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# Heterotrimeric G Protein–Coupled Signaling in Plants

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## Abstract

Investigators studying G protein–coupled signaling—often called the best-understood pathway in the world owing to intense research in medical fields—have adopted plants as a new model to explore the plasticity and evolution of G signaling. Much research on plant G signaling has not disappointed. Although plant cells have most of the core elements found in animal G signaling, differences in network architecture and intrinsic properties of plant G protein elements make G signaling in plant cells distinct from the animal paradigm. In contrast to animal G proteins, plant G proteins are self-activating, and therefore regulation of G activation in plants occurs at the deactivation step. The self-activating property also means that plant G proteins do not need and therefore do not have typical animal G protein–coupled receptors. Targets of activated plant G proteins, also known as effectors, are unlike effectors in animal cells. The simpler repertoire of G signal elements in *Arabidopsis* makes G signaling easier to manipulate in a multicellular context.

### Keywords

G protein signaling; regulator of G protein signaling; heterotrimeric G proteins

## INTRODUCTION

### The New G Signaling Paradigm

Recently, a fifth Nobel Prize was awarded to researchers working in the field of G protein–coupled signal transduction (in glucose metabolism, in this case) (48, 53). Much of the seminal work for these Nobel Prizes was accomplished over the past 40 years, so it is no wonder that it has been said many times that this pathway is the best understood in the world. G protein–coupled signaling is taught in many high schools, and certainly every college biology major is familiar with at least the basic principles of this pathway.

However, what we have learned and taught is but one version of G signaling, a version influenced by the enormous anthropocentric focus on human health and disease. Can plants tell us something new about G signaling? In the past 10 years, research on plant G proteins has revealed a fundamental difference between plant and animal G protein activation and led to the conclusion that the animal paradigm for G activation is probably limited to one small corner of the eukaryotic kingdom. Studies using rice and *Arabidopsis* have revealed the

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molecular plasticity of G signaling and pointed to novel mechanisms that control the activation state. Plants—and now other eukaryotes beyond vertebrates and yeast—are telling us that there is still much to learn about G signaling.

In this review, we describe the established paradigm for G signaling, show where and how plant G signaling differs, and convey the significance of these differences. We begin with the textbook view of G signaling (Figure 1*a*).

In animals and fungi, as well as some amoebae (like the slime mold), a seven-transmembrane (7TM) cell surface receptor is in complex with the heterotrimeric complex tethered to the cytoplasmic face of the membrane. The G protein complex, comprising Gα, Gβ, and Gγ subunits, is in its resting state with GDP bound to the Gα subunit. The details of this nucleotide binding are provided below (see Structural Basis for Rapid Nucleotide Exchange). The 7TM G protein–coupled receptor (GPCR) binds its cognate ligand, which causes a conformational change in the orientation of the transmembrane spans. This new protein surface is recognized by the Gα subunit at the cytoplasmic face of the membrane. Because the G protein complex is intimately coupled, this change in GPCR conformation causes Gα to release its GDP nucleotide, enabling the binding of a GTP.

Let us pause for a moment to emphasize this point. In the textbook paradigm, we are taught that this release of GDP is the rate-limiting step in G protein activation occurring at basal rates (i.e., without a GPCR) that either are too low to measure or have a slow $k_{cat}$ of approximately 0.01 $min^{-1}$. Slow nucleotide exchange in the absence of an active GPCR occurs in animal and fungal Gα subunits but not in plant G proteins.

Returning to the animal paradigm: GTP binding causes a conformational change in the Gα subunit that disrupts interaction with the Gβγ dimer and separates them, although the extent of physical separation may vary. The Gβγ dimer is tethered to the membrane by a covalently attached prenyl group while the Gα subunit is delimited there by a myristyl group. The freed and therefore activated Gβγ dimer and $G\alpha^{GTP}$ subunits are now able to interact with other specific target proteins, which in the G protein field are also called effectors. Two classical examples of effectors in animals are (*a*) adenylyl cyclase, which generates the secondary messenger cAMP, and (*b*) specific isoforms of phospholipase C (26) that generate inositol trisphosphate and diacyl glycerol. Secondary messengers amplify the signal; GPCR coupling to the G complex provides the selectivity in signaling, and agonist binding to the GPCR provides the specificity and sensitivity in signaling.

Signaling requires both activation and deactivation. Deactivation in animals is not rate limiting and is described as the intrinsic property of either the particular type of Gα subunit or the signaling complex. For example, among different types of Gα subunits in humans, deactivation occurs by hydrolyzing the GTP to GDP at intrinsic rates between ~0.01 and ~3.5 $min^{-1}$ (36). Because nucleotide phosphate hydrolysis is far faster than GDP release, the steady-state pool of activated Gα subunits is related to the amount of agonist binding to its GPCR and sets the rate for reactivation by the agonist-occupied GPCR. But in some pathways, the inherent hydrolysis rate is not fast enough for the overlying physiology [e.g., in human vision (110)], in which case GTPase-accelerating proteins (GAPs)—specifically

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known as regulator of G protein signaling (RGS) proteins—speed deactivation (96). There are at least 37 RGS proteins in humans, falling into 10 basic architectures, none of which contain transmembrane domains, although many contain domains that permit membrane localization, such as lipid or GPCR binding (88).

RGS proteins do one other thing in animals worth mentioning so as to contrast below with plants. Paradoxically for an inhibitor of signaling, RGS proteins increase and sharpen signal amplitude (131). The current explanation, coined kinetic scaffolding (118), involves an RGS-dependent reset rate that is faster than the diffusion of the Gα subunit from its receptor. Whether dynamic scaffolding occurs in plants and specifically in plant G signaling is not known.

## Plant G Proteins Are GPCR-Independent and Therefore Self-Activating

The two key biochemical differences that make plant G signaling seemingly "upside down" relative to the animal paradigm are that (*a*) in vitro plant Gα subunits exchange guanine nucleotides in the absence of a GPCR, and (*b*) the intrinsic hydrolysis rate is extremely slow [$k_{cat}$ = 0.05 $min^{-1}$ for *Arabidopsis* Gα (AtGPA1)]. In fact, with excess GTP in vitro, the *Arabidopsis* Gα subunit is 99% bound with GTP (40). The combination of these two properties—rapid nucleotide exchange and slow hydrolysis—was termed self-activating or GEF-less G protein activation (where GEF stands for guanine nucleotide exchange factor). Therefore, the regulation of G signaling must take place by either speeding nucleotide hydrolysis or slowing nucleotide exchange (see sidebar Plants Do Not Have Canonical GPCRs).

As in animal cells, the GTP-bound form of Gα is the active state in plants. The argument supporting this is as follows: If the opposite were true, and the inactive state for G signaling were GTP bound, then regulation would occur by signal stimulation of hydrolysis. However, this is not the case. This was shown by increasing the pool of active G proteins and observing a phenotype (11, 13, 39, 107–109) and by showing that GTP disrupts heterotrimer formation, as it does in animals (42). This work also indicates that although plant G proteins constitutively bind GTP without a GPCR, in the plant cell, the GTP-bound pool is regulated. We are left with one conclusion: Regulation must occur by inhibiting deactivation, inhibiting the nucleotide hydrolysis reaction, and/or inhibiting an inhibitor of nucleotide exchange.

To sum up: In animals, the presence of a signal (e.g., light, hormones, protein activators, and ions) stimulates the production of the activated G protein. In plants, in contrast, the presence of a signal inhibits deactivation of constitutive G activation (Figure 1*b*).

## Structural Basis for Rapid Nucleotide Exchange

The Gα-subunit structure reveals two distinct domains (Figure 1*c*): a Ras domain highly similar to the structure of the monomeric GTP-binding protein Ras, and a helical domain composed of all helices. Until the plant Gα-subunit structure and function were studied, the only function ascribed to the helical domain was an interaction with the GoLoco motif from RGS14 (46). The Ras domain and the domain linkers contain the residues that contact GPCRs (in animals), RGS proteins, and the Gβ subunit, as well as residues that form the guanine nucleotide-binding pocket and hydrolyze GTP (90). Most important for the

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explanation of why plant Gα subunits are self-activating, the nucleotide-binding pocket is located between these two domains (Figure 1*c*).

The overall structure of the *Arabidopsis* Gα subunit (AtGPA1) is highly similar to the previously reported structures of activated forms of vertebrate Gα subunits (16, 76). Indeed, the root mean square deviation is only 1.8 Å for 307 of the equivalent residues. This was an astounding finding at the time, raising the question of how it is possible that two proteins with essentially the same three-dimensional structure could be so different biochemically. The answer lies in the fourth dimension, namely, protein dynamics. AtGPA1 exhibits more dynamic motion than mammalian $G\alpha_{i1}$, owing mainly to two helices in its helical domain, consistent with the fragmented appearance of the electron density for these two α helices in the AtGPA1 crystal (40, 41). One of these helices (helix A in Figure 1*c*) serves as a spine, providing rigidity through the domain and affecting motion in the overall molecule. Molecular dynamic simulations predicted that AtGPA1 has increased motion between the Ras and helical domains, with the predominant form of the two-body motion being like the opening and closing of a clamshell (41). Helical domain–swapping experiments showed that this domain alone from either the plant or the animal Gα subunit is necessary and sufficient to confer the slow or rapid nucleotide exchange property (40). The discovery that a single domain controls the molecular dynamics of the entire molecule was new, and a function for the helical domain was finally discovered; plant G protein research had proven that it has much to offer.

The *Arabidopsis* structure and the new role of the helical domain bear directly on our recent understanding of GPCR activation of G proteins, the crux of the shared 2012 Nobel Prize in Chemistry. Brian Kobilka and colleagues solved the sought-after structure of a GPCR in complex with a G protein empty of its nucleotide, and showed that the nucleotide-free conformation of the Gα subunit is with the Ras domain in contact with the receptor (no surprises there) and that the helical domain is stretched out in a position that maximizes the opening of the nucleotide-binding pocket (15, 84, 125). This nucleotide opening driven by the helical domain is the lesson learned from the *Arabidopsis* Gα structure (40, 41). If the helical domain imparts the intrinsic dynamic property of the subunit, then it is possible that the ligand-bound receptor engages the Gα subunit, largely through its grasp of the Ras domain. Hypothesis: The energy of motion of the entire molecule is then translated to the helical domain, much as grabbing the handles of a rigid-body jackhammer causes dynamic motion to spread to the second body, one's own. This hypothesis on how a receptor might cause nucleotide loss may be controversial among GPCR-Gα aficionados because, like the chicken and the egg, it is unclear whether (*a*) the receptor ejects the nucleotide and the two domains then spread apart, or (*b*) the receptor causes the two domains to spread apart first, thus allowing the nucleotide to leave. Although either interpretation is possible at this point, the data on molecular dynamics of plant, animal, and plant–animal hybrid Gα subunits (40, 41) persuade us that the latter scenario is more likely.

That the C- and N-terminal regions of Gα subunits contact the GPCR was confirmed by extensive biochemical analyses (78), so it was not surprising to find these contacts in the crystal structure (84). This contact interface might be the "grabbing the jackhammer handles" analogy mentioned above. Plant Gα subunits, despite being encoded by a single

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gene in most species, have C-terminal regions that are not conserved (95). Because plant Gα subunits are orthologous, the lack of conservation of the terminal regions suggests that there is no core function and that plant Gα subunits do not couple physically to a receptor in the way that animal Gα subunits do. Supporting this suggestion is the observation that placement of a fluorescent protein tag at the C terminus apparently does not disrupt its function (120). Therefore, if coupling occurs between a plant Gα and a receptor, it does so differently from how this coupling occurs in animals.

## Evolutionary Support for the Lack of Plant GPCRs

The 7TM topology is the conserved feature of GPCRs. However, conservation at the amino acid sequence level is poor among GPCRs, even within individual species. This lack of conservation has befuddled and obstructed bioinformaticists trying to reconstruct GPCR evolutionary history (21, 92). Sequence-based methods for GPCR homology failed to enlighten, and therefore algorithms that do not depend on sequence alignments were created to approach the problem; these algorithms were used first to identify candidate orphan GPCRs in lower metazoan groups such as insects (45) and then later to identify candidate 7TM receptors in plants (25, 60, 68, 69). There is little doubt that plants contain 7TM proteins with the topology of an animal GPCR, but there is no sequence-based evidence that any of these proteins have homology to a bona fide GPCR (see sidebar The Importance of Plants in Solving the Evolution of G Signaling).

Others have noted that candidate plant GPCRs are related to proteins that were drummed out of the GPCR corps. Here are a few examples: Heptahelical proteins 1–5 (HHP1–5) were presented as plant GPCR candidates because they share some sequence similarity to the human progestin and adipoQ receptors (PAQRs) (25, 94). However, human PAQRs have no homology to GPCRs (94); rather, they have significant similarity to hemolysin III (3) and are not 7TM proteins (127). Although PAQRs stimulate inhibitory G protein pathways (98–100), they do so by acting as ceramidases (49, 117), which produce sphingolipids (70), well-known ligands for GPCRs (89). G-COUPLED RECEPTOR 2 (GCR2) and GPCR-TYPE G PROTEIN (GTG) were also proposed to be plant GPCRs (57, 80). GCR2 is homologous to the prokaryotic enzyme lanthionine synthase (4, 14, 66). GTG1 and GTG2 likely contain eight-transmembrane domains, which explains how split-ubiquitin complementation was observed with a cytoplasmic Gα subunit when the other half of split ubiquitin was placed on the N terminus, which would be extracellular on an animal GPCR (80). Instead, GTGs are Golgi ion transporters (34, 62). CAND2, -6, -7, and -8 were also proposed to be plant GPCRs (25). CAND6 and CAND7 are homologous to human GPR107 and GPR108, and CAND2 and CAND8 are similar to human GPR175/TPRA40 (1, 77, 116). These human proteins are not GPCRs (77, 94).

There is one exception to the statement that proposed plant GPCRs share sequence similarity to animal proteins that are not GPCRs. The plant 7TM protein GCR1, the first proposed plant GPCR, has weak sequence similarity to the *Dictyostelium* cAMP receptor cAR1. However, several troubling observations challenge the idea that GCR1 is a plant GPCR. First, the weak homology to cAR1 is hard to interpret because the homologs are found in organisms that lack G proteins, and thus cAR1-homologous genes clearly have a function

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that does not involve G proteins (112). Second, in plants, some loss-of-function phenotypes of GCR1 are unlinked to G proteins (12). Finally, the evidence that GCR1 interacts with the *Arabidopsis* Gα subunit (AtGPA1) has been called into question. For example, Johnston et al. (37) pointed out that the in vitro translation and yeast complementation assays used to reach the conclusion that AtGPA1 and GCR1 physically interact had design flaws. Taken together, the current evidence does not support GCR1 having an activating role for plant G proteins, and therefore GCR1 is not a typical GPCR.

## MECHANISMS FOR REGULATING THE ACTIVE STATE OF G PROTEINS

### The Core Components of Plant G Signaling

Most plants have one Gα subunit, one Gβ subunit, and three to five Gγ subunits. For example, rice has one canonical Gα subunit, one Gβ subunit, and five Gγ subunits (103), and *Arabidopsis* has one canonical Gα subunit (AtGPA1) (61), one Gβ subunit (AGB1) (124), and three Gγ subunits (AGG1, AGG2, and AGG3) (10, 63, 64). Loss-of-function mutants of *GPA1* and *AGB1* display altered sugar sensing, seedling development, and stomatal closure (19, 121). *gpa1* mutants have a lower stomatal density (128), whereas *agb* mutants have a higher density. *agb1* mutants have more lateral roots, whereas *gpa1* mutants have fewer. *agb1* mutants are less resistant to many pathogens (58, 104, 105). The *agg1 agg2 agg3* triple mutant displays all of the *AGB1* null mutant phenotypes inventoried so far (10, 101).

Gγ subunits exhibit an extraordinary level of structural diversity and show important differences from their animal counterparts (103). Whereas all animal Gγ subunits are less than 100 amino acids, AGG3 homologs can be two to four times the average mammalian size. Some plant Gγ subunits lack the isoprenylation motif at their C terminus, a conserved feature of all animal Gγ subunits and an essential part for membrane anchoring. There are three classes of Gγ subunits based on their structures (103): Type A Gγ subunits are the prototypical, small Gγ subunits containing a C-terminal CaaX isoprenylation motif (where CaaX means cysteine, then any two aliphatic residues, and then any residue). Type B Gγ subunits are similar to type A but lack the CaaX motif. Type C Gγ subunits have two well-defined regions: an N-terminal domain with high similarity to classic Gγ subunits and a C-terminal domain highly divergent and enriched in cysteine residues (103). *Arabidopsis* AGG1 and AGG2 are both type A, and AGG3 is type C. The rice genome encodes a type B protein [Gγ2 (RGG2)] and three type C homologs [grain size 3 (GS3), DEP1, and G protein γ subunit type C 2 (OsGGC2)].

### Regulation by the Receptor GAP AtRGS1

Having dismissed plant GPCRs, we must search for something else that controls G activation in plant cells (Figure 2). In most plants, this role is performed by a receptor GAP (113). GAPs increase the intrinsic rate of nucleotide hydrolysis; in essence, they speed the G proteins back to their resting “off” state. Receptor GAPs have the capacity to control G activation in cells with self-activating G proteins such as plant cells, but the mechanism is significantly different from GPCRs in animal cells. The prototypical receptor GAP is *Arabidopsis* regulator of G protein signaling 1 (AtRGS1), a hybrid protein with a 7TM domain at the N terminus connected to a cytoplasmic RGS box with a short hinge sequence

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located N terminal to the box and a regulatory domain located C terminal to the box (13). As with GPCRs in animal cells, trafficking of plant receptor GAPs is an important part of signal transduction. In mammals, the internalization of GPCRs causes signal desensitization by uncoupling them from their cognate G proteins (52). In correlation with receptor occupancy by their ligand, GPCRs are phosphorylated at the C-terminal region by kinases, such as G protein receptor kinases not found in plants. In many cases, the phosphorylated GPCRs are recognized by β-arrestin, which functions as an adaptor that connects GPCRs to the endocytic machinery by recruiting clathrin. Some GPCRs are recycled back to the membrane, and some are targeted to lysosomal degradation through ubiquitination (87).

The plant receptor GAP is also trafficked rapidly from the plasma membrane to the endosome upon ligand occupancy but with the opposite consequence (81, 114). Instead of desensitizing G signaling in animals, endocytosis of plant receptor GAPs probably causes G activation. The mechanism for this unusual form of G activation has been solved. AtRGS1 is phosphorylated at the C terminus after directly or indirectly binding its ligand (D-glucose). Phosphorylation is essential for AtRGS1 endocytosis. WITH NO LYSINE (K) kinases (WNKs) phosphorylate AtRGS1 for endocytosis. Because plants lack the clathrin recruiter β-arrestin, the link between phosphorylated receptor GAPs and clathrin is unknown. The receptor GAP internalizes but leaves the G protein complex at the plasmamembrane; that is, it becomes physically uncoupled, allowing the plant G protein to self-activate (114). Because loss-of-function mutations in *RGS1* do not confer constitutive sugar signaling, the story is more complex. One explanation is that sugar signaling through activated AtGPA1 at the plasma membrane also requires an origin of signaling through AtRGS1 at the endosome (114). Signaling by a plasma membrane receptor at the endosome is an exciting new topic (31), and we anticipate that plant receptor GAPs will contribute to the new understanding.

Mathematical modeling of plant G activation revealed two important network properties: (*a*) The amount of receptor GAP leaving the plasma membrane is sufficient to cause G activation, and (*b*) this unusual network architecture for plant G signaling imparts an emergent property, namely, the ability for a plant cell to detect both the dose and duration of signaling, termed dose–duration reciprocity (23). Modeling also illuminated the mechanism: Two kinases with different dynamics were predicted to serve as the critical gears, with those shown to be WNK1 acting slowly on glucose binding and redundant WNK8/10 acting rapidly.

## Other Expected Mechanisms of Regulation of G Activation

As mentioned, although all plant G proteins are self-activating, some plants lack receptor GAPs, indicating that some other molecule fills the role of G activator. Two examples are worth discussion: cereals and liverworts. The rate-limiting step for the cereal rice was once controversial (33, 86) but has since been shown unequivocally to be at GTP hydrolysis, meaning rice G signaling is self-activating (113). However, rice lacks RGS proteins. Moreover, a key residue necessary for tight interaction between RGS proteins and their Gα substrate is missing in cereal Gα subunits. This suggests that a mutation occurred in the ancestor of cereal Gα subunits that weakened the regulatory effect of a receptor GAP on G activation, and ultimately the receptor-GAP gene was lost. Evidence for this scenario has

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been found in foxtail millet (*Setaria italica*), a close relative of rice. *S. italica* has a remnant of the receptor-GAP gene with transposon insertions, representing a snapshot of the evolution of receptor GAPs in cereals. Cereals share an evolutionary history, and other cereals completely lost RGS function. Moreover, there must have been something in place of a receptor GAP in the cereal ancestor such that the loss of RGS function was not counters-elective. It is interesting that loss-of-function mutations in the Gα-subunit gene confer several different traits in rice than they do in *Arabidopsis* (111). Rice therefore likely represents an excellent model to discover new G activation mechanisms. Rice is also a model for engineering synthetic regulation of G signaling because rice Gα (RGA1) can serve as a good substrate for the *Arabidopsis* receptor GAP (AtRGS1) (113).

Another useful model is liverwort (*Marchantia polymorpha*). Like other higher plants, *M. polymorpha* encodes a Gα subunit that rapidly exchanges guanine nucleotides (113). However, unlike in other plants, nucleotide hydrolysis is extremely fast, almost as fast as exchange. It is hard to imagine how the active state is regulated in *M. polymorpha*. Given their phylogenetic positions, understanding *Arabidopsis*, rice, and liverwort not only will pave the way for new G regulation mechanisms, but also will provide an excellent platform for understanding how the basic body plan of plants evolved: G activation is critical in development, and the body plans of the liverworts, dicots, and cereals differ greatly.

Without the need for or presence of GPCRs in cereals and liverwort, and given that these species lack receptor GAPs, we speculate that some molecule engages the GDP-bound state and that this engagement is regulated. In animals, a GDP dissociation inhibitor (GDI) engages the inactive Gα subunit, but no plant GDIs are yet known.

## EFFECTORS

Effectors are targets of activated Gα subunits and Gβγ dimers. Adenylyl cyclase, which generates cAMP, and phospholipase Cβ, which generates inositol trisphosphate and diacyl glycerol, are the two classic examples in animals. But it is not likely that plant biologists can apply their rich understanding of G protein activation of these two effectors to plant cells. A canonical adenylyl cyclase is not encoded by plant genomes, cAMP levels are extremely low, and the natural role of cAMP in plants, if any, is controversial (24, 54). An unusual plant adenylyl cyclase was reported (71), but there has been no evidence that this putative adenylyl cyclase activity is regulated by G proteins. Although plant genomes include genes encoding phospholipase C proteins, they are different from the effector subtype of phospholipase Cβs in animals (26), and the two reported interactions with plant Gα subunits await elucidation (44, 67). Given that, in plant cells, phosphatidic acid may be more important as a secondary messenger (74, 97) than the classic inositol trisphosphate is (7), the report that PLDα, the enzyme producing phosphatidic acid, is regulated by AtGPA1 (130) was exciting until closer examination revealed problems with the data (37) that have not been resolved. G proteins regulate $K^+$ flux; in animal cells, this occurs via activation of G protein–coupled inwardly rectifying potassium channels, for which genes are seen only in the animal lineage. The activation mechanism is understood at the level of atomic structures (126). Plant cells might use a similar mechanism, but it remains elusive (121). Other

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candidate plant G protein effectors have been reported and are discussed in greater detail elsewhere (111).

The lack of known effector homologs in plants prompted an international consortium of plant G signaling researchers (47) to seek plant effectors ab initio and to create the Web-based *Arabidopsis* G-Signaling Interactome Database (http://bioinfolab.unl.edu/AGIdb). This searchable database provides more than 500 unique protein pairs. The *Arabidopsis* G protein interactome is distinct among interactome data sets in many ways: (*a*) Although it is not a complete list, it is the exhaustive result of an interaction screen that interrogated nine different plant cell cDNA libraries multiple times; (*b*) deep filtering and in vivo interaction confirmation eliminated false positives; (*c*) the interactome was well correlated with the expression patterns; and (*d*) this interactome includes the G protein phenotypes of insertion mutations in the genes encoding the protein nodes. A true test of an interactome is whether the data point to new hypotheses that are then experimentally validated.

The core of the interactome is defined by 68 proteins, each connected by at least two interacting partners. Among many G protein interactors (22, 29, 50, 73, 106, 119, 122), some function as potential G protein effectors. For example, thylakoid formation 1 (THF1) interacts with Gα and acireductone dioxygenase 1 (ARD1), and N-Myc-downregulated like 1 (NDL1) interacts with Gβγ. THF1 localizes on the outer plastid membrane, in particular where the membrane extends into a long protrusion called a stromule (28, 65). Stromules associate with the plasma membrane, and AtGPA1 and THF1 interact at these sites, as determined by fluorescence resonance energy transfer analysis, but the biochemical activity of THF1 and the effect of AtGPA1 on THF1 action are not yet known.

ARD1 is an unusual metalloenzyme because its catalytic function in methionine salvage and/or ethylene production depends on whether the coordinated metal in its active site is iron or nickel (43). The plant ARD1 contains iron, and therefore ARD1 catalyzes reduction of acireductone coming indirectly from *S*-adenosyl methionine into α-ketoacid, which is converted back to methionine (82). AGB1 enhances ARD1 activity, and loss of ARD1 confers reduced cell division and ethylene content, as observed in the *agb1* mutant (22).

NDL1 and its homologs NDL2 and NDL3 interact with AGB1. NDL proteins regulate root and shoot development in *Arabidopsis* (72). The mechanism involves establishment and maintenance of the auxin distribution pattern in the root through control of two polar auxin transport streams. A feedback mechanism with AGB1, auxin, and sugars operates in a feedback loop to control NDL1 steady-state levels.

The *Arabidopsis* G protein interactome reveals many new avenues for research. The high proportion of cell wall–modifying enzymes in the interactome led to the new finding that G proteins regulate cell wall xylose (47), which was confirmed and extended to a possible mechanism for altered pathogen resistance (18), lending credence to the value of discovery-based research like studies of the interactome. Several transcription factors are in the interactome. One of these, MYC2, was no surprise. MYC2 and AGB1 operate in a genetically defined pathway in fungal resistance, probably through a scaffolding protein such as one of the ARD proteins (discussed above), which are physical partners to

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bothMYC2 and AGB1 (47). The interactome also connects the dots between various signaling pathways and G signaling.

## CROSSTALK AND BOTTLENECKS IN G SIGNALING

After the loss-of-function alleles of the G proteins became available in 2001 (109), a flurry of reports claimed that the plant G protein coupled numerous signals to various cell behaviors (38). At that time, plant G signaling was expected to follow the animal paradigm: Researchers assumed that a large set of plant GPCRs recognized a large set of signals, all of which funneled through the G protein nexus to cause whatever change was noted as aberrant in the mutant (Figure 3*a*). This notion changed when it became clear that plants do not have typical GPCRs, but rather have a single 7TM receptor GAP. With just a single receptor, the rethinking was that there is one ligand, one 7TM receptor GAP, and one cell behavior that manifests differently depending on the cell type. Although no direct biochemical proof exists, ample indirect data support the idea that the agonist for this 7TM receptor GAP or its coreceptor is D-glucose or its metabolite (6, 13, 27).

If we follow this line of reasoning, then sugar modulates other signaling networks. In essence, G signaling could be a sensor of nutrient status, and it is easy to imagine how altered nutrient sensing in a G protein mutant would impinge on a cell's ability to sense other signals, such as stress, light, and defense. The idea is that plant G proteins mediate sugar sensing, and the information of low or high sugar is integrated among other signals to alter cell behavior (Figure 3*b*). To illustrate this concept, take red-light-dependent *Arabidopsis* seed germination. For seeds that lack the Gβ subunit and thus falsely report the nutrient status to the radicle, one expects the seeds to have altered red-light sensitivity, which they do (8). This and many similar examples (6, 8, 20, 79, 108, 122) suggest that G signaling does not directly couple a multitude of signals, as in the animal paradigm; rather, a single signal modulates a multitude of other signal pathways, acting as a molecular rheostat (Figure 3*b*).

The molecular rheostat explanation solves the problem of the signaling bottleneck caused by a single G protein complex (or a small number of complexes) and explains why so many plant signaling pathways are modified but not lost when G proteins are genetically ablated. But is this view too narrow? Is it biased? Is it not possible that other signals modulate sugar signaling or activate G signaling through the one receptor GAP that plant cells have? Because sustained activation occurs in *Arabidopsis* through a phosphorylation event of the C-terminal domain of AtRGS1, for example, it is conceivable that some (or all) of the 400 receptor kinases in plant cells phosphorylate the receptor GAP and thus activate G signaling. This means that many signals merge upstream of the G protein complex to control one cell behavior (Figure 3*c*). Crosstalk between GPCRs and receptor kinases has been observed in animal cells for some time, and explanations of potential mechanisms are ample (17). The evidence for receptor kinases in plant G signaling has also been in plain view for a decade. The first screen for additional alleles of the *erecta* kinase mutant gene (rounded leaves) generated the first recessive allele of *AGB1* (51). Both *erecta* and *agb1* mutants are hypersusceptible to fungal pathogens (58). Consistent with a joint role for receptor kinases and G proteins in pathogen defense, pathogen-associated molecular patterns such as the

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flg22 and elf18 peptides induce G protein expression (132), and *agb1* mutants are insensitive to these receptor kinase ligands with regard to oxidative burst induction (32, 59). Null mutations in *GPA1* are also insensitive to flg22 (129). A weak allele of *agb1* also suppresses the cell death phenotype caused by loss of function of the receptor kinase BAK1-INTERACTING RECEPTOR-LIKE 1 (BIR1) (56). Crosstalk was also proposed in the maize shoot meristem, where Gα may genetically and biochemically link with CLAVATA receptor kinases (5). Because cereals lack the receptor GAP gene, some receptor kinases may directly regulate the G protein complex. Thus, although the idea that receptor kinases and plant G proteins work together is not new, we still await a mechanism.

## G PROTEIN–MEDIATED SUGAR SIGNALING AND CELLULAR BEHAVIORS

Loss-of-function mutations in core G protein elements confer many phenotypes (111). In *Arabidopsis*, Gα mutations have developmental effects such as fewer stomata (128), altered leaf morphology, short hypocotyls (109), and altered signal transduction (38). Loss of AGB1 confers even stronger phenotypes in many cases. *agb1* null mutants are profoundly sensitive to many pathogens (18, 35, 56, 75, 83, 102, 105) and have short hypocotyls, altered leaf shape, and excess lateral roots and stomata (128). Both AtGPA1 and AGB1 operate in programmed cell death (55, 123). In rice, Gα mutations confer disease susceptibility (93), decreased seed size, and short internodes (2). Altered expression of Gβ confers many of the same phenotypes as loss of Gα, but with the addition of increased programmed cell death (115); for example, loss of RGA1 abolishes ethylene-induced cell death (91).

The altered cellular behaviors underlying many, if not all, of these phenotypes are cell proliferation and programmed cell death. This suggests that G proteins are involved in cellular decisions that shift the balance between life and death, analogous to nutrient sensing and target of rapamycin (TOR) signaling (30). Although this effect has not been seen in rice, in *Arabidopsis*, G protein mutations confer altered sugar sensing. This was originally observed using a screen called the green seedling assay. In this assay, seedlings are grown for up to two weeks on agar plates supplemented with high sugar doses, which arrest growth and turn wild-type cotyledons yellow. The surviving green mutants are called sugar insensitive, but given the harsh conditions and long duration, they should really be considered stress mutants. Not surprisingly, genetic screens using this assay identified many mutations in genes known to operate in stress physiology (85). For example, *gpa1* and *agb1* mutants are hypersensitive to high sugar, and *rgs1* and constitutively active Gα mutants are resistant to it (13). Fortunately, this assay was replaced by a reporter assay based on a remarkably small set of sugar-induced, G protein–dependent, rapidly expressed genes (27). The prototype gene reporter is called *TBL26* and encodes an unknown protein. *TBL26* expression is significantly reduced in all G protein mutants, indicating that G proteins mediate sugar signaling. The connection between sugar sensing and signaling and cell-proliferation and programmed-cell-death behavior makes perfect sense, and the ancestral role for G signaling may have been this basic process of life.

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## Glossary

| | |
|---|---|
| **G protein–coupled receptors (GPCRs)** | receptors that sense molecules outside the cell and then activate interior signal transduction pathways and, ultimately, cellular responsesl |
| **Effectors** | proteins, usually enzymes, that are bound by activated G proteins to cause a change in the activity of the effector protein |
| **GTPase-accelerating proteins (GAPs)** | proteins that, in general, bind to activated G proteins and stimulate their GTPase activity, thereby terminating the signaling events |
| **Regulator of G protein signaling (RGS) proteins** | GAPs that use heterotrimeric Gα subunits as their substrates |
| **Guanine nucleotide exchange factors (GEFs)** | proteins that activate G proteins by stimulating the release of GDP to allow binding of GTP |
| ***Arabidopsis* regulator of G protein signaling 1 (AtRGS1)** | the prototype for 7TM-containing RGS proteins |
| **Receptor kinases** | transmembrane proteins with an intracellular kinase domain and an extracellular domain that binds ligands |
| **Pathogen-associated molecular patterns** | molecules associated with groups of pathogens that are recognized by cells of the innate immune system |

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### PLANTS DO NOT HAVE CANONICAL GPCRs

In vitro, animal G proteins bind GDP, and removal of this nucleotide to allow GTP to bind requires a receptor having GEF activity. Plant G proteins spontaneously release GDP and bind GTP in vitro, and thus are self-activating. Self-activation removes the requirement for a receptor GEF. Plants do not need and therefore do not have GPCRs. This idea is difficult for many to grasp because plants have 7TM proteins. There are approximately 50 proteins in *Arabidopsis* and rice that potentially have the same topology as human GPCRs (25, 69), but topology and sequence do not solely define a GPCR. These GPCR "look-alikes" are not plant GPCRs, and we should avoid calling them plant GPCRs.

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## THE IMPORTANCE OF PLANTS IN SOLVING THE EVOLUTION OF G SIGNALING

At present, molecular phylogenetics has classified eukaryotes into six monophyletic supergroups: Opisthokonta, *Amoeba*, Archaeplastida, Chromalveolata, Rhizaria, and Excavata. Opisthokonta includes animals and fungi, and Archaeplastida includes plants and green algae. These two supergroups, divided near the eukaryotic root, render animals and plants as a paired model to deduce the ancestral state of eukaryotes. Two firsts in G protein research came from *Arabidopsis*: (*a*) the self-activating Gα, characterized by combined fast GDP/GTP exchange and slow GTP hydrolysis properties, and (*b*) the receptor GAP 7TM-AtRGS1, which combines a 7TM region (presumably involved in perceiving extracellular ligands and possibly partnered with a coreceptor) with an RGS domain that accelerates the intrinsically slow GTP hydrolysis by Gα. Self-activating Gα and 7TM-RGS proteins are found in an excavate (*Trichomonas vaginalis*) and a chromalveolate (*Ectocarpus siliculosus*) but not in opisthokonts or amoebae. Archaeplastida, Chromalveolata, Rhizaria, and Excavata have few or no GPCR-homologous genes, which implies that G protein regulation by the self-activating property represents the ancestral state and was inherited within those clades. Indeed, canonical G protein effectors are also seen only in the animal lineage. Understanding plant G proteins will solve the fascinating mystery of how organisms evolved elaborate G protein networks, and may also contribute to finding new pharmacological targets against evolutionarily diverged protozoa.

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## SUMMARY POINTS

1. G protein–coupled signaling in plants is profoundly different than it is in animals, even though both plant and animal cells contain the same G protein core elements.
2. Plant G proteins are self-activating; specifically, they bind GTP without the need for a G protein–coupled receptor (GPCR).
3. Plants do not have canonical GPCRs.
4. In most plants, regulation of the activation state is at the back reaction, GTP hydrolysis.
5. A new protein architecture comprising a seven-transmembrane (7TM) domain and a regulator of G protein signaling (RGS) domain was first identified in plants, and the prototype protein, *Arabidopsis* RGS1, serves as the regulatory point of G activation.
6. The well-characterized targets of G proteins in animals (also called effectors) do not exist in plants. Plant effectors have been identified and are prompting new areas of intense investigation.
7. The primary function of G signaling in plants is nutrient sensing, and this information impacts signaling by several plant hormones, light, pathogen-associated molecular patterns, and probably other signals.

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## FUTURE ISSUES

1. Because regulation of G signaling is different in plants than it is in animals, we cannot borrow molecular mechanisms and structures from our colleagues working on animal G signaling. To advance research on plant G signaling, we must solve the atomic structures of the core elements. This information is critical for engineering nutrient sensing.
2. With the availability of many genomes, G protein core element atomic structures, and interaction networks, we have the opportunity for the first time to deduce the evolution of a signaling pathway. Plants will be extremely informative in determining how G signaling networks were assembled, from the base of the tree of life to humans.
3. Plants are ideal for studying developmental plasticity and the role of the environment in developmental outcome. An important example is how drought changes root system architecture. An underlying template for developmental plasticity is the methylome, but the environmental signal transduction to methylome changes is unclear. Because plant G proteins control drought-directed root architecture, the first opportunity to understand how environment controls plasticity is at hand.
4. Sugar sensing is the primary function of plant G signaling, but loss of G signaling affects signaling in pathogen resistance, development, and cell behavior. Plant cells sense their nutrient status and use that information to attenuate or strengthen other signal pathways. This complexity can be resolved by overlaying our knowledge of protein–protein interaction networks and the genetic relationships of the encoding genes.
5. Signal integration may be the reason that water use efficiency and photosynthetic output were not amenable to single-gene manipulation. For example, added genes to increase biomass may be compensated by contradictory information on the cell's need for more biomass. One possible solution is to engineer the nutrient-sensing pathway to allow new functionalities to operate without negative allostery.

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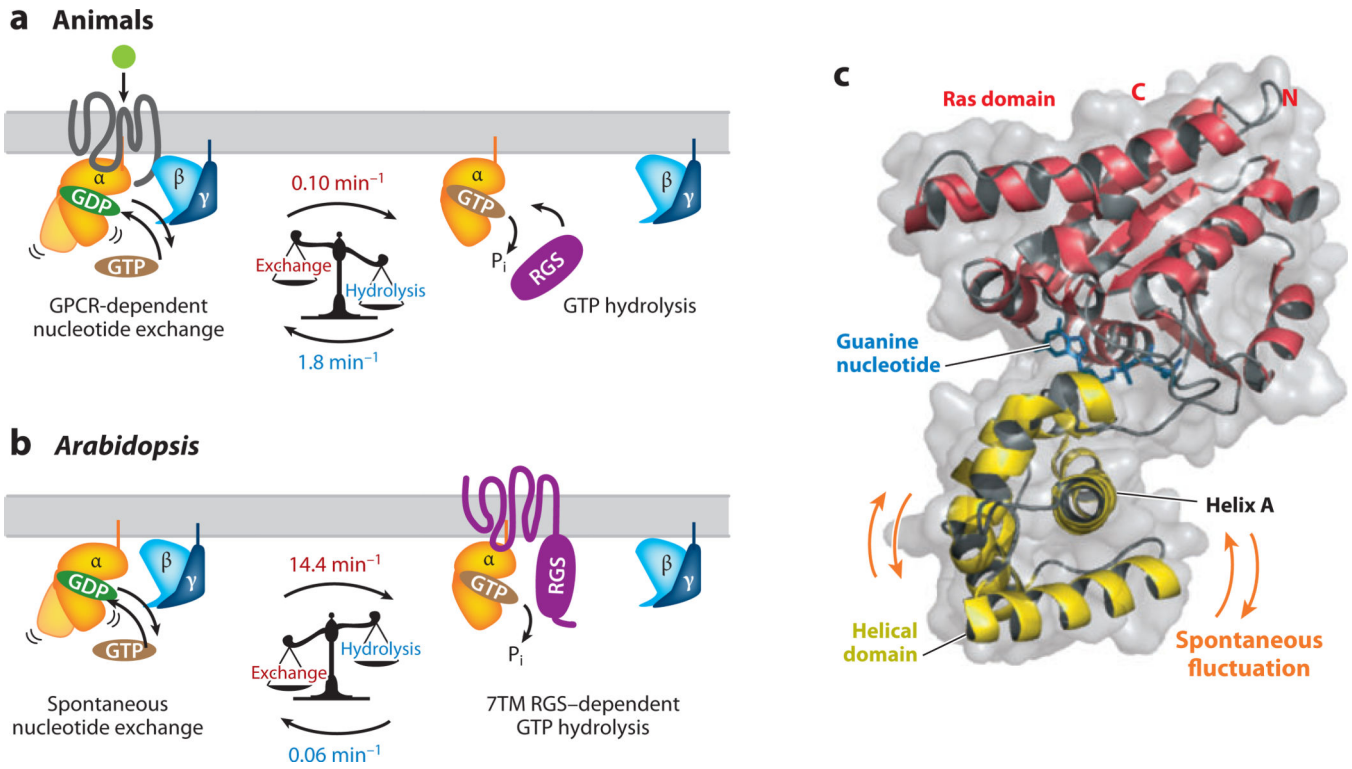


**Figure 1.**

Intrinsic properties and regulatory systems of animal and plant G proteins. (*a*) The animal model. An animal G protein forms an inactive heterotrimer in the steady state. Ligand-bound G protein–coupled receptors (GPCRs) promote nucleotide exchange on the Gα subunit, and GTP-bound Gα separates from the Gβγ dimer. Both the GTP-bound Gα and the freed Gβγ regulate the activity of the effectors. Gα hydrolyzes GTP, returns to the GDP-bound state, and then re-forms the inactive heterotrimer with Gβγ. Regulator of G protein signaling (RGS) proteins accelerate GTP hydrolysis by Gα. The numbers ($min^{-1}$) beside the black arrows show the intrinsic rates of GDP/GTP exchange and GTP hydrolysis. (*b*) The *Arabidopsis* model. The *Arabidopsis* Gα protein, AtGPA1, spontaneously exchanges its GDP for GTP without GPCRs but does not readily hydrolyze GTP without GTPase-accelerating proteins (GAPs). A seven-transmembrane (7TM) RGS protein, AtRGS1, constitutively promotes the intrinsically slow hydrolysis reaction by AtGPA1. (*c*) A structural basis for the self-activating property of AtGPA1 (Protein Data Bank 2XTZ). The Ras domain (*red*) has similarity to small GTPases. It contains sites for binding to guanine nucleotides, effectors, and RGS proteins. The helical domain (*yellow*) shields the guanine nucleotide (*blue*) bound on the Ras domain. Ligand-bound GPCRs in animals or spontaneous fluctuations in *Arabidopsis* change the orientation of the helical domain, leaving the guanine nucleotide exposed, which leads to dissociation from the Ras domain. Blue arrows indicate spontaneous fluctuation of the helical domain, which confers the self-activating property of AtGPA1. Models in panels *a* and *b* adapted from Reference 9.

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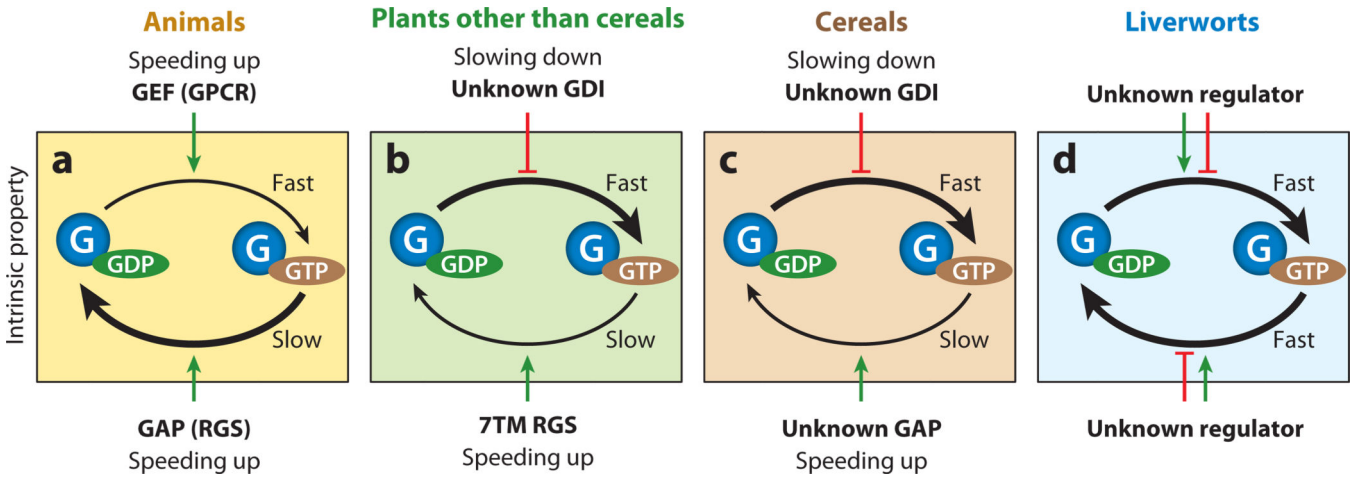


**Figure 2.**

Models of potential regulators of G proteins. The thin curved arrows represent rate-limiting reactions, and the thick curved arrows represent non-rate-limiting reactions. The regulatory molecules that operate on these reactions are shown above and below the curved arrows. The active G protein is shown as a "G" with a bound GTP. The inactive G protein is bound by GDP. (*a*) In animals, activation of G proteins is regulated by a guanine nucleotide exchange factor (GEF) that speeds up the release of bound GDP. (*b*) In plants other than cereals, a seven-transmembrane (7TM) regulator of G protein signaling (RGS) protein speeds up the rate-limiting reaction of hydrolysis. Plants may also utilize a GDP dissociation inhibitor (GDI), which slows nucleotide exchange. (*c*) Cereals lack canonical RGS proteins; therefore, if the rate-limiting GTP hydrolysis is regulated, it is by an unknown mechanism and protein. (*d*) In liverworts, both nucleotide exchange and hydrolysis are fast. The mechanism for regulating the active state of G proteins is unknown and without precedent.

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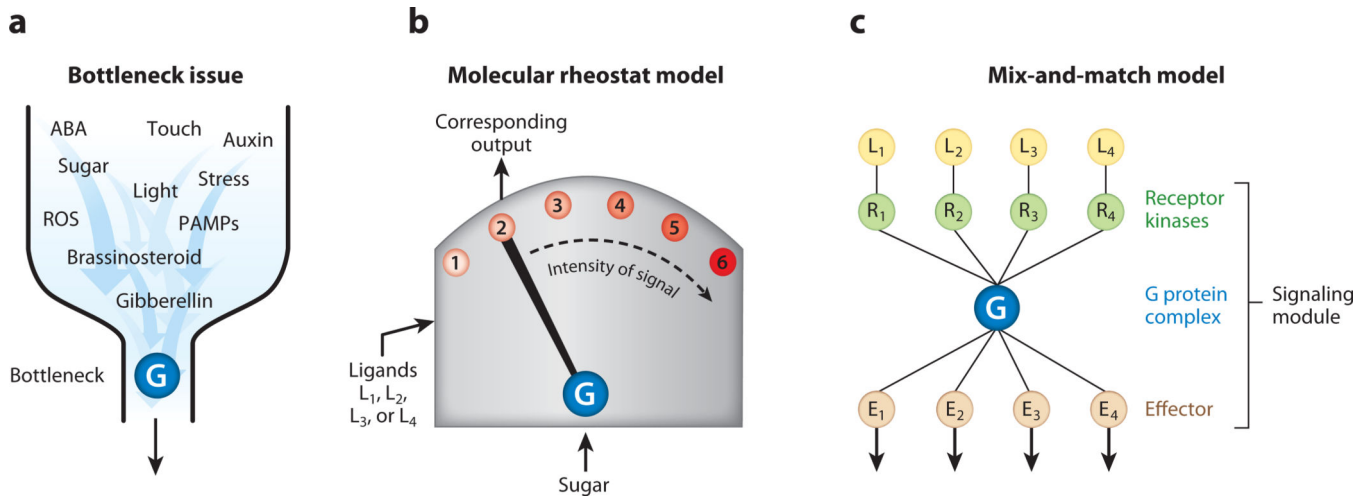


**Figure 3.**

Two models for integrating comprehensive G signals. (*a*) A bottleneck issue in plant G signaling. Plant G proteins process multiple signaling inputs, despite the small repertoire of the G signaling complex. How plant cells sort these inputs out to the appropriate signaling pathways remains unknown. Abbreviations: PAMP, pathogen-associated molecular pattern; ROS, reactive oxygen species. (*b*,*c*) Two models that fit the observations. The molecular rheostat model (panel *b*) modulates different physiological functions. G proteins sense the nutrient status—in this case, the sugar concentration, depicted as the input at the bottom of the rheostat. The nutrient status determines the activation level of the G proteins (the operating arm of the rheostat), then alters the cellular responses in multiple physiological events (the contacts of the rheostat). This model allows G proteins to affect many physiological events without a direct coupling to specific receptors. Only one signaling pathway is shown, but the concept is applicable to others as well. In the mix-and-match model (panel *c*), phosphorylation and endocytosis of *Arabidopsis* regulator of G protein signaling 1 (AtRGS1) cause sustained activation of G signaling by physically uncoupling the seven-transmembrane (7TM) receptor GTPase-accelerating protein (GAP) from the self-activating G protein. In this model, different receptor kinases may indirectly activate G signaling by phosphorylating the 7TM receptor GAP and causing endocytosis of AtRGS1. In this model, each receptor may form a signaling complex with a specific effector of G signaling. This allows a small number of G protein complexes to control various pathways and cellular responses through a single G protein complex.