Plants: the latest model system for G-protein research

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In humans, heterotrimeric G proteins couple stimulus perception by G-protein-coupled receptors (GPCRs) with numerous downstream effectors. By contrast, despite great complexity in their signal-transduction attributes, plants have a simpler repertoire of G-signalling components. Nonetheless, recent studies on *Arabidopsis thaliana* have shown the importance of plant G-protein signalling in such fundamental processes as cell proliferation, hormone perception and ion-channel regulation.

Keywords: *AGB1*; *Arabidopsis thaliana*; *GCR1*; *GPA1*; G-protein-coupled receptor; heterotrimeric G protein

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Introduction

Metazoans have evolved a myriad of methods to sense and respond to their environment. One evolutionarily ancient mechanism involves the heterotrimeric guanine-nucleotide-binding (G) proteins, which are composed of α -, β - and γ -subunits. Heterotrimeric G-protein complexes link ligand perception by G-protein-coupled receptors (GPCRs) with downstream effectors (Fig 1).

Genes that encode G-protein-signalling elements have been identified in slime moulds, fungi, plants and animals. Several recent reviews have focused on the plant physiological responses that are linked to G-protein action (Ma, 1994; Fujisawa *et al*, 2001; Assmann, 2002; Jones, 2002). The goal of this short article is to systematically compare the composition of G-protein-signalling elements in plants with those in mammals, and to discuss the two best-characterized fundamental G-protein-related processes in plants: ion-channel regulation and cell proliferation.

The G-protein model

GPCRs and the G-protein heterotrimer. G-protein signalling begins with the alteration of the conformation of a GPCR by agonist binding (Pierce *et al*, 2002). GPCRs have seven transmembrane (7TM)-spanning domains with an extracellular amino-terminus and cytosolic domains that are coupled to the $G\alpha$ -subunit of the

G-protein heterotrimer in a way that influences the activation state of the α -subunit (Fig 1). In essence, the GPCR is a guanine-nucleotide-exchange factor (GEF) that promotes the exchange of GDP for GTP in the associated $G\alpha$ -subunit. In this view, the binding of agonists and inverse agonists (Fig 1) allosterically modifies the GEF and its subsequent action on its enzymatic substrate, the $G\alpha$.

The $G\alpha$ -subunit contains a Ras-like domain that has a GDP/GTP-nucleotide-binding site and GTP-hydrolase activity. In the GDP-bound form of $G\alpha$, the N-terminal helix and three 'switch' regions of $G\alpha$ interact with a seven-bladed propeller structure in the β -subunit ($G\beta$). On activation by a GPCR, the $G\alpha$ protein changes conformation to a structure that allows GTP binding (Morris & Malbon, 1999). Consequent reorientation of the switch regions in the Ras domain disrupts the tight interaction between $G\alpha$ and $G\beta$, which results in the separation of $G\alpha$ from the tightly associated $G\beta/\gamma$ -subunit dimer. $G\alpha$ and/or $G\beta\gamma$ then interact with downstream-effector molecules (Table 1). The intrinsic GTPase activity of $G\alpha$ eventually results in GTP hydrolysis, during which a reorientation of the switch regions promotes the

Fig 1 | The heterotrimeric G-protein cycle. This simplified model highlights elements of the pathway that are found in both metazoans and plants. The repertoire of each element in plants is greatly reduced compared with metazoans. GPCR, G-protein-coupled receptor; RGS, regulator of G-protein signalling. Figure modified with permission from Assmann, 2002.

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reassociation of $G\alpha$ with $G\beta\gamma$. Therefore, $G\beta\gamma$ activity is indirectly controlled by $G\alpha$ activation.

Regulatory elements. In mammals, many types of regulatory proteins (Table 1) modulate the basic GTPase cycle. The phosphorylation of GPCRs by protein kinases A and C reduces the coupling between the GPCR and $G\alpha$, which leads to desensitization (Ferguson, 2001). Another set of kinases—the GPCR kinases, known as ‘GRKs’—phosphorylate activated GPCRs. This phosphorylation enhances receptor affinity for arrestin proteins, one function of which is to intervene with signal transduction through the promotion of both receptor internalization and ubiquitylation (Rockman *et al*, 2002). In some situations, arrestins also have a positive role by functioning as scaffolding elements that recruit effector proteins to the signalling complex (Pierce *et al*, 2002).

At the level of the heterotrimer, regulator of G-protein signalling (RGS) proteins are GTPase-activating proteins (GAPs) for the $G\alpha$ -subunits, which typically result in reduced signal strength and/or accelerated termination of the signal after ligand removal from the GPCR. Another class of proteins, the phosphatases, interferes with G-protein signalling by sequestering $G\beta\gamma$ complexes, and so prevents the reassembly of a functional heterotrimer (Schulz, 2001).

G-protein-signalling genes in the human genome

GPCRs. There are at least 800 GPCRs in humans that share 25% or more sequence identity within a subfamily but show little or no sequence similarity between subfamilies (Pierce *et al*, 2002). This makes the cross-kingdom identification of GPCRs problematic.

$G\alpha$ genes. The human genome contains 17 $G\alpha$ genes, with known $G\alpha$ splice variants leading to a total of 23 $G\alpha$ -subunits. The $G\alpha$ s are divided into four subfamilies by functional and sequence attributes: $G\alpha_s$, $G\alpha_i$, $G\alpha_o$ and $G\alpha_{12/13}$. Members of each $G\alpha$ subfamily interact with hallmark-effector proteins (Table 1).

$G\beta$ and $G\gamma$ genes. The human genome encodes five $G\beta$ genes and at least 12 $G\gamma$ genes. The number of downstream effectors that have been shown to interact with $G\beta\gamma$ continues to grow, and includes isoforms of phospholipase C (PLC) and A_2 (PLA_2), certain Ca^{2+} and K^+ channels, and some isoforms of adenylyl cyclase (Ford *et al*, 1998; Morris & Malbon, 1999).

Modifiers and effectors. The human genome contains more than 30 genes that encode RGS proteins (Fig 1). RGS proteins accelerate the intrinsic GTPase activity of the $G\alpha$ -subunit by stabilizing the transition state of the GDP+P form through a physical interaction between the switch regions of $G\alpha$ and a domain in the RGS protein called the RGS box. This interaction effectively attenuates $G\alpha$ signalling by reducing the pool of activated $G\alpha$ -subunits. The human genome also contains at least four arrestin genes, with possible additional splice variants, at least seven GPCR kinase genes, numerous PKA and PKC genes, and at least four phosphatase isoform genes (Table 1). The diversity of ligands, GPCRs and GPCR regulatory mechanisms—along with the ability of GPCRs to oligomerize, to interact with more than one $G\alpha$, and to interact with many $G\beta\gamma$ complements and effectors (Table 1)—confers great versatility and complexity to G-protein signalling in humans (Albert & Robillard, 2002; Pierce *et al*, 2002). By contrast, the situation in plants is much simpler because, as described in the next section, there are far fewer G-protein signalling elements.

G-protein-signalling genes in the plant genome

GPCRs. The fully sequenced genome of the dicot angiosperm *Arabidopsis thaliana* encodes one gene product, GCR1, which has approximately 20% identity in short segments in its 7TM domain to the *Dictyostelium discoideum* (slime mould) cyclic AMP receptor CAR1 (Josefsson & Rask, 1997; Plakidou-Dymock *et al*, 1998; Kanyuka *et al*, 2001). GCR1 physically interacts with the plant $G\alpha$ -subunit GPA1 (Pandey & Assmann, 2004), but a ligand for GCR1 has not been identified. Given that even human GPCRs do not show significant sequence conservation between subfamilies, searches on the basis of sequence homology alone could fail to detect true plant GPCRs. The 15 members of an *Arabidopsis* gene family designated mildew resistance O (MLO) encode proteins that do not share sequences with any known GPCR, but have a predicted 7TM domain structure that has been confirmed biochemically for one MLO from oat (Devoto *et al*, 2002). A direct interaction between an MLO and $G\alpha$ has not yet been shown. Whether plant GPCRs have intrinsic basal activity that is suppressed by inverse agonists and enhanced by agonists (Fig 1) is not yet known.

$G\alpha$ genes. Only one canonical $G\alpha$ gene has been found in the genomes of *Arabidopsis* (GPA1; Ma *et al*, 1990), rice (RGA1; Ishikawa *et al*, 1995) and other diploid angiosperms (Assmann, 2002). Encoded plant $G\alpha$ proteins have ~30–40% identity to non-plant $G\alpha$ -subunits, with the closest sequence similarity to the $G\alpha_i$ subfamily (B. Temple and A.M.J., unpublished data). Intrinsic GTPase activity has been shown for these plant $G\alpha$ -subunits, although rates are lower than for typical mammalian $G\alpha$ s (Iwasaki *et al*, 1997; Seo *et al*, 1997; Aharon, 1998; Chen *et al*, 2003).

$G\beta$ and $G\gamma$ genes. Only one $G\beta$ gene (AGB1) has been identified in *Arabidopsis* (Weiss *et al*, 1994). Sequence similarity between the single plant protein and the various mammalian $G\beta$ proteins is ~40%. The two candidates for *Arabidopsis* $G\gamma$ -subunit genes, AGG1 and AGG2 (Mason & Botella, 2000, 2001) have only 20% overall identity to an alignment of the sequences of the 12 human $G\gamma$ proteins. However, other evidence supports the two proteins having a $G\gamma$ function: the two plant candidate $G\gamma$ s are a similar size to mammalian $G\gamma$ s, they interact with AGB1 in a yeast two-hybrid assay and they have a predicted carboxy-terminal prenylation sequence.

The plant heterotrimer. Despite low sequence identity between plant and human subunits, modelling and experimentation robustly support a genuine G-protein complex (Ullah *et al*, 2003; Kato *et al*, 2004). Many residues that occupy positions at the $\alpha\beta$ and $\beta\gamma$ interfaces of the plant G-protein subunits are conserved. Conserved residues in plant $G\alpha$ -subunits are found in the switch regions and the nucleotide-binding motifs. Genetic evidence that is consistent with a genuine heterotrimeric complex has been provided by a subset of phenotypes that are identical in $G\alpha$ and $G\beta$ loss-of-function mutants (Ullah *et al*, 2003).

Modifiers and effectors. Despite the apparent simplicity of G-protein-coupled signalling elements in plants, G proteins have been implicated in many plant phenomena (Assmann, 2002). Work with mutants has implicated G proteins in ion-channel regulation, control of seed germination, light responses (Okamoto *et al*, 2001, but, see also Jones *et al*, 2003), cell division and elongation, and responses to the phytohormones abscisic acid (ABA), gibberellic acid (GA) and auxin (Ma, 1994; Fujisawa *et al*, 2001; Jones, 2002). Pharmacological studies have also implicated heterotrimeric G proteins in plant interactions with symbiotic bacteria (Pingret *et al*, 1998; Kelly & Irving, 2003).

Table 1 | Components of heterotrimeric G-protein signalling

Component	Humans	Plants
Ligands		
Small molecules	For example, adenosine, ATP, calcium, catecholamines, glutamate, histamine, odorants, retinal and serotonin	Although plant cells contain some of these small molecules, none have been shown to bind to a GPCR
Lipids and lipid metabolites	For example, S1P, lysophosphatidic acid and eicosanoids	S1P is used as a signal in G-protein-based pathways in guard cells (Ng <i>et al.</i> , 2001; Coursol <i>et al.</i> , 2003)
Peptides	For example, thyrotropin, angiotensin, bradykinin, glucagons, calcitonin and parathyroid hormone	None of these peptides are known. So far, the receptors for plant-peptide ligands are leucine-rich repeat and cysteine-rich receptor kinases (Ryan <i>et al.</i> , 2002; Shin-Han & Bleeker, 2003), not GPCRs
Proteins	For example, thrombin, leutinizing hormone and follicle-stimulating hormone	Protein ligands are not known
Receptors		
Seven-span polytopic membrane proteins	A total of ~800, of which ~150 are known to be coupled by G proteins or have an identified ligand	The number of seven transmembrane proteins is not known; there are only a few candidates
Heterotrimeric G proteins (couplers)		
G α -subunit	17 genes with 6 splice variants, 23 G α in total comprising 4 groups: G α_q , G α_s , G α_i and G $\alpha_{12/13}$	One canonical G α subunit and three unusual forms (Ma <i>et al.</i> , 1990; Lee & Assmann, 1999)
G β -subunit	Five	One (Weiss <i>et al.</i> , 1994)
G γ -subunit	12	Two (Mason & Botella, 2000, 2001)
Selected modifiers/interactors		
RGS	At least 21 currently	One (Chen <i>et al.</i> , 2003)
Arrestins	At least four genes with splice variants	No obvious homologue. However, one protein cross-reacts with anti-arrestin serum (Nato <i>et al.</i> , 1997)
GPCR kinases	At least seven isoforms	No obvious homologue
Phosducin	One phosducin, and three homologues, two of which might not interact with G $\beta\gamma$	<i>Arabidopsis</i> has at least one gene that encodes a putative phosducin-like protein
Selected effectors		
Pirin	Pirins have not been shown to interact with a G-protein subunit	Pirin interacts with GPA1 in a yeast two-hybrid assay. Pirin mutants share some G-protein mutant phenotypes (Lapik & Kaufman, 2003)
AC and GC	Many GPCRs are coupled by G proteins to activate these cyclases	There is at least one possible GC (Ludidi & Gehring, 2003). A possible AC shows similarity to a fungal AC (Moutinho <i>et al.</i> , 2001). Neither of these cyclases has been shown to be coupled by a G protein
PL C β and PLC ϵ	The β -isoform of PLC is activated by G α_q . PLC ϵ is activated by G $\beta\gamma$	<i>Arabidopsis</i> has seven <i>PLC</i> genes, but based on sequence, none appear to encode the α , β or ϵ isoform (Mueller-Roeber & Pical, 2002)
PLA $_2$	Several cytosolic isoforms of PLA $_2$, calcium dependent (cPLA $_2$) and independent (iPLA $_2$). cPLA $_2$ is activated by heterotrimeric G proteins	The <i>Arabidopsis</i> genome contains 10 genes that encode the calcium-independent cytosolic isoform class (Holk <i>et al.</i> , 2002)
PLD	Two <i>PLD</i> genes with splice variants. PLD is activated directly by the small (Ras-like) GTPases	There are 12 <i>PLD</i> genes plus two splice variants in <i>Arabidopsis</i> . PLD may be directly regulated by a heterotrimeric G protein (Wang, 2002)
PI3K	Several PI3K isoforms in animals but only PI3K γ is thought to be stimulated by G $\beta\gamma$	Plants have PI3K, although it is not clear if the PI3K γ isoform is present (Mueller-Roeber & Pical, 2002). The products of PI3K have a role in guard-cell physiology (Jung <i>et al.</i> , 2002)
Potassium channels	There are four subunits of K $^+$ channels known as GIRK1–4, which are coupled by heterotrimeric G proteins	There are no clear homologues to GIRKs. However, K $^+$ channel activity is regulated by a heterotrimeric G protein in guard cells (Wang <i>et al.</i> , 2001)
Calcium channels	G-protein-coupled Ca $^{2+}$ channel currents are referred to as N-, P/Q-, R- and L-types	There is evidence that G α increases the mean open-pore probability of at least one calcium channel (Aharon <i>et al.</i> , 1998)
PDE	There are at least 20 isoforms of cGMP PDE alone	No obvious homologues
Rho GEF	There are at least two isoforms that are activated by G $\alpha_{12/13}$	No obvious Rho GEF homologues, although <i>Arabidopsis</i> does contain GEFs to other small GTPases (Vernoud <i>et al.</i> , 2003)
TUBBY proteins	At least four genes	There are at least eight TUBBY homologues, but modulation by G proteins has not been tested (Lai <i>et al.</i> , 2004)

AC, adenylyl cyclase; GC, guanylyl cyclase; GPCR, G-protein-coupled receptor; PDE, phosphodiesterase; PI3K, phosphatidylinositol-3-kinase; PL, phospholipase; RGS, regulator of G signalling; Rho GEF, small GTPase guanine-nucleotide exchange factor; S1P, sphingosine-1-phosphate.

Among the modulatory proteins, the *Arabidopsis* genome probably encodes a single RGS box-containing protein RGS1 (Chen *et al*, 2003). RGS1 is distinct from mammalian RGS proteins, which are cytosolic. The protein contains a predicted 7TM domain, which indicates that it could be ligand regulated, followed by the RGS box in its cytosolic C-terminal half.

Plants undoubtedly have as yet undiscovered G-protein modulators and effectors that are unique to the 'green' way of life. One such example might be a pirin protein. In humans, pirin serves as a transcriptional cofactor (Lapik & Kaufman, 2003). As shown in Table 1, G-protein effectors in plants also include a subset of those described in humans. Some of these effectors, including PLD, K⁺ channels and anion channels, have been confirmed by genetic or direct biochemical tests to function downstream of the G protein. Other candidates are included in the table on the basis of either their analogy to mammalian pathways or their known role in plant pathways that show G-protein involvement.

G proteins and ion channels: plants versus mammals

Calcium channels. In neuroendocrine and cardiac tissue, L-type Ca²⁺ currents are regulated indirectly by secondary messenger G-protein-based pathways (Carbone *et al*, 2001). A direct physical interaction between Gβγ, N-type and P/Q-type Ca²⁺ channels occurs in neurons (Catterall, 2000).

In plants, wild type or constitutively active recombinant Gα-subunits from tomato enhance the mean open probability of hyperpolarization-activated Ca²⁺ channels in the plasma membrane (Aharon *et al*, 1998). Because this phenomenon was observed in isolated membrane patches, a membrane-delimited pathway of channel regulation must be responsible, although a direct interaction has not been shown. Ca²⁺ channel open probability is also increased by fungal elicitors, which are pathogen-derived molecules that signal to the plant that it is under attack. This effect is abolished by a non-hydrolysable form of GDP and is mimicked by a non-hydrolysable form of GTP, which indicates that pathogen sensing could be mediated by G-protein-dependent Ca²⁺ channel regulation (Gelli *et al*, 1997). Consistent with a role for Gα in the response to pathogens, the rice Gα mutant *d1* shows reduced initiation of the defence response and increased infectivity after inoculation with avirulent rice blast fungus (Suharsono *et al*, 2002). **Potassium channels.** In mammals, direct interaction between G proteins and K⁺ channels in the membrane has been detected in an important class of heterotetrameric ion channels called the 'G-protein-activated inwardly rectifying potassium' (GIRK) channels (Mark & Herlitze, 2000). Cardiac GIRK currents are enhanced by sphingosine-1-phosphate (S1P), which is a phospholipid metabolite that interacts with a distinct subfamily of GPCRs that have recently been renamed the S1P receptors (Himmel *et al*, 2000; Spiegel & Milstein, 2003).

Basal activity of GIRK channels is inhibited by GαGDP or the GDP-bound heterotrimer through the direct interaction of Gα with the channel molecule. On receptor activation, formation of the GTP-bound form of Gα both alleviates this basal inhibition and releases free βγ-subunits, which stimulate channel activation by promoting GIRK interaction with phosphatidylinositol 4,5-bisphosphate (PIP2; Peleg *et al*, 2002; Mirshahi *et al*, 2003).

In plant guard cells, in which K⁺ channel regulation is crucial, substantial genetic and electrophysiological evidence indicates that the plant hormone ABA inhibits inwardly rectifying K⁺ channels through a heterotrimeric G protein (Assmann, 2002, and references

therein). Gα-subunit-null (*gpa1*) mutants of *Arabidopsis* are insensitive to ABA-mediated inhibition of whole-cell inward K⁺ currents, and this contributes to ABA insensitivity of stomatal opening (Fig 2A; Wang *et al*, 2001). Accordingly, leaves of these plants lose water through stomatal pores to the atmosphere at greater rates than wild-type plants. As for cardiac GIRKs, the K⁺ channels of guard cells are also modulated by S1P through a G-protein-coupled pathway. S1P inhibits the guard cell inward K⁺ channels in wild-type but not in Gα-null plants (Coursol *et al*, 2003). Consistent with this observation, S1P production through sphingosine kinase activity is stimulated by ABA (Coursol *et al*, 2003).

The secondary messengers that couple GPA1 to K⁺ channel regulation in guard cells have not been identified. However, PLD and PLC are candidate effectors, as both enzymes are stimulated by ABA and produce metabolites—phosphatidic acid and inositol 1,4,5-trisphosphate, respectively—that inhibit inwardly rectifying K⁺ channels (reviewed in Assmann, 2002). The α-isoform of *Arabidopsis* PLD binds directly to GPA1 and increases its GTPase activity, which indicates that PLD acts as a GAP. Reciprocally, GDP-bound GPA1 inhibits PLD activity (Lein & Saalbach, 2001; Zhao & Wang, 2004). Therefore, ABA stimulation of PLD activity in guard cells could conceivably occur by loss of GDP–Gα inhibition of PLD, concurrent with G-protein activation.

Anion channels in *Arabidopsis* guard cells are activated by both ABA and S1P. S1P activation is eliminated in the *gpa1*-null lines, which indicates obligate signalling through an intact G protein (Coursol *et al*, 2003). However, ABA-mediated activation of anion channels seems to act through a bifurcating pathway, only one branch of which is Gα- (and S1P-) dependent, whereas the other branch uses elevation in cytosolic pH as a signalling intermediate (Wang *et al*, 2001). G-protein-mediated regulation of anion channels has been reported rarely in mammalian systems, which indicates either that this is a plant-specific pathway or that such channel regulation is difficult to detect in mammalian systems. Because not all ABA responses in the guard cell are obligately coupled by GPA1, there must be distinct and separable pathways that are initiated by this single plant hormone, only some of which are mediated by a heterotrimeric G protein.

The role of *Arabidopsis* RGS in ion-channel regulation awaits evaluation. However, the putative GPCR GCR1 is known to modulate signalling 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 & Assmann, 2004). These results indicate that GCR1 functions as a negative regulator of these responses.

G proteins and cell division: plants versus mammals

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

Fig 2 | Selected phenotypes of plant G-protein mutants. (A) Guard-cell pairs on the surface of wild-type or *gpa1-2* $G\alpha$ -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). (Reprinted with permission from Wang *et al*, 2001 © (2001) American Association for the Advancement of Science.) (B) The dwarf rice variety *d1* has a mutation in the $G\alpha$ gene *RGA1*. (Reprinted with permission from Ueguchi-Tanaka *et al*, 2000 © (2000) National Academy of Sciences, USA.) (C) Ectopic *GPA1* expression in cultured tobacco cells causes a premature advance in the nuclear cycle (Ullah *et al*, 2001). Note the larger nuclei (white arrow) in cells overexpressing $G\alpha$ (C) versus control cells (D). (E) *agb1* ($G\beta$) mutants show phenotypes in the fruit (top two panels), flower morphology (middle four panels) and flowering stems (bottom two panels), which indicates that $G\beta$ operates throughout development. (Reprinted with permission from Lease *et al*, 2001; Ullah *et al*, 2003 © (2001, 2003) American Society of Plant Biologists.) (F) $G\beta$ 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 primordia. (Reprinted with permission from Ullah *et al*, 2003 © (2003) American Society of Plant Biologists.)

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 (Fig 2B; 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 & 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 & 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 (Fig 2C,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 *et al*, 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 (Fig 2E).

In mammalian cells, $G\alpha$ -subunits have been identified as oncogenic determinants (Morris & Malbon, 1999), whereas $G\beta\gamma$ subunits have not. By contrast, in some plant organs, $G\beta\gamma$ 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 $G\beta\gamma$ 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 $G\beta\gamma$ -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* (*GPA1^{QL}* 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 $G\alpha$ -subunit, whereas lateral root stem cells are negatively regulated by the $G\beta\gamma$ -subunits (Fig 2F). 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 $G\alpha$ - and $G\beta\gamma$ -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 ironed out, the studies described above confirm that the plant heterotrimeric G proteins are essential in at least two processes that are fundamental to the existence of all multicellular life forms: ion homeostasis and cell proliferation. What is not yet clear is whether these are separate pathways or whether modulation of cell proliferation exploits changes in ion flux in plant cells. Because *Arabidopsis* probably has only two heterotrimer combinations and a fraction of the mammalian receptors and effectors, it could provide a simpler system in which to understand how these effectors are modulated in multicellular organisms.

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