Trends in Plant Science

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Feature Review

Moving beyond the arabidopsis-centric view of G-protein signaling in plants

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Heterotrimeric G-protein-mediated signaling is a key mechanism to transduce a multitude of endogenous and environmental signals in diverse organisms. The scope and expectations of plant G-protein research were set by pioneering work in metazoans. Given the similarity of the core constituents, G-protein-signaling mechanisms were presumed to be universally conserved. However, because of the enormous diversity of survival strategies and endless forms among eukaryotes, the signal, its interpretation, and responses vary even among different plant groups. Earlier G-protein research in arabidopsis (*Arabidopsis thaliana*) has emphasized its divergence from Metazoa. Here, we compare recent evidence from diverse plant lineages with the available arabidopsis G-protein model and discuss the conserved and novel protein components, signaling mechanisms, and response regulation.

Highlights

Heterotrimeric G-proteins are key conduits that connect signal perception by receptors to their cognate effectors in eukaryotic cells.

Extensive research in animals and fungi has established a common mechanistic model of G-protein signaling, which has been extended to plants, using arabidopsis (*Arabidopsis thaliana*) as a representative species.

Several inherent knowledge gaps in the proposed mechanisms and recent information from other plant species necessitate redefining this model and moving beyond the established arabidopsis-centric paradigm.

A brief history of G-protein signaling in plants

Initial studies of plant G-protein signaling were inspired by mammalian and yeast G-protein research and used pharmacological compounds, such as GTPase inhibitors, GTPγS, and cholera and pertussis toxin, to observe their effects on plant responses [1,2]. Although many of these chemicals led to a response, their lack of specificity, stability, and uptake by plants remained a concern [3–11]. Molecular cloning of genes coding for the Gα and Gβ proteins from arabidopsis (*Arabidopsis thaliana*), rice (*Oryza sativa*), tomato (*Solanum lycopersicum*), and maize (*Zea mays*) proved the existence of heterotrimeric G-protein components in plants [12–17]. The functional characterization of G-proteins by genetic means began with identification of the Gα loss-of-function mutant (*d1*) in rice and its potential role in gibberellic acid (GA) signaling [16,18–20]. The first arabidopsis G-protein mutants were isolated during the early 2000s [12,13]. The availability of unparalleled genetic resources in arabidopsis allowed for elaborate characterization of G-protein signaling in this species, which laid the foundation for the development of a G-protein-signaling model in plants, somewhat distinct from metazoan systems [21–35].

Classic G-protein-signaling model in arabidopsis

Signaling pathways regulated by heterotrimeric G-proteins, their cognate receptors, regulators, and effectors have been elegantly described in metazoan systems (Box 1 and Figure 1). The arabidopsis genome encodes one canonical Gα (GPA1), one Gβ (AGB1), and two canonical Gγ (AGG1, AGG2) proteins, implying a markedly reduced repertoire of G-proteins in plants. Similarly to mammalian Gα proteins, GPA1 is catalytically active and binds/hydrolyzes GTP; Gβ and Gγ proteins are obligate dimers; and the regulator of G-protein signaling (RGS) protein accelerates the GTPase activity of the Gα-protein [24,36]. The 3D structure of GPA1 almost fully overlaps with that of a human Gα protein, even though the proteins share relatively little sequence similarity [37]. Furthermore, the interaction of a human RGS protein can accelerate the GTPase activity of GPA1 and vice versa [36]. In a multitude of signaling and development pathways, loss-of-function mutants of arabidopsis genes encoding Gα, Gβ, and Gγ exhibit

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Box 1. G-protein-signaling mechanism

The Gα, Gβ, and Gγ subunits of heterotrimeric G-proteins, along with a guanine nucleotide, constitute a molecular switch that transduces environmental and hormonal signals from the GPCRs to effector proteins. In the resting stage, the Gα protein is GDP bound and remains associated with the Gβ and Gγ subunits. Signal perception by a cognate GPCR causes a change in its conformation so that the bound GDP is released and Gα binds to GTP. GTP-bound Gα dissociates from the Gβγ dimer; thus, both these components (Gα and Gβγ) are free to bind to their effector proteins, resulting in signal propagation. The inherent GTPase activity of Gα hydrolyzes the bound GTP to GDP, resulting in its association with the Gβγ and reconstitution of the heterotrimer. In addition to this simple switch-like on–off mechanism, G proteins also act as molecular timers because specific stages determine the speed and amplitude of signal propagation. Guanine nucleotide disassociation inhibitor (GDI) proteins (e.g., Gβγ dimers) inhibit the rate of GDP release from Gα, whereas guanine nucleotide exchange factors (GEFs), such as GPCRs, regulate the rate of GDP/GTP exchange. The regulator of G-protein signaling (RGS) and specific phospholipase C (PLC) enzymes accelerate the rate of GTP hydrolysis and are known as GTPase-activating proteins (GAPs). GDI, GEF, and GAP proteins have a vital role in fine-tuning the signal propagation.

similar (Gα-mediated signaling) or opposite (Gβγ-mediated signaling) phenotypes [38]. The phenotypes of plants lacking *GPA1* are generally opposite to those of plants lacking *RGS1*, as expected based on the role of RGS as a GTPase activity-accelerating protein (GAP). G-protein-coupled receptor 1 (GCR1), a protein that shows some similarity to non-plant G-protein-coupled receptors (GPCRs), interacts with GPA1 and is involved in the regulation of G-protein-dependent pathways [39]. These observations suggested that the basic framework of the heterotrimeric G-protein core has remained largely unchanged during more than 1 billion years of evolution.

In vitro biochemical characterization of the Gα proteins from arabidopsis (and a few other plant species) demonstrated that these have exceptionally fast GTP binding, coupled with very slow

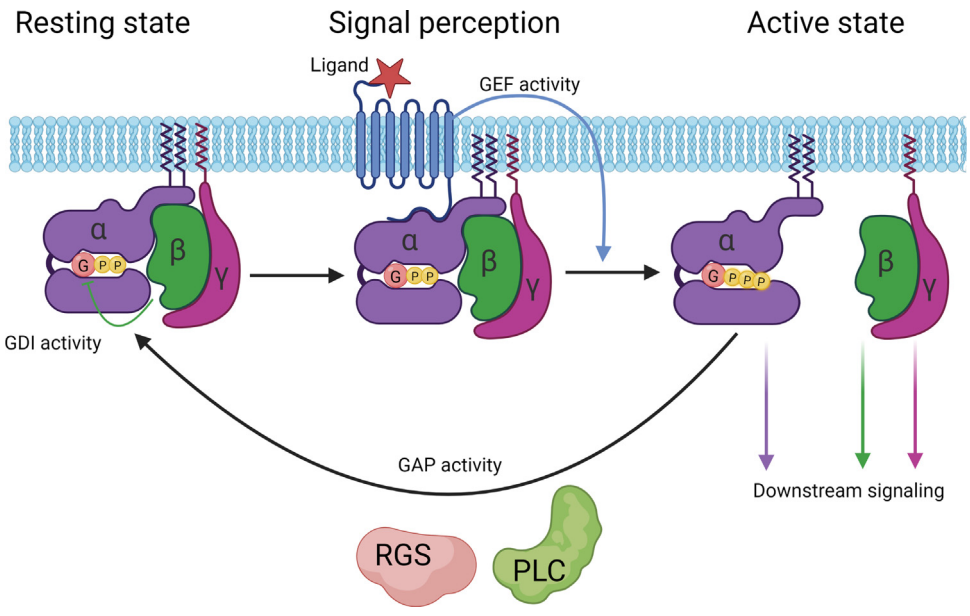

Figure 1. Classic G-protein signaling mechanism. The G-protein heterotrimer, comprising one subunit each of the Gα, Gβ, and Gγ proteins, switches between the inactive and active forms depending on the nucleotide-binding status of Gα. GDP to GTP exchange on Gα causing its activation is facilitated by ligand binding to a cognate G-protein-coupled receptor (GPCR), which acts as a guanine nucleotide exchange factor (GEF). Inherent GTP hydrolysis by Gα is aided by the GTPase activity-accelerating proteins (GAPs), such as regulator of G-protein signaling (RGS) and specific phospholipase C (PLC). Figure created using BioRender (biorender.com).

GTPase activity (an order of magnitude slower than the slowest mammalian Gα) [36,40–42]. Based on the quantification of GTP binding, GTP/GDP exchange, and GTP hydrolysis rates of GPA1, it was proposed that plant Gα proteins are inherently GTP bound, that is, they are self-activated and, thus, do not require a guanine nucleotide exchange factor (GEF) activity-possessing GPCR [40–42]. These studies also proposed that GTPase activity is the rate-limiting step of the plant G-protein cycle (opposite to mammalian models, where GDP–GTP exchange is the rate-limiting step); consequently, the GAP activity of RGS1 is the central regulator of plant G-protein-signaling pathways. A four-state model has been proposed to explain the RGS1-dependent regulation of dynamic signal inputs in arabidopsis [43].

However, the extrapolation of this arabidopsis-centric model to other crop species has yielded many surprises. We now know that the presence of specific components, the complexity of signaling networks, and their potential regulation and usage vary significantly within different plant groups, necessitating modification of this existing model.

Expanse, diversity, and loss of G-protein constituents in plants

One of the stark differences observed during the early days of plant G-protein research was the apparent paucity in the number of core G-protein components. The fully sequenced genomes of arabidopsis and rice have genes encoding only one Gα and one Gβ protein each, which was generalized to be the situation in the entire plant lineage [44]. Furthermore, the rice genome lacks an RGS-coding gene, which was extended as a general rule to differentiate the G-protein repertoire in monocots (without RGS) versus eudicots (with RGS) [45]. Work in recent years demonstrated that both these generalizations were myopic and do not represent the true picture of G-protein components in plants.

The diversity of G-protein components in plants is attributed to the presence of several unique variants of G-protein subunits and their multiplicity [24]. These include variants of the canonical Gα protein, the extra-large Gα (XLGα), and of the Gγ proteins, the type I, type II, and type III Gγ (also known as type/group A, B, and C Gγ, respectively) (Figure 2). The XLGα proteins, as the name suggests, are larger Gα proteins and have a 300–500 amino acid N-terminal extension

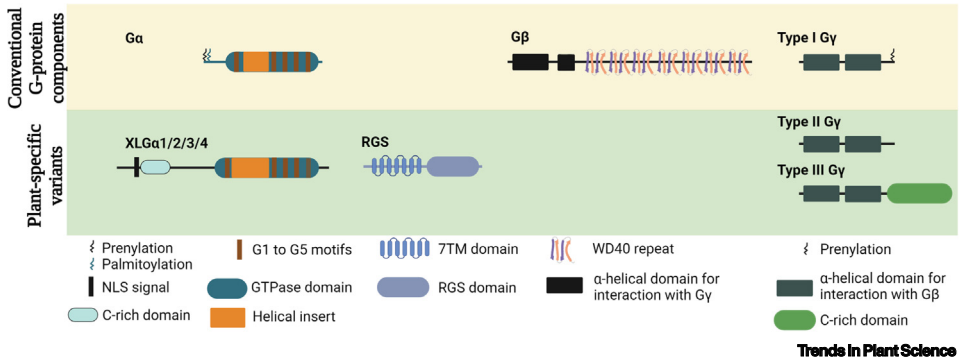

Figure 2. Canonical and plant-specific G-protein components. Plant Gα proteins share several conserved features with mammalian Gα, such as myristoylation and palmitoylation sites for membrane anchoring, the GTPase domain containing G1–G5 motifs and a helical insert between the G1 and G2 motif. XLGα1, XLGα2, XLGα3, and XLGα4 are plant-specific variants of canonical Gα. XLGα are large proteins with a nuclear localization signal (NLS) and cysteine-rich (C-rich) domain. The plant regulator of G-protein signaling (RGS) proteins have a seven-transmembrane (7TM) domain, attached to the RGS domain. Only canonical Gβ are present in plants, which have an α-helical domain, used for interaction with Gγ, and seven WD40 repeats. Type I Gγ are canonical proteins with an α-helical domain for interaction with Gβ, and a prenylation site for membrane anchorage. Plant-specific variants include type II and type III Gγ. Type II Gγ is similar to type I but lacks the prenylation motif, while type III Gγ has acquired a C-terminal C-rich domain. Figure created using BioRender (biorender.com).

fused with a Gα-like domain. The proteins are distinct from the extra-large Gα proteins found in mammals, which are a result of the alternative splicing of a Gα-coding gene [46,47]. Plant *XLGα* proteins are coded by distinct genes and may be nuclear localized [48]. In contrast to canonical Gα proteins, XLGα proteins are present in multiple copies in most diploid plant species; for example, arabidopsis and rice have one canonical Gα but three and four XLGα proteins, respectively [27,49–52]. Although XLGα proteins were identified in arabidopsis during the early days of G-protein signaling using biochemical approaches [53,54], the focus on following the metazoan G-protein model hindered their acceptance as core trimeric G-proteins. This was also aided by their proposed nuclear localization, an altered GTPase domain lacking a few amino acids identified to be critical for G-protein activity based on metazoan studies [43,55], and subtle developmental phenotypes of the complete loss-of-function mutants in arabidopsis, even though specific phenotypes, such as abscisic acid (ABA) responsiveness and root phenotypes of *xlg* mutants, were similar to those of *agb1* mutants [48,56]. The first concrete evidence of XLGα proteins working with the Gβ protein came from the reference moss species, *Physcomitrium* (formerly *Physcomitrella*) *patens*. This moss presented a unique opportunity because it does not have a canonical Gα protein and, thus, enables evaluation of the role of XLGα without the confounding effects of having two types of Gα protein interacting with the same Gβ. *P. patens* mutants lacking the *XLGα* or *Gb* gene shared similar phenotypes: they grew slower, their gametophytes did not elongate as much as those of wild-type (WT) moss, and they did not form sporophytes [57].

Gγ proteins in plants are diverse and classified into three groups: type I, which are the canonical metazoan-type Gγ; type II, which are similar to type I but lack the C-terminal prenylation motif found in all type I Gγ; and type III, which are found only in vascular plants [58]. The type III Gγ are modular proteins with an N-terminal Gγ domain with a C-terminal cysteine-rich extension of 75–500 amino acids [59–61]. Although only one type III Gγ is present in arabidopsis (AGG3), most plants have multiple copies of these proteins (e.g., DEP1, GS3, and GGC2 in rice). In plants with multiple type III Gγ proteins, the Gγ domain is of similar length, whereas the C-terminal cysteine-rich region can vary in length [62]; for example, the rice proteins GS3, GGC2, and DEP1 have 117, 223, and 306 amino acid-long C-terminal regions, respectively [63,64]. The diversity is further magnified because many plant species are recent polyploids and maintain multiple copies of G-protein components in their genome (i.e., no significant losses occurred during allopolyploidy), resulting in elaborate plant G-protein complexes. For example, the soybean genome has 16 Gα (four canonical and 12 XLGα), four Gβ, and 14 Gγ proteins, which may give rise to 896 different trimeric combinations [58,65]. Just in terms of subunit numbers, it is similar to the human genome, which encodes 23 Gα, five Gβ, and 12 Gγ proteins [66,67].

These nonconventional G-proteins regulate diverse signaling and development pathways, with varying degrees of functional overlaps with the canonical G-protein components. For example, in arabidopsis, using quadruple mutants lacking the three *XLGa* and the canonical *Ga* genes, it has been demonstrated unequivocally that the XLGα proteins primarily mediate plant immune responses, while canonical Gα regulates most developmental phenotypes, with varying degrees of functional overlap [27,68]. Different Gγ proteins also regulate distinct responses; that is, type I and II Gγ proteins typically mediate biotic stress responses, while type III Gγ proteins are primarily involved in modulating abiotic stress responses and grain size control [60,64,69–72].

One point that is debated in the field is whether these expanded networks due to duplicated genes truly add to the diversity of signaling mechanisms. The multiplicity that has emerged from recent genome duplications results in very similar proteins. For example, the four soybean Gα proteins are more than 90% identical at the protein sequence level [65]; however, they exhibit

differences in their rates of GTP binding and hydrolysis [65,73], which is reflected in their biological function. Knockdown of one subgroup of soybean Gα (GmGα 2 and 3) led to stronger nodulation phenotypes compared with the other subgroups (GmGα 1 and 4) [74]. Furthermore, cross-complementation of arabidopsis and yeast *gpa1* mutants with the four soybean Gα proteins showed that GmGα 2 and 3 were able to restore the arabidopsis *gpa1* mutant phenotype [75], whereas GmGα 1 and 4 were able to restore the yeast *gpa1* mutant phenotypes [76]. This suggests that, although recent, such duplications can provide selectivity to the protein function. The ability of soybean GmGα 1 and 4 to complement the yeast *gpa1* mutant also implied that they could be activated by a canonical GPCR system, which was not considered a possibility based on complementation studies with arabidopsis GPA1 [76].

Another unique aspect of plant G-proteins is the loss of specific core components in different lineages. Initial studies in arabidopsis identified all proteins of the heterotrimer, RGS1, and one GPCR-like protein (GCR1), which implied a one-to-one relationship with the metazoan proteins, albeit in a significantly reduced quantity [44]. *P. patens* was the first species identified that did not have a Gα and RGS homolog, but a functional Gβ protein [57], suggesting that the constituents and signaling mechanisms do not necessarily follow the signaling models proposed based on studies in arabidopsis.

A detailed evolutionary analysis of G-proteins throughout the plant lineage has confirmed this hypothesis (Figure 3). It is now established that many green algae, especially Chlorophyta, have lost all G-protein components and the presence of G-proteins in algal lineages is sporadic [77]. This was unexpected because, due to their presence in all opisthokonts, this signaling complex

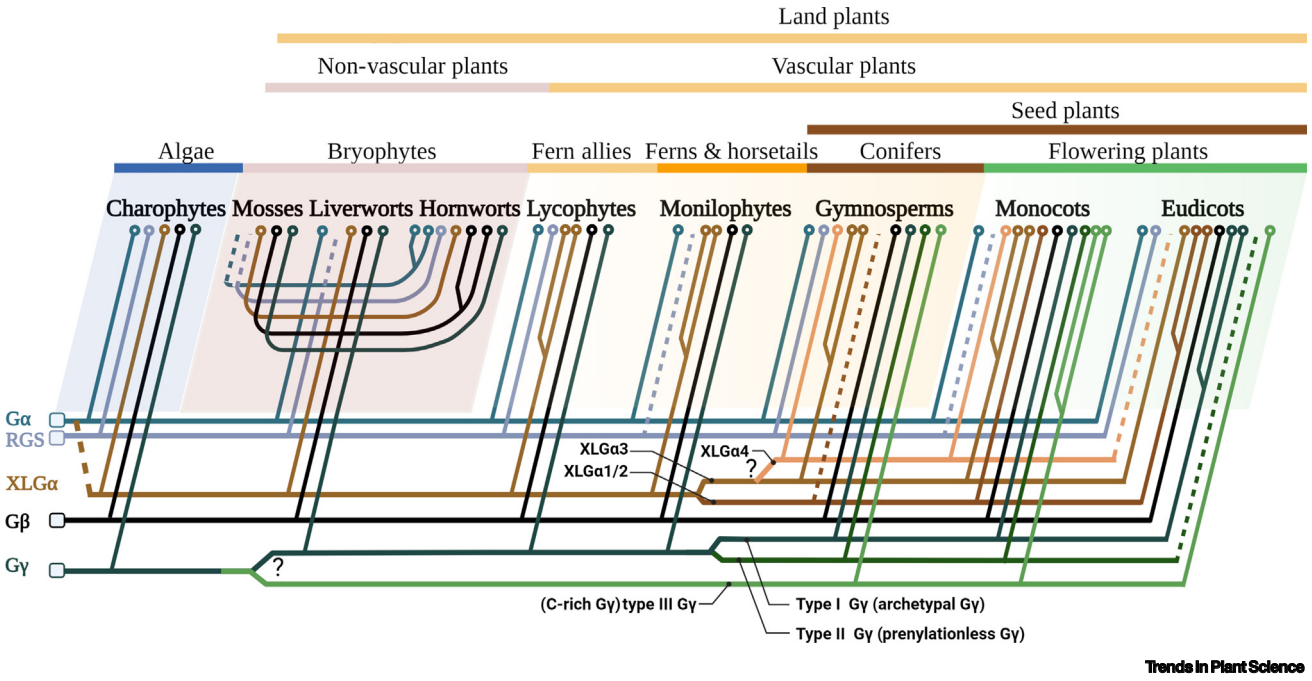

Figure 3. Evolutionary history of plant G-protein components. The gene tree of each G-protein component is represented with different colored lines and labels. The splits in the horizontal and vertical lines represent ancient and lineage-specific gene duplications, respectively. The duplicated gene clades are also labeled. Broken lines mark the absence of a component in many but not all species. The presence of a component is marked by a circle at the tip of the line. The ambiguity in gene duplication timeline is labeled by a question mark. The cladogram is drawn based on [77]. The divergence of XLG3 and a new clade, named XLG4, is depicted as per [52]. However, the timeline of this event occurring before the origin of gymnosperms is poorly supported. Similarly, Gγ divergence before the origin of land plants remains ambiguous. Common names used to represent different plant lineages are marked at the top. Figure created using BioRender (biorender.com). Abbreviation: RGS, regulator of G-protein signaling.

was thought to be ubiquitous in eukaryotes [67]. Another surprising observation was that not only *P. patens*, but also many other species, including the entire group of Bryopsida mosses, have lost their Gα protein [77]. Intriguingly, the XLGα proteins, which appear for the first time in Charophyte algae (pre-dating land plants), are present in all plant lineages [78,79]. The Gβ proteins remain constant in both number and structure (no variants identified, to date) but Gγ diverged at some point between the emergence of land plants and vascular plants and underwent changes in protein domains, which led to three distinct subtypes [77]. *XLGα* and *Gγ* also exhibit many lineage-specific gene duplications. This distribution of G-proteins supports an alternative model in which XLGα, Gβ, and Gγ form the minimal core of the heterotrimer in all land plants [77]. Intriguingly, RGS appears to be under relaxed selection and is lost frequently in many plant groups [36,77].

Essential versus nonessential roles of G-proteins in plants

Another striking revelation of studying plant G-proteins beyond arabidopsis is the identification of their essential role in regulating plant life. In arabidopsis, G-proteins are involved in several developmental signaling pathways and regulate cell division and expansion, ion channel activities, responses to several endogenous signals, and the external environment [23,29,32,80]. However, G-proteins are nonessential for arabidopsis. The loss of all Gα, Gβ, or Gγ proteins, individually or in different combinations, causes several phenotypic changes, but the plants survive and complete their life cycles [27,51,68]. This had led to the hypothesis that 'plant' G-proteins mainly modulate different pathways to achieve optimal growth [81].

Research with several plant species has now confirmed that the roles of these proteins are not only modulatory, but essential for completion of the life cycle in several plant lineages. As mentioned earlier, *P. patens* lacking a functional XLGα or one of the two Gβ genes never develop a sporophyte, the only diploid tissue of the moss [57]. Furthermore, rice and maize plants (and probably other monocots) lacking all *XLGa* genes or the *Gb* gene are seedling lethal [32,82,83]. Seedling lethality was also reported recently for tomato Gβ mutants [84], suggesting that the phenotypes of G-protein mutants in arabidopsis and other closely related species, for example, *Camelina sativa* [85] are an exception to the norm. The underlying mechanisms of essential versus non-essential roles of G-proteins in distinct lineages are not yet fully explored but have been proposed to be dependent on altered regulation of plant immune responses by G-proteins [82,84]. The G-protein subunits themselves are highly similar, structurally and functionally, within the plant lineage. An arabidopsis *XLGa* or *Gb* can restore the phenotypes of *P. patens XLGa* and *Gb* mutants, respectively [57]. Similarly, a *Ga* gene from rice or *Brachypodium distachyon* (brachypodium) can fully complement all arabidopsis Gα (*gpa1*) mutant phenotypes [86]. These observations suggest that the distinct effects are not due to the intrinsic differences in the proteins per se, but that their developmental signaling networks differ among different lineages.

Conserved and nonconserved features of the metazoan model of G-protein signaling in plants

The plant G-protein model (Figure 2) explains G-protein signaling, to some extent in arabidopsis, and continues to be discussed as the universal G-protein regulatory mechanism in plants [28,43]. However, the lack of an RGS protein in many plants already questions its general applicability. Interestingly, the G-protein cycle itself does not differ between plants with or without an RGS protein [87]. A comparative analysis of two monocot models, brachypodium (no *RGS* gene in the genome) and *Setaria viridis* (setaria) (with an *RGS* gene in the genome), demonstrated that the loss of *Ga* function resulted in shorter plants with broader cells, leaves, and seeds in both species. *RGS* present in setaria is functional, as demonstrated by its overexpression in setaria

(native) or brachypodium, which resulted in plant phenotypes similar to suppression of their respective *Ga* genes [87]. Furthermore, the brachypodium *Ga* gene fully complements the phenotypes of arabidopsis *gpa1* mutants [86].

The role of RGS in plants remains enigmatic. It is present in all eudicots but is frequently lost in other plant lineages [77], without any known effect on plant fitness. However, when present, it is functional and involved in regulating important plant traits [87–91]. It is also notable that the presence of the RGS-coding gene in plant genomes is linked with the presence of Gα-coding genes. There are no instances where an RGS-coding gene is present in the genome in the absence of a Gα-coding gene [77], although the reverse is not true. The role of RGS proteins in the context of XLGα proteins is also perplexing. XLGα proteins, which constitute the core of the plant G-protein trimer, exhibit substantially reduced GTP binding and almost no GTPase activity, and their interaction with the RGS protein is debatable [28,32,92–94].

Canonical GPCR-like proteins present a somewhat similar situation. Several proteins that 'appear' similar to canonical metazoan GPCRs are present in plants [95]. Of these, GCR1 remains the most well-characterized protein in the context of G-protein signaling. GCR1 and its homologs in a few other plant species have been shown to have critical roles in the regulation of G-protein-coupled pathways, based on genetic [39,96–101] and transcriptomics analyses [102–104], and a recent report showed its binding with ABA and gibberellin [105]. However, its role as a canonical GPCR with GEF activity, similar to that of metazoan GPCRs, remains to be established. Additionally, the absence of some of the well-established effectors of mammalian G-proteins, such as adenyl cyclases, β arrestins, or GPCR kinases, necessitates the exploration of alternative signaling mechanisms, not necessarily regulated by the classic GPCR/RGS module.

Plant-specific, noncanonical signaling mechanisms

Two major recent developments corroborate the idea that plant G-protein signaling is regulated by mechanisms other than those universally established: (i) several studies have demonstrated that plant G-proteins are regulated by receptor-like kinase (RLK)-mediated phosphorylation/dephosphorylation-based signaling mechanisms; and (ii) nucleotide exchange may not be the central regulatory mode of G-proteins in plants, with nucleotide exchange-independent activation also having a role.

Phosphorylation-dependent regulation of G-protein signaling

G-proteins are physically and genetically coupled with RLKs and receptor-like proteins (RLPs) prevalent in plants [25]. Plant G-protein and RGS have been identified as phosphoproteins in several nontargeted studies. Furthermore, multiple RLKs phosphorylate specific G-protein components under *in vitro* conditions, suggesting a key role of these modifications [89,106].

In recent years, several studies have shown regulation of the G-protein cycle by RLKs by phosphorylation/dephosphorylation-based mechanisms. During regulation of the immune response in arabidopsis, the well-established receptor complex FLS2/BAK1/BIK2 has been proposed to regulate G-protein signaling, in a guanine nucleotide-dependent manner but in the context of the unusual biochemistry of plant G-proteins [32,92,93]. In this model, during the resting stage, the G-protein trimer (either Gα or XLGα with Gβγ) is associated with the receptor complex and maintained as such by the GAP activity of RGS1. Signal perception by the receptor (FLS2) causes a change in its interaction with BAK1 and activates BIK1, which phosphorylates RGS1, causing its dissociation from the receptor complex [92,93,107]. In an alternate model, BAK1 directly phosphorylates RGS1, which is then released from the receptor complex and endocytosed [89,106,108]. The removal of RGS1 from the complex releases Gα, which, due to

its spontaneous GTP binding, dissociates from Gβγ. Both freed entities can interact with downstream effectors to transduce the signal. This mechanism, in general, still depends on the canonical 'on/off' status of the G-protein heterotrimer, but, in contrast to activation by a classical GPCR, is regulated by the removal of the deactivator protein (RGS1) from the complex [32]. The relevance of this mechanism to plant species that do not have an RGS homolog is unclear. A recent report in rice suggests that phosphorylation of XLG proteins by plasma membrane RLKs promotes their nuclear localization and further regulation of defense responses by nuclear protein kinases [109].

Another example of phosphorylation-based regulation is demonstrated during nodule development in soybean. Gα proteins are negative and Gβγ proteins are positive regulators of nodulation [74]. During nodule development, a two-pronged approach of deactivating Gα and making Gβγ available for signaling has been demonstrated. On the one hand, the nod factor receptor, NFR1, phosphorylates and activates the RGS proteins, which deactivate Gα (i.e., inactivation of the negative regulator) [110]. On the other hand, an additional RLK of the nod factor receptor complex, SymRK, directly phosphorylates Gα proteins. Phosphorylated Gα cannot bind to the Gβγ dimer, thus setting the dimer free to interact with downstream effectors (i.e., the availability of positive regulators) [111]. Given that the role of XLGα during nodule development is not yet known, it is possible that either the freed Gβγ becomes exclusively available to XLGα or that XLGα is also phosphorylated, and the free Gβγ primarily regulates downstream events.

Additional examples of the involvement of RLKs in G-protein signaling include the interaction of maize Gα (Ct2) with the Clavata receptor signaling module for shoot apical meristem development [112–114], arabidopsis G-proteins with Feronia for stomatal aperture control and salinity responses [115,116], interaction of BRI/BAK1 in the sugar response [117], and of zygotic arrest 1 (ZAR1) and AGB1 during asymmetric cell division in zygotes [118]. However, the mechanistic details of these physiological observations or genetic interactions have not yet been fully established.

Nucleotide exchange-independent mechanism

Nucleotide binding-, exchange-, and hydrolysis-dependent activation/deactivation are the defining characteristics of G-proteins. Nonetheless, the idea of nucleotide exchange-independent activation has been discussed in mammalian models for some time [119]. Activation of the G-protein cycle by inducing conformational changes in Gα or in Gβγ by proteins other than classic GPCRs has been demonstrated [120,121]. However, such mechanisms are not the norm and may exist only under specific conditions. For the most part, until recently, the classic mammalian model was also expected to be the mechanism for plant G-protein signaling. In fact, most arabidopsis G-protein signaling models are based on the exceptionally fast nucleotide binding and exchange activity of the Gα protein combined with its slow rate of GTP hydrolysis [40,42,122], although the limitations of such a model, beyond arabidopsis, are also already clear. There are additional inconsistencies, even when considering arabidopsis Gα. For example, a constitutively active Gα (GPA1^{Q222L} or GPA1^{CA}), which can bind to GTP but not hydrolyze it, can still bind to the Gβγ dimer [123,124]. Furthermore, in humans, an analogous mutation results in the expected overactivation of the G-protein cycle, resulting in higher cAMP levels and uncontrolled cell growth. The phenotypes of arabidopsis that express constitutively active Gα or lack an RGS protein are subtle, and do not align well with the proposed central roles of these proteins [125]. The role of constitutively active Gα proteins in rice and maize is confounding, compared with arabidopsis. In rice, the expression of constitutively active Gα produced longer grains compared with WT plants, supporting continuous signaling, but, when expressed in rice Gα mutants, restored the phenotype to WT levels, and did not overcompensate for it [126,127]. In maize, a

constitutively active Gα only partially complemented the mutant phenotypes, implying that it is a weak allele of Gα [128]. Contrary to arabidopsis, the maize constitutively active protein version does not interact with Gβγ proteins [128]. The XLGα proteins add additional complication to this scenario because their nucleotide binding, exchange, and hydrolysis is poorly characterized, even *in vitro*.

Recent work in arabidopsis using a point mutant version of GPA1, GPA1^{S52C}, a protein variant that is unable to bind or hydrolyze GTP due to a substitution in its GTP-binding site, provides credence to the existence of an alternative mechanism. Genetic complementation of a *gpa1* mutant with the GPA1^{S52C} variant restored most plant phenotypes to the WT level, suggesting no role of GTP-binding or hydrolysis in these responses [49,124]. A structure–function study with arabidopsis XLG2 suggests that the protein is not in a nucleotide-bound state 'in planta' and functions only by sequestering the Gβγ from other Gα proteins [49].

More compelling data for the guanine nucleotide-independent role of G-proteins are from soybean, during regulation of nodulation signaling [111]. Two of the sites phosphorylated by SymRK are vital for GTP-binding by Gα proteins. Thus, phosphorylation of Gα makes it unable to bind (or hydrolyze) GTP. However, in this case, contrary to that reported for arabidopsis GPA1, the phosphorylated protein cannot bind Gβγ and, therefore, frees it for signal propagation [111].

Roles of G-proteins in regulating important signaling and developmental processes for their use in future agricultural modifications

The study of G-proteins in arabidopsis determined their roles in modulating several signaling and developmental pathways and set the foundation for future research. In eudicots, G-proteins have been studied for their agronomically important roles in rice, soybean, tomato, cotton [84,129–132], *Camelina*, pea [133,134], and *Brassica* [135–138] species (Figure 4). In soybeans, specific subunits of G-proteins and RGS proteins regulate nodule development and, consequently, their ability

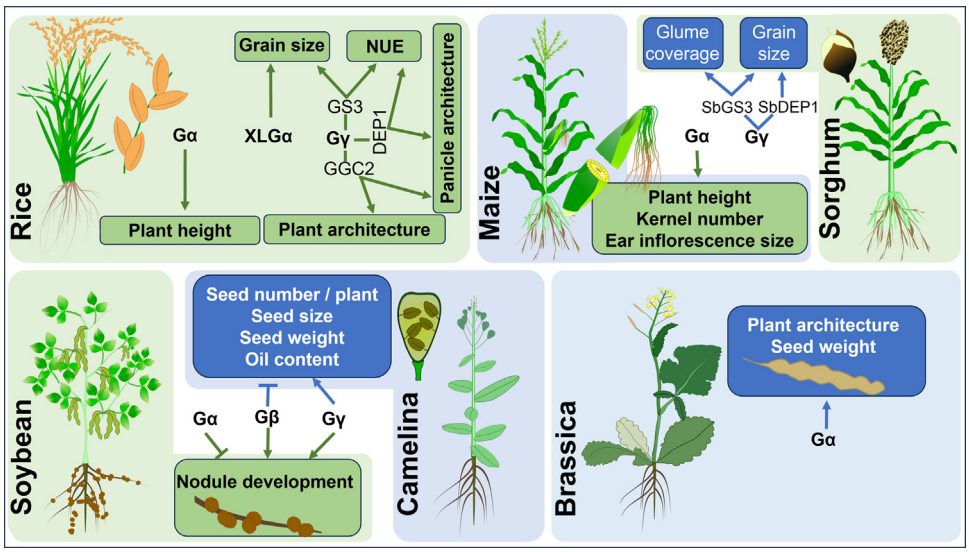

Figure 4. Summary of agronomic traits affected by G-proteins in plants. The illustration shows G-protein-dependent agronomic traits in different crop plants. Positive or negative regulations are denoted by lines ending with an arrow head or bar, respectively. G-protein components with no known effects as yet are not shown. The number of plants and agronomic traits detailed are example only and do not represent the complete list. Abbreviation: NUE, nitrogen-use efficiency.

to fix nitrogen, which is important for sustainable agriculture. In *Camelina*, overexpression of arabidopsis *AGG3* led to higher seed yield, more oil, and improved stress tolerance. Interestingly, knocking down the *Camelina Gb* gene increased the oil content of the seeds [85]. In tomato, pea, and *Brassica*, specific subunits of G-proteins are involved in regulating responses to several biotic and abiotic stresses. The underlying mechanism for these responses remains mostly unknown, but, in tomato, a low light-dependent sensing of glucose by the RGS protein and consequent modulation of the G-protein cycle have been demonstrated to regulate response to bacterial pathogens [132]. Although these studies have highlighted the potential of modulating G-protein signaling to improve future agriculture, their true impact is illustrated only when evaluating them in crop plants, especially rice. Several studies have highlighted the roles of rice G-protein subunits, especially the Gα subunit, RGA1, in regulating stress and developmental responses and nutrient-use efficiency [139,140]. However, the identification of type III Gγ proteins as some of the most critical grain size-related quantitative trait loci (QTL), has transformed the field for evaluating the agronomic potential of G-protein signaling in plants. In fact, type III Gγ proteins were identified as the underlying QTL for grain size regulation (*grain size 3*, *GS3*) and panicle architecture (*Dense and Erect Panicle 1*, *DEP1*) before their discovery in arabidopsis and their functional assignment as plant-specific Gγ proteins [63,141–143]. Rice *GS3* and *DEP1* are some of the most extensively researched genes for their biotechnological applications. The favorable allele of *GS3* is highly enriched in a set of cultivated accessions (34%) compared with in a set of wild accessions (4%) [141]. A survey of rice literature revealed more than 100 publications, ranging from the discovery of these genes as major QTL for several agronomic traits, their application in breeding, an artificial positive selection of specific alleles in domesticated varieties, GWAS and haplotype analysis, RNAi- and CRISPR-based gene editing, and the expression of specific domains, to multiyear field trials [64,71,72,143–168]. The overall conclusion is that *GS3* is a key regulator of grain size. Rice varieties carrying a wild-type *GS3* allele produce grains of normal length. Rice varieties carrying the complete loss-of-function allele eliminating the entire GS3 protein produce long grains, whereas rice varieties, which express a truncated protein (*i.e.,* an intact Gγ domain but no C-terminal region) produce very short grains [72]. In addition to its role in controlling grain size, *GS3* has been implicated in improving nitrogen use efficiency (NUE) in the long grain *japonica* rice varieties, and in improving cold tolerance and seed quality [158,162,169]. A recent study identified the role of *GS3* in thermotolerance using QTL analysis and named it *thermo-tolerance 2* (*TT2*), expanding its functional repertoire [170]. The second type III Gγ gene in rice, *DEP1,* was identified as a key determinant of panicle architecture [63,171,172]. Specific substitutions in *DEP1* led to erect panicles with more branches and seeds, or smaller panicles with fewer branches and fewer seeds. The phenotypes appear to depend on whether mutations remove the entire protein or only the C-terminal region, leaving the Gγ domain intact [150,173]. *DEP1* has also been identified as a major QTL for NUE in rice and implicated in several abiotic stress responses [63,171,172,174–176]. Mutations in *GGC2*, the third type III Gγ homolog in rice, also result in altered plant architecture, including changes in panicle and seed morphology [72,145,177]. *GGC2* has been proposed to have overlapping function with *DEP1*. Several groups have now generated mutants in different combinations of specific alleles of *GS3*, *DEP1*, and *GGC2* in rice, to uncover their redundant versus specific roles in grain size regulation [71,72,150,178]. These studies suggest a complex interaction of these three Gγ subunits with the sole Gβ subunit of rice [72].

The opposite phenotypes caused by distinct mutations in *GS3* or *DEP1* have been explained based on the modular nature of type III Gγ proteins. The consensus in the rice G-protein field is that the C-terminal region of the protein acts as a negative regulator of the Gγ domain function. Mutations that cause deletion of the C-terminal region abolish this negative regulation, allowing the Gγ domain to function. By contrast, mutations that remove the entire protein result in the loss of the Gγ domain function. Consequently, C-terminal versus full-length deletions result in

opposite phenotypes [71,72,179]. Attempts to identify signaling modules and downstream effectors of these proteins have led to disparate mechanisms, ranging from Ca^{2+}/CaM-dependent pathways, MAP kinases, lipid signaling, ubiquitin proteasome-mediated inhibition, interaction with several transcription factors, and more [71,159,161,164,170,174,180–183].

The sorghum homolog of *DEP1* (*SbDEP1)* has been identified as a possible locus responsible for grain size differences between different landraces of sorghum [184], whereas the *GS3* homolog (*SbGS3*) has been identified as the gene underlying QTL *qTGW1a*, which is a negative regulator of seed size [185]. The same locus has been identified as the causal gene that controls the glume coverage in sorghum seeds and was named *Glume coverage 1* (*GC1*) [181]. Analysis of 915 diverse accessions of sorghum for glume coverage and its relationship to domestication identified *GC1* as the main cause of, and stable locus for, this trait. Transgenic expression of truncated versions of *GC1* (*SbGS3*) in sorghum resulted in seeds with reduced glume coverage. Surprisingly, this study did not identify any strong association between these variations and grain size. A recent study has identified the GS3 homolog of sorghum as the locus responsible for tolerance to alkaline soil (Alkaline Tolerance 1, AT1). The genotypes with truncated alleles of AT1 (*at1* allele, C-terminal truncation) caused increased sensitivity, whereas the complete gene knockout conferred tolerance to alkaline soil, respectively, in several crop species. The protein is proposed to function via affecting aquaporin phosphorylation, thereby controlling oxidative stress [186].

A few studies in wheat, barley, and maize assessed the roles of the homologs of type III Gγ proteins with varying degrees of success. A 12-year field study in barley showed that the loss of function of *HvDEP1* resulted in consistent effects on stem elongation and grain size but conferred either a significant increase or decrease in harvestable yield depending on the environment [187]. In retrospect, such results appear obvious given that these proteins regulate responses to both environmental and endogenous developmental cues. In wheat, a survey of *DEP1* sequences in species with normal, compactoid, and compact spikes did not identify any specific changes that correlated with the phenotype [188]. Some association has been seen in kernel size and *GS3* variation in maize, but unlike rice, the gene does not appear to be under any positive selection [189,190].

Knockout mutants of canonical Gα in grasses, such as rice, brachypodium, setaria, and maize, are semi-dwarf, which in itself is a desirable agronomic trait [125]. However, these mutants also have associated nonpreferred phenotypes, especially low yield, which has restricted their use in agricultural practices. Nevertheless, by introducing a constitutively active biochemical variant of canonical Gα in maize [114], which can bind to, but not hydrolyze GTP, several beneficial traits were observed. $CT2^{CA}$ acted as a weak allele of *CT2* and led to a higher spikelet density and kernel row number, larger ear inflorescence meristems (IMs), and more upright leaves, causing an improved yield [128].

Concluding remarks and future perspectives

The study of G-proteins from multiple plant species in recent years has established the diversity of signaling components, mechanisms, and regulatory pathways. At the level of G-protein components, it is clear that these are lost in several algal lineages, necessitating a redefinition of their proposed ubiquitous presence in all eukaryotes. Furthermore, XLGα proteins appear to be the key component of the heterotrimer with the Gβγ proteins in plants. RGS proteins maintain their basic biochemistry, that is, deactivating the canonical Gα, and are active and relevant when present, but their loss is frequent and tolerated without any major consequences. This observation is even more pertinent in the context of the unusual activation/deactivation mechanisms of plant G-proteins and the possibility of a nucleotide exchange-independent protein function. Overall, it

Outstanding questions

How are plant Gα proteins activated?

What is the relevance of GTP binding and hydrolysis if these activities are not involved in response regulation?

Why are some canonical G-proteins lost in specific plant groups?

Why do plants without an RGS protein not have any fitness penalties?

How is specificity achieved in plant G-protein signaling?

What are the downstream effectors of plant G-proteins?

Why are G-proteins essential for many (e.g., rice) but not all plants (e.g., arabidopsis)?

What is the significance of the long and varied cysteine-rich region of type III Gγ proteins?

How do G-proteins integrate developmental and stress-mediated responses?

appears that the G-protein core has been modified and rewired to suit specific needs in the green plant lineage. While the core biochemical properties of individual G-protein components are maintained, these might not be utilized in accordance with the established mammalian model.

It is also evident that G-proteins are essential for many but not all plant groups and are involved in regulating key agronomic traits. Moreover, small differences in their biochemistry or the presence/absence of specific domains have tremendous effects on plant phenotypes, but the magnitude of these effects differs even among a few species studied to date. These observations suggest that not only their study in additional plant species, but also a better understanding of their signaling mechanisms is needed to realize their full potential (see also Outstanding questions).

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Declaration of interests

The authors have no conflict of interest to declare.

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