (95) (**Figure 2*c***). The importance of RGS phosphorylation during nodule formation was corroborated by using a phosphomimic version of the RGS protein. Introduction of the phosphomimic RGS in a soybean mutant lacking an active NFR1 (*nod49*) resulted in partial restoration of nodule formation, implying that at least one role of activated NFR1 is to phosphorylate RGS proteins, thereby regulating the G-protein cycle (95). Our unpublished data show that another RLK involved in symbiosis, SymRK, also interacts with the NFR1/G protein/RGS complex (S. Roy Choudhury & S. Pandey, unpublished data). SymRK phosphorylates the Gα protein at a site that is important for its GTP-binding. Phosphorylated Gα can no longer bind GTP but is also

unable to interact with the Gβγ. The net effects of the phosphorylation of RGS and Gα proteins by two different RLKs are the unavailability of an active Gα protein and the availability of free Gβγ, both of which promote nodule formation. While this mechanism does not exactly address the activation of G proteins (because repression of Gα is required for nodule formation), it does uncover a novel, yet-unexplored mechanism for the regulation of the G-protein cycle by plant-specific RLKs.

Additional examples of the involvement of RLKs in G-protein signaling include the interaction of FERONIA (FER) with the *Arabidopsis* AGB1 during the control of stomatal aperture and during salinity response (141, 142), the interaction of BRI and BAK1 proteins with G proteins during sugar-responsive growth and development (86), and zygotic arrest 1 (ZAR1) and AGB1 interaction during asymmetric cell division in zygotes (140). However, the mechanistic details of these physiological observations or genetic interactions have not been fully established. Multiple additional RLKs have been shown to interact with different G-protein subunits, many of which can also phosphorylate G proteins or the RGS protein in vitro (116–118). It is an exciting possibility that G-protein activation/deactivation in plants is controlled by a phosphorylation/dephosphorylation-based mechanism. All plant G-protein subunits and RGS proteins are phosphoproteins, each with multiple potential phosphorylation sites (13, 117, 118; S. Pandey, unpublished data). This is certainly going to be an active area of research and may provide important clues to the signaling mechanisms of G proteins in plants. Furthermore, given the conservation of many G-protein phosphorylation sites between plant and mammalian proteins, deeper knowledge of plant processes may also help inform unexplored G-protein regulatory mechanisms in metazoan systems.

Finally, in the context of the previous discussion, should plant RLKs be considered GPCRs? If the defining feature of a GPCR is its ability to couple with the Gα protein and change its activity, then regardless of the topology of the proteins or their ability to facilitate GDP-to-GTP exchange, these single TM domain–containing RLKs certainly fulfill the criteria. Current evidence suggests that RLK phosphorylation–dependent activation/deactivation of the G-protein cycle is the norm for plant G-protein signaling mechanisms.

3.2. G-Protein Deactivation Mechanisms in Plants

Deactivation of the G-protein cycle is an intrinsic part of its signaling mechanism because the Gα proteins are GTPases. Efficient and precisely regulated GTP hydrolysis ensures that the G proteins are available for the next round of activation by a receptor, allowing for sustained signaling. Because the GTPase activity of Gα proteins is significantly slower than the GDP-to-GTP exchange rate, the role of the GAPs in keeping the cycle synchronized becomes central to its efficient functioning. RGS proteins and specific phospholipases represent proteins with GAP activity.

3.2.1. Regulator of G-protein signaling proteins in plants. The unique 7TM RGS proteins of plants were the first GAPs identified that could accelerate the GTPase activity of plant Gα proteins (18). Their biochemical activity has been verified using in vitro assays where purified RGS domain can increase the GTPase activity of Gα proteins by at least an order of magnitude (18, 102). Furthermore, *rgs1* null mutants of *Arabidopsis* exhibit the expected opposite phenotypes compared with the *Arabidopsis gpa1* null mutants in multiple hormonal and developmental signaling pathways (17, 42, 127). Similarly, knockdown of Gα or RGS proteins in soybean hairy roots leads to opposite phenotypes during nodule development (95). These data confirm the in planta role of RGS proteins as deactivators of G-protein signaling. The regulatory role of RGS proteins as GAPs for Gα proteins has also been established during immune signaling in *Arabidopsis*, as discussed previously.

The critical role of RGS proteins during regulation of the G-protein cycle implies that all species that possess Gα proteins also possess RGS proteins. This relationship holds true for all metazoans, and evolutionary analysis suggests that there is a direct correlation between the number of Gα proteins and the number of RGS proteins in any given species (1). While this correlation also holds true for the basal plants, including green algae, bryophytes, gymnosperms, basal angiosperms, and all eudicots, many monocot plants are an exception to this rule. All monocots analyzed to date have one or more Gα protein homologs in their genome, but many monocots, especially widely studied reference organisms, such as rice, maize, *Brachypodium distachyon*, sorghum, and wheat, lack an RGS homolog (32, 124). Given the critical roles of RGS during regulation of G-protein signaling, this discovery was surprising.

A series of earlier studies analyzing a limited number of plant species proposed that monocots, with the exception of *Setaria viridis*, do not possess an RGS homolog in their genomes. These studies also suggested that the *RGS* gene was lost in the monocot lineage due to evolutionary coadaption of specific amino acids in the Gα and RGS proteins (122, 125). As extensive genomic and biochemical data have accumulated, it is clear that this early hypothesis was not supported.

Detailed evolutionary analysis of RGS-encoding genes from all available plant genomes confirmed that plants representing all monocot orders possess an RGS-encoding gene, but the gene is lost from many species. The loss is apparently random and likely happened multiple times during evolution (32). Detailed analysis of the amino acid sequences of the Gα and RGS proteins from multiple monocot and dicot species found no correlation between the presence of specific amino acids and their coevolution in RGS:Gα protein pairs. In fact, the interaction interface between the RGS:Gα protein pairs is conserved across kingdoms (32). Under in vitro conditions, the RGS protein from plants can accelerate the GTPase activity of human Gα protein and vice versa. Although it is not known why selection pressure on *RGS* genes is relaxed in some monocots, whenever the proteins are present, they are biochemically functional (32). Furthermore, the Gα proteins from plants lacking an inherent RGS protein (e.g., rice, maize, *B. distachyon*) can still interact with and be deactivated by RGS proteins from other species. Thus, the availability and analysis of extensive new data fail to support the hypothesis of adaptive coevolution between these proteins (32).

3.2.2. Phospholipases as GTPase activity–accelerating proteins for Gα proteins. The absence of RGS proteins in certain plant species and the importance of GAP activity for plant Gα proteins suggests that other proteins must take over the GAP function. True orthologs of PLCβ proteins, the other established GAPs from mammalian systems, are not found in plants. However, recent work suggests that another group of plant-specific phospholipases, the PLDα proteins, can act as GAPs in plants. *Arabidopsis* PLDα1, the most highly expressed member of the PLDα family, interacts with and accelerates the GTPase activity of GPA1 (80, 96, 146). All plants possess multiple PLDα homologs, which show unique expression patterns and involvement in specific signaling and development pathways. The diversity of PLDα proteins may offer corresponding diversity in the response regulation that is mediated by G proteins. Moreover, plants also possess additional families of phospholipases, many of which interact with Gα and/or Gβ proteins (24, 29, 48, 75, 93). Although the roles of most phospholipases as GAPs for Gα have not yet been explored, it is conceivable that if such a regulation is not restricted to the PLDα family, multiple cell-specific, tissue-specific, or signal-dependent regulatory modules comprising Gα and phospholipases can exist in plants and expand the diversity of regulatory modes even further.

3.2.3. Interaction between the regulator of G-protein signaling and phospholipase Dα1 proteins. Recent research in *Arabidopsis* shows that the two GAPs of its Gα protein,

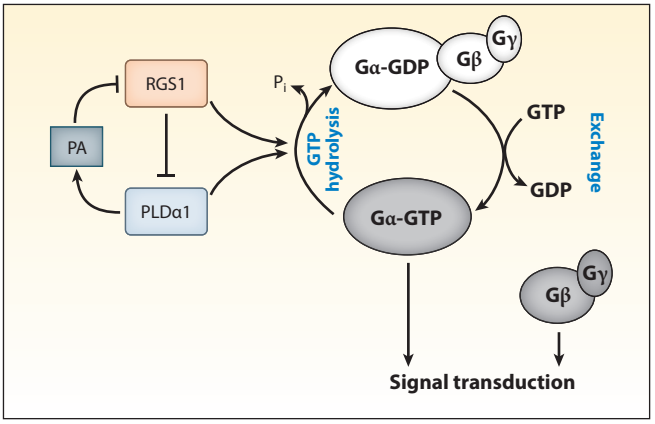

Figure 3

Deactivation of the G-protein cycle by the concerted activities of RGS1 and PLDα1 in *Arabidopsis*. Both RGS1 and PLDα1 act as GAPs for the Gα protein GPA1. RGS1 inhibits the phospholipase activity of PLDα1, whereas PA, the main product of PLDα1 activity, binds with and inhibits the GAP activity of RGS1. Abbreviations: GAP, GTPase activity–accelerating protein; GPA1, *Arabidopsis* Gα; PA, phosphatidic acid; PLDα1, phospholipase Dα1; RGS1, Regulator of G-protein signaling 1. Figure adapted with permission from Reference 98.

RGS1 and PLDα1, exhibit genetic and biochemical interactions and function to fine-tune signal–response coupling (80, 81). Such regulation offers another level of precise control of the G-protein cycle. Both RGS1 and PLDα1 interact with GPA1 and accelerate its GTPase activity. The two proteins also interact with each other in a double negative regulatory loop (**Figure 3**). RGS1 interacts with PLDα1 and inhibits its phospholipase activity (97). Moreover, phosphatidic acid, the key product of PLDα1 phospholipase activity, binds with and inhibits the GAP activity of RGS1 (98). These physical and biochemical interactions regulate specific physiological responses. Such complex interactions are thought to control the availability of free, active Gα protein with the utmost precision, thereby defining the amplitude and duration of the G-protein cycle and providing for specificity of response regulation. Again, if additional phospholipases were to work in a similarly integrated manner, it would provide enormous plasticity to the modulation of plant G-protein signaling (81, 85, 112). As has been suggested, if the deactivation of Gα proteins is more critical in plants than in animals due to their self-activation, multiple mechanisms may exist for fine-tuning this phase of the G-protein cycle.

3.3. Additional Regulatory Mechanisms of G Proteins

Along with the mechanisms described previously, e.g., phosphorylation-dependent regulation of RGS protein and its ability to control the G-protein signaling in plants (116–118), recent work points to the existence of additional regulatory modules. The involvement of mitogen-activated protein (MAP) kinases is central to mammalian and yeast G-protein signaling pathways, and, in plants, several MAP kinases have been identified as interactors or regulators of G proteins (7, 73, 105, 143). During defense signaling in response to a specific protease, an unknown receptor signals through G proteins and MAP kinases in *Arabidopsis* (19). Various protein phosphatases also must be involved in these regulatory processes, dephosphorylating the G-protein subunits or RGS proteins. Plants possess multiple protein phosphatases (more than 100 in *Arabidopsis*) and two of

them, ABI1 and PP2C52, have been reported to interact with G-protein subunits and to have a role during the regulation of specific signaling pathways (6, 74, 114).

Recent evidence also points to the role of ubiquitination during G-protein signaling. In immune signaling via the FLS2 receptor, the G proteins directly interact with two plant U-box proteins, PUB25 and PUB26, which mark the BIK1 protein for degradation via the proteasome pathway. G proteins inhibit the activity of PUB25/PUB26, thereby stabilizing BIK1, which is central to the activation of immune signaling (131). The XLGs also interact with two additional E3 ligases, PUB2 and PUB4, and regulate cytokinin signaling in *Arabidopsis*, especially during the development of stamens and tapetum (132). Another regulatory mechanism has been explored in the context of D-glucose-mediated RGS protein internalization in *Arabidopsis*, similar to what is reported for the mammalian GPCRs (127). These and potentially additional regulatory modes are only beginning to be explored and will certainly provide many new exciting research opportunities in the future.

4. AGRONOMIC IMPORTANCE OF PLANT G PROTEINS

Given the importance of G proteins in regulating almost all aspects of growth, development, and response to biotic and abiotic stresses, it seems likely that they control important agronomic traits. Indeed, there are multiple examples of a direct effect of G proteins in controlling yield.

As mentioned previously, the rice homologs of the *Arabidopsis* group III Gγ protein AGG3 were independently identified as major quantitative trait loci (QTLs) for panicle branching, seed size, and seed number by classical genetic methods. Rice dense and erect panicle 1 (DEP1) controls panicle branching and erectness, thereby directly affecting yield (39, 51). Similarly, multiple naturally occurring mutations in grain size 3 (*GS3*) modulate rice grain length and could explain about 79% of the variation between short-grain versus long-grain varieties (22, 70). In fact, the *AGG3* gene itself was also identified as an organ size regulator in a genetic screen in *Arabidopsis*, independent of its discovery as a Gγ protein (57). Targeted experiments using *Arabidopsis* plants overexpressing or lacking *AGG3* exhibited larger or smaller floral organs, respectively (16, 57). Overexpression of *AGG3* in *C. sativa*, an important oil seed crop, resulted in early and prolonged flowering and a significant increase in branching, seed number, and seed size, leading to an impressive increase in overall yield (100).

Based on the positive correlation observed between the *AGG3* expression level and improved yield in *Arabidopsis* and *Camelina* and unique agronomic traits of various naturally occurring *GS3* or *DEP1* alleles in rice, it was expected that type III Gγ proteins would positively regulate grain yield. However, in contrast to dicots, the regulation of different phenotypes in the monocot lineage seems to be more complex. Although the *GS3* gene is responsible for grain size determination, different mutations in the same gene result in shorter or longer grains. Furthermore, the environmental effect is huge; depending on growth conditions, larger or smaller seeds as well as higher or lower yields have been reported for rice plants expressing different alleles of *GS3* (10, 22, 70, 108). Targeted overexpression of *AGG3* in *S. viridis* (a monocot reference organism) also revealed that the effect of this gene on seed size and number is highly affected by growth conditions (47).

Similar to the situation with *GS3*, *DEP1* also has a complex role in controlling panicle branching or erectness because different naturally occurring mutations result in distinct phenotypes. Transgenic *Setaria* plants overexpressing *AGG3* did not show a significant change in either branching or panicle erectness under greenhouse growth conditions (47), although overexpression of the same gene in *Camelina* did result in significantly more branching (100). Similarly inconsistent results were obtained in a long-term field study in barley and in wheat, suggesting a complex genotype and environmental effect on the function of this protein (108, 129, 134). The lineage-specific

regulation of G-protein pathways in plants is an active area for future research. Interestingly, the same *DEP1* gene, which is responsible for panicle branching, density, and erectness, was also identified as a major QTL for NUE in rice (107). *Setaria* plants overexpressing *AGG3* did exhibit better growth in low-nitrogen conditions during early development (47).

The unusual nature of the group III Gγ proteins and their roles in affecting important agronomic traits also raise the question of whether the regulation is controlled by the G-protein cycle or whether regulation is an independent function of these proteins. During regulation of NUE, the role of DEP1 depends on the G-protein cycle, although—unusually in this case—DEP1 also interacted with rice Gα in addition to its usual interaction partner, Gβ (107, 108).

A recent report demonstrating the role of maize Gα in SAM development directly confirms the importance of G-protein activity in regulating critical agronomic traits. Maize *ct2* mutant plants expressing $CT2^{CA}$ exhibited phenotypes of a weak *ct2* mutant allele. This resulted in several useful traits, such as erect leaves, higher spikelet density, and increased kernel row number in the transgenic plants without the negative effects of a strong *ct2* allele, which results in fasciated ears and extremely dwarf plants (41, 123, 126, 135). It can be envisioned that by engineering specific alleles of *Gα* (and potentially *XLG* genes) exhibiting precisely modulated GTP-binding or GTP-hydrolysis activities one can produce crops with desirable traits.

5. UNANSWERED QUESTIONS AND FUTURE DIRECTIONS

Significant progress has been made during the past few years to understand G-protein signaling mechanisms in plants and the roles G proteins have in affecting critical agronomic traits. G-protein research has entered a fascinating phase where the key players have been established, critical pathways have been identified, and mechanistic details are beginning to emerge. There remain many unanswered questions, which are sure to keep researchers busy for years to come.

One pivotal question has to do with the difference in G protein–regulated pathways in monocots versus eudicots, with the caveat that data are available from only a handful of species. Although G-protein sequences from different plant species are highly similar and the proteins exhibit similar biochemical properties in vitro, it is obvious that their regulation is vastly different. Single, double, or higher-order mutants in G proteins in *Arabidopsis* (and potentially other eudicots) exhibit differences in growth and development but are viable and fertile. Their overall architecture is unchanged. In contrast, monocot plants lacking a functional canonical *Gα* gene are severely dwarf with significantly altered architecture. Furthermore, monocots lacking the *Gβ* gene (complete nulls) or all *XLG* genes are seedling lethal, a phenotype not observed in dicot *Gβ* or *XLG* mutants. Moreover, in the moss *P. patens*, G proteins are required for completion of the life cycle. A moss lacking *XLG* or *Gβ* exhibits slower growth and reduced elongation and never forms sporophytes, which is the only diploid stage of the life cycle. The reasons for these altered phenotypes and their link to specific plant lineages remain completely unknown.

Similarly, why the RGS proteins are under relaxed selection pressure in one particular plant lineage is not known. Extensive data mining and evolutionary analysis have failed to determine any particular pattern of loss for this protein in specific plants. While it is possible that other proteins can substitute for the RGS protein function, it does suggest altered or rewired regulation of the G-protein cycle in these plants. In *Arabidopsis*, RGS1 interacts with and regulates PLDα1 activity. PLDα1 homologs are present in all plants; so how the regulatory circuit involving RGS1/Gα and PLDα is wired in plants lacking an RGS homolog remains to be investigated.

Incidentally, the differences in the phenotypes of the G-protein mutants between dicot and monocot plants are not related to the missing *RGS* gene in the examined monocot plants (rice, maize). The phenotype of *Gα* mutants from plants such as rice, maize, and *Brachypodium*, which

do not have *RGS* in their genomes, is identical to the *Gα* mutant from *Setaria*, a monocot plant with an *RGS* gene in its genome (N. Bhatnagar, A. Vijayakumar, D. Hackenberg & S. Pandey, unpublished data).

The activation mechanisms of G proteins remain to be established for additional pathways. The roles of protein kinases and phosphatases and proteasome-mediated degradation pathways are only beginning to be explored. As more and more mechanistic information becomes available, it will certainly lead to precise genome editing of agronomically important plants for optimized growth under abiotic or biotic stress conditions and also to improved yield under changing climate conditions.

SUMMARY POINTS

1. G-protein complexes in plants comprise both conventional and plant-specific variants.
2. G-protein complexes span the entire plant lineage, from green algae to angiosperms, and are significantly more expansive and diverse than what was previously proposed.
3. The ways in which G proteins influence plant growth and development differ greatly between evolutionarily distinct plant groups.
4. The activation and deactivation mechanisms of plant G proteins may be different from the established paradigm in metazoan systems.
5. There is mounting evidence that receptor-like kinases may acts as receptors in G protein–regulated pathways in plants.
6. Phosphorylation and dephosphorylation have emerged to be the key G-protein regulatory mechanisms in plants.
7. G proteins control many agronomically important traits and may be key targets for future molecular breeding needs.

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Errata

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