Heterotrimeric G-proteins in plant development

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1. ABSTRACT

Signaling through heterotrimeric G-proteins (G-proteins) is a conserved mechanism found in all eukaryotes. In plants, the repertoire of G-protein signaling complex is much simpler than in metazoans. Specifically, the genome of the model plant, Arabidopsis, encodes only one canonical Galpha, one Gbeta, and two Ggamma subunits. Similarly, only one Regulator of G-protein Signaling (RGS) protein is encoded by the Arabidopsis genome, and no *bona fide* G-protein-coupled receptor (GPCR) together with its ligand has been unequivocally identified. Nonetheless, several proteins, including AtPIRIN1, $PLD\alpha1$, PD1, and THF1, have been shown to physically interact with the Arabidopsis heterotrimeric G-protein alpha subunit (GPA1), and are potential downstream effectors for GPA1. The smaller repertoire of the heterotrimeric G-protein complex in plants offers a unique advantage over its counterpart in mammals for dissecting their roles in development. The analyses of loss-of-function alleles and gain-of-function transgenic lines of G-protein subunits and signaling components suggest that the G-proteins play regulatory roles in multiple developmental processes ranging from seed germination and early seedling development to root development and organ shape determination. Future studies are expected to reveal more components of the heterotrimeric G-protein signal transduction pathways, and to identify the mechanisms by which G-proteins regulate phenotypic and developmental plasticity.

2. INTRODUCTION

The heterotrimeric G-proteins (G-proteins) form classical signal transduction complexes conserved in all eukaryotes. Heterotrimeric G-proteins consist of three subunits, namely G-protein α ($G\alpha$), β ($G\beta$), and γ ($G\gamma$) subunits. In the canonical G-protein signaling paradigm, the G-proteins transduce signals from a specific class of transmembrane receptors designated as G-protein-coupled receptors (GPCRs), all of which contain seven-transmembrane (7TM) domains (1). Ligand binding to a GPCR activates the G-protein-mediated signaling pathway by promoting the exchange of $G\alpha$-bound GDP for GTP. The $G\beta\gamma$ dimer then dissociates from the $G\alpha$. The activated $G\alpha$ (GTP-bound) and the $G\beta\gamma$ dimer (freely released) activate downstream effector proteins. One of the important mechanisms for deactivating the G-protein signaling pathway is through the Regulator of G-protein Signaling (RGS) proteins which preferentially bind the activated (GTP-bound) form of $G\alpha$ and accelerate its intrinsic GTPase activity (2). The heterotrimeric G-protein complexes transduce diverse signals in mammals including cellular perception of photons, odorants, tastants, neurotransmitters, and hormones (1-4).

The heterotrimeric G-protein subunits appear to be conserved across plant species. Comparative analysis of the completely-sequenced genomes of Arabidopsis and rice reveals that the heterotrimeric G-protein complex in both monocots and eudicots is much simpler than its counterpart

in mammals, since the genomes of Arabidopsis and rice each encode only one canonical Gα, one Gβ, and two Gγ subunits. The Arabidopsis genome also encodes a unique RGS protein, which contains 7TM domain (5). On the other hand, no *bona fide* GPCR together with its ligand has been unequivocally identified in any plant species, although two 7TM-containing proteins, GCR1 and AtRGS1, have been shown to physically interact with the Gα in Arabidopsis (5, 6). Biochemical and genetic screens have identified several proteins that are putative downstream effectors for Gα and Gβ in plants. Analyses of loss-of-function alleles and gain-of-function transgenic lines of G-protein subunits in rice and Arabidopsis suggest that the G-proteins play regulatory roles in diverse developmental processes, and function in a cell-type- or developmental stage-specific manner (7).

Since the isolation and characterization of the first loss-of-function alleles of the Gα subunit in Arabidopsis (8, 9), G-protein signaling has become an increasingly attractive topic in the field of plant biology. It has been frequently reviewed in the last several years (10-19), and has been highlighted in the Connections Map Overview of Science's Signal Transduction Knowledge Environment (Science's STKE) (20-22). In this article, I will briefly review the subunits and known signaling components of the heterotrimeric G-protein complex in plants. Then, I will specifically focus on the role of the heterotrimeric G-proteins in plant development. For details of other aspects of plant heterotrimeric G-proteins, readers are referred to the abovementioned review articles and references therein.

3. HETEROTRIMERIC G-PROTEIN SUBUNITS IN PLANTS

Protein sequence homologs of Gα have been found and characterized in Arabidopsis, rice, maize, tomato, tobacco, pea, soybean, spinach, lotus, lupin, wild oat and alfalfa (12), while protein sequence homologs of Gβ have been found and characterized in Arabidopsis, rice, maize, pea, and tobacco. Homologs of Gγ have also been identified and characterized in Arabidopsis, rice, and pea. In Arabidopsis, the Gα subunit is encoded by a single gene, *GPA1* (23); the Gβ subunit is encoded by a single gene, *AGB1* (24); and the Gγ subunits are encoded by two genes, *AGG1* and *AGG2* (25, 26). Similarly, the rice genome encodes only one canonical Gα (RGA1) (27), one Gβ (RGB1) (28), and two Gγ (RGG1 and RGG2) subunits (29). These subunits are predicted to form only two possible G-protein heterotrimers in Arabidopsis or rice, in striking contrast to the situation in mammals, where theoretically >1000 heterotrimers can be formed.

Molecular modeling revealed that critical motifs found in mammalian heterotrimeric G-proteins are largely conserved in Arabidopsis G-protein subunits as well (17, 30). The physical interaction between GPA1 and AGB1 has also been confirmed at both the biochemical and cellular levels (31-33). AGG1 and AGG2 were identified from a yeast two-hybrid screen in which AGB1 was used as bait (25, 26). Further, the physical interactions between AGB1 and AGG1/AGG2 were confirmed at the cellular level (33).

Therefore, the formation of AGB1-AGG1 or AGB1-AGG2 dimers is also assured. The *in vivo* assembly of the Arabidopsis heterotrimers, GPA1-AGB1-AGG1/AGG2, has been demonstrated using fluorescence resonance energy transfer (FRET) imaging (33). The formation of the G-protein heterotrimers has also been demonstrated in rice and pea biochemically (29, 34). Therefore, molecular modeling, biochemical, and cellular evidence support the view that plant heterotrimeric G-protein subunits can form authentic heterotrimers. The significance of the assembly of heterotrimers has also been evaluated genetically in root development (31).

4. HETEROTRIMERIC G-PROTEIN SIGNALING COMPONENTS IN PLANTS

The heterotrimeric G-proteins act as critical molecular switches. In the classical model of G-protein signaling, the G-proteins receive input signals from upstream 7TM GPCRs, and act through downstream effector proteins. These upstream GPCRs and downstream effectors are referred to here as G-protein signaling components. There are several dozens of genes in the Arabidopsis genome encoding 7TM proteins (35), and among these proteins, GCR1 and AtRGS1 have been shown to physically bind GPA1 (5, 6). AtRGS1 was shown to play an important role in sugar sensing (5, 36, 37), while GCR1 was shown to negatively regulate plant hormone abscisic acid (ABA) signaling (6). However, no ligand has been unequivocally identified for either GCR1 or AtRGS1, although it was implied that D-glucose is likely a ligand for AtRGS1 (37). Recently, Liu et al. (2007) proposed GCR2 as an ABA-signaling GPCR (38). However, analysis of the GCR2 amino acid sequence in robust transmembrane prediction systems predicts that GCR2 is unlikely to be a 7TM protein (39, 40). Because a 7TM domain is the structural hallmark of GPCRs, it is doubtful that GCR2 is an authentic GPCR. Instead, GCR2 shows significant sequence similarity with bacterial lanthionine synthetases (39, 40).

AtRGS1 is a unique 7TM protein because it contains a C-terminal RGS domain (5). AtRGS1 preferentially binds the activated (GTP-bound) form of GPA1, and executes its GAP (GTPase Accelerating Protein) activity on GPA1. AtRGS1 genetically complemented the yeast RGS deletion mutant, *sst2Δ*, suggesting that AtRGS1 can indeed function as an RGS protein. However, AtRGS1 contains an N-terminal 7TM domain, a structural hallmark of typical GPCRs, which raises the possibility that AtRGS1 may be a ligand-regulated GAP, GEF (Guanine-nucleotide Exchange Factor), or dual GAP and GEF. This idea will be further discussed below.

Four proteins, including AtPIRIN1, THF1, PD1, and PLDα1, have been shown to physically interact with Arabidopsis Gα, GPA1, and are thus candidate downstream effectors for GPA1. Three of these four proteins were identified in yeast two-hybrid screens. AtPIRIN1 is a member of the cupin protein superfamily (41). PD1 is a cytosolic prephenate dehydratase (42). Both AtPIRIN1 and

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Table 1. Heterotrimeric G-protein subunits and signaling components in Arabidopsis

Gene	Encoded Protein	Functions	References
Heterotrimeric G-protein subunits			
GPA1	Gα	Modulates cell proliferation in hypocotyl, root and leaf; negatively regulates abscisic acid (ABA) and D-glucose signaling in seed germination, early seedling development and ABA-induced gene expression; promotes ABA-and sphingosine-1-phosphate (S1P)-inhibited stomatal opening; positively regulates gibberellin (GA)- and brassinosteroid (BR)-stimulated seed germination; promotes blue light-induced synthesis of phenylpyruvate and phenylalanine and gene expression; positively modulates ozone response; negatively regulates defense response to necrotrophic fungus; regulates organ shape	5, 6, 8, 9, 23, 30, 31, 36, 40, 42, 43, 48, 50-52, 81-87
AGB1	Gβ	Modulates cell proliferation in hypocotyl, root and leaf; negatively regulates ABA and D-glucose signaling in seed germination, early seedling development and ABA-induced gene expression; positively regulates GA- and BR-stimulated seed germination; positively modulates methyl jasmonate signaling in seed germination, early seedling development and gene expression; negatively modulates responses to acute ozone treatment; positively regulates defense responses to necrotrophic fungi; modulates tunicamycin-induced cell death; regulates organ shape	24, 30, 31, 45, 50-52, 70, 83-85, 87, 88
AGG1	Gγ1	Negatively modulates acropetal auxin polar transport in roots; promotes methyl jasmonate-inhibited root elongation; negatively regulates the responses to high concentrations of D-glucose and mannitol in seed germination; regulates organ shape	25, 52, 89
AGG2	Gγ2	Negatively modulates basipetal auxin polar transport in roots; negatively regulates the response to high concentrations of D-glucose in seed germination	26, 52
Putative G-protein-coupled receptors			
GCR1	A 7TM protein; weak sequence homology with the cAMP receptor, CAR1, of the slime mold	Physically binds GPA1; negatively regulates ABA signaling in seed germination, early seedling development and gene expression; negatively regulates ABA inhibited stomatal opening and ABA-promoted stomatal closure; positively regulates GA- and BR-stimulated seed germination; promotes blue light-induced gene expression; promotes cell division in tobacco BY-2 suspension cells	6, 50, 51, 81, 90-94
AtRGS1	A 7TM protein; contains an C-terminal RGS domain	Attenuates cell elongation in hypocotyls; attenuates cell division in primary roots; up-regulates the expression of ABA biosynthesis genes; positively regulates D-glucose sensing in high concentrations of D-glucose-inhibited hypocotyl elongation, root elongation and cotyledon greening	5, 36, 37, 54, 55
RGS Protein			
AtRGS1	A 7TM RGS	Preferentially binds GTP-bound GPA1; exhibits GTPase accelerating protein (GAP) activity on GPA1; attenuates cell elongation in hypocotyls; attenuates cell division in primary roots; up-regulates the expression of ABA biosynthesis genes; positively regulates D-glucose sensing in high concentrations of D-glucose-inhibited hypocotyl elongation, root elongation and cotyledon greening	5, 36, 37, 54, 55
Gα-interacting proteins			
AtPIRIN1	A member of the cupin protein superfamily	Negatively regulates ABA signaling in seed germination and early seedling development; mediates blue light-induced gene expression	41, 81
PLDα1	A major isoform of phospholipase D	Produces phosphatidic acid (PA); positively regulates ABA-inhibited stomatal opening and ABA-promoted stomatal closure; exhibits GAP activity on GPA1	44, 86
THF1	A plastid protein; no significant sequence homology with other proteins	Acts downstream of GPA1 to regulate D-glucose sensing	43
PD1	A cytosolic prephenate dehydratase	Regulates blue light-mediated synthesis of phenylpyruvate and phenylalanine and gene expression	42, 81
Gβ-interacting proteins			
		Besides Gα and Gγ, no other proteins have been shown to physically interact with Gβ.	

D1 were identified in a similar yeast two-hybrid screen using GPA1 as bait (41, 42). THF1, on the other hand, a plastid protein that shares no significant sequence homology with any known proteins, was identified in a yeast two-hybrid screen using a constitutively activated form of GPA1 ("GTPase dead") as bait (43). PLDα1 is a major isoform of phospholipase D (PLD), and was identified in a biochemical assay where it binds GPA1 (44).

No other proteins (except Gα and Gγ) have been shown to interact with the Gβ subunit in plants. However, SGB1, a Golgi-localized hexose transporter, has been shown to be genetically coupled to Arabidopsis Gβ (AGB1) in regulating cell division in the hypocotyls, and in sugar sensing (45).

Table 1 lists all heterotrimeric G-protein subunits and known signaling components identified thus far in Arabidopsis. Future studies are expected to identify more components in the heterotrimeric G-protein signaling complex.

5. THE FUNCTIONS OF HETEROTRIMERIC G-PROTEINS IN PLANT DEVELOPMENT

The functional characterization of the heterotrimeric G-proteins in plant development has been mainly conducted in the model plants Arabidopsis and rice. Based on studies using loss-of-function alleles and gain-of-function overexpression lines of the heterotrimeric G-protein subunits and signaling components, the heterotrimeric G-proteins have been shown to modulate hormonal and stress responses, and play regulatory roles in diverse developmental processes. Among those developmental events, the actions of the heterotrimeric G-proteins have been characterized in seed germination, early seedling development, root development and organ shape determination.

5.1. Seed germination

Seed germination represents the first developmental process in which the life cycle of a plant resumes. This process involves the activation of the dormant embryo and the protrusion of the embryonic root through the seed coat, and is regulated by both environmental factors and biological stimuli. Among biological stimuli, the plant hormones gibberellin (GA) and brassinosteroid (BR) promote seed germination, whereas ABA and high concentrations of D-glucose inhibit germination.

The most direct and compelling evidence for the involvement of G-proteins in seed germination came from the study of the rice *dwarf1* (*d1*) mutant which was initially identified as having a defect in response to GA. Map-based cloning revealed that the defect in *d1* mutant was in fact due to a loss-of-function mutation in the gene encoding Gα (46). In rice aleurone cells, the *d1* mutant displayed a dramatically reduced sensitivity to GA in the transcription of *α-amylase*, which encodes a key enzyme required for seed germination (47). Similarly, the expression of *OsGAMYB*, which encodes a GA-inducible transcription factor that positively regulates the expression of *α-amylase*, was also significantly reduced upon GA induction in *d1* mutants compared with that in wild-type. Because GA is the major activator for seed germination, these results suggested that the heterotrimeric G-proteins play a critical role in GA signaling during seed germination. However, at high GA concentrations, expression of the *α-amylase* gene was still induced by GA in *d1* mutants, implying that Gα may only participate in a high-affinity GA signaling pathway (47). A similar hyposensitivity to GA was found in Arabidopsis Gα (*GPA1*) mutants (48). Loss-of-function alleles of *GPA1* were found to be hyposensitive to GA, whereas seeds over-expressing *GPA1* were hypersensitive to GA during germination. *gpa1* mutants are also hyposensitive to the plant hormone brassinolide (BL) (48), another activator of seed germination (49). A similar combination of GA and BL hyposensitivity was also found in the loss-of-function allele of *AGB1* (50). Therefore, the heterotrimeric G-proteins can be viewed as positive regulators of GA and BL signaling in seed germination.

ABA is a major inhibitor of seed germination, where it antagonizes the action of GA. Since G-protein mutants are hyposensitive to GA, it was expected that these mutants might be hypersensitive to ABA. Indeed, *gpa1* and *agb1* single and double mutants are hypersensitive to ABA in seed germination assays (40, 48, 51). The loss-of-function alleles of *AGG1* and *AGG2* were also recently isolated and characterized (52). As would be predicted from the behavior of other G-protein loss-of-function mutants, *agg1* and *agg2* single and double mutants were found to be hypersensitive to high concentrations of D-glucose or the non-metabolizable osmotic agent, mannitol. High concentrations of D-glucose inhibit seed germination, likely through the regulation of endogenous ABA levels (53). Consistent with their roles in seed germination, both *GPA1* and *AGB1* mRNAs can be detected in dry seeds and imbibed seeds, as well as germinating seeds (40, 51). The expression of some ABA marker genes was also up-regulated in *gpa1* and *agb1* mutants (40, 51). Therefore, the G-proteins are interpreted as negative regulators of ABA signaling in seed germination (Figure 1A).

Loss-of-function alleles of *GCR1,* a gene encoding a putative 7TM GPCR in Arabidopsis, also displayed an ABA hypersensitivity phenotype similar to that observed in *gpa1* and *agb1* mutants (51). In addition, the *gcr1 gpa1* double mutant phenocopied the *gpa1* mutant, and the *gcr1 agb1* double mutant phenocopied the *agb1* mutant, in terms of ABA hypersensitivity during seed germination. Furthermore, the *gcr1 gpa1 agb1* triple mutant also phenocopied the *agb1* mutant, and, as in the *gpa1* and *agb1* mutants, the transcript levels of some ABA marker genes were up-regulated in *gcr1* mutant backgrounds (6, 51). Taken together, these results suggest that GCR1 functions in a common pathway with GPA1 and AGB1, and that GCR1 works upstream of GPA1 and AGB1 to negatively regulate ABA responses in seed germination. Because the *agb1* mutant displayed greater ABA hypersensitivity than the *gpa1* or *gcr1* mutants, AGB1 is likely the predominant regulator of the G-protein complex in controlling ABA signaling during seed germination. On the other hand, the *gpa1*, *agb1*, and *gcr1* mutations had additive/synergistic effects on responses to GA and BL in seed germination (50), which suggests that GCR1 may also act independently of the heterotrimeric G-proteins to modulate GA and BL signaling in seed germination.

Loss-of-function alleles of *GCR2* were reported to be insensitive to ABA in seed germination (38), but *gcr2* mutants did not display consistent ABA insensitivity in seed germination under other conditions (40). Therefore, the exact nature of ABA insensitivity of *gcr2* mutants in seed germination awaits further investigation. If GCR2 indeed acts as an ABA-signaling GPCR as proposed (38), it is unclear how GCR2 passes signals to the heterotrimeric G-protein subunits to regulate ABA response in seed germination. As discussed above, the heterotrimeric G-protein complex has been consistently shown to be a negative regulator of ABA signaling in seed germination (Figure 1A). Consistent with this conclusion, loss-of-function alleles of *AtRGS1*, an inhibitor of G-protein signaling, were hyposensitive to ABA whereas over-expression of *AtRGS1* conferred ABA hypersensitivity in seed germination (54, 55). It was shown that binding of ABA to GCR2 promoted the dissociation of the GCR2-GPA1 complex, and presumably the activation of the G-protein signaling. It, therefore, remains mysterious how the proposed activator (GCR2) and a known inhibitor (AtRGS1) of G-protein signaling would have the same impact on the heterotrimeric G-protein complex in ABA signaling in seed germination.

All G-protein subunit and signaling component mutants shown to have altered sensitivity to ABA also have similar altered sensitivity to high concentrations of D-glucose. These include *gpa1*, *agb1*, *agg1*, *agg2*, *gcr1* and *Atrgs1* (48, 51, 52, 54, 55). Interestingly, the sensitivity of the *agb1*, *agg1* and *agg2* mutants to D-glucose was also found to be dependent on light intensity. Greater D-glucose
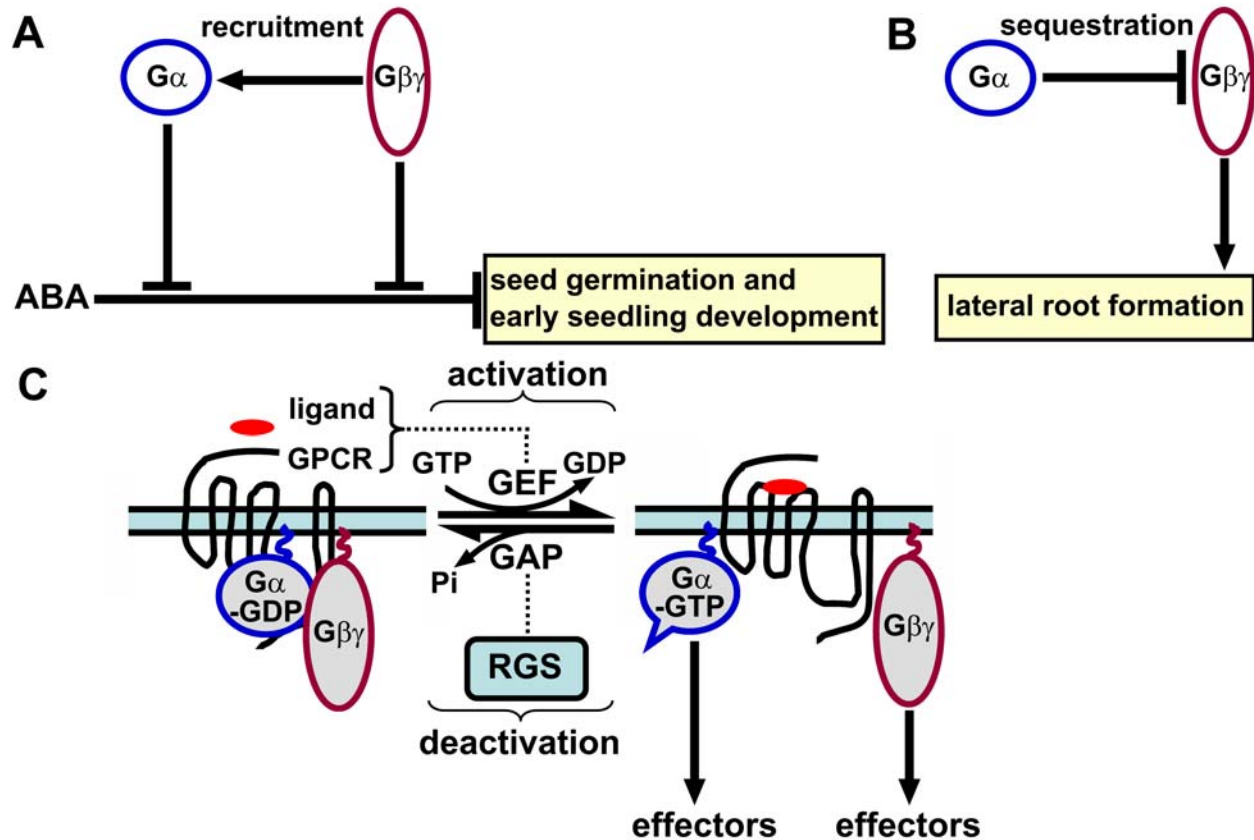
Figure 1. Modes of action of Arabidopsis heterotrimeric G-proteins. Arrows indicate positive regulation and blunted arrows indicate negative regulation. (A) Mode of action of Arabidopsis heterotrimeric G-protein subunits in the modulation of abscisic acid (ABA) signaling in seed germination and early seedling development. A similar phenotype of ABA sensitivity in seed germination and early development has been observed between *gpa1* and *agb1* mutants. Both mutants are hypersensitive to ABA in the ABA-inhibited seed germination and early seedling development. Therefore, GPA1 (Gα) is interpreted to act in a positive coordination with or independently of AGB1 (Gβ). Presumably AGB1 is required for the recruitment by GPA1 to associate the heterotrimer into the complex with the GPCR. The heterotrimeric G-protein complex negatively regulates ABA signaling in seed germination and early seedling development. (B) Mode of action of Arabidopsis heterotrimeric G-protein subunits in the modulation of lateral root formation. An opposite phenotype of lateral roots is observed between *gpa1* and *agb1* mutants. *gpa1* mutants have fewer whereas *agb1* mutants have more lateral roots. Therefore, AGB1 (Gβ) is interpreted as the predominant subunit of the heterotrimer in this developmental pathway, and acts downstream of GPA1 (Gα) to negatively modulate the formation of lateral roots. Presumably GPA1 acts by sequestering AGB1. (**C**) A classical model of G-protein signaling. In Arabidopsis, Gα is encoded by a single gene, *GPA1*. Gβ is encoded by a single gene, *AGB1*. Gγ is encoded by two genes, *AGG1* and *AGG2*. Two seven-transmembrane (7TM) proteins, AtRGS1 and GCR1, have been shown to physically bind GPA1. AtRGS1 contains a C-terminal RGS domain, preferentially binds the GTP-bound GPA1 and exhibits GTPase accelerating protein (GAP) activity on GPA1. No ligand or guanine nucleotide exchange factor (GEF) activity has been identified for either AtRGS1 or GCR1. Four proteins, AtPIRIN1, PLDα1, PD1, and THF1 have been shown to physically interact with GPA1, and are putative effectors of GPA1. No proteins, besides GPA1, AGG1, and AGG2, have been shown to physically interact with AGB1.

hypersensitivity could be observed in these mutants under low light irradiation levels (63 μmol·m^{-2}·s^{-1}) than at high light irradiation (150 μmol·m^{-2}·s^{-1}). Compared with *agg1* mutants, *agg2* mutants displayed a lower level of hypersensitivity to high concentration of D-glucose (52), but a more pronounced difference was observed when mannitol was used (52). *agb1*, *agg1*, and *agg1 agg2* mutants were significantly hypersensitive to mannitol, whereas *agg2* mutants only had weak or near wild-type response to mannitol. Based on these results, it was proposed that Gβγ1 is mostly involved in the osmotic component of the response to D-glucose, whereas Gβγ2

likely plays a role in D-glucose sensing (52). These findings imply that the two Gγ subunits may condition functional selectivity of Gβγ dimer signaling in Arabidopsis. This will be further discussed below in the context of the role of the heterotrimeric G-proteins in root development.

Loss-of-function alleles of *AtPIRIN1*, a gene encoding a GPA1-interacting protein that is a member of the cupin protein superfamily (41), also displayed ABA hypersensitivity in seed germination, raising the possibility that AtPIRIN1 may function as an effector for GPA1 in the

modulation of ABA signaling in seed germination. However, the roles of the other three known putative downstream effectors of GPA1, THF1, PD1, and PLDα1, in seed germination have yet to be assessed, and the description of the G-protein signaling pathway(s) acting during seed germination is still far from complete. Therefore, the exact effector(s) of G-proteins involved in this process remains unclear.

5.2. Early seedling development

Once seeds are germinated, they undergo a series of developmental processes leading to the establishment of a seedling. In Arabidopsis, early stages of seedling development include the growth and development of root and hypocotyl, and the greening of cotyledons, leaves, and hypocotyls. In etiolated seedlings, greening of the cotyledons or other organs does not occur, but another specific process, the formation of an apical hook, is observed. This apical hook formation is believed to have protective role for the apical meristem.

The formation of an apical hook in the dark results from a differential growth process on opposite sides of the hypocotyl (56), and involves a complex interplay of hormones and light. The differential cell elongation in the apical hook is established and maintained mainly by ethylene and auxin, but also involve other hormones, such as GA (57, 58). The subsequent opening of the apical hook is dramatically promoted by white light irradiation, but both the *gpa1* and *agb1* mutants display a partially-opened hook when grown in the dark (8, 30). Microscopic examination revealed that the partially-opened hook of *gpa1* seedlings was due to expansion of adaxial cells in the hook region (8). Notably, the *gpa1* mutants could still respond to 1-aminocyclopropane-l-carboxylic acid (ACC), an ethylene biosynthesis immediate precursor, by closing its hook, which would suggest that the heterotrimeric G-proteins may not be coupled to the ethylene signaling pathway in regulating apical hook formation (48). The exact mechanism by which the heterotrimeric G-proteins participate in regulation of hook formation remains unknown, and deserves further investigation.

The number of epidermal cells in a single cell file from the base to the top of the Arabidopsis hypocotyl is determined during embryogenesis, and such a cell file typically contains approximately 20 cells. Following embryogenesis, cortical or epidermal cell divisions are absent or insignificant in the elongating hypocotyl of either dark- or light-grown seedlings (59). The hypocotyl is therefore a model system for study of cell division defects occurring during embryogenesis, and for study of cell elongation process during hypocotyl growth, on the other hand. In addition to their other phenotypic traits, *gpa1* and *agb1* etiolated seedlings also have short hypocotyls (8, 30), a phenotype that was found to be due to reduced cell division in the hypocotyl epidermal cells. Compared with *gpa1* mutants, *agb1* mutants have even shorter hypocotyls (31, 50). In addition, the *gpa1 agb1* double mutant phenocopies the *agb1* single mutant hypocotyl phenotype (31). These results suggested that AGB1 likely works in the same pathway, and downstream of, GPA1 to regulate cell

division in hypocotyls. However, while displaying reduced axial cell division, the *agb1* mutant also displays increased circumferential cell division in hypocotyl cells (30), indicating that AGB1 may also function in a GPA1-independent pathway to regulate circumferential cell division in the hypocotyls of Arabidopsis seedlings. Interestingly, etiolated seedlings of the loss-of-function alleles of *AtRGS1* have extended hypocotyls, compared with wild-type (5), but microscopic examination revealed that the longer hypocotyl phenotype of *Atrgs1* mutant was due to increased cell elongation of hypocotyl epidermal cells, rather than increased cell division. This observation implies that the heterotrimeric G-protein complex may also have a role in regulating cell elongation in hypocotyl epidermal cells.

Light-irradiation promotes chlorophyll synthesis during the greening of cotyledons, leaves, and other organs in plants, but both ABA and high concentrations of D-glucose inhibit the greening of these organs in Arabidopsis seedlings. These responses, particularly the greening of cotyledons, have been used as an efficient bioassay to study ABA signaling and D-glucose sensing. Similar to their respective responses in seed germination, the *gpa1* and *agb1* mutants displayed hypersensitivity to ABA and high concentrations of D-glucose in the greening of cotyledon assay, whereas the *Atrgs1* mutant was hyposensitive (5, 36, 37, 40). The hyposensitivity of the *Atrgs1* mutant to high concentrations of D-glucose was found to depend on the presence of a functional GPA1, because the *Atrgs1 gpa1* double mutant phenocopied the *gpa1* single mutant's D-glucose hypersensitivity phenotype (37). Further, over-expression of a mutated AtRGS1, AtRGS1(E320K), in which a point mutation introduced into the RGS domain of AtRGS1 disrupts the Gα-binding interface and eliminates AtRGS1's GAP activity, failed to confer the D-glucose hypersensitivity that was typically observed in plants over-expressing the wild-type form of AtRGS1 (37). These results demonstrated that AtRGS1's GAP activity is critical for D-glucose sensing through the heterotrimeric G-proteins.

The ABA and glucose sensitivities of G-protein mutant seedlings were also assessed by using another assay in which the primary root growth was measured in the presence or absence of ABA, or of high concentrations of D-glucose (43, 51). The results obtained using this assay were comparable to those obtained in the greening of cotyledon assay. By analyzing *gpa1*, *agb1*, and *gcr1* single, double and triple mutants, it was concluded that GPA1, AGB1, and GCR1 are all negative regulators of ABA signaling and D-glucose sensing during primary root growth (51).

A loss-of-function allele of *THF1*, a gene encoding a GPA1-interacting protein that is localized in the plastid, was also shown to be D-glucose hypersensitive in the D-glucose inhibition of root growth assay, whereas seedlings over-expressing *THF1* were hyposensitive (43). Further, the *gpa1 thf1* double mutant phenocopied the *thf1* single mutant's glucose hypersensitivity. These results suggest that THF1 operates downstream of GPA1 in the G-protein-coupled glucose-sensing pathway (43).

5.3. Root development

The roles of the heterotrimeric G-proteins in root development have been demonstrated using loss-of-function alleles and over-expression transgenic lines in Arabidopsis. *gpa1* mutants had normal primary roots length but produced fewer lateral roots, whereas *agb1* mutants had longer primary roots and produced more lateral roots (30, 31). Since root growth phenotypes were also observed in *Atrgs1*, *agg1* and *agg2* mutants (5, 31, 52), it appears that the heterotrimeric G-proteins participate in the establishment of root architecture. Consistent with their roles in root development, *GPA1* and *AGB1* were expressed at a higher level in roots than in shoots (31), and were also detected in the meristem of the primary root and in the primordia of lateral roots (52, 60).

Based on the analysis of lateral root formation in *gpa1* and *agb1* single mutants and transgenic lines over-expressing *GPA1* and *AGB1* in wild-type background, it was hypothesized that AGB1 acts downstream of GPA1 to negatively regulate lateral root formation (30). According to the classical model of G-protein signaling, Gα sequesters Gβ and Gβ is required for the recruitment of the Gα-containing heterotrimer to the GPCR. Therefore, it is possible that other modes of action of the heterotrimeric G-proteins in regulating root cell division may also exist. For example, the phenotypes observed in loss-of-function alleles of Gα could also be due to the activation of freely-released Gβ. In addition, the functionality of the intact heterotrimer could not be assessed from this study, although the formation of such heterotrimer has been confirmed both by molecular modeling (30) and by FRET imaging (33).

In a subsequent study, the roles of GPA1 and AGB1 in cell division in both the primary roots and in the formation of lateral roots were assessed using *gpa1* and *agb1* single and double mutants, and transgenic lines over-expressing *GPA1* or *AGB1* in *agb1* or *gpa1* mutant backgrounds, respectively (31). The advantage of this approach was that a sequestration role of Gα on Gβ was eliminated in plants over-expressing *AGB1* in the *gpa1* mutant background. Similarly, the requirement of Gβ for Gα's recruitment was eliminated in plants over-expressing *GPA1* in the *agb1* mutant background. Based on the analyses of cell production in the primary roots and the formation of lateral roots in these mutants and transgenic lines, it was proposed that GPA1 and AGB1 have differential roles in regulating cell division in roots (31). These results supported the previous model in which AGB1 acts downstream of GPA1 to inhibit lateral root formation (Figure 1B), and extended it to include a new mode of action of the heterotrimeric G-proteins in which the intact heterotrimer negatively regulates cell division in the root apical meristem (RAM) whereas the activated form of GPA1 promotes cell division in the RAM (31). Because the *Atrgs1 gpa1* double mutant phenocopied the *gpa1* single mutant's root phenotype, it was also proposed that AtRGS1 acts in the same pathway with GPA1, and functions through GPA1 to regulate cell division in roots (31).

The exact position at which the heterotrimeric G-proteins influence the cell cycle in root cells is unclear. Because over-expression of *GPA1* in synchronized tobacco BY-2 cells shortened the G1 phase of the cell cycle and promoted the formation of nascent cell plate (8), it is possible that GPA1 mainly regulates cell cycle at the G1-to-S transition phase in root cells. Consistent with this notion, the transcript of *GPA1* is strongly induced by auxin in a lateral root induction system at a time point where pericycle founder cells were at the G1-to-S transition (61, 62).

Auxin is the major regulator for cell division and lateral root formation in plant roots. The *gpa1* and *agb1* single and double mutants respond to auxin treatment by producing larger numbers of lateral root (30, 51, 52), suggesting that neither GPA1 nor AGB1 is required for auxin signaling in the activation of pericycle founder cells. Therefore, G-proteins are interpreted to have modulatory role in auxin-regulated root cell division. If G-proteins are not directly coupled to auxin signaling pathway, then what would be the possible mechanism by which G-proteins regulate auxin-induced lateral root formation? Recent research has shed light on a possible mechanism upon the isolation and characterization of loss-of-function alleles of *agg1* and *agg2* (52). It was found that similar to *agb1* mutants, both *agg1* and *agg2* mutants also produced more lateral roots than wild-type. When compared with *agb1* mutants, *agg1* and *agg2* single mutants had fewer lateral roots. However, the number of lateral roots in *agg1 agg2* double mutants was equivalent to that in *agb1* mutants, suggesting that *agg1* and *agg2* mutants have additive effective on lateral root formation and that a complete action of AGB1 in lateral root formation may require both AGG1 and AGG2. Results from auxin induction assays indicated that similar to *agb1* mutants, *agg1* and *agg2* single and double mutants responded to auxin and produced more lateral roots, supporting the notion that the heterotrimeric G-proteins are not directly coupled to the auxin signaling pathway. Analyses of lateral root formation in these mutants in the presence of the auxin polar transport inhibitor, N-1-naphthylphthalamic acid (NPA), applied at the shoot-root junction (to block polar auxin transport from the shoot) or at the root tip (to block basipetal auxin polar transport from the root tip) suggested that Gβγ1 acts within the central cylinder to attenuate signaling from acropetally transported auxin, whereas Gβγ2 affects the action of basipetally transported auxin within the epidermis and/or cortex (52). Consistent with this model, *AGG1* in roots was mainly expressed in the stele whereas *AGG2* expression was found in the cortex and epidermis but not in the stele (52). *AGB1*, on the other hand, was expressed in all these cell types. These results indicated that the heterotrimeric G-proteins attenuate auxin polar transport to regulate auxin-induced lateral root formation. These findings also suggested that differential deployment of the two heterotrimeric G-protein γ subunits could be providing functional selectivity in Gβγ dimer signaling, and allows definition of an alternative mode of action of the heterotrimeric G-proteins in auxin-regulated lateral root formation. It has been shown that such functional selectivity is also operating in other processes, such as in

seed germination (as discussed above) and in defense against necrotrophic fungi (52).

5.4. Organ shape determination

gpa1 and *agb1* mutants exhibit a range of morphological defects (30), suggesting that the heterotrimeric G-proteins may participate in the regulation of organ morphogenesis. The round rosette leaf shape of *gpa1* and *agb1* mutants, which is a characteristic phenotypic trait of these mutants, is just one of many morphological differences that could be defined by phenomics profiling (30).

The leaf shape in *gpa1* and *agb1* mutants is similar to that observed in *rotundifolia3* mutants (63). *Rotundifolia3* encodes a member of the cytochrome P450 family, CYP90C1, that is potentially involved in BR biosynthesis (64-66). Microscopic examination revealed that *gpa1* mutants contained fewer but larger epidermal cells in the leaf, suggesting that GPA1 controls leaf shape by regulating cell division and possibly also cell expansion (8). The increased cell size of leaf epidermal cells is likely a compensation effect for decreased cell division, since an analogous compensation effect between cell division and cell expansion has also been observed in other cases, such as for CDC2a kinase, a key regulator of the Arabidopsis cell cycle (67), and for ABP1, a putative auxin receptor that regulates auxin-induced cell expansion (68). Both *gpa1* and *agb1* mutants have similar defects in leaf morphology, whereas *gpa1 agb1* double mutants are more similar to *agb1* mutants than to *gpa1* mutants (31). It thus remains unclear which subunit of the heterotrimeric G-protein complex is the predominant factor regulating leaf shape, or what is the exact mechanism of such regulation by the heterotrimeric G-proteins. It is known that G-proteins control cell polarity and asymmetric cell division in *Drosophila* neuroblasts (69), which makes it tempting to speculate that G-proteins might also control polarity-dependent cell proliferation during leaf morphogenesis.

Although both *gpa1* and *agb1* mutants have round rosette leaves, *agb1* mutants can be distinguished from *gpa1* mutants by the shape of their siliques. In fact, the first allele of *AGB1*, *agb1-1*, was identified in a genetic screen for *erecta*-like mutants (70), since siliques in both *erecta* and *agb1-1* mutants are wider and have blunt tips. *ERECTA* encodes a leucine-rich repeats, serine/threonine receptor-like protein kinase (71). The *agb1* mutant is similar to the *erecta* mutant in that both mutants have round rosette leaves, but more dramatically, their silique morphology appears to be identical (70). Epistatic analysis indicated that *AGB1* and *ERECTA* may work in parallel pathways to regulate silique shape, because the silique of *agb1 erecta* double mutants was significantly shorter than in either the *agb1* or the *erecta* single mutants (70). However, other aspects of the mutant phenotypes, such as silique width and pedicel length in the *agb1 erecta* double mutant were not significantly different from those in either single mutant, implying that *AGB1* and *ERECTA* may also share functions in a common developmental pathway.

Because *AGB1* and *ERECTA* did not significantly regulate each other at the transcriptional level, and a physical interaction between AGB1 and ERECTA proteins has yet to be demonstrated, the specific functional relationship between AGB1 and ERECTA in regulating organ shape remains unknown.

Other notable phenotypes in *agb1* mutants include a modest shortening of floral bud length, and increased fruit and seed weights (30, 70), and among these, the *gpa1* and *agb1* mutants exhibited some similarities and some differences (30). For example, the fruit and seed weights of *gpa1* mutants are greater than those of wild-type. However, the sepals of *gpa1* mutants are longer than wild type, whereas they are shorter than wild type in *agb1* mutants (30). According to the classical "recruitment" model, in which the recruitment of Gα to the GPCR requires a functional Gβ, a phenotype shared by *gpa1* and *agb1* mutants generally implies that GPA1 acts positively with AGB1 to control the developmental process(es) governing the given phenotypic trait. A contrasting phenotype between *gpa1* and *agb1* mutants, on the other hand, generally implies that AGB1 is the predominant subunit controlling that development pathway, since the model predicts that Gα normally sequesters Gβ, and thus blocks the interaction between Gβ and its effectors.

In young seedlings, *agb1* mutants can also be distinguished from wild-type and *gpa1* mutants by the shape of the cotyledons (31, 50). *agb1* mutants exhibit larger and rounder cotyledons, a feature that was not observed in *gpa1* mutants. It is possible that, as in rosette leaves, such morphological defects may be due to reduced cell division and increased cell expansion in epidermal cells, but no cellular level examination of the abnormal cotyledon shape in *agb1* mutants has been reported.

In other plant species, G-proteins have also been shown to regulate many aspects of development. For example, in rice it has been shown that Gα (RGA1) regulates seed size, seed shape and stem elongation (72, 73), and in tobacco, Gβ regulates anther shape, pollen development and inflorescence architecture (74).

6. MODES OF ACTION OF HETEROTRIMERIC G-PROTEINS IN PLANT DEVELOPMENT

The field of heterotrimeric G-protein signaling in the plant kingdom has developed largely within the theoretical framework that was established in mammals. Therefore, it has been believed that the plant heterotrimeric G-proteins will be coupled to upstream GPCRs and to downstream effector proteins. In this scenario, upon ligand occupancy, the specific GPCR undergoes a conformational change which results in a subsequent conformational change in Gα and thereby triggers the exchange of Gα-bound GDP for GTP. This activation of Gα (GTP-bound) promotes the dissociation of Gβγ dimer from Gα. Both GTP-bound Gα and freely-released Gβγ dimer can then potentially interact with downstream effectors. In this model, the GPCR acts as a GEF that promotes the
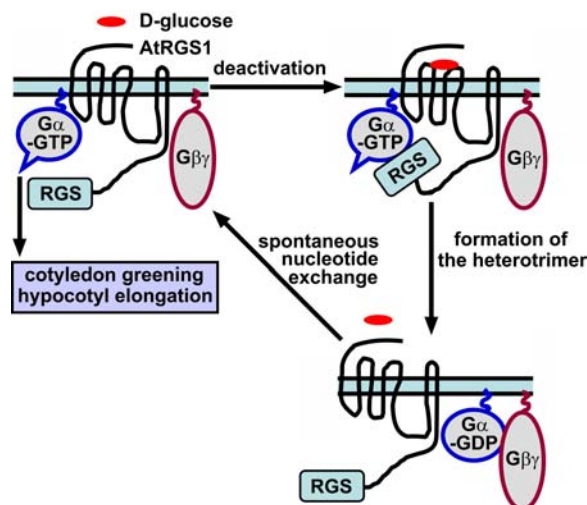
Figure 2. Mode of action of Arabidopsis heterotrimeric G-proteins in D-glucose sensing. AtRGS1 is a seven-transmembrane (7TM) RGS protein, with an N-terminal 7TM domain and a C-terminal RGS domain. AtRGS1 preferentially binds GTP-bound GPA1, and exhibits its GTPase accelerating protein (GAP) activity on GPA1. GPA1 is a unique and unusual Gα because of a rapid nucleotide exchange and the slowest GTPase activity. Therefore, GPA1 is believed to be constitutively active (GTP-bound). D-glucose induces the interaction between AtRGS1 and GPA1, but a direct binding of D-glucose to AtRGS1 has not been demonstrated. The GAP activity is an essential regulatory function of AtRGS1, and the GTP hydrolysis, rather than GDP release, is the rate-limiting step in the guanine nucleotide cycle of GPA1 in D-glucose sensing.

nucleotide exchange activity of Gα. The GTP-bound Gα is returned to its inactive form (GDP-bound) through its intrinsic GTPase activity, which can be accelerated by the RGS proteins (possessing GAP activity on Gα). Because heterotrimeric G-protein subunits, putative GPCRs, and an RGS protein have all been identified in plants, and the assembly of the G-protein heterotrimers has been confirmed in Arabidopsis, rice and pea, it was reasonable to assume that a classical G-protein signaling model operates in plant cells (Figure 1C).

However, there is only one canonical Gα, one Gβ, and two Gγ subunits in both Arabidopsis and rice, and only a single RGS protein has been identified in Arabidopsis (5) and other plant species (37). The pea genome appears to encode an additional Gα, with two Gα, one Gβ, and two Gγ subunits (34) but, overall, the repertoire of heterotrimeric G-protein signaling complex is much simpler in plants than in mammals. Because only two heterotrimeric complexes, Gα/Gβ/Gγ1 and Gα/Gβ/Gγ2, are predicted to form in Arabidopsis and rice, plants may also have need of far fewer GPCRs than mammals. Indeed, only a few dozen proteins in Arabidopsis are predicted to have 7TM structures, based on the analyses using robust transmembrane prediction systems (35), which makes them much less abundant than their mammalian homologs. Even amongst these structural candidates, however, no *bona fide*

GPCR together with its ligand has been unequivocally identified in plants. Furthermore, no GEF activity has been demonstrated for any putative 7TM GPCRs, including GCR1 and AtRGS1. Therefore, it remains unclear how well, if at all, the classical mammalian model represents the nature and exact operation of G-protein signaling complexes in plants.

Recently, the discovery of a unique and unusual GTPase activity associated with the sole Gα in Arabidopsis, GPA1, has shed light on what could be an alternative mode of action of the heterotrimeric G-proteins in plant cells (37). Biochemical analysis revealed that GPA1 is constitutively GTP-bound, displaying high spontaneous nucleotide exchange rate coupled with slow GTP hydrolysis. GTPγS binding by GPA1 was shown to be 22-fold faster than the most rapidly exchanging Gα subunit previously described, namely human GαoA, whereas GPA1's GTPase activity was found to be the slowest ever described for heterotrimeric Gα (37). Taken together, the rate of GTP hydrolysis by GPA1 is over two orders of magnitude slower than the rate of nucleotide exchange, which means that under steady state conditions, 99% of the cellular GPA1 protein is GTP-bound, in striking contrast to the situation with Gα in mammals, where about 10% is predicted to be GTP-bound. Thus, it has been proposed that GTP hydrolysis, rather than GDP release, may be the rate-limiting step in the guanine nucleotide cycle of GPA1 (Figure 2). It was demonstrated that the acceleration of GPA1's GTPase activity by AtRGS1 represents a critical step in the regulation of G-protein-mediated D-glucose sensing in Arabidopsis (37).

If this model of heterotrimeric G-protein signaling is valid in Arabidopsis, the GEF activity of GPCR that is so important in mammalian G-protein signaling may not be required for the activation of G-protein signaling in plant cells, because plant Gα is already constitutively GTP-bound (Figure 2). The implication is that plant heterotrimeric G-proteins may act effectively in the absence of cognate GPCR. This would be consistent with the observation that no candidate GPCRs in Arabidopsis have been shown to possess GEF activity toward the sole Gα, GPA1, and the fact that no G-protein-coupled-receptor kinases (GRKs) or arrestins have been found in plants. GRKs control the desensitization of mammalian GPCRs by phosphorylating ligand-occupied GPCRs, which triggers arrestin binding and leads to GPCR internalization (75-78). If there is no GPCR substrate in plants, presumably there would also be no need for GRKs or arrestins in plant cells. It is noteworthy that there are also no Activator of G-protein Signaling (AGS) protein homologs encoded in the Arabidopsis genome. Some mammalian AGS proteins, such as AGS1, possess GEF activity on some Gα types (79, 80), but again, AGS proteins may not be required for heterotrimeric G-protein signaling in plants if Gα is already constitutively activated.

7. PERSPECTIVE

Analyses of the loss-of-function alleles of heterotrimeric G-protein subunits or signaling components,

and transgenic lines over- or under-expressing G-protein subunits or signaling components, have revealed important information about the roles of the heterotrimeric G-protein complex in plant development. However, the molecular mechanisms by which the heterotrimeric G-proteins regulate these processes are still largely unknown. In addition, the heterotrimeric G-proteins are required for correct basal expression of some genes, such as auxin-regulated genes (30) and ABA-regulated genes (40, 51). It has yet to be established how the G-proteins-coupled signal at the plasma membrane is transduced to the nucleus to induce activation or suppression of gene expression. Recently, a signaling cascade has been proposed for the blue light signal transduction pathway in which GCR1, GPA1, AtPIRIN1, and a nuclear factor Y complex form a signaling chain to perceive and transduce the blue light signal and control gene expression in etiolated seedlings (81). Such a signal chain was also proposed to operate in the ABA inhibition of seed germination process (81). On the other hand, the discovery of the unique and unusual biochemical properties of the Arabidopsis Gα protein offers an alternative model for the mechanism by which the heterotrimeric G-proteins operate in plant cells. While this model and its predictions need to be tested further, the fact remains that the number of known components in the heterotrimeric G-protein signaling complex in plants is still very limited. There is an urgent need to identify new components in this signaling complex, and to elucidate the molecular mechanisms by which the heterotrimeric G-proteins exert their influences on developmental and phenotypic plasticity in plants.

8. ACKNOWLEDGEMENTS

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Abbreviations: ABA: abscisic acid, ACC: 1-aminocyclopropane-l-carboxylic acid, AGB1: Arabidopsis heterotrimeric G-protein β subunit, AGG1: Arabidopsis heterotrimeric G-protein γ subunit 1, AGG2: Arabidopsis heterotrimeric G-protein γ subunit 2, AtRGS1: Arabidopsis regulator of G-protein signalling 1, BR: brassinosteroid, BL: brassinolide, *d1: dwarf1* mutant, FRET: fluorescence resonance energy transfer, G protein: heterotrimeric GTP-binding protein, Gα, Gβ, Gγ: heterotrimeric G protein α, β, or γ subunit, GA: gibberellin, GAP: GTPase accelerating protein, GEF: guanine-nucleotide exchange factor, GPA1: Arabidopsis heterotrimeric G-protein α subunit, GCR1: Arabidopsis G-protein-coupled receptor 1, GCR2: Arabidopsis G-protein-coupled receptor 2, GPCR: G-protein-coupled receptor, NPA: N-1-naphthylphthalamic acid, PLD: phospholipase D, RAM: root apical meristem, RGA1: rice G protein α subunit, RGB1: rice G protein β subunit, RGG1: rice G protein γ subunit 1, RGG2: rice G protein γ subunit 2, RGS: regulator of G-protein signaling protein, S1P: sphingosine-1-phosphate

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