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Physiological Regulation of G Protein-Linked Signal Transduction in Plants

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G-proteins represent a class of molecules that can bind guanine nucleotides (GDP or GTP) and are involved in signal transduction. The G-protein-linked signal transduction is well established in animals where it is involved in biochemical events such as vision and hormone action. Plants, like animals, use signal transduction pathways based on heterotrimeric guanine nucleotide-binding proteins (G-proteins) to regulate many aspects of developmental processes and cell signaling. Some components of G-protein signaling are highly conserved between plants and animals and some are not. By contrast, despite great complexity in their signal-transduction attributes, plants have a simpler repertoire of G-signaling components. Nonetheless, recent studies have shown the importance of plant G-protein signaling in such fundamental processes as cell proliferation, hormone perception and ion-channel regulation.

Keywords: *G-proteins, signal transduction, cAMP, G-protein coupled receptors, plant hormones, phytochrome*

INTRODUCTION

All cells, whether belonging to unicellular or multicellular organisms, have the capacity to communicate with their surroundings by detecting and responding to a wide range of stimuli. Extracellular signals such as hormones, neurotransmitters, growth factors and even light are detected by interaction with specific receptors present on the plasma membrane of the target cell, triggering biochemical processes that produce intracellular events.

Signal transduction is vital to the coordination and growth particularly of complex multicellular eukaryotes, since organisms must be able to respond to external stimuli. In biology signal transduction refers to any process by which a cell converts one kind of signal or stimulus into another, most often involving ordered sequences of biochemical reactions inside the cell that are carried out by enzymes, activated by second messengers resulting in what is thought of as a "signal transduction pathway".

The G-protein model of signal transduction

GPCRs and the G-protein heterotrimer. The G-protein family is involved in a wide variety of signal-transducing events controlling important processes like sensory transduction and cell division and differentiation (Gibbs et al., 1985; Barbacid, 1987; Kleuss et al., 1994). The guanine nucleotide-binding protein (G-protein) superfamily shares a similar biological function and a common structural core. G-proteins are present in both prokaryotes and eukaryotes. They are involved in signal transduction, where external stimulation of a G-protein coupled receptor generates a signal that is transduced to the cytosolic side of the membrane.

G-protein signaling begins with the alteration of the conformation of a GPCR by agonist binding (Pierce et al., 2002). The largest gene family in animals encodes heptahelical transmembrane proteins that physically interact with a heterotrimeric G-protein. These polytopic membrane proteins are termed G-protein-coupled receptors (GPCRs) and their ligands are as diverse as is the GPCR family itself.

GPCRs have seven transmembrane (7TM)-spanning domains with an extracellular amino-terminus and cytosolic domains that are coupled to the Gα-subunit of the G-protein heterotrimer in a way that influences the activation state of the α-subunit (Figure 1). In essence, the GPCR is a guaninenucleotide-exchange factor (GEF) that promotes the exchange of GDP for GTP in the associated Gα-subunit.

Rhodopsin is a familiar example of a prototypical GPCR. It is the gateway of vision operated by a light-assisted change in the geometry of the retinal chromophore buried deep within the bundle of seven transmembrane (TM) helices. It is known that light induces a photoisomerization of a *cis* retinal moiety, which is covalently bound (within its heli-

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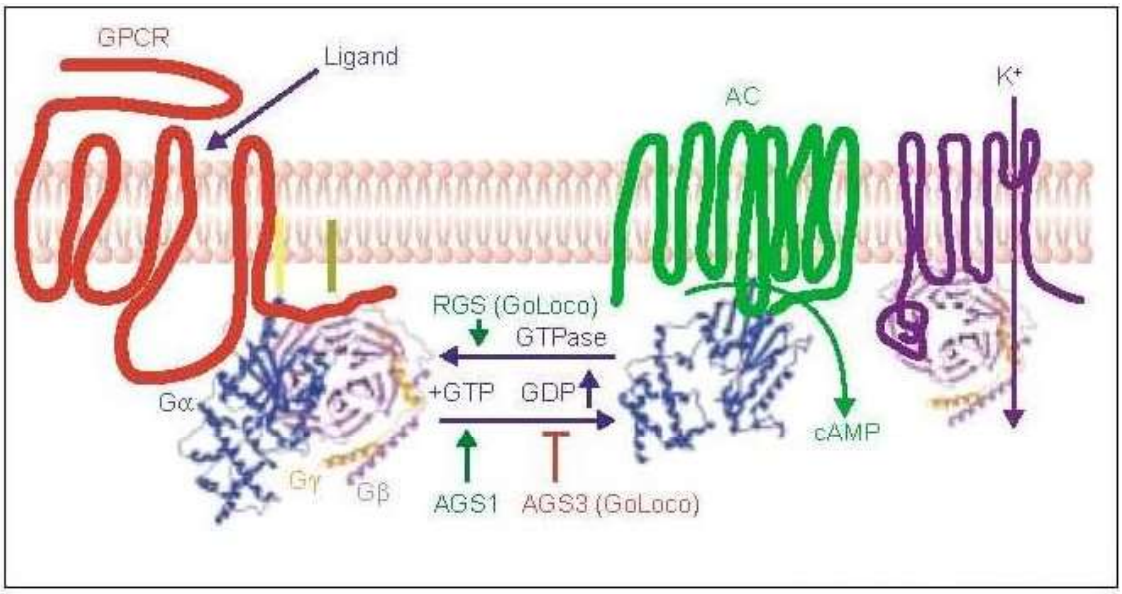

Figure 1. Classical model for G-protein-coupled signaling in animal cells. The binding of a ligand to its cognate receptor induces a conformational change that is perceived cytoplasmically. The ribbon structure of the three subunits of a heterotrimeric G-protein complex is shown to be associated with the receptor. The G-protein is tethered to the bilayer and kept proximal to the receptor by lipid modification of its **α** and **γ** subunits (green and yellow bars). The activated receptor promotes GDP for GTP exchange, which dissociates the Gα from the Gβγ subunit. Both Gα and Gβγ can then activate downstream targets, such as adenyl cyclase (AC) and ion channels, to cause changes in the levels of secondary messengers. The effect of the G-protein subunits on their targets can be either positive or negative, although only stimulation is shown. The intrinsic GTPase of the Gα subunit hydrolyses GTP to GDP and thus returns the G-protein complex to its resting state. Regulators of G-protein signaling (RGS) can facilitate the Gα GTPase (GAP) activity by interactions at their GoLoco domains. Some effectors exert GAP activity. Activities that alter the activated state of the G-protein complex independently of receptor activation are provided by AGS proteins. AGS1 facilitates GTP exchange whereas AGS3 acts as an inhibitor of GDP dissociation. AGS3 stimulates G-coupled pathways in which Gβγ is involved because AGS3 can displace the Gβγ subunit from the complex, enabling it to interact with its effectors.

cal core) to rhodopsin, to the all *trans* retinal form. The light-induced *cis*-3-*trans* isomerization of retinal is thought to induce rigid body movements of the TM helices forcing a transition of rhodopsin to the active metarhodopsin II (MII) state. Formation of the latter is accompanied by small but significant tertiary structural changes inthe solvent-exposed cytoplasmic interhelical loops thereby opening high affinity sites for the binding and activation of several signaling proteins. Similarly, small molecules such as serotonin, peptides such as somatostatin, and even large proteins such as thrombin bind to their cognate GPCRs and induce certain cytoplasmic conformations through shifts in helix positions that translate into specific loop conformations. These changes of loop conformation appeared in three cytoplasmic loops C2, C3 and C4 which certain conserved amino-acids are involved in G-protein of visual cascade transducin activation (Natochin et al., 2003).

The Gα-subunit contains a Ras-like domain that has a GDP/GTP-nucleotide-binding site and GTP-hydrolase activity. In the GDP-bound form of Ga, the N-terminal helix and three switch regions of Gα interact with a seven-bladed propeller structure in the β-subunit (Gβ). On activation by a GPCR, the Gα-protein changes conformation to a structure that allows GTP binding (Morris and Malbon, 1999). Consequent reorientation of the switch regions in the Ras-domain disrupts the tight interaction between Gα and Gβ, which results in the separation of Gα from the tightly associated Gβ/Gγ-subunit dimer. When Gα is released, the interaction between Gα and its cognate effector occurs along the same interface between Gα and Gβ, Gα and/or Gβγ then interact with downstream-effector molecules. The intrinsic GTP-ase activity of Gα eventually results in GTP hydrolysis, during which a reorientation of the switch regions promotes the reassociation of Gα with Gβγ and readying Gα for another cycle of activation by its cognate GPCR. Therefore, Gβγ activity is indirectly controlled by Gα activation. Similar interaction cycles are repeated over and over for each of the thousands of signals using the GPCR pathway.

Diversity

The multiplicity of signals and their intracellular transduction raises the central question: how can so many signals, each recognized independently by a separate GPCR, specifically couple to only a dozen or fewer effectors via G-proteins? Specificity in signal coupling in metazoans is accomplished by two mechanisms. First, some G-proteins are able to recognize a specific GPCR and a specific effector. Second, promiscuous G-proteins are sequestered in signaling rafts that contain a specific GPCR, the cognate effector and all of the other components that operate on a particular pathway.

Animals have 23 different Gα, 6 Gβ, and 12 Gγ subunits, potentially assembling more than a thousand different G-proteins (Vanderbeld and Kelly, 2000). Given differences in the expression of G-proteins among different cell types and the known exclusion of some subunit pairs, we can more conservatively estimate that more than a hundred heterotrimeric complexes exist in a cell. Gα forms four subfamilies, Gs, Gi, Gq, and G12, on the basis of their sequence. In contrast to animals, *Arabidopsis* and rice have single canonical Gα - GPA1, RGA1, respectively, and single Gβ subunits AGB1 or RGB1, respectively, and possibly just two Gγ subunits AGG1 or RGG1 (Mason and Botella, 2000; Kato et al., 2004) and AGG2 or RGG2 (Ma et al., 1990; Weiss et al., 1994). The *Arabidopsis* Gα-subunit is roughly 30% identical to mammalian Gα-subunits of the Gi subfamily, and essentially all of this conservation lies in the few critical domains (Figure 2). GPA1 is most similar to the member of Gi subfamily called Gz. Like Gz, GPA1 lacks the carboxy-terminal cysteine that is targeted for ribosylation by pertusis toxin. GPA1 shares slightly more identity with Gz than with other members of the Gi subtype, and contains a Gz-specific myristolization motif. Gz plays a role in cell proliferation and death via its control of potassium channeling, thus it is possible that GPA1 operates in an analogous way.

The theoretical models (Figure 2B) of the *Arabidopsis* G-protein heterotrimer monomers based on the mammalian templates (Figure 2A) are "valid" structures overall. The final theoretical structures for *Arabidopsis* Gα and Gβ are nearly as compatible with the *Arabidopsis* sequences as the experimentally determined mammalian structures of Gα and Gβ are with the mammalian sequences. Although the overall structures are valid, there are some minor differences between the *Arabidopsis* structures and the mammalian structures caused by insertions in the *Arabidopsis* proteins. The insertions generally are small, with an average size of 5.0 residues for 5 inserts in the *Arabidopsis* Gα-subunit and an average size of 2.3 residues for 10 inserts in the *Arabidopsis* Gβ-subunit. The unpredicted conformations are colored green in Figure 2B.

Are there G-protein coupled receptors in plants?

In animal most of G-protein coupled receptors (GPCRs) are proteins composed of a single chain, which possesses seven hydrophobic regions of sufficient length to span the plasma membrane. It was previously shown that plant G-proteins may interact with plant receptors such as phytochrome, and auxin binding proteins. In fact, these receptors clearly do not belong to the family of GPCRs. To date no GPCR has been isolated from plant tissues to be

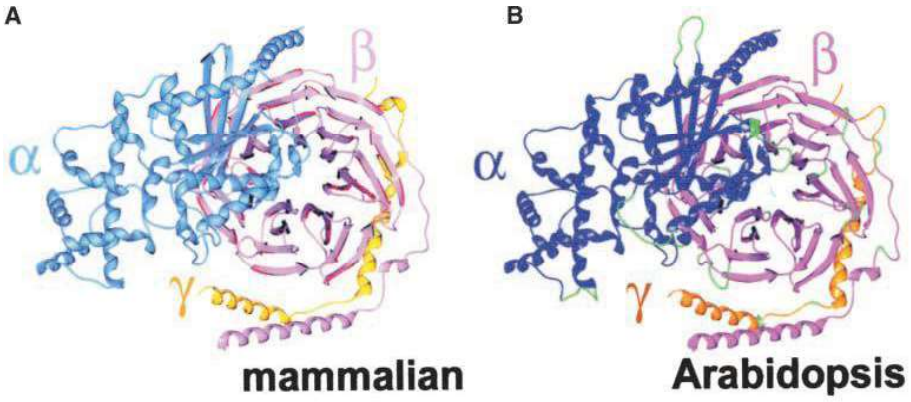

Figure 2. Modeling the *Arabidopsis* Heterotrimeric G-Protein Complex.
(A) and **(B)** Homology models were built for the GPA1, AGB1, and AGG1 deduced protein sequences from *Arabidopsis* using the Insight II molecular modeling system from Accelrys, Inc. The macromolecular structures were built using an experimentally determined structure of a mammalian G-protein heterotrimer (PDB access code 1GOT.pdb) as the template. The structure of the mammalian heterotrimer used as the template is shown in **(A)**, and the predicted structure of the *Arabidopsis* G-protein heterotrimer is shown in **(B)**. The α-monomers are shown in cyan, the β-monomers are shown in magenta, and the γ-monomers are shown in gold.

directly coupled by G-proteins. Furthermore, in contrast to the thousand or more heptahelical transmembrane proteins in animals, plants have only a few candidates confirmed to be heptahelical to date.

In a last decade there were few cases of cloning proteins which possess hydrophobic heptahelical transmembrane spanning regions.

These proteins share some sequence identity to animal GPCRs. The cloned from *Arabidopsis* seven transmembrane protein was similar to cAMP receptor from *Dictyostelium discoideurn* and author philogenetically include it to rhodopsin family (Josefsson and Rask, 1997). Cloned another 7-transmembrane plant receptor (Plakidou-Dymock et al., 1998) was identified in root and leaves of *Arabidopsis* and it was encoded with single copy of gene. Expressed by this gene protein has effect on sensitivity of plant to cytokinins.

One of these proteins, called MLO1, confers resistance to powdery mildew when present in its recessive form, but the mechanism of resistance is unknown. Recent evidence indicates that disease resistance conferred by *mlo* is not dependent on a G-protein (Devoto et al., 1999; Kim et al., 2002). However, the possibility remains that MLO is coupled by a G-protein in another signaling pathway because the function of this putative orphan receptor is unknown.

The gene cloned by Josefsson (Josefsson and Rask, 1997) from *Arabidopsis* called G-coupled receptor1 (GCR1) shares some sequence identity to animal GPCRs of the rhodopsin/serotonin family. GCR1 has a predicted heptahelical structure but this has not yet been confirmed by direct analyses. Overexpression of GCR1 modifies the cell cycle in a manner that is difficult to interpret (Colucci et al., 2002; Zhao and Wang, 2004). Specifically, M phase appears to be uncoupled from S. On the other hand, *gcr1* loss-of-function mutants do not share any of the G-protein mutant phenotypes, suggesting either that GCR1 is not coupled by GPA1 or that the GCR1 function is redundant (Chen et al., 2004).

Receptor-independent, G-protein signaling occurs in animals. Using a functional screen in yeast, Lanier's group (Cismowski et al., 2001) found three proteins (activator of G-protein signaling-1–3 [AGS1–3]) that are capable of activating G-protein signaling in the absence of a cognate receptor. Perhaps the most interesting of these is AGS3, which has subsequently been shown to be a guanine dissociation inhibitor (GDI) (Natochin et al., 2000, 2001). AGS3 binds the GDP-bound form of Gα to release Gβγ via a protein interaction involving a GoLoco motif. In yeast, this interaction directly activates a mitogen-activated protein (MAP) kinase pathway (Elion, 2000). However, there are not evidence AGS3 homologs or GoLoco containing proteins exist in plants.

Thus, we are left with only three possible conclusions: in contrast to animals, plants couple only one or a few heptahelical receptors by a G-protein to downstream effectors; and/or receptor-independent G-protein signaling is the primary mechanism in plants; and/or plants couple nonheptahelical receptors. Although the jury is still out, some interesting facts shed light on this problem. First, the carboxy-terminal domain of all plant G-protein orthologs is nearly 100% conserved, whereas in animals this region is poorly conserved due to the diversity of Gα–receptor interactions. Complete conservation in sequence among plant Gα carboxy-terminal domains suggests that there is a single or only a few receptors with which plant Gα can interact. Second candidate as potential receptors for Gα activation can be non membrane spanning protein like plant photoreceptors phytochromes. Previous observation indicate that special analogue of GTP - Gpp(NH)p activate that fractions of cAMP related enzymes which isolated from irradiated by red light seedlings, while enzyme isolated from etiolated seedlings wasn't sensitive to Gpp(NH)p (Gasimov and Fedenko, 1992; Fedenko and Gasimov, 1993). Third, indirect observations are consistent with G-coupling to nontraditional receptors like brassinosteroid (BR) (Ullah et al., 2002) and BR receptor-like kinase. These recent observations raise the exciting possibility that G-protein couples one or more of the more than 400 receptor-like kinases in plants (Mason and Botella, 2001).

G-proteins and plant hormones

The action of plant hormones at the cellular level is still poorly understood. However, some hormone receptors have been characterized and there is evidence that G-proteins could be involved in plant hormone mediated signalling.

Arabidopsis gpa1 mutants, which lack GPA1, have reduced cell division during hypocotyl and leaf formation (Ullah et al., 2001). The overexpression of GPA1 causes ectopic cell divisions, including massive overproliferation of meristem formation at high GPA1 expression levels. Overexpression of pea Gα stimulates cell division in yeast (Kim et al., 2002). These observations suggest that GPA1 couples a signal that controls cell division. A likely candidate is auxin. Auxin increased GTPγS binding to microsomal membranes of *Daucus carota* (Zbell et al., 1990) and to rice aerobic coleoptiles (Zaina et al., 1990). Auxin was also shown to increase GTPase activity in rice coleoptile membranes (Zaina et al., 1991). However, auxin-induced cell division occurs in mutants that lack

either Gα or Gβ, indicating that auxin cannot be coupled directly by a G-protein (Ullah et al., 2003). However, although G-protein mutants respond to auxin, they have dramatically altered auxin sensitivity. It is therefore possible that some other, as yet unknown, G-protein-coupled pathway interacts with auxin signaling in a way that controls auxin sensitivity.

Ca^{2+}-dependent swelling of mesophyll protoplasts from dark-grown wheat was shown to be induced by auxin, gibberelic and abscisic acid. The swelling could be inhibited by GDPβS, indicating the involvement of G-proteins in the process (Bossen et al., 1991). Unlike auxin signaling, an abscisic acid (ABA) signaling pathway appears to be directly coupled by a G-protein. Wang et al. (2001) demonstrated that ABA inhibition of light-induced stomatal opening is completely lacking in *gpa1* mutants. Consistent with this loss of ABA responsiveness, ABA does not inhibit inward K^+-channels or activate pH-independent anion channels in *gpa1* mutants (Figure 3). Interestingly, ABA-induced stomatal closure that is mediated by a pH change remains unaffected by the loss of GPA1 function, indicating that there are ABA pathways that are independent of G-protein in guard cells (Wang et al., 2001; Assmann, 2002).

The putative GPCR GCR1 is known to modulate signaling in guard cells in an unexpected manner: *gcr1*-knockout plants are hypersensitive to both ABA and S1P in stomatal aperture responses, and also show hypersensitivity to ABA in root-growth assays and foliar gene expression (Pandey and Assman, 2004). These results indicate that GCR1 functions as a negative regulator of these responses.

Not only a specific cell type contain multiple signaling mechanisms for one hormone, such as ABA, but different cell types can also have different mechanisms for the same hormone (Ullah et al., 2002). For example, although the guard cells of *gpa1* mutants are ABA insensitive, *gpa1* seeds have wild-type sensitivity to ABA but are 100-fold less sensitive to gibberellic acid (GA) and completely insensitive to brassinosteroid (BR). Seeds that overexpress GPA1 are a million-fold more sensitive to GA than wild-type seeds but still require GA for germination. One interpretation of these loss- and gain-of-function results is that GA signaling in seed germination is not directly coupled by G, but rather that some other G-coupled pathway crosstalks in a way that controls GA sensitivity. This indirect effect on a pathway via control of sensitivity is a reoccurring theme. Because it is known that BR regulates GA sensitivity, and that seeds that have reduced GA levels will fully germinate when treated with BR, it is possible that a BR pathway coupled by a G-protein is the sought after pathway that controls GA sensitivity (Figure 3A). Consistent with this, Ullah et al. (2002) have shown that BR synthesis and response mutants have the same reduced GA sensitivity as *gpa*1 mutants, and that BR was completely ineffective at rescuing the germination of *gpa*1 seeds when GA levels were reduced.

G-proteins and photosignal transduction

The classic example of the molecular mechanism of photosignal transduction by heterotrimeric G-protein to a downstream effector is vision in animals where the alpha subunit of the cognate heterotrimeric complex, transducin, couples the activated heptahelical membrane receptor rhodopsin to its cGMP phosphodiesterase effector in rod photoreceptor cells (Baylor, 1996). Plant cells are also light sensitive, especially in the red (R)/far-red (FR) light spectral region due to its highly light-sensitive family of photoreceptors called phytochrome. Therefore, an obvious question has been whether phytochrome light perception is similarly coupled by a heterotrimeric G-protein to an unidentified downstream effector.

In early 1990s it was shown the involvement of G-proteins in the phytochrome response. It was shown both red and far red light inhibits GTPγS binding in *L.paucicostata* (Hasunuma et al., 1987). In contrast, in *A.sativa* microsomal membranes (Romero et al., 1991) and in *Medicago sativa* plasma membranes (Muschietti et al., 1993), red light stimulated GTPγS binding while far red light reversed the effect of red light. Some phenotypes of a tomato (*Lycopersicon esculentum*) phytochrome mutant could be rescued to wild type by pertussis and cholera toxins, agents that stabilize the activated form of the G-protein subunit by different means (Neuhaus et al., 1993; Bowler et al., 1994). Microinjection of phytochrome A into aurea cells restored phytochrome-mediated effects. Injection of *Pertussis* toxin or GDPβS (which keep the G-proteins in their trimeric inactive form) with phytochrome A blocked the response. Injection of high GTPγS concentrations (30-100 mM) or coinjection of cholera toxin and low GTPγS concentrations (1 mM) produced an intracellular response indistinguishable from that mediated by phytochrome A.

Our observations have shown red light dependent response of cAMP phosphodiesterase to the action of GTP analog – Gpp(NH)p (Fedenko and Gasimov, 1993; Gasimov and Fedenko, 1992). Addition of Gpp(NH)p to reaction mixture resulted in increasing of phosphodiesterase activity from irradiated by relight maize seedlings, while enzyme from etiolated seedlings was insensitive to the action Gpp(NH)p. And recent our studies indicated that red light induces sensitivity of adenilylcyclase isolated from etiolated sorghum seedlings to the

action of Gpp(NH)p while far red light did not effect sensitivity of adenilylcyclase to Gpp(NH)p (Gasimov, 2008). These observations led to conclude that a heterotrimeric G-protein was positioned downstream of phytochrome (very likely phytochrome-B) in the light signal transduction pathway and upstream of a cNMP mediated step, in analogy to light perception in visual cascade.

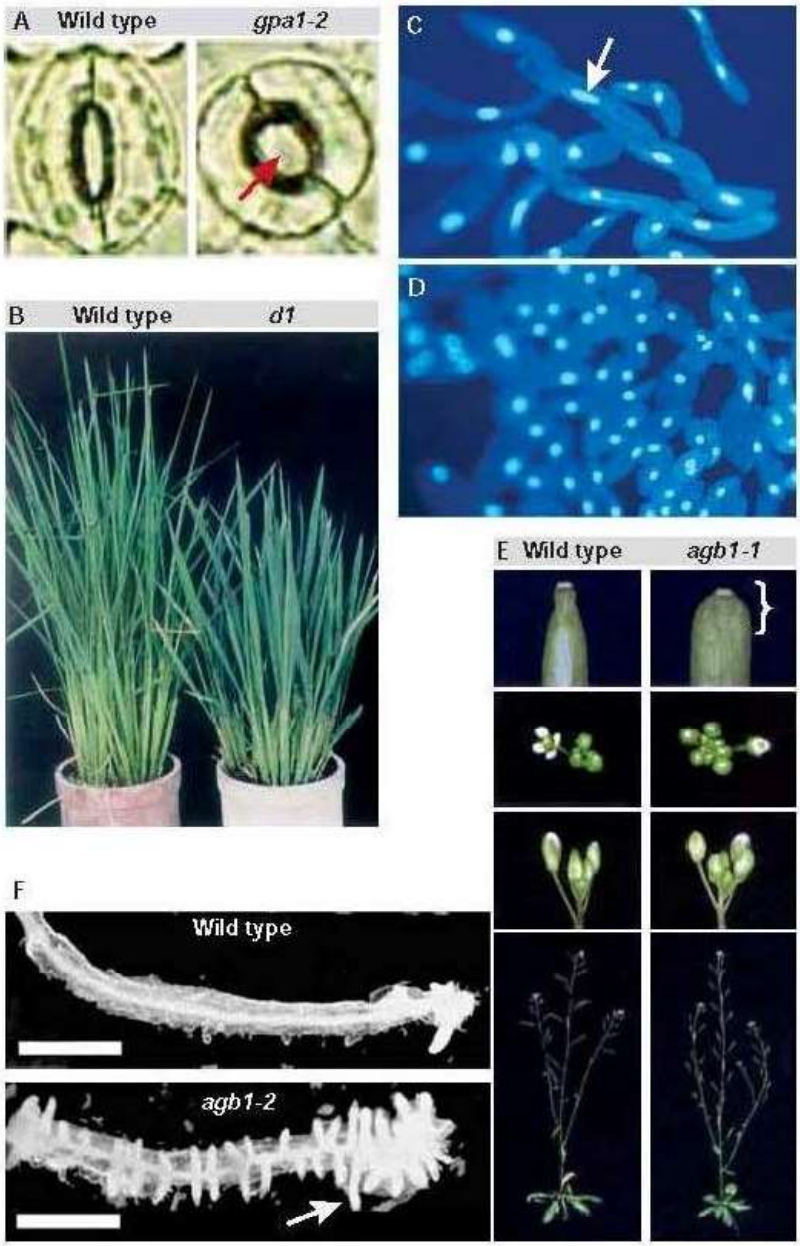

Figure 3. Selected phenotypes of plant G-protein mutants. (**A**) Guard-cell pairs on the surface of wild-type or *gpa1-2* Gα-null mutant leaves. *gpa1-2* guard cells fail to respond to the stress hormone abscisic acid (ABA) and consequently the pore of the stomate is open (red arrow). (**B**) The dwarf rice variety *d1* has a mutation in the Gα gene *RGA1*. (**C**) Ectopic *GPA1* expression in cultured tobacco cells causes a premature advance in the nuclear cycle. Note the larger nuclei (white arrow) in cells overexpressing Gα (**C**) versus control cells (**D**). (**E**) *agb1* (Gβ) mutants show phenotypes in the fruit (top two panels), flower morphology (middle four panels) and flowering stems (bottom two panels), which indicates that Gβ operates throughout development. (**F**) Gβ mutants have increased cell division in lateral root meristems, which leads to excessive root formation (white arrow) after treatment with the phytohormone auxin. AGB1 acts as a repressor of cell division in lateral root primordial.

Several other labs used pharmacological approaches in different systems and came to the same conclusion. Microinjection of GDPβS blocked R-induced protoplast swelling, whereas GTPγS induced swelling in darkness (Bossen et al., 1991). Cholera toxin was shown to increase the steady-state mRNA levels of the light-regulated gene, CAB (Romero and Lam, 1993).

More recently, Okamota and colleagues took a gain-of-function approach to test this hypothesis and concluded with all previous authors that a heterotrimeric G-protein is involved in phytochrome-mediated signal transduction (Okamota et al., 2001). The authors reported that *Arabidopsis* ectopically overexpressing the α-subunit of the heterotrimeric G-protein, regardless of the G-activation state, was hypersensitive to R and FR. And recent studies in the lab Jones (Jones et al., 2003) indicated the role of the single canonical heterotrimeric G-protein in R and FR control of hypocotyl growth using a loss-of-function approach. Single- and double-null mutants for the GPA1, AGB1 genes encoding the α- and β-subunit of the heterotrimeric G-protein, respectively, have wild-type sensitivity to R and FR. Ectopic overexpression of wild type and a constitutive active form of the α-subunit and of the wild-type β-subunit had no effect that can be unequivocally attributed to altered R and FR responsiveness. These results preclude a direct role for the heterotrimeric G complex in R and FR transduction in *Arabidopsis* leading to growth control in the hypocotyls (Jones et al., 2003).

Although heterotrimeric G-proteins appear to be involved in the phytochrome signal transduction pathway, the mechanism of interaction between the molecules is not clear. Phytochrome is a cytosolic protein and it is not structurally related to G-protein coupled receptors which are integral membrane proteins. However upon red light activation the phytochrome B precipitate in membrane fraction which can explain the way of signal transmission from photoreceptor to G-protein. On the other hand it is possible that an intermediate may transduce the signal from Pfr to G-proteins.

G-proteins and cell division

Congruent with their role in mammalian cells, G-proteins also regulate cell proliferation in plants (Ullah et al., 2001). During seed germination, massive cell proliferation occurs and the evidence supports a role for G-proteins in this process. For example, the plant hormones GA and brassinosteroid (BR) promote seed germination, whereas ABA inhibits seed germination and seedling development, and promotes seed dormancy. Seeds with ectopic overexpression of GPA1 are hypersensitive to GA (Colucci et al., 2002; Ullah et al., 2002), and overexpression of GCR1 reduces seed dormancy and promotes cell division (Apone et al., 2003). Conversely, ***gpa1***- and ***gcr1***-null lines show reduced seed germination in response to exogenous GA and BR (Chen et al., 2004). However, *gcr1/gpa1*double-mutants have an additively or synergistically attenuated response to GA and BR, which indicates that GCR1 has a role in seed germination that is independent of the heterotrimeric G-protein (Chen et al., 2004). Similarly to *Arabidopsis*, seeds of rice RGA1 antisense lines show reduced physiological and transcriptional responses to GA (Figure 3B) (Ueguchi-Tanaka et al., 2000).

Seeds that are mutant for *gpa1* show moderately enhanced sensitivity to ABA inhibition of germination, and seeds that lack the GPA1 interactor PIRIN1 are also hypersensitive to ABA, which indicates that PIRIN1 might be an effector in this response (Lapik and Kaufman, 2003). This is in contrast to the reduced ABA sensitivity of *gpa1* guard cells, which indicates that specific cell types might use GPA1 in different ways in response to an identical signalling molecule; a phenomenon that is also observed in mammalian G-protein pathways (Albert and Robillard, 2002). GPA1 and AGB1 are strongly expressed in meristems, in which the maintenance of a stem-cell population allows indeterminate growth (Huang et al., 1994; Kaydamov et al., 2000). Seedlings of *gpa1*-knockout lines have short hypocotyls that result from a decreased number of cells. *gpa1* mutants also show a reduced number of epidermal cells in leaves and reduced expression of a mitotic reporter, whereas GPA1-overexpressing plants show ectopic cell division in the epidermis (Ullah et al., 2003; Jones et al., 2004). Ectopic GPA1 expression in cells mimics an auxin-induced advance in the nuclear cycle (Figure 3C and D). The rice mutant *d1* has reduced GA sensitivity in internode elongation, which accounts for its dwarf phenotype, but shows wild-type growth responses in other vegetative organs (Ashikari et al., 1999; Ueguchi-Tanaka, 2000). Considering the central importance of G-proteins in many hormone-mediated cell-division pathways, it is not surprising that these mutants have pleiotropic phenotypes (Figure 3E). In mammalian cells, Gα-subunits have been identified as oncogenic determinants (Morris and Malbon, 1999), whereas Gβγ subunits have not. By contrast, in some plant organs, Gβγ seems to be the active form in controlling cell proliferation, albeit in the opposite direction. *Arabidopsis* lines that lack *AGB1* develop excessive lateral roots, whereas overexpression of *AGB1* results in the suppression of cell division stimulated by the plant hormone auxin (Ullah et al., 2003). These results indicate that free Gβγ is a negative regulator of auxin-induced cell division in the lateral root meristem. Consistent with this hypothesis, the overexpression of wild-type GPA1, which is

expected to sequester Gβγ-subunits, promotes lateral-root formation in response to auxin, whereas in *gpa1*-knockout lines this activity is reduced (Ullah et al., 2003). By contrast, in the primary root meristem, increasing the levels of active GPA1 either through the ectopic expression of a GTPase-deficient GPA1 (GPA1QL mutant) or loss-of-function of RGS1 promotes cell proliferation. Clearly, plant heterotrimeric G-proteins function in a cell-type-dependent manner. Specifically, primary root stem cells are positively regulated by the activated Gα-subunit, whereas lateral root stem cells are negatively regulated by the Gβγ-subunits (Figure 3F). The simplest model for this specificity follows the classic model of differential coupling mediated through one type of effector/receptor pair in one cell type and a different pair in another.

However, it remains plausible that there is only one set of receptor–effector coupling involving both the Gα- and Gβγ-subunits, perhaps antagonistically, and that the specificity is manifest through the balance of these subunits in different cells.

CONCLUSIONS AND FUTURE PROSPECTS

Although many details remain to be studied, the researches described above confirm that the plant heterotrimeric G-proteins are essential in at least three processes that are fundamental for the existence of all multicellular organisms: ion homeostasis, cell proliferation and photomorphogenesis. Plants probably have only two heterotrimer combinations G-proteins and analogues of the mammalian receptors and effectors. Therefore it could provide a simpler system in which to understand how these effectors are modulated in multicellular organisms. However there is not evidence confirming coupling of plant GCR and plant Gα-proteins similar in animal mechanism, but it is evident in this review that, numerous processes at all stages of plant development are modulated by heterotrimeric G-proteins.

Some of the many signals proposed to be coupled directly by G-proteins in plants may actually lie on pathways that are only indirectly regulated by G-proteins. The final integration of G-protein signalling emerges as physiological regulation. Once molecular biology completes the identification of the member(s) of the GPCR (GCR), G-protein subunits, effectors, and interacting proteins, the task at the cellular level will be to explore how regulation cell proliferation and plant morphogenesis goes on. The pace and intensity of the effort suggest that much will be gained well before decade has passed.

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