Plant heterotrimeric G protein function: insights from *Arabidopsis* and rice mutants

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Heterotrimeric G proteins have been implicated in a wide range of plant processes. These include responses to hormones, drought, and pathogens, and developmental events such as lateral root formation, hypocotyl elongation, hook opening, leaf expansion, and silique development. Results and concepts emerging from recent phenotypic analyses of G-protein component mutants in *Arabidopsis* and rice are adding to our understanding of G-protein mechanisms and functions in higher plants.

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Abbreviations

ABA	abscisic acid
AGB1	*Arabidopsis* G protein β subunit 1 gene
AGG1	*Arabidopsis* G protein γ subunit 1 gene
BL	brassinolide
BR	brassinosteroid(s)
d1	*dwarf1* mutant
G protein	heterotrimeric GTP-binding protein
Gα,Gβ,Gγ	heterotrimeric G protein α, β, or γ subunit
GA	gibberellin(s)
GAP	GTPase-activating protein
GCR1	*Arabidopsis* G protein-coupled receptor 1 gene
GPA1	*Arabidopsis* G protein α subunit 1 gene
GPA1^{QL}	GTPase-deficient version of GPA1
GPCR	heptahelical G-protein-coupled receptor
O_3	ozone
Pirin1	*Arabidopsis* cupin domain protein 1
PLC	phospholipase C
PLD	phospholipase D
RGA1	rice G protein α subunit 1gene
RGG1	rice G protein γ subunit 1 gene
RGS	regulator of G-protein signaling protein
S1P	sphingosine-1-phosphate

Introduction

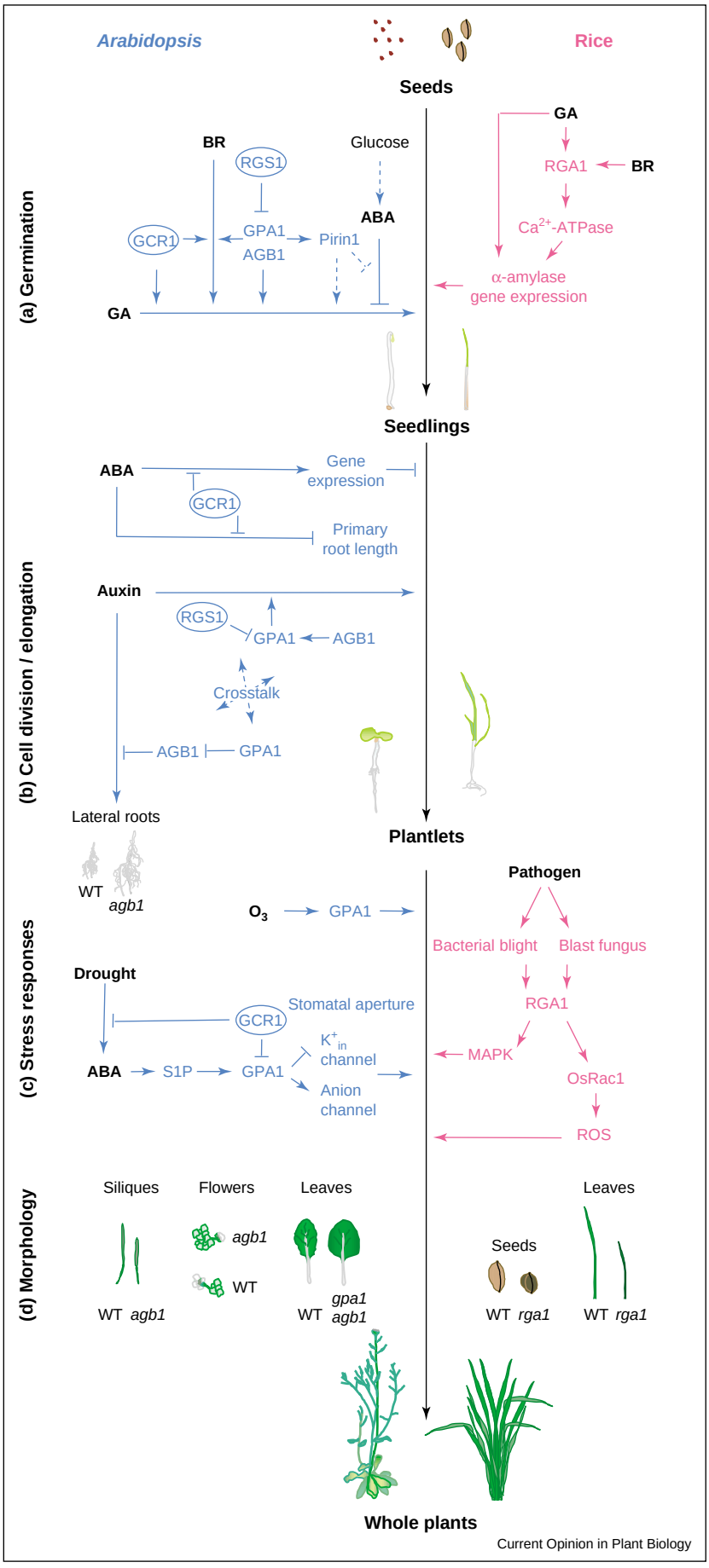
Heterotrimeric GTP-binding proteins (G proteins) provide a key mechanism by which a specific signaling cascade is switched on or off to translate an incoming signal into a specific cellular response. In recent years, much has been learned about the diversity of signal transduction through plant G proteins thanks to the identification and mutation of genes in *Arabidopsis* and rice (*Oryza sativa*) that encode specific G-protein components. These components include the α, β, and γ subunits of the G protein heterotrimer, possible heptahelical G-protein-coupled receptors (GPCRs), and regulator of G-protein signaling proteins (RGS). Such studies are revealing two crucial concepts. First, some physiological responses are predominantly mediated by Gα, whereas others are predominantly mediated by Gβγ. Second, the particular role of any given G-protein component in plant developmental processes [1,2,3••] and responses to biotic and abiotic stresses [4–7,8••] can differ in a cell-type- or developmental-stage-specific manner. Thus, one mutant can even show opposite phenotypic responses to the same stimulus, depending on the particular cell or tissue under study. To highlight these concepts, in this review, we discuss the latest genetic studies on plant G-protein signaling from an 'organ' point-of-view (Figure 1). The reader may also be interested in reviews on plant heterotrimeric G proteins that have emphasized comparisons with mammalian systems [9–13].

The heterotrimeric G-protein paradigm

The G protein itself consists of three different subunits, α, β, and γ (respectively named Gα, Gβ, and Gγ), which form a heterotrimeric complex in the inactive state. Binding of an agonist (i.e. an activating ligand) to its specific GPCR leads to the conversion of an inactive G protein to its active conformation. The GPCR acts as a guanine nucleotide exchange factor, causing Gα to exchange GDP for GTP. As a result, Gα–GTP separates from the Gβγ dimer and both Gα–GTP and the Gβγ dimer separate from the receptor and can activate downstream effectors. Subsequent to signal propagation, the GTP that is bound to Gα is hydrolyzed to GDP, thereby inactivating Gα and allowing its re-association with the Gβγ dimer to reform the inactive heterotrimeric complex. RGS proteins act as GTPase-activating proteins (GAPs) for Gα, typically attenuating signaling by hastening the return of the G protein to the resting state.

G-protein components in *Arabidopsis* and rice

Candidate genes that encode polypeptides that are similar to mammalian G-protein components have been isolated from several higher plant species (summarized in [11]). In *Arabidopsis* and rice, Gα is encoded by a single-


Arabidopsis
Rice
Seeds
(a) Germination
BR
RGS1
Glucose
ABA
GCR1
GPA1
AGB1
Pirin1
GA
GA
RGA1
BR
Ca2+-ATPase
α-amylase
gene expression
Seedlings
(b) Cell division / elongation
ABA
Gene
expression
GCR1
Primary
root length
Auxin
RGS1
GPA1
AGB1
Crosstalk
AGB1
GPA1
Lateral roots
Plantlets
WT
agb1
(c) Stress responses
O3
GPA1
Pathogen
Bacterial blight
Blast fungus
RGA1
Drought
Stomatal aperture
GCR1
K+in
channel
ABA
S1P
GPA1
Anion
channel
MAPK
OsRac1
ROS
(d) Morphology
Siliques
Flowers
Leaves
Leaves
agb1
WT
WT
agb1
Seeds
WT
gpa1
agb1
WT
rga1
WT
rga1
Whole plants
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copy gene, designated *GPA1* or *RGA1*, respectively [14,15]. Gβ is likewise encoded by a single-copy gene, designated *AGB1* or *RGB1*, respectively [16,17]. Two Gγ genes were recently isolated from *Arabidopsis* and rice: *AGG1* or *RGG1* [18,19•] and *AGG2* or *RGG2* [19•,20]. No plant gene has been found that is highly homologous to metazoan GPCRs. However, in *Arabidopsis*, *GCR1* is a likely candidate as a GPCR-encoding gene because it encodes a protein that has some GPCR sequence similarity and a predicted heptahelical structure that is the hallmark of *bona fide* GPCRs [8••,21,22]. Finally, it appears that the *Arabidopsis* genome contains only one member of the RGS family, *RGS1* [23••]. Transgenic plants that ectopically and/or conditionally express each of the above-described components, except the Gγ subunit genes, have been described recently (Table 1), and various single, double, and triple mutants have been generated (Table 1).

On the basis of both modeling [3••] and experimentation, the basic paradigm of mammalian G-protein signaling described above also appears to operate in plants [13]. Gα, Gβ, Gγ, GCR1 and RGS1 can all be found at the plasma membrane of plant cells [1,8••,19•,22,23••,24,25]. In yeast two-hybrid assays and co-immunoprecipitation experiments, Gβ interacts tightly with both Gγ subunits in both *Arabidopsis* and rice [18,19•,20]. In rice, gel-filtration experiments have confirmed that Gβγ dimers associate with Gα. This association is disrupted by a non-hydrolysable form of GTP, GTPγS, which is expected to maintain the activated conformation of Gα [19•]. Pandey and Assmann [8••] used *in planta* and *in vitro* co-immunoprecipitation as well as split-ubiquitin yeast two-hybrid assays to provide the first conclusive evidence that the putative GPCR, GCR1, physically interacts with Gα. RGS1 interacts with both a constitutively active GPA1 ($GPA1^{QL}$, the GTPase-deficient version of GPA1) and wildtype GPA1, and the carboxy-terminal domain of AtRGS1 has been shown to exert GAP activity on a yeast Gα [23••].

Striking differences also exist, however, between the G-protein components of plants and those of other eukaryotic organisms: the sequence similarity of the relevant genes and proteins is limited, and a much smaller number of genes encode each of the different components in plants than in other eukaryotes [13].

G-protein signaling in seeds

Seed germination is a complex phenomenon that is modulated by numerous signals, including gibberellins (GA), abscisic acid (ABA), brassinosteroids (BR), ethylene, light, and sugars, some acting in concert and others in opposition [26]. Current models of seed germination in non-graminaceous species suggest that BR act downstream of GA to promote germination. Both ABA and sugars inhibit germination, and ethylene negatively regulates ABA's effects.

In the absence of stratification, *gpa1-1* and *gpa1-2* mutant seeds exhibit delayed germination [27•], suggesting that they are more dormant than wildtype seeds. Consistent with this phenomenon, *gpa1* mutants exhibit moderately increased sensitivity to the inhibition of germination by ABA and sugars [2,27•]. Many of these phenotypes are also observed in *Arabidopsis* T-DNA insertional mutants

(Figure 1 Legend) Highly simplified model of physiological processes during plant development that involve G proteins based on the phenotypes of *Arabidopsis* and rice G-protein-component mutants. The black time-line symbolizes plant developmental stages from seeds to whole plants (not drawn to scale). To the left (blue) and to the right of this line (pink), the model describes physiological processes that involve G proteins in *Arabidopsis* and rice, respectively. Arrows represent activation and T-bars inhibition in the signaling pathways. Dotted arrows signify putative pathways that still require experimental confirmation. (a) Germination. In *Arabidopsis*, GCR1 may positively regulate seed germination by coupling BR promotion of GA-stimulated germination. GCR1 also can act independently of GPA1 and AGB1 in a pathway to regulate GA-stimulated germination [23••]. RGS1 antagonizes the activation of GPA1 [23••]. Pirin1 may positively regulate seed germination by overcoming the negative effect of ABA or by activating germination-promoting pathways [27•]. In rice, RGA1 may work in a high-sensitivity GA pathway that regulates the induction of Ca^{2+}-ATPase and α-amylase, leading to seed germination [32]. RGA1 may also be a component of BR signaling [33]. In addition, there may be an alternative GA pathway that also induces α-amylase but does not involve RGA1 [32]. **(b)** Cell division/elongation. During seedling growth, GPA1, AGB1 and GCR1 may act in the inhibition of primary root development by ABA ([8••]; S Pandey, SM Assmann, unpublished). Furthermore, GCR1 negatively regulates ABA-induced gene expression [8••]. AGB1 and GPA1 activate cell division in both hypocotyls and leaves [1,2,3••,37] whereas RGS1 antagonizes the activation of GPA1 in apical root meristems [23••]. Auxin treatment also increases GPA1 transcript levels and decreases AGB1 transcript levels (not shown in figure, [3••]). During lateral root formation, AGB1 functions downstream of GPA1 and inhibits auxin-induced cell division, and GPA1 inhibits AGB1 function [3••]. **(c)** Stress responses. According to the leaf curling phenotype, GPA1 promotes the O_3 sensitivity of *Arabidopsis* plants [6]. Drought stress and ABA treatment inhibit stomatal opening and promote stomatal closure. ABA triggers S1P formation, which is coupled by GPA1 to inhibit plasma membrane inwardly rectifying K^+ channels and to activate slow anion channels, resulting in the inhibition of stomatal opening and the promotion of stomatal closure [4,45••]. The GPCR-like protein GCR1 directly binds to GPA1 and negatively controls ABA- and S1P-regulation of stomatal apertures [8••]. In rice, responses to avirulent rice blast fungus, including the accumulation of transcripts for the small GTPase, OsRac1, are attenuated in the *RGA1* mutant *d1*. OsRac1 acts as a key molecular switch for multiple signaling pathways, such as the production of reactive oxygen species that lead to disease resistance. Expression of constitutively active OsRac1 in the *d1* mutant restores defense signaling [5]. In response to virulent strains of bacterial blight, lesions are more severe in the *d1* mutant than in wildtype plants [7]. **(d)** Morphology. In *Arabidopsis*, both GPA1 and AGB1 modulate leaf development and shape [1,39]. *gpa1* and *agb1* mutants exhibit rounded lamina. AGB1 is also involved in flower and fruit development [1,3••,39]. In *agb1*, the floral buds at the inflorescence apex are more tightly clustered, the siliques are shorter, and the silique tips are more blunt than those of wildtype plants. In rice, RGA1 modulates plant stature by regulating internode and panicle elongation, and also influences the color of leaf blades and sheaths and grain shape [32].

Table 1

Mutant and transgenic lines for Gα, Gβ, GCR1 and RGS1.

Name/allele	Ecotype/ cultivar	cDNA	Status of transcript/ translation product	Phenotype comparison with wildtype ecotypes
Mutant loss of function for Gα subunit in *Arabidopsis* (*A. thaliana*)				**Phenotypes of *gpa1* mutants**
gpa1-1	Ws	T-DNA insertion in 7th intron. Wisconsin KO *Arabidopsis* facility α population.	Lacks full-length transcript [1].	Less sensitive to GA and BL stimulation of germination [1,2]. More sensitive to the GA biosynthesis inhibitor paclobutrazol [2,22]. Hypersensitive to ABA and sugar inhibition of germination [2,27•]. In darkness, partial de-etiolation: open hook; shorter hypocotyls caused by reduced cell division [1].
gpa1-2	Ws	T-DNA insertion in 8th exon. Wisconsin KO *Arabidopsis* facility α population.	Lacks full-length transcript [1].	Primary root forms fewer lateral root primordia [1]. Less sensitive to auxin promotion of lateral root formation [3••]. Rounded lamina shape [1,37]. Leaf cells are fewer and larger [1]. Longer sepals and pedicels [1]. Less sensitive to O3 [6]. More water loss [4].
gpa1-3	Col-0	T-DNA insertion in 9th exon. Salk collection.	Lacks full-length transcript [37].	Insensitive to ABA inhibition of stomatal opening [4]. Insensitive to ABA inhibition of inward K^+ channels [4]. Insensitive to S1P promotion of stomatal closure [45••].
gpa1-4	Col-0	T-DNA insertion in 12th intron. Salk collection.	Lacks full-length transcript [37].	Altered sensitivity to ABA activation of slow anion channels [4]. Insensitive to S1P activation of slow anion channels [45••].
Mutant loss of function for Gα subunit in rice (*Oryza sativa*)				**Phenotypes of Daikoku *dwarf1* (*d1*) mutants (DK22, HO541, CM1361-1, T65d1, *rga1*)**
DK 22	Nipponbare	Point mutation of G598 to T in 8th exon.	Stop codon generated [31,40]. Protein null [19•].	Shorter and rounded grains [30,31]. Reduced GA and BL stimulation of gene expression [32,33].
HO 541	Nipponbare	Spontaneous mutant: deletion of 833 basepairs between 1st exon and intron.	*RGA1* transcript null [30].	Shorter and darker green leaves, more compact panicle [5,30–32,38].
CM 1361-1	Kinmaze	Insertion between nucleotides 354–355.	Predicted protein lacks GTP-, effector- and receptor-binding regions [31].	Shorter internodes — may be due to a decrease in the number of cells per internode [31]. Reduced GA stimulation of internode growth [32]. Normal GA stimulation of second leaf sheath elongation [32].

Table 1 (Continued)

Name/allele	Ecotype/ cultivar	cDNA	Status of transcript/ translation product	Phenotype comparison with wildtype ecotypes
T65d1	Taichung 65	Deletion of nucleotides 1003–1004.	Stop codon generated before third effector-binding region [32].	Reduced hypersensitive response to infection by rice blast fungus [5].
rga1	Nipponbare	Antisense suppression.	*RGA1* transcript null line [31].	Increased sensitivity to infection by virulent strain of bacterial blight [7].
HO 532 HO 533 HO 537	Nipponbare	Spontaneous mutants.	[30]	Not used for phenotypic analysis.
HO 538 HO 552 FL2	Nipponbare	Marker line derived from HO 538.	[30]	Not used for phenotypic analysis.
ID 1	Shiokari	Deletion of nucleotides 1003–1004.	[31]	Not used for phenotypic analysis.
CM392; 1729; 248; 723; 1232	Kinmaze	Induced by *N*-methyl-*N*-nitrosourea.	[5,30]	Not used for phenotypic analysis.
DKT 1	Taichung 65	Point mutation of A1075 to T.	[31]	Not used for phenotypic analysis.
DKT 2	Taichung 65	Deletion between nucleotides 932–979.	Predicted to lack GTP-binding region [31].	Not used for phenotypic analysis.
Mutant gain of function for Gα subunit in *Arabidopsis*				**Phenotypes of GPA1 overexpressors**
Q222L or GPA1*	Col	35S promoter::point mutation A1264 to T derived from GPA1 cDNA.	Mutation disables GTPase activity, leading to constitutively active Gα [1,3••,37].	No effect on auxin-induced cell division in lateral roots [3••]. No auxin effect on hypocotyl length [3••].
$GPA1^{QL}$	Ws	35S promoter::constitutive form of GPA1 cDNA.	Overexpression of constitutively active Gα ($GPA1^{QL}$) [23••].	Increased hypocotyl length caused by increased cell elongation [23••]. Longer primary roots caused by increased cell production [23••].
cGα	Ws	DEX inducible promoter:: constitutive form of GPA1 cDNA.	Overexpression of constitutively active Gα ($GPA1^{QL}$) [54].	Under low light condition, shorter hypocotyls are caused by a reduction in cell elongation, also smaller cotyledons and increased stomatal density in hypocotyl [54].
wGα	Ws	DEX inducible promoter:: GPA1 cDNA.	Overexpression of full-length GPA1 protein [54].	Under low light condition, shorter hypocotyls are caused by a reduction in cell elongation, also smaller cotyledons and increased stomatal density in hypocotyl [54].
gpa1 (GPA1)	Col-0	DEX inducible promoter:: GPA1 cDNA.	Complementation in *gpa1* background [3••,37].	Hypersensitive to GA stimulation of germination [2]. In darkness, shorter hypocotyls [37]. Hypocotyl hypersensitive to auxin-induced adventitious root formation [3••].
35S::GPA1–GFP	Col	35S promoter:: GPA1 cDNA fused with GFP.	Overexpression of fluorescent GPA1 [23••]	
GOX	Col	DEX-inducible promoter:: GPA1 cDNA.	Overexpression of GPA1 [2,37].	Mimics *agb1-2* lateral root phenotype: more lateral roots [3••].
GOX1	Tobacco cells *Nicotiana tabaccum* cv. BY2	DEX inducible promoter:: GPA1 cDNA.	Transformed BY2 cells overexpressing GPA1 [1,49].	Shorter cell cycle [1]. Higher PtdIns–PLC activity [49]. Higher Ins(1,4,5)P_3 content [49].

Table 1 (Continued)

Name/allele	Ecotype/ cultivar	cDNA	Status of transcript/ translation product	Phenotype comparison with wildtype ecotypes
Mutant gain of function for Gα subunit in rice				**Phenotypes of RGA1 overexpressors**
QL/*d1* or Q223L	Nipponbare	35S promoter:: point mutation Q223 to L derived from RGA1 cDNA.	Expression of constitutively active Gα in *d1* background [19•].	Active form GTP–Gα presents free from Gβ or Gγ subunits [19•].
Mutant loss of function for Gβ subunit in *Arabidopsis*				**Phenotypes of *agb1* mutants**
agb1-1 or *elk4*	Col	Ethyl methanesulfonic acid mutagenized. Missense mutation: failure to splice out the 1st intron.	Mutant transcript slightly larger because of splicing failure. Stop codon generated [39].	Less sensitive to GA and BR stimulation of germination [22]. More sensitive to the GA biosynthesis inhibitor paclobutrazol [2,22]. Hypersensitive to sugar inhibition of germination [2]. In darkness, partial de-etiolation: open hook; shorter hypocotyls caused by reduced cell division [1]. Hypocotyl hypersensitive to auxin-induced adventitious root formation [3••]. Primary root forms more lateral root primordia [3••].
agb1-2	Col-0	T-DNA insertion in 4th exon. Salk collection.	Lacks full-length transcript [3••].	More sensitive to auxin promotion of lateral root formation [3••]. Rounded lamina shape and presence of islands of small cells that create a crinkly surface [3••,39]. Shorter flowers and sepals [39]. Shorter and thicker siliques [39].
Mutant gain of function for Gβ subunit in *Arabidopsis*				**Phenotypes of AGB1 overexpressors**
agb1-1 (AGB1)	Col	Transformation with genomic fragment containing *AGB1* gene and promoter.	Complementation in *agb1-1* background [39].	
agb1-2 (AGB1)	Col-0	DEX inducible promoter:: AGB1 cDNA.	Complementation in *agb1-2* background [3••].	Decreased auxin-induced lateral root formation relative to *agb1* [3••].
BOX	Col-0	DEX inducible promoter:: AGB1 cDNA.	Overexpression of AGB1 [3••,37].	
Mutant loss of function for GCR1 in *Arabidopsis*				**Phenotypes of *gcr1* mutants**
gcr1-1	Col-0	T-DNA insertion in 8th intron. Salk collection.	Lacks full-length transcript [22].	Less sensitive to GA and BR stimulation of germination [22]. Hypersensitive to ABA inhibition of germination [8••].
gcr1-2	Col-0	T-DNA insertion in 6th exon. Salk collection.	Lacks full-length transcript [22].	Hypersensitive to the GA inhibitor paclobutrazol [22]. Increased ABA promotion of ABA-regulated gene expression [8••].
gcr1-3	Ws	T-DNA insertion in 2nd intron. Wisconsin *Arabidopsis* KO facility BASTA population.	Lacks full-length transcript [8••].	Decreased water loss [8••]. Increased resistance to drought stress [8••]. Hypersensitive to ABA and S1P inhibition of stomatal opening [8••].
gcr1-4	Col	T-DNA insertion in 3rd intron. SAIL collection of TMRI.	Lacks full-length transcript [8••].	Hypersensitive to ABA and S1P promotion of stomatal closure [8••]. Flowers slightly earlier [22].

Table 1 (Continued)

Name/allele	Ecotype/ cultivar	cDNA	Status of transcript/ translation product	Phenotype comparison with wildtype ecotypes
Mutant gain of function for GCR1 in *Arabidopsis*				**Phenotypes of GCR1 overexpressors**
gcr1-3 (GCR1)	Ws	DEX inducible promoter:: GCR1 cDNA fused with FLAG tag.	Expression in *gcr1-3* background [8••].	GCR1–FLAG immunoprecipitates with GPA1 [8••].
35S::GCR1-GFP	Col	35S promoter::GCR1 cDNA fused with GFP.	Overexpression of fluorescent GCR1 [22].	
GCR1-over-expressing lines	Col	35S promoter::GCR1 cDNA.	Overexpression of GCR1 [29].	Lacks seed dormancy [29]. Increased expression of germination associated genes [29]. Early flowering [29].
GCR1-over expressing BY2 cells	Tobacco cells *Nicotiana tabaccum* cv. BY2	35S promoter::GCR1 cDNA.	Transformed BY2 cells overexpressing GCR1 [29,49].	Increased DNA synthesis [29]. Higher PtdIns–PLC activity [49]. Higher Ins(1,4,5)P_3 content [49].
Mutant loss of function for RGS1 in *Arabidopsis*				**Phenotypes of *rgs1* mutants**
rgs1-1	Col-0	T-DNA insertion in 6th intron. Salk collection.	Lacks full-length transcript [23••].	Mimics $GPA1^{QL}$ phenotype under darkness: longer hypocotyls caused by increased cell elongation [23••]. Longer primary roots caused by increased cell production in light [23••].
rgs1-2	Col-0	T-DNA insertion in 9th intron. Salk collection.	Lacks full-length transcript [23••].	Insensitive to 6% D-glucose inhibition of seedling growth [23••].
Mutant gain of function for RGS1 in *Arabidopsis*				**Phenotypes of RGS1 overexpressors**
35S::RGS1-GFP	Col	35S promoter::RGS1 cDNA fused with GFP.	Overexpression of fluorescent RGS1 [23••].	
ROX	Col-0	DEX inducible promoter:: RGS1 open reading frame.	Overexpression of full length RGS1 protein [23••].	Mimics *gpa1* mutant hypocotyl phenotype under darkness: shorter hypocotyl [23••]. Hypersensitive to 6% D-glucose inhibition of seedling growth [23••].
Loss of function double/triple mutants in *Arabidopsis*				**Phenotypes double/triple mutants**
gpa1-4 agb1-2	Col-0	Cross between *gpa1-4* and *agb1-2*.	[37]	Less sensitive to GA and BR stimulation of germination (same sensitivity as *agb1* mutant [22]). Shorter hypocotyls and partially opened hooks [37]. Rounded lamina shape [22]. Less sensitive to O_3 [6].
gcr1-2 gpa1-4	Col-0	Cross between *gcr1-2* and *gpa1-4 agb1-2*.	[22,37]	Less sensitive to GA and BR stimulation of germination (additive or synergistic effect of mutations [22]). *gpa1* phenotype under darkness: shorter hypocotyl and partially opened hook [22]. *gpa1* leaf morphology: rounded lamina shape [22]. Less sensitive to GA and BR stimulation of germination (additive or synergistic effect of mutations [22]).

Table 1 (Continued)

Name/allele	Ecotype/ cultivar	cDNA	Status of transcript/ translation product	Phenotype comparison with wildtype ecotypes
agb1-2 gcr1-2	Col-0	Cross between *gpa1-4 agb1-2* and *gcr1-2*.	[22]	*agb1* phenotype under darkness: shorter hypocotyl and partially opened hooks [22]. *agb1* leaf morphology: rounded lamina shape [22]. Less sensitive to GA and BR stimulation of germination (additive or synergistic effect of mutations [22]).
agb1-2 gcr1-2 gpa1-4	Col-0	Cross between *gpa1-4 agb1-2* and *gcr1-2*.	[22]	*gpa1* and *agb1* phenotype under darkness: shorter hypocotyl and partially opened hooks [22]. *gpa1* and *agb1* leaf morphology: rounded lamina shape [22].
agb1-1 er-105	Col	Cross between *agb1-1* and receptor-like kinase erecta mutant, *er-105*.	[39]	Shorter petiole, shorter lamina than either *agb1* or *er105* single mutant, suggesting that ER and AGB1 function in parallel pathways controlling these characteristics [39].
d1 slr	Nipponbare	Cross between *d1* and GA insensitive mutant *slr*.	[32]	*SLR* is epistatic to *D1* supporting RGA1 involvement in GA signaling [32].

35S, cauliflower mosaic virus 35S promoter; BOX, AGB1 overexpressing lines; Col, Columbia; DEX, dexamethasone; *elk*4, erecta like 4 mutant; *er-105*, receptor-like kinase erecta mutant; GFP, green fluorescent protein; GOX, GPA1 overexpressing lines; Ins(1,4,5)P_3, inositol-1,4,5-trisphosphate; PtdIns-PLC, phosphatidylinositol-phospholipase C; ROX, RGS1 overexpressing lines; SAIL, Syngenta Arabidopsis Insertion Library; *slr*, slender rice mutant; TMRI, Torrey Mesa Research Institute; Ws, Wassilewskija.

of Pirin1, a cupin-domain protein that has been identified as a GPA1 interactor in yeast two-hybrid assays [27•].

Because *gpa1* seeds have wildtype ABA concentrations [2], the results described above presumably reflect either an increased sensitivity to ABA or a decreased sensitivity to stimulatory signals such as GA. In support of the latter hypothesis, the germination of *gpa1* and *agb1* seeds is significantly less sensitive to exogenous GA and significantly more sensitive to the GA-synthesis inhibitor paclobutrazol than the germination of wildtype seeds [2,22]. Ullah *et al.* [2] speculate that GPA1 controls the sensitivity of the GA pathway because although the overexpression of GPA1 in *Arabidopsis* confers a million-fold increase in the GA sensitivity of seed germination, the requirement for GA is not abolished. If GPA1 directly coupled the GA response, then the ectopic expression of *GPA1* would be expected to confer GA independence, which is not the case. Ullah *et al.* [2] further suggest that BR controls GA sensitivity in a GPA1-dependent manner, because brassinolide (BL) rescue of germination in seeds treated with paclobutrazol is complete for wildtype seeds but only partial for *gpa1* and *agb1* seeds [2,22,28].

Like *gpa1* mutants, *gcr1* mutants exhibit reduced sensitivity toward GA and BR in seed germination, whereas *GCR1* overexpression reduces seed dormancy [22,29]. Under some but not all conditions, seeds of *gcr1 gpa1* and *gcr1 agb1* double mutants have additive or synergistic germination responses to GA and BR, which is unexpected if GCR1 were to function upstream of the G protein. Thus, under some conditions, GCR1 appears able to act independently of the heterotrimer in regulating seed germination [22].

Seeds of the rice *dwarf1* (*d1*) mutant, a null mutant [19•] of the rice Gα subunit, *RGA1*, exhibit a morphological phenotype consisting of short, round grains [30,31]. Observations in rice are also consistent with a role for G proteins in GA-based signaling pathways and the control of transcription in the seed. *d1* mutants exhibit reduced GA induction of α-amylase gene expression and enzyme activity in their aleurone cells and reduced expression of the GA-induced genes *OsGAMYB* and *GA-Ca^{2+} ATPase* [32]. Gα may also be a component of BR signaling in rice because BL-stimulated expression of a novel BL-enhanced gene is weaker in *d1* mutants than in wildtype seedlings [33]. Ueguchi-Tanaka *et al.* [32]

suggest that there may be two separate GA-signaling pathways in rice, with either high or low sensitivity to GA, and that RGA1 may mediate the former pathway. Both this model and the 'GPA1 modulation' model described above [2,32] are consistent with the current data from both *Arabidopsis* and rice. Hence, additional experimentation, including determination of the ABA sensitivity of *d1* seeds, will be required to distinguish between these two possibilities.

G-protein signaling in roots

Root growth and architecture involves a balance between cell production in the apical and lateral root meristems and the subsequent elongation of those cells. One advantage of the root as a model system for development is that it is possible to measure rates of cell production and elongation as well as the number of lateral root primordia quite precisely, thereby making it possible to quantify exactly what has changed in the roots of loss- and gain-of-function G-protein mutants. The formation of lateral root meristems originates from a set of founder cells that differs from that used to form the primary meristem [34,35]. Therefore, it would not be surprising if the molecular mechanisms that underlie the initiation of lateral and primary root meristems were different. Studies on root meristem formation and maintenance using G-protein mutants are beginning to reveal these mechanistic differences.

Primary root

The primary root growth of wildtype plants and that of *gpa1* and *gcr1* single mutant seedlings appears to be identical in the absence of exogenous hormone treatment [3••,8••]. By contrast, *rgs1* mutants have longer primary roots because of their increased cell production rate in the primary root meristem [23••]. *rgs1* cells, which lack GAP activity, are predicted to have a greater steady-state pool of activated Gα. This prediction is consistent with the observation that the expression of a transgene that encodes $GPA1^{QL}$ also causes accelerated cell production by the primary root meristem [23••]. This suggests that Gα plays a role in modulating cell division in the primary root meristem. The lack of a large effect of the *gpa1* null mutation on primary root growth suggests that the type of modulation that GPA1 exerts may be an increase over a basal state, a state that does not require Gα.

In response to exogenous treatment with plant growth regulators such as ABA and auxin, primary root elongation is retarded and/or the direction of primary root growth changes. The primary root elongation of *gpa1*, *agb1*, or *gcr1* single mutants, as well as of double and triple combinations of these mutants, is more sensitive to inhibition by ABA than that of wildtype plants ([8••]; S Pandey, SM Assmann, unpublished). However, the auxin inhibition of primary root length in *gpa1* and *agb1* mutants is the same as that in wildtype plants [3••], indicating that the dependency of growth inhibition on G proteins differs depending on the hormonal stimulus.

Lateral and adventitious roots

While the growth of the primary root of *gpa1* mutants is like that of wildtype plants under many conditions, the number of lateral roots is greatly increased in *agb1* mutants and is decreased in *gpa1* mutants [3••]. Opposite to its inhibitory effect on the primary root, auxin is a key activator of lateral root initiation [36]. In the presence of auxin, *agb1* plants form more lateral roots, whereas *gpa1* plants form fewer lateral roots, compared to wildtype plants [3••]. As is expected if the auxin-induced phenotype for the proliferation of lateral roots is AGB1-dependent, ectopic expression of GPA1 (which is expected to sequester AGB1 into the heterotrimeric complex) also yields an *agb1*-like phenotype. The expression of $GPA1^{QL}$ has no effect on this phenotype, a finding that is inconsistent with GPA1 acting as a positive modulator of cell division in the lateral root meristem. Thus, Ullah *et al.* [3••] propose that free Gβγ directly attenuates auxin-induced cell division in lateral roots, as opposed to Gα acting to stimulate this process.

G-protein signaling in shoots

As for seed germination and root development, several differences have been observed between G-protein-component mutants and wildtype plants during the development of above-ground organs in seedlings and mature plants [10–12].

When grown in darkness, *gpa1* and *agb1* seedlings have shorter hypocotyls than wildtype plants because of a reduction in cell number, and these seedlings exhibit partially opened hooks [1,3••,37]. These phenotypes were also observed in *gcr1 gpa1* double, *agb1 gcr1* double, and *agb1 gpa1 gcr1* triple mutants [22]. By contrast, *rgs1* mutant seedlings have a longer etiolated hypocotyl as a result of increased cell elongation. This mutant phenotype is similar to that observed in plants that express $GPA1^{QL}$, consistent with the premise that, in plants as in animals, RGS proteins oppose Gα activation [22].

When grown in light, *gpa1* and *agb1* mutants have rounded rosette leaves [1]. The round-leaf phenotype is also found in *Arabidopsis gcr1 gpa1* and *agb1 gcr1* double mutants and *agb1 gcr1 gpa1* triple mutants [22]. Because *gcr1* single mutants have wildtype phenotypes for both hypocotyl and rosette-leaf development, GCR1 may not act as the GPCR that is responsible for control of these developmental pathways [22].

The rice Gα (*d1*) mutants also exhibit an altered shoot morphology, consisting of broad, dark green leaves and compact panicles [5,30,31,32,38]. One notable contrast between *Arabidopsis* and rice Gα mutants, however, is that the rice mutants are dwarf but the *Arabidopsis*

mutants are not. Dwarf phenotypes are often associated with GA insensitivity, and GA induction of internode elongation is significantly reduced in the *d1* mutants [31]. However, the GA responsiveness of the elongation of the second leaf sheath is similar in *d1* mutants and wildtype plants [32]. This selective impairment of GA signaling in *d1* mutants suggests cell specificity in GA response, with some pathways being only marginally dependent on Gα.

During the reproductive phase of plant development, *agb1-1* mutants have a floral phenotype consisting of shorter flowers and thicker siliques, but this phenotype is not shared by *gpa1* mutants [1,3••,39]. Constitutive overexpression of *GPA1* reduces silique length, producing a phenotype that is similar to that of *agb1*. This evidence is consistent with the idea that silique length is controlled by released Gβγ [3••]. *gpa1* sepals and pedicels are longer, whereas *agb1* sepals are shorter, than those of wildtype plants [3••], findings that are again consistent with a Gβγ-dependent pathway. Transformants that overexpress *GCR1* flower earlier [29], but *gcr1* null mutants typically do not flower later, than wildtype plants [22].

Stress responses

G proteins are implicated in several stress-signaling pathways in plants. In mature leaves, G proteins transmit signals to molecules, including small GTPases, ion channels, and phospholipases, that are effectors in the responses to various biotic and abiotic stress conditions, including pathogen elicitation, ozone treatment and water deficit.

There are no reports as yet on pathogen signaling in *Arabidopsis* G-protein mutants, but some defense signaling pathways in rice appear to rely on RGA1. Upon infection with a virulent strain of bacterial blight (*Xanthomonas oryzae* pv. *Oryzae* [*Xoo*]), symptom development in *d1* mutants is more severe than that in wildtype plants [7]. By contrast, infection with virulent strains of rice blast fungus (*Magnaporthe grisea*) produces identical lesions in *d1* mutants and wildtype plants. *d1* mutants exhibit a highly reduced response, however, upon inoculation with avirulent rice blast [5,7]. Expression of a constitutively active OsRac1 in *d1* mutants restores defense signaling and resistance, suggesting that RGA1 functions upstream of this small GTPase [5]. Yet, in a *d1* mutant cell line treated with the oligosaccharide elicitor chitin, the elicitation of defense responses such as extracellular alkalinization, generation of reactive oxygen species, phytoalexin accumulation and the induction of specific genes does not differ from that of wildtype cells [40,41]. Taken together, these studies indicate that the extent of G-protein coupling of responses to both avirulent and virulent pathogens is pathogen- and elicitor-specific.

Like pathogen infection, exposure to high ozone (O_3) levels results in foliar lesions, and O_3 responses share signaling pathways and gene expression patterns with the hypersensitive response [42]. *gpa1* null mutants and the double mutant *gpa1-4 agb1-2* respond differently to O_3 compared to wildtype plants, and to *gcr1* and *rgs1* single mutants. The major difference observed among these mutant genotypes is an O_3-resistant phenotype of the *gpa1* lines, indicated by lack of leaf curling in response to O_3 [6].

One of the phenomena commonly observed following O_3 exposure is a reduction in stomatal apertures [43], a response that is also evoked by ABA. *gpa1* mutants exhibit reduced O_3 sensitivity at the whole-leaf level. At the single (guard)-cell level, *gpa1* mutants also exhibit aspects of ABA insensitivity, including reduced ABA inhibition of guard cell inward K^+ channels and altered ABA-promotion of slow anion currents [4]. Recently, the lipid metabolite, sphingosine-1-phosphate (S1P), has been described as a secondary messenger for ABA responses [44,45••]. The guard cells of *gpa1* mutants show insensitivity to inhibition of stomatal opening by either ABA or S1P. However, ABA still induces wildtype levels of stomatal closure in *gpa1* [4], whereas stomatal closure in this genotype is insensitive to S1P. This difference implies that the S1P response is obligatorily mediated by GPA1, whereas there is a parallel or backup pathway for ABA induction of stomatal closure that is independent of GPA1 [45••].

gcr1 mutant guard cells exhibit hypersensitivity to ABA and S1P in both inhibition of stomatal opening and promotion of stomatal closure [8••], which would be unexpected if GCR1 were to transmit the ABA signal to GPA1. Pandey and Assmann [8••] therefore proposed that GCR1 acts as a negative regulator of GPA1-mediated ABA responses in guard cells. Consistent with this phenomenon, *gcr1* mutant plants have higher expression levels of some known drought- and ABA-regulated genes after exogenous ABA treatment and exhibit improved recovery following drought stress [8••].

Many enzymes, including phosphatidylinositol-phospholipase Cs (PLCs; reviewed in [46]) and phospholipase Ds (PLDs; [47,48]), act as effectors of the ABA response during the regulation of stomatal aperture. These phospholipases also have been identified recently, albeit not yet in guard cells, as intracellular effectors of G protein signaling. For instance, using tobacco BY2 cells that overexpressed GCR1, Apone *et al.* [49] concluded that GCR1 regulates DNA synthesis through activation of PLC. In *Arabidopsis*, PLDα1 directly binds GPA1 via a motif similar to the DRY motif that is present in many mammalian GPCRs [50••]. Binding inhibits PLDα1 activity and is relieved upon GTP addition, suggesting that, *in vivo*, G protein activation leads to the activation of PLDα1 [50••]. Thus, it will be of interest to assess PLC and PLD activity in guard cells in which the levels of G-protein components are altered.

Conclusions: with few G-protein complexes in plants, GPCRs and effectors must specify signal transduction

As is evident from the phenotypes described in this review, numerous processes at all stages of plant development are modulated by heterotrimeric G proteins. Many of these phenotypes appear upon null mutation of the Gα subunit genes *GPA1* or *RGA1*, implying dependency upon Gα coupling to downstream effectors. However, some phenotypes, notably lateral root proliferation and altered silique morphology, are present in *agb1* mutants but are either absent or opposite in *gpa1* mutants, implying a dependency on Gβγ-coupled signaling. But is that the whole story? Plausibly, the different phenotypes of *gpa1* and *agb1* mutants could reflect differences in the relative levels of the released subunits from the heterotrimeric complex, different fluxes of signaling through Gα (as opposed to Gβγ) in the different cell types or organs, and/or a different relative balance in positive or negative feedback. To sort out these issues, it will be necessary to determine the effect on a given trait of quantitatively altered ratios of Gα to Gβγ, rather than of the two extremes of ratios of 0 or ∞ that are created by single null mutations. Given the plethora of G-protein-related phenotypes in combination with the dearth of heterotrimeric G-protein subunits in plant genomes, one might well predict that plants will be found to have evolved novel and abundant mechanisms for coupling G protein components with downstream effector molecules.

In *Arabidopsis*, seeds and light-grown *gpa1* seedlings show increased sensitivities to ABA and sucrose together with decreased sensitivities to BL [2,27•], suggesting that identical G-protein-based signaling pathways may operate in seed germination and early seedling development. In mature rosette leaves, however, *gpa1* guard cells exhibit reduced rather than enhanced sensitivity to ABA. In rice, internode sensitivity to GA is strongly reduced in *d1* mutants, yet GA-regulation of leaf-sheath elongation is scarcely affected. These differential sensitivities indicate that the roles of GPA1 must be cell- and tissue-specific, presumably reflecting cell- and tissue-specific effectors and/or GPCRs. Cell-specific mechanisms for G-protein-coupled signaling have precedent in animal systems (e.g. [51]).

By parallel reasoning, one might expect a proliferation of cell-specific GPCRs in plants. However, if this is true, the plant GPCRs must be defined by functionality rather than by sequence similarity; *GCR1* is the sole candidate GPCR to be identified in *Arabidopsis* on the basis of homology criteria and its sequence similarity to known GPCRs is limited. The observations that GCR1 is not implicated in many of the pathways that are affected by mutation of *GPA1* and/or *AGB1*, and that the ABA-related phenotypes of *gcr1* mutants are opposite to those of *gpa1* mutants, further highlight our lack of knowledge about components that function upstream of plant G-protein heterotrimers. Signals may be transduced either via novel GPCRs or through proteins that transmit signals to G proteins independently of GPCRs [51,52]. Furthermore, plant-specific 'unconventional' G proteins, such as *A. thaliana* Extra Large G Protein1 (XLG1), a protein that has significant similarity to Gα subunits and exhibits GTP-binding capability [11,53], could potentially partner with components of G-protein pathways. Thus, the future is bright for model plant systems such as *Arabidopsis* and rice to contribute new insights regarding this ubiquitous eukaryotic signaling paradigm.

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