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GTP-binding proteins in plants: new members of an old family

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Abstract

Regulatory guanine nucleotide-binding proteins (G proteins) have been studied extensively in animal and microbial organisms, and they are divided into the heterotrimeric and the small (monomeric) classes. Heterotrimeric G proteins are known to mediate signal responses in a variety of pathways in animals and simple eukaryotes, whiole small G proteins perform diverse functions including signal transduction, secretion, and regulation of cytoskeleton. In recent years, biochemical analyses have produced a large amount of information on the presence and possible functions of G proteins in plants. Further, molecular cloning has clearly demonstrated that plants have both heterotrimeric and small G proteins. Although the functions of the plant heterotrimeric G proteins are yet to be determined, expression analysis of an *Arabidopsis* Gα protein suggests that it may be involved in the regulation of cell division and differentiation. In contrast to the very few genes cloned thus far that encode heterotrimeric G proteins in plants, a large number of small G proteins have been identified by molecular cloning from various plants. In addition, several plant small G proteins have been shown to be functional homologues of their counterparts in animals and yeasts. Future studies using a number of approaches are likely to yield insights into the role plant G proteins play.

Introduction

GTP-binding regulatory proteins (for simplicity, referred to as G proteins hereafter) are members of a large family of guanine nucleotide binding proteins found in all eukaryotes. On the basis of subunit composition and size, G proteins have been classified as heterotrimeric or small (monomeric) G proteins. Heterotrimeric G proteins, consisting of α, β, and γ subunits, generally relay information from membrane receptors to intracellular effectors [16, 17, 59, 144, 155]. One of the best studied heterotrimeric G proteins is the G protein (G_s) that mediates the hormonal stimulation of adenylate cyclase, and is widespread in animal cells [59]. In this case, the binding of the signal epinephrine to its receptor triggers the activation of G_s, which then activates adenylate cyclase. The synthesis of cAMP in turn leads to a cascade of protein phosphorylations and dephosphorylations, which regulate the activity of a variety of proteins. Another well characterized example of heterotrimeric G protein is the transducins (G_t), which transmit visual signals in vertebrates [59]. The G_t-coupled receptor is the light-activated rhodopsin, and the G_t-activated

effector is cGMP phosphodiesterase. Therefore, G_t mediates the light-activated hydrolysis of cGMP, which in turn regulates Na^+/Ca^{2+} channels in the photoreceptors, altering membrane potentials [160]. In animals, extensive biochemical and pharmacological studies have accumulated a large amount of information on many additional heterotrimeric G proteins and their functions [59, 144]. In recent years, molecular cloning has identified genes for previously known G proteins, as well as genes for new types of heterotrimeric G proteins in both mammals and other animals [87, 155]. Furthermore, a combination of genetic, biochemical and molecular analyses have uncovered G protein functions in simple eukaryotes [16, 17, 87, 155]. The sizes of the α subunits range from 35 to 45 kDa, those of the β and γ subunits are generally of 35–36 and 8–10 kDa, respectively. In addition, recent biochemical studies have uncovered novel proteins that are much larger (66–74 kDa) than known Gα's, yet have some characteristics of Gα subunits, such as GTP-binding, cross-reactivity with antisera against a conserved Gα peptide, and interaction with G-protein-coupled membrane receptors [73, 79, 80, 125, 156]. It is not known, however, how structurally similar these new GTP-binding proteins are to known Gα subunits, and whether they interact with either known or novel G$\beta\gamma$ subunits.

The small G proteins include a large number of molecules, from 20 to 30 kDa in size, and have very diverse functions [63, 64]. The discovery that the proto-oncogene *ras* encodes a small GTP-binding protein opened a new chapter in the history of G proteins. Although the precise biochemical function of mammalian *ras* is still not clear, increasing evidence suggests that it mediates signals received by membrane-receptor tyrosine kinase(s) and transmits them through other proteins to protein kinases, in particular the MAP kinase cascade [64, 109, 149, 164]. Genetic and molecular studies in invertebrate animals have also provided strong support for this signalling pathway, which is important for the regulation of specific cellular differentiations [145, 157]. Molecular, genetic and biochemical analyses of the Ras proteins in the yeast *Saccharomyces cerevisiae* have demonstrated an important role for Ras in the regulation of cellular growth; in particular, Ras regulate the activity of adenylate cyclase in yeast, and much (but not all) of the Ras function is mediated by cAMP in yeast [177, 178]. A variety of studies in recent years have identified a large number of different, but related, small G proteins functioning in a variety of processes, from signal transduction to controlling protein secretion, from regulating cytoskeletal functions to organizing membranes [63, 64].

Since the late 1980s, biochemical, physiological and molecular approaches have been successfully used to demonstrate the presence of G proteins in plants. For comparison, this article will summarize briefly G proteins in animals and simple eukaryotes, and focus the remainder of the discussion on the recent results on G proteins in plants. Other GTP-binding proteins, including tubulins and translation factors, will not be discussed here, although they perform important cellular functions. Other recent reviews on plant G proteins offer different emphases and perspectives [86, 94, 163].

G protein structures and functions in animals and simple eukaryotes

Heterotrimeric G proteins

Heterotrimeric G proteins were first identified in mammalian signal transduction pathways [87, 155]. In addition to the aforementioned G_s, there are three inhibitory G proteins ($G_{i(1-3)}$) which mediate the hormonal inhibition of adenylate cyclase activity. Furthermore, there are two transducins, G_{t1} and G_{t2}, which transmit visual signals to membrane potentials in rod and cone photoreceptor cells, respectively. The genes encoding the α subunits of these G proteins have been isolated [87]; the G_i's and G_t's are more similar to each other at the amino acid sequence level than they are to G_s (Fig. 1). Another Gα, G_o, was discovered which is most similar to G_i in sequence. Other mammalian Gα genes have been isolated that are structurally and functionally re-

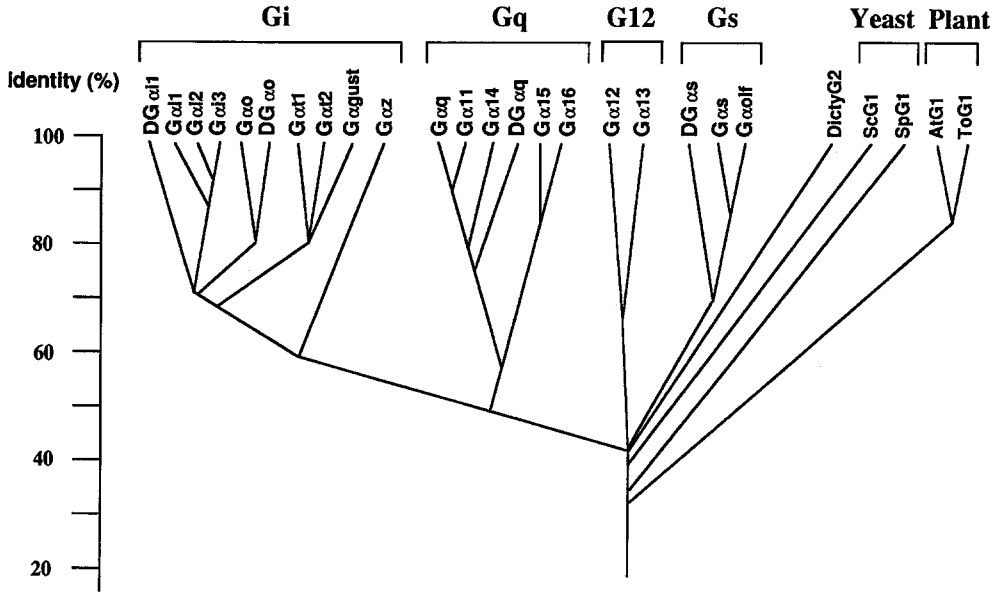

Fig. 1. Comparison of heterotrimeric G protein α subunits.The similarity tree is based on the percentage of amino acid sequence identity, and is modified from Fig. 2 of Simon *et al.* [155]; the additional amino acids in G_s, G_{olf}, and the yeast ScG1 were not included in the comparison, as described in Lochrie and Simon [103]. The DG's are from *Drosophila melanogaster*, DictyG2 is from *Dictyostelium discoideum*, ScG1 from *Saccharomyces cerevisiae*, SpG1 from *Schizosaccharomyces pombe*, AtG1 from *Arabidopsis thaliana*, ToG1 from tomato, and the other from mammals.

lated to G_s (G_{olf}, for olfactory response) or G_t (gustducin, for taste sensation) [111, 155]. Moreover, two new classes of Gα have been isolated recently: the G_q and the G_{12}/G_{13} classes [2, 158, 159, 179]. At least some of these new G proteins are involved in the regulation of phospholipase C [155]. Figure 1 shows the amino acid similarity between different Gα proteins. Genes encoding homologues of all major types of mammalian Gα proteins (Fig. 1) have also been isolated from *Drosophila* [36, 129, 132, 135–137, 158], and genes for the G_s and G_o types of Gα have been isolated from *Caenorhabditis elegans* [51, 102]. Among simple eukaryotes, the yeasts *S. cerevisiae* and *Schizosaccharomyces pombe* each have two Gα genes [40, 76, 115, 118, 119, 124]. *Dictyostelium* has as many as eight different α subunits, Gα1-Gα8 [52, 62, 92, 133, 182].

Heterotrimeric G protein α subunits have conserved consensus regions for GTP binding and GTPase activity [17, 87, 155]. A conserved arginine residue is found in all known Gα's, and it can serve as a site for ADP ribosylation by cholera toxin; however, only some Gα's are known to be modified by cholera toxin while others are not [155]. ADP ribosylation by cholera toxin blocks GTPase activity, resulting in an activated form of the α subunit (Fig. 2A). Some α subunits, including G_i's and G_t's, have a site (a cysteine residue near the C-terminus) for a similar modification by pertussis toxin. The ADP ribosylation by pertussis toxin interferes with the interaction between Gα and the receptor, and blocks the exchange of GDP for GTP, rendering the protein unable to be activated by the receptor (Fig. 2A, see below for the mechanism of G protein action).

Molecular cloning has identified several mammalian genes encoding at least 4 different β subunits and 5 γ subunits [74, 155]. Genes for β subunits have also been isolated from invertebrate animals squid [146], *Drosophila* [184, 185] and *C. elegans* [165]. In addition, the budding yeast *S. cerevisiae STE4* and *STE18* genes were found to encode β and γ subunits, respectively

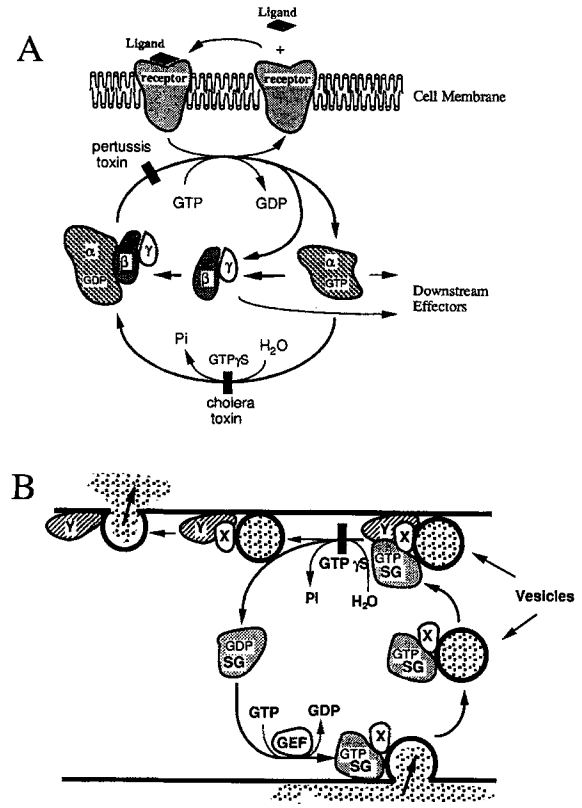

Fig. 2. Mechanisms of G protein actions. A. Heterotrimeric G proteins composed of the α, β and γ subunits. The α and/or $\beta\gamma$ subunits may interact with a variety of effectors. B. Small G proteins (SG) involved in secretion. Proteins X and Y are membrane-associated factors, and GEF is the guanine-nucleotide exchange factor, which interacts with the G protein. Solid bars indicate blockage by GTPγS or toxins.

[174], and *Dictyostelium* has one Gβ subunit involved in development [101]. The $\beta\gamma$ subunits have long been recognized to interact with the α subunit, thereby regulating its activity. In recent years, increasing evidence indicates that the $\beta\gamma$ subunits may also directly regulate effectors, and play a role in receptor interaction [74]. For example, studies indicate that G protein action in the pheromone response pathway in yeast involves the interaction of $\beta\gamma$ subunits with the effector [40, 174, 175]. In mammalian cells, the $\beta\gamma$ subunits have been shown to activate phospholipase A_2 in photoreceptors [78], K^+ channels in heart muscle [104], and the phospholipase C isoform β_2 from human granulocytes [22, 74, 84]. In addition, the $\beta\gamma$ subunits can activate type II adenylate cyclase in the presence of activated G_s, while they prevent the activated G_s from activating type I adenylate cyclase [98]. Not all of the β and γ subunits are functionally similar; in fact, different β and γ subunits act to distinguish different receptors under certain conditions [89, 90].

Receptors and effectors of the heterotrimeric G proteins

Heterotrimeric G proteins are coupled to receptors of the seven-transmembrane-segment class. A large number of receptors in this class have been identified using pharmacological, biochemical and molecular techniques [30, 42, 155]; they include the mammalian hormone and neurotransmitter receptors (adrenergic, serotoninergic, muscarinic, dopamine receptors, and so on), rhodopsin and the color vision opsins, a large family of odorant receptors, yeast pheromone receptors, and the *Dictyostelium* cAMP receptor. Since there are many more known receptors than known G proteins, different receptors likely interact with the same G protein. For example, there are three different color rhodopsins and only one color transducin, the signals received by all three rhodopsins are thought to be mediated by the same transducins. Furthermore, in the yeast mating response, both the a-factor and the α-factor receptors activate the same heterotrimeric G protein [70].

There are also numerous downstream effectors regulated by heterotrimeric G proteins [30, 155]. The first known G protein effectors are adenylate cyclases, which are regulated by G_s, and G_i. In addition, in olfactory epithelial cells, a specific isoform of adenylate cyclase [6, 130] acts downstream of G_{olf}, which is very similar to G_s [81]. As mentioned earlier, in the visual response, the effector of transducins is cGMP phosphodiesterase. Another class of effectors includes the isoforms of phospholipase C, which may be regulated by G_i, G_o and the G_q class of G proteins [155]. There has been evidence for regulation of

phospholipase A_2 by transducins [78]. Finally, several types of K^+ channels are regulated by G_i's and G_o, some Ca^{2+} channels are regulated by G_s and G_o, and Na^+ channels are regulated by G_{i3} and G_s [18, 30]. In *Dictyostelium*, a guanylate cyclase has been shown to be regulated by $G\alpha 2$ [52]. Note that the same G protein may regulate different effectors: G_s can both activate adenylate cyclase and open Ca^{2+} channels, G_i can inhibit adenylate cyclase while opening K^+ channels, and *Dictyostelium* $G\alpha 2$ can activate both a guanylate cyclase and a phospholipase C [30, 52]. As many G protein-mediated responses are yet to be characterized at the molecular level, other effectors and G protein-effector interactions are certain to be uncovered.

Small G proteins

On the basis of amino acid sequence similarity, small G proteins have been grouped into several subfamilies [43, 63, 64]. All of the small G proteins have consensus regions for GTP-binding, which are related in varying degrees to those found in the α subunits of heterotrimeric G proteins [17]. Members of each subfamily share characteristic additional residues in the GTP binding consensus regions in addition to the residues that are conserved in all G proteins [17]. The most extensively studied small G proteins are in the ras subfamily which includes the products of several *ras* proto-oncogenes, and homologues in invertebrate animals and yeasts. The activity of the ras protein is directly regulated by guanine nucleotide exchange factors (GEFs), which stimulate the exchange of bound GDP for GTP, thus activating ras [11]. In addition, GTPase activating proteins (GAPs) stimulate the intrinsic GTPase activity of ras, leading to faster hydrolysis of the bound GTP and reducing ras activity [11]. Increasing evidence indicates that membrane receptor tyrosine kinases mediate the regulation of ras activity by extracellular signals, through the 'adaptor proteins' (e.g., the mammalian Grb2 protein) which bind to the activated, autophosphorylated receptor and to GEFs [149]. In addition, genetic, molecular and biochemical studies have uncovered a conserved kinase cascade (MAP kinase cascade) which functions downstream of ras [122]. Ras homologues in *Drosophila* and *C. elegans* are involved in signal transduction during development [145, 157]. In yeast, Ras proteins regulate cell growth by controlling the activity of adenylate cyclase and thus the level of cAMP [63, 178].

A second group of small G proteins consists of several mammalian rab proteins, the yeast YPT1 and SEC4 proteins, and related proteins; these are involved in vesicular transport in the secretory pathway [63, 64, 152]. Extensive biochemical and genetic studies indicate that the rab/ypt subfamily of small G proteins associate with membrane vesicles (probably interacting with membrane proteins) and shuttle between donor and acceptor membrane structures. It is believed that the binding of GDP or GTP stabilizes alternate conformations of these small G proteins, allowing them to associate with protein(s) on one structure or another. Furthermore, the bound GTP is hydrolyzed only when stimulated by the appropriate GAP protein. Therefore, GTP binding and hydrolysis drive a unidirectional transport of vesicle content (see below and Fig. 2B). The members of the Ypt/Rab subfamily are regulated by their cognate GEFs, GAPs, and a third type of regulator: the guanine-nucleotide dissociation inhibitors (GDIs), which inhibit GDP dissociation from rab [11].

In addition to the ras and rab/ypt subfamilies, another group includes the mammalian rho and rac, and the yeast CDC42 proteins, which function in cell polarity and cytoskeletal function [63, 64]. There are three other types of small G proteins [64]. One is represented by the mammalian and yeast ARFs, which are known to facilitate ADP ribosylation of α subunits by cholera toxin *in vitro*. More recently, ARF has been implicated in secretion, where its proposed function is to control the assembly of secretory vesicle coat proteins in a GTP-dependent manner [64]. In the yeast *S. cerevisiae*, another small G protein, CIN4, was found to be involved in chromosome segregation [14]. Although the sizes of the ARF and

CIN4 proteins lead to their classification as small G proteins, they resemble the larger heterotrimeric α subunits in two ways [14]. Firstly, the GTP-binding consensus regions of ARF and CIN4 are more similar to those of Gα's than to those of ras and rho. The second feature concerns the position and nature of lipid modification; ARF and CIN4 both contain a glycine as the second residue from the N terminus, where all known α subunits have a glycine. The glycine residues in ARF and some Gα's are known to be myristoylated [20, 82]. In contrast, most small G proteins such as ras do not have this glycine residue, but are isoprenylated (farnesylated for ras) at a cysteine residue very close to the C terminus [16]. Some small G proteins are also palmitoylated at a cysteine residue in the C-terminal half of the protein [21]. Other small G proteins include the mammalian Ran protein and its homologues and the *S. cerevisiae* Sar1 protein [17, 140]. The Ran protein is involved protein targeting to the nucleus and affects mitosis [116, 140]. The Ran protein lacks both the N-terminal glycine and the C-terminal cysteine residues; therefore, its post-translational modification, if any, is different from both Gα/ARF and ras. The Sar1 protein functions in vesicular transport [17].

Mechanisms of G protein activation

Much has been learned about the mechanisms of some mammalian heterotrimeric G proteins, particularly G_s and transducins [9, 16, 59, 155], which have served as models for other G proteins. Briefly, heterotrimeric G proteins function through a cycle of reactions and protein-protein interactions (Fig. 2A). At the resting (inactive) state, the α subunit binds GDP and associates with the $\beta\gamma$ subunits to form a complex. Upon the binding of a ligand, a cell surface receptor is activated, and it catalyzes the exchange of the bound GDP on Gα for a GTP, which causes a conformational change of the α subunit, resulting in its dissociation from the $\beta\gamma$ complex. The active GTP-bound α subunit then regulates its effector(s), such as adenylate cyclases or K^+ channels, leading to a cascade of downstream events. Often the GTP-bound α subunit is the known species which activates downstream events, while the $\beta\gamma$ complex acts as an inhibitor of the α subunit. However, as mentioned before, several studies clearly demonstrate that the $\beta\gamma$ subunits can also directly interact with downstream effectors [74, 98]. The hydrolysis of GTP to GDP and phosphate by the intrinsic GTPase of the α subunit returns the α subunit to its inactive conformation and the GDP-bound α subunit re-associates with the $\beta\gamma$ subunits. Non-hydrolyzable GTP analogues, such as GTPγS, and mutations reducing the GTPase activity of the α subunit prolong the active state of heterotrimeric G proteins. In addition, pertussis toxin uncouples the receptor from its G protein and thus blocks signal transduction, and cholera toxin blocks the GTPase activity of the α subunit and fixes it in an activated form [87, 155].

Genetic and biochemical evidence suggests that the mechanisms of the ras proteins and close homologues are similar to those of the heterotrimeric G proteins [16, 17, 63, 178]. Mutations which lead to oncogenic and constitutively active forms of ras are found to alter two kinds of residues: (1) those important for GTP binding, and (2) those required for GTPase activity. The first class of mutant ras proteins are enhanced for GTP binding, and the second kinds of mutant ras proteins are defective in GTP hydrolysis [43, 63]. In other words, increased GTP binding by ras leads to a more active ras; therefore, the GTP-bound form is active, while the GDP-bound form of ras is inactive. However, the GDP/GTP exchange and GTP hydrolysis, that is the interconversion between the active and inactive states, is controlled differently for ras proteins than for heterotrimeric G proteins. Instead of activated transmembrane receptors as in the case of heterotrimeric G proteins, the GDP/GTP exchange of ras requires a different type of protein factor, known as the guanine nucleotide exchange factors, which are regulated by receptor tyrosine kinases. In addition, ras proteins have a very low intrinsic GTPase activity, which can be substantially increased by GAPs (GTPase-activating proteins). The larger

Gα proteins, on the other hand, contain an insertion of more than 100 amino acid residues between the first and second consensus regions for GTP binding. Since Gα's have a higher intrinsic GTPase activity than small G proteins, it has long been proposed that this extra region has a GTPase activating activity [17]. This has recently been demonstrated experimentally by using two truncated proteins: one contains just the 'insertion' region and can activate the GTPase activity of the other which contains the remainder of the protein [108]. Furthermore, the crystal structure of the transducin α subunit indicates that the GAP-like region folds into a domain separate from the GTPase domain of the protein [123].

In contrast to heterotrimeric G proteins and ras, another mechanism is likely to operate in the action of small G proteins involved in secretion, such as the yeast Sec4 and Ypt1, and mammalian rab proteins [15, 63, 166]. For these proteins, the GTP-bound form is required for a portion of a cyclical traffic of cellular membranes, and one GTP is required for one cycle (Fig. 2B). GTP hydrolysis is necessary for the cycle to be completed. Models have been proposed which postulate that the GTP-bound form of the small G protein associates with some factor(s) during part of the cycle, while the GDP-bound form associates with others. Since the normal function of these small G proteins requires the continuous cycling of GDP/GTP exchange and GTP hydrolysis, the binding of non-hydrolyzable GTP analogues, and mutations reducing GTPase activity (either in the G proteins or in the GAPs), inhibit the function of these small G proteins.

Biochemical studies of plant G proteins and their involvement in plant signalling

Detection of GTP-binding proteins

Plant cells respond to a variety of signals both from the environment (light, humidity, temperature, gravity and pathogens) and from other cells (hormones, nutrients). However, little is known about the mechanisms of signal transduction in plants. Many have thought that due to the conserved nature of G proteins, they may play important roles in plant signal transduction pathways, as they do in animals and simple eukaryotes. There are several ways to detect G proteins *in vitro*. One of the widely used methods to detect G proteins is a GTP-binding assay, usually using one of the non-hydrolyzable GTP analogues, such as GTPγS. In general, GTP-binding assays detect both heterotrimeric and small G proteins, and other GTP-binding proteins; therefore, binding activities present in plant extracts can be quite complex [46]. Consequently, they are usually used for preliminary studies, and additional, more specific assays are often needed to demonstrate that G proteins are present. Nevertheless, in a relatively well defined system, for example a partially purified membrane fraction, GTP-binding assays can still be very informative when one performs proper controls such as binding using ATP and treatment with specific signals. Furthermore, a filter assay for GTP binding by renatured proteins has been very successfully used to identify small G proteins. Since both the α subunit of heterotrimeric G proteins and small G proteins have intrinsic GTPase activities, the GTPase assay is another way these proteins may be detected. Indeed, assays for GTPase activity have been used to provide evidence for the presence of G proteins in plant cells (see below and Table 1). This method is also limited by its lack of specificity for particular types of G proteins.

In addition to GTP-binding and GTPase activity assays, a method for preferentially detecting heterotrimeric G proteins is the use of bacterial toxins that covalently link an ADP-ribose moiety to a particular amino acid residue (ADP ribosylation) of some α subunits of heterotrimeric G proteins. The most frequently used toxins are pertussis and cholera toxins [58]. The susceptible Gα subunits may be labeled using these toxins and radioactive NAD^+, which serves as the donor of the ADP-ribose group. G protein α subunits sensitive to pertussis toxin, including G_i's and transducins, have a cysteine near the carboxy terminus (usually at the fourth position from the end), thus ADP ribosylation by pertussis toxin is

Table 1. Biochemical detection of GTP-binding proteins.

Plant	Tissue	Sizes[a]	Assays[b]	References
Arabidopsis thaliana	leaf, root	36, 31	anti-Gα, GTPγS (s)	[10]
Arabidopsis thaliana	leaf	33	anti-Gi	[173]
Avena sativa (oat)	etiolated seedling	24	GTP (f), anti-Gα, CTX	[143]
Chlamydomonas reinhardtii	eyespot	24	GTP (s), GTPase, anti-Gα	[91]
Cucurbita pepo (zucchini)	etiolated hypocotyl	33, 50	GTPγS (s), anti-Gs	[77]
Cucurbita pepo (zucchini)				[45]
Commelina communis	leaf, root	38, 34	anti-Gα	[10]
Dunaliella salina		28	GTP (f), anti-YPT1	[141]
Dunaliella saline		29, 30	GTP (f)	[141]
Glycine max (soybean)	cultured cells	45	anti-Gα, CTX	[99]
Hordeum vulgare (barley)	aleurone	32, 36	anti-Gα	[168]
Hordeum vulgare (barley)	aleurone	22, 24	GTP (f), anti-ras	[168]
Lemna paucicostata		?	GTP (s)	[65]
Oryza sativa (rice)	coleoptile	28, 30	GTP (f), anti-Gα	[189]
Pisum sativum (pea)	plumules	21	GTP (f), anti-ARF	[112]
Pisum sativum (pea)	plumule nucleus	27, 28, 30	GTP (f)	[29]
Pisum sativum (pea)	leaf chloroplast	24	GTP (f)	[148]
Pisum sativum (pea)	etiolated seedlings	25, 37	anti-Gi	[173]
Pisum sativum (pea)	etiolated seedlings	43	anti-GPA1	[173]
Pisum sativum (pea)	etiolated seedlings	40	anti-Gi/Go, PTX	[169]
Pisum sativum (pea)	etiolated epicotyl	?	GTP (s)	[67]
Spinacea oleracea (spinach)	leaf	?	GTPγS (s)	[114]
Vicia faba (broad bean)	leaf, root	37, 31	anti-Gα	[10]
Zea mays (maize)	root	27, 34	GTPγS (s), purification	[8]

[a] The sizes in kDa were estimated from protein gels; therefore, the sizes of proteins were not known when the GTP-binding activities detected in solution assays.

[b] GTP or GTPγS indicate the binding of labeled nucleotide on filter (f) or in solution (s); western experiments were indicated by the antiserum used: Gα, a conserved Gα peptide; GPA1, a peptide specific for GPA1 from *Arabidopsis*; YPT1, from *S. cerevisiae*; Gi, Go, Gs, and ARF are from mammals. CTX and PTX indicate labeling with cholera and pertussis toxins, respectively.

a sensitive assay for a subset of G protein α subunits. ADP ribosylation by cholera toxin is considerably more complex [58]. Although the arginine residue that is ADP-ribosylated is conserved among all known G protein α subunits, only $G\alpha_s$ has been well documented as being efficiently modified by cholera toxin, while others may be modified under some circumstances [58]. Furthermore, ADP ribosylation by cholera toxin is greatly stimulated by a soluble factor, called the ADP ribosylation factor (ARF), which is itself a small G protein, and is activated by the binding of GTP or GTP analogues [58]. Proteins other than G proteins can also serve as substrates for ADP ribosylation by cholera toxin, but they are ribosylated considerably more slowly than G_s and dominate the labeling pattern only if they are very abundant [58]. The effect of ARF and the possibility that proteins other than G proteins can be modified by cholera toxin make its use less desirable and more prone to artifacts. It must be emphasized that the toxins are only useful to identify the presence of certain classes of G proteins in an extract. Therefore, there might be G proteins which are not substrates for the toxin-catalyzed ADP ribosylations.

Immunoblot procedures using antisera against peptides from known G proteins have also been very powerful. A number of groups have used antibodies raised against a conserved peptide of the α subunit of heterotrimeric G proteins (see below). These analyses in combination with GTP-binding studies provide strong evidence for the presence of heterotrimeric G proteins in

plants. The limitation of the antibody studies is that it requires cross-reactivity between the antibodies and the plant proteins. Since there are known animal α subunits which contain amino acid divergence in the conserved region used to raise the antibodies, it would not be surprising if some plant Gα proteins also contain amino acid changes in the same region. Another problem is that not all cross-reacting proteins are G proteins. Nevertheless, with other independent assays, analyses using antibodies have produced very valuable information on potential plant G proteins.

Using one or more of these *in vitro* biochemical techniques, GTP-binding activities and proteins have been detected in a variety of plant species (Table 1). By using ^{35}S-labeled GTPγS binding in solution, GTP-binding activities were detected in the thylakoid membranes of spinach (*Spinacea oleracea*) leaves [114], and in membranes of rice coleoptile [190]. GTP-binding and GTPase activities were found in the eyespot of the green alga *Chlamydomonas reinhardtii* [91], and in membrane extracts from maize roots [8]. GTP-binding activities and substrates for ADP ribosylation catalyzed by pertussis toxin were detected in gel filtration fractions of extracts from pea (*Pisum sativum*) and *Lemna paucicostata* [65–67]. In addition, a filter assay for GTP binding has been used to uncover small G proteins in the green alga *Dunaliella salina* [113, 141], in microsomes of zucchini (*Cucurbita pepo*) hypocotyl [45], and in the chloroplast outer envelope membrane [148] and nuclear envelope from pea [29]. Furthermore, an ARF-like protein was detected in the cytosol of pea plumule cells [112].

The combination of GTP-binding studies and immunological analysis with antibodies raised against known G protein α subunits have detected potential heterotrimeric G protein subunits from *Arabidopsis*, broad bean (*Vicia faba*), *Commelina communis* [10], zucchini (*Cucurbita pepo*) [77], and barley (*Hordeum vulgare*) [168]. By using a GTP-binding assay, western blot analysis using anti-Gα antibodies, and ADP ribosylation with cholera toxin, Romero *et al.* identified a 24 kDa GTP-binding protein in oat (*Avena sativa*) etiolated seedlings [143], although this protein is smaller than any known heterotrimeric G protein α subunits, and is within the size range of known small G proteins. Similarly, using anti-Gα antibodies and a filter GTP-binding assay, two small GTP-binding proteins were identified in rice coleoptile membranes (28 and 30 kDa) [189], and in barley aleurone protoplasts (22 and 24 kDa, also recognized by anti-ras antibodies) [168]. It is possible that the anti-Gα antibodies cross-react with small G proteins, and the conditions used for ADP ribosylation by cholera toxin allow the modification of small G proteins. The definitive identification and characterization of these proteins must await further molecular studies.

G protein involvement in plant signalling pathways

Studies of known G proteins indicate that the interaction of G proteins with receptors, effectors, and GTP/GDP occur at particular points of a cycle (Fig. 2); therefore, alteration at one point in the cycle affects the subsequent point(s). This property of G proteins has been exploited to learn possible G protein functions in individual signalling pathways. GTP analogues and the cholera and pertussis bacterial toxins described previously are useful tools to probe the involvement of G proteins in various cellular processes. GTP analogues, particularly GTPγS, are used frequently due to the relative ease with which they can probe G protein functions. However, GTP analogues affect both heterotrimeric and small G proteins; therefore, conclusions from studies using GTP analogues are not definitive. The bacterial toxins, particularly pertussis toxin, are more specific, and they are used for analyses of heterotrimeric G proteins. G proteins that are involved in mediating extracellular signals, such as the heterotrimeric G proteins, are usually activated by a receptor-ligand complex. The activation usually involves the exchange of a bound GDP for a GTP. Therefore, the presence of a G protein in a signalling pathway can often be detected as a stimulation of GTP binding by the signal. If at least a portion of the signalling pathway can be reconstituted *in vitro*, then the effect

of the signal on GTP binding can be characterized using the GTP-binding assays described above. In addition, heterotrimeric G proteins are usually activated by binding to GTP. This activation is attenuated by the hydrolysis of GTP to GDP and phosphate due to the intrinsic GTPase activity of the α subunit. For small G proteins, the intrinsic GTPase activity is greatly stimulated by the GTPase activating protein (GAP). Because GTP analogues such as GTPγS and GMP-PNP are not hydrolyzable, they are more potent activators of G proteins. If a signal is known or suspected for a particular cellular process, such as response to light, then guanine nucleotides may be used to mimic the signal in generating the response.

The use of bacterial toxins can also probe G protein function in cellular processes. Cholera toxin can ADP-ribosylate both GTP- and GDP-bound forms [58], and cholera toxin-catalyzed ADP ribosylation inhibits intrinsic GTPase activity, prolonging the activated state of the α subunit. In contrast, pertussis toxin-catalyzed ADP ribosylation only occurs on the GDP-bound heterotrimeric form, and the modification uncouples the G protein from the receptor [59]. Therefore, pertussis toxin keeps the G protein in the inactive state. If a signal activates a G protein, then the ADP ribosylation catalyzed by pertussis toxin should be reduced. Further, a positive effect of cholera toxin on some cellular response would suggest that an activated G protein is involved in promoting the response, while a positive effect of pertussis toxin suggests that an activated G protein can inhibit the response.

A number of studies have implicated GTP-binding proteins in light-stimulated signalling pathways, using both GTP analogues and bacterial toxins. In *Lemna*, a single 8 h period of darkness induces flowering. It was found that, when the extracts were prepared from *Lemna* plants that had been in darkness for 8 h, GTP binding was inhibited by about 20% by red or far-red light, as compared to the binding in the dark, but not affected by blue light [66]. This suggests that the red/far-red receptor phytochromes may be involved in the regulation of one or more G proteins. However, it is puzzling that in this case similar inhibition was seen with red light and far-red light, which have opposite effects on phytochromes [134]. In more recent studies, it was found that GTP binding by pea nuclear membranes was stimulated by a 2 min exposure of red light, and such stimulation was eliminated if the red-light exposure was followed by a 4 min exposure of far-red light [29]. Similarly, far-red light-reversible, red light-stimulated GTP binding was observed with membranes from etiolated oat seedlings [143]. These results strongly suggest that one or more GTP-binding proteins are activated by red light via phytochrome, since the far-red reversibility is a characteristic of phytochrome signalling. In other studies, it was found that the GTP analogues GTPγS (30–100 μM intracellular) and Gpp(NH)p (50–100 μM intracellular) mimicked the effects of the light receptor phytochrome A on light-dependent synthesis of anthocyanin and the expression of a reporter gene (*GUS*) under the control of a light regulated *cab* gene promoter, suggesting that a G protein may be involved in phytochrome signal transduction [121]. In this case, cholera toxin alone had only a small effect on the light responses; however, cholera toxin in combination with a low concentration (1 μM) of GTPγS, which has no effect by itself, produced an effect similar to that of phytochrome A or 30–100 μM GTPγS [121]. These results are consistent with a role for a cholera toxin-sensitive G protein that is put into a prolonged activated state by ADP ribosylation. In addition to red light, blue light has also been observed to stimulate GTP binding in etiolated oat seedlings [143]. Moreover, blue light stimulates a GTPase activity, as well as a GTP-binding activity, in plasma membranes of etiolated pea seedlings [169]. A 40 kDa protein in these membranes was ADP-ribosylated by pertussis toxin in the absence but not presence of GTP and blue light; in addition, a protein of the same size (presumably the same protein) cross-reacted with antisera which detect transducin or G_i/G_o α subunits [169]. These results suggest that a heterotrimeric G protein in etiolated pea seedlings may be involved in blue light signal transduction.

Although the results discussed above certainly suggest that light signals can be mediated by heterotrimeric G proteins in plants, it is not known how light activates G proteins. The red light receptor phytochromes appear to be soluble proteins from their sequences [154]; furthermore, recent isolation of an *Arabidopsis* blue-light response gene (*HY4*) suggests that a blue light receptor is also a soluble protein that is similar to photolyases [1]. It is possible that these non-transmembrane photoreceptors interact with other proteins, possibly membrane-associated ones, which in turn interact with G proteins. Alternatively, the plant photoreceptors may directly interact with G proteins; this would represent the direct contact of G proteins with entirely new types of receptors. In either case, light signalling in plants is likely to provide new insights into G protein functions.

Plant cells also respond to a number of plant hormones. In one study, the auxin, indole-3-acetic acid (IAA), was observed to enhance GTPγS binding in rice coleoptile; further, GTPγS caused a reduction in auxin binding [190]. These findings suggest that auxin stimulates the exchange of the GDP bound on a G protein for a GTP. The effect of GTPγS may be explained in two ways: first, it is possible that the activated G protein due to the binding of GTPγS desensitizes the auxin receptor; alternatively, the auxin receptor may require the association with a GDP-bound G protein to interact with the ligand, auxin.

Biochemical studies have also suggested the involvement of GTP-binding proteins in the regulation of downstream events. It has been found that GTPγS affects K^+ currents in the guard cells of broad bean (*Vicia faba*) leaves [49]. In these guard cells, GTPγS was found to reduce an inward K^+ current, while GDPβS enhanced the current [49]. Since GTPγS activates while GDPβS inhibits G proteins, these results suggest that one or more G proteins negatively regulate K^+ inward currents. In addition, it was found that cholera toxin inhibits the inward K^+ current in guard cells, further supporting the idea that a G protein negatively regulates the K^+ currents [49]. Pertussis toxin also inhibits K^+ current in guard cells [49]. In general, pertussis toxin blocks the activation of G proteins; therefore, the inhibition by pertussis toxin on K^+ currents in guard cells suggests that a second G protein acts in these cells to positively regulate the K^+ current. Since reduced K^+ uptake inhibits stomatal opening, these results suggest that GTPγS and the toxins would inhibit stomatal opening in broad bean guard cells under these conditions. In contrast, both GTPγS and pertussis toxin induce stomatal opening in the epidermis of *Commelina communis* [97]. Again, similar effects of GTPγS and pertussis toxin suggest more than one protein may be involved. It is not clear why opposite effects were seen in these two systems; it is possible that the different conditions and techniques favor one G protein over another, since more than one G protein seems to be involved in both cases. However, GDPβS had no effect on stomatal opening in *Commelina communis* [97], indicating that the situation in this case is rather complex, and that additional studies are need before the involvement of G protein(s) can be ascertained. G proteins have also been implicated in the regulation of an outward K^+ current in broad bean mesophyll cells; GTPγS and cholera toxin, but not pertussis toxin, inhibit the outward K^+ current in these cells [100]. In addition, GTP and GTP analogues have been shown to affect swelling of wheat protoplasts [13] and the formation of inositol phosphate derivatives in *Acer pseudoplatanus* [41], indicating the possible involvement of GTP-binding proteins. In cultured French bean cells, both cholera and pertussis toxins enhance the response to a fungal elicitor by the cells [12], suggesting possible G protein participation in this signalling pathway. Recently, a 45 kDa protein in cultured soybean cells that is recognized by an anti-Gα antiserum and labeled by ADP ribosylation with cholera toxin has been suggested to be involved in the elicitation of the defense responses [99]. Finally, in cultured soybean cells, both cholera and pertussis toxins were found to stimulate the expression of a *cab* gene, which normally depends on phytochrome for expression, and rendered the *cab* gene expression light-independent [142].

In summary, biochemical and physiological experiments have produced an impressive amount of evidence for the involvement of G proteins in plant signalling processes. Even though more studies are needed before one can learn the nature of these G proteins, it is very encouraging that a variety of pathways seem to employ G proteins.

Molecular analyses of G proteins in plants

Isolation of genes encoding heterotrimeric G protein subunits

Although biochemical studies have produced much evidence for the existence of G proteins in plants and their involvement in plant signalling pathways, none of these proteins have been identified or purified. Therefore, little is known about these proteins at the molecular level. However, using molecular approaches, a number of cDNAs have been isolated that encode putative heterotrimeric G protein subunits (Table 2). Using PCR with degenerate oligonucleotides based on conserved peptides among known G protein α subunits, a gene (*GPA1*) was isolated from *Arabidopsis thaliana* which encodes a protein with 36% identity to mammalian G_i and transducins, and contains GTP-binding consensus regions for heterotrimeric G protein α subunits [107]. The isolation of *GPA1* provided a clear demonstration that heterotrimeric G protein(s) are present in plants. Subsequently, a homologue (*TGA1*) of *GPA1* was isolated from tomato using low-stringency hybridization with *GPA1* as the probe [106]; the two predicted proteins are 84% identical. Both *GPA1* and *TGA1* were shown to be single-copy genes by Southern analyses. Furthermore, PCR and low-stringency procedures have failed to identify additional genes encoding G protein α subunits (H. Huang and H. Ma, unpublished). More recently, a single homologue of *GPA1* has been isolated from each of soybean (L. Romero and E. Lam, pers. comm.), lotus (C. Poulsen, pers. comm.) and maize (C.D. Han and R. Martienssen, pers. comm.). These genes all encode proteins that are very similar (greater than 75% amino acid sequence identity) to GPα1, the product of *GPA1*. In addition, a 43 kDa protein was detected in pea membranes using an antiserum raised against a C-terminal peptide of GPα1 [173]. These results indicate that *GPA1*

Table 2. Plant heterotrimeric G proteins and related proteins identified by molecular cloning.

Plant[a]	Predicted proteins	Protein size (kDa)	Most similar[b] animal protein (%)	Expression and/or function	References
Heterotrimeric G proteins					
α subunits					
Arabidopsis thaliana	GPα1	44.6	Gt1 (36)	Expressed in all major organs	[107]
Lycopersicon esculentum (tomato)	TGα1	44.9	Gt1 (34)		[106]
β subunits					
Arabidopsis thaliana	AGβ1	41.0	β2 (44)	Expressed in many organs	[171]
Zea mays	ZGβ1	41.7	β2 (42)	Expressed in many organs	[171]
WD-40 proteins					
Arabidopsis thaliana	COP1	111.8	β3 (29)[c]	Light signal transduction	[38]
Chlamydomonas reinhardtii	Cblp	35.1	MHC12.3 (66)	Constitutively expressed	[150]
Nicotiana tabacum (tobacco)	arcA	35.8	MHC12.3 (67)	Auxin-regulated	[75]

[a] Homologues of GPα1 have been isolated from *Glycine max* (soybean; L. Romero and E. Lam, pers. comm.); *Lotus japonicus* (C. Paulsen, pers. comm.); and *Zea mays* (C.D. Han and R. Martienssen, pers. comm.).

[b] The Gt1 (rod transducin), β2 and β3 proteins are from man, and the MHC12.3 protein is from chicken.

[c] The percent identity is only for the WD-40 domain of COP1.

(and its homologues) is a conserved gene found in all flowering plants. Although the α subunits identified from plants are probably homologues of each other, their low levels of sequence identity to those from animals and simple eukaryotes make it unlikely that the plant Gα's are functional homologues of any of the non-plant ones. The apparent uniqueness of *GPA1* (and homologues) suggests that it has a non-redundant function in plants, and the fact that it is highly conserved in many plants suggest that its function is important. Furthermore, the uniqueness of *GPA1* and its homologues suggests that if there are other Gα genes in plants, they must be quite different from *GPA1*, such that they can not be detected through hybridization or PCR.

Do plants also have G protein β and γ subunits? One is tempted to say yes, since all other organisms that have α subunits also have β and γ subunits. All known Gβ's from animals and simple eukaryotes contain 7 repeats of a motif called WD-40, which is characterized by the dipeptide tryptophan-aspartate and is about 40 amino acids long [54]. The WD-40 motif is also found in a variety of proteins with diverse functions, including regulation of cell cycle [54], RNA splicing [33], cytoskeletal function [35], and transcriptional repression [180]. A chicken protein, MHC12.3, of unknown function also contains several WD-40 motifs [60]. Although there is no evidence that any of these non-Gβ WD-40 proteins interacts with Gα or Gγ, it is known that the yeast WD-40 protein TUP1 interacts with another transcriptional repressor SSN6 (also called CYC8) [181], which contains a different type of repeats [151]. Three genes have been isolated from plants which encode WD-40-containing proteins. Two of the predicted proteins (Cblp and arcA) are very similar to each other (68% identity), and to the chicken MHC12.3 protein (>65%), but have only about 25% of sequence identity to known β subunits [75, 150]. Therefore, these plant WD-40-containing proteins may have any of a number of functions that are not related to G proteins. The third plant WD-40 protein (COP1) has a large non-WD-40 N-terminal domain, including two zinc fingers, in addition to the C-terminal WD-40 domain [37]. These features and the phenotypes of *cop1* mutants have led to the hypothesis that COP1 may be a transcriptional repressor [37], as is the yeast TUP1 protein. Recently, cDNAs encoding proteins with a much higher degree of similarity (42% or more) to animal β subunits have been isolated from maize (*ZGB1*) and *Arabidopsis* (*AGB1*) [171]. This indicates that plants have at least one pair of α and β subunits. It is most likely that G protein γ subunit(s) is(are) also present in plants. Interestingly, like *GPA1*, both *ZGB1* and *AGB1* appear to be single-copy, suggesting that, if other Gβ genes exist in these plants, they also must have very different sequences.

A detailed analysis of the Arabidopsis GPA1 *expression pattern*

In order to gain more information on the function of GPα1, detailed analyses of its spatial and temporal expression were carried out using a fusion between *GPA1* and the reporter gene *uidA* (encoding a β-glucuronidase), and using immunolocalization studies with specific antibodies directed against a peptide from the C terminal region of GPα1 [72, 172]. The results show that the *GPA1* gene product is expressed in nearly all tissues examined and during all stages of plant development. The level of *GPA1* expression, however, varies in different tissues and at different stages. In germinating seeds, GPα1 level is high in the cotyledons and at the root tip. In young seedlings, the highest level of GPα1 is detected in the shoot and root apical meristems as well as the lateral root meristems and leaf primordia. As the plants develop vegetatively, GPα1 level remains very high in the meristems and primordia, and in the root elongation zones, and decreases as the rosette leaves and cauline leaves mature. In mature leaves and roots, the GPα1 levels are high in the vascular tissues, particular phloem, but lower in the leaf mesophyll cells, and not detectable in the epidermis. During early flower development, GPα1 is present at high levels in the floral meristem and floral organ primordia, and the level

Table 3. Plant small G proteins identified by molecular cloning.

Plant	Protein	Size[a] (kDa)	Homologue[b] (% identity)	Expression and/or function	References[c]
Arabidopsis thaliana	Ara	24.2	Rab11 (55)		[110]
	Ara2	24.0	Rab11 (63)		[3]
	Ara3	23.8	Rab8 (58)		[3]
	Ara4	24.0	Rab11 (57)		[3]
	Ara5	21.6*	Rab1 (75)		[3]
	Arf1	20.6	Arf1 (88)		[138]
	Rab2a		Rab2	Expressed preferentially in pollen	[127]
	Rab2b		Rab2		[127]
	Rab6	23.1	Rab6 (72)	Complements a yeast *ytp6* mutation	[7]
	Rab11	24.0	Rab11 (66)		[186]
	Rha1	21.7	Rab5 (62)	Expression high in root and callus	[4]
				Expressed primarily in guard cells and root tips	[162]
	Sar1	22.0	ScSar1 (63)	Suppresses a yeast *set12* mutation	[31]
Brassica napus	Bra	24.4	Rab11 (55)		(a)
Chlamydomonas reinhardtii	yptC1	22.6	Rab1 (81)	Complements a yeast *ypt1* mutation	(b)
	yptC4	23.6	Rab2 (79)		(b)
	yptC5	23.1	Rab7 (67)		(b)
	yptC6	24.2	Rab11 (76)		(b)
Glycine max	sRab1	22.4	Rab1 (75)	Complements a yeast *ypt1* mutation	[28]
				Membrane biogenesis during root nodulation	
	sRab7	23.1	Rab7 (61)		[28]
Lycopersicon esculentum	Rab1A	20.1*	Rab1 (77)		(c)
	Rab1B	22.5	Rab1 (74)	Complements a yeast *ypt1* mutation	(c)
	Rab1C	22.6	Rab1 (78)	Complements a yeast *ypt1* mutation	(c)
	Sar1	22.0	ScSar1 (62)	Expressed in several organs	[34]
	Ypt2	23.9	Rab8 (58)	Expressed in apical meristem	[53]
Nicotiana plumbaginifolia	Np-ypt3	24.2	Rab11 (68)	In stem and root, high in flowers	[32]
	Rhn	21.8	Rab5 (60)	High in roots, flowers, lower in stems	[161]
Nicotiana tabacum	Nt-rab5	22.0	Rab5 (62)	Expressed in stem and root, high in flowers	[32]
	Rgb1	22.4	Rab1 (79)		(d)
	Rgb2	21.8	Rab5 (62)		(d)
Oryza savita	Rgp1	24.9	Rab11 (55)	Expression reduced in 5azaC-induced dwarf	[147]
	Rgp2	23.9	Rab11 (63)	Expressed in several organs	[187]
	ric1	22.4	Rab1 (76)		[88]
	ric2	24.0	Rab11 (70)		[88]
Pisum sativum	pra1	23.9	Rab11 (56)	Expressed highly in leaves and roots	[117]
	pra2	24.6*	Rab11 (52)	Expressed at a low level in leaves	[117]
	pra3	24.9	Rab11 (57)	Expressed at moderate levels in leaves and roots	[117]
	pra4	24.0	Rab11 (67)	Expressed highly in roots, less in leaves	[117]
	pra5	24.1	Rab11 (67)	Expressed at low levels in leaves and roots	[117]
	pra6	24.0	Rab11 (66)	Expressed at moderate levels in leaves and roots	[117]
	pra7	24.2	Rab11 (64)	Expressed highly in roots, less in leaves	[117]
	pra8	22.4	Rab1 (75)	Expressed at moderate levels in leaves and roots	[117]
	pra9A	22.5	Rab1 (79)	Expressed highly in roots, less in leaves	[117]
	pra9B	22.5	Rab1 (77)	Expressed highly in roots, less in leaves	[117]
	pra9C	22.6	Rab1 (75)	Expressed highly in roots, less in leaves	[117]
	Rho1	22.5	Rac2 (59)	Expressed in all organs of seedling	[183]
	Rab	23.0	Rab7 (69)	Expressed in pod	[44]
Vicia faba	Gnrp1	22.6	Rab1 (75)		(e)
	Gnrp2	22.9*	Rab11 (69)		(e)

Table 3. (Continued)

Plant	Protein	Size[a] (kDa)	Homologue[b] (% identity)	Expression and/or function	References[c]
	Gnrp3	24.0	Rab11 (67)		(e)
	Gnrp4	25.2	Rab11 (56)		(e)
Vigna aconitifolia	vRab7	23.1	Rab7 (61)	Membrane biogenesis during root nodulation	[28]
Volvox carteri	yptV1	22.5	Rab1 (81)	Complements a yeast *ypt1* mutation Expressed throughout development	[48]
	yptV2	24.2	Rab8 (53)		[47]
	yptV3	22.3	None –	Expressed throughout development	[47]
	yptV4	23.7	Rab2 (79)	Expressed throughout development	[47]
	yptV5	23.1	Rab7 (66)	Expressed throughout development	[47]
Zea mays	yptm1	23.3	Rab1 (65)	Complements a yeast *ypt1* mutation	[128]
	yptm2	22.5	Rab1 (79)	Complements a yeast *ypt1* mutation	[128]
	yptm3	23.0	Rab2 (79)		[127]
	yptm4		Rab1		[127]

[a] If the MW value was provided by the reference, then it is used here; if it was not, then it was calculated using Intelligenetics Software. An asterisk next to a value indicates that the sequence was incomplete, and a blank indicates that the sequence was not available.

[b] The same mammalian or yeast protein is listed for each member of a subgroup. The following are the homologues (see Fig. 3 for references): human Rab1, Rab3, and Rab6; dog (*Canis familiaris*) Rab2, Rab4, Rab5, Rab7, Rab8, Rab11, Rac2 and Rho1; Bovine Arf1; and yeast (*Saccharomyces cerevisiae*) Sar1. The percent identity was calculated based on an alignment using the Pileup program of the GCG Package (version 7) [57].

[c] In addition to published references, others are listed as follows: (a) Y. Park, H. Kang, J. Kwak, H. Lee, and H. Nam, submitted to GenBank; (b) S. Fabry, pers. comm.; (c) A. Loraine, pers. comm.; (d) J.A. Napier and P.R. Shewry, submitted to GenBank; (e) G. Saalbach and J. Thielmann, submitted to GenBank.

decreases as the organs mature. Later during flower development, *GPA1* expression is concentrated in the vascular tissues, in the carpel wall, in the microspore tetrads, and in the ovules. During pollination, a high level of GPα1 is found in the growing pollen tube, and after pollination, *GPA1* is expressed highly in embryos until the late curved stage, but not in mature embryos. Therefore, GPα1 is present at high levels in all actively proliferating cells, as well as in the cells that have begun to differentiate, but lower in most fully differentiated cells. Among the differentiated cells, those in the vascular tissues have very high levels of GPα1, as do the carpel and silique walls. These tissues are all involved in nutrient transport.

The fact that GPα1 is present at very high levels in the undifferentiated cells of meristems and organ primordia, and in cells during the early phase of organ differentiation, and that its level reduces as organs become fully differentiated, suggests that GPα1 is involved in promoting active cell division, and that its function is reduced in differentiated cells. This is parallel to the function of some mammalian G proteins in regulating cell division and differentiation [61, 153]. Constitutively activated mutant G_s and G_i have been associated with human tumors [96, 105], indicating that active Gα promotes cell division. On the other hand, the level of the $G\alpha_{i2}$ protein has been observed to decline during differentiation of a cell line [56]. Furthermore, active $G\alpha_{i2}$ blocks while $G\alpha_{i2}$ antisense RNA stimulates this differentiation [170]. Other studies showed that $G\alpha_s$ antisense oligonucleotides stimulate the differentiation of fibroblasts to adipocytes [167]. G proteins (G_q and G_{i2}) have also been shown to mediate the effect of growth factors on DNA synthesis and calcium flux [95]. Recently, the human $G\alpha_{12}$ gene, when overexpressed, was found to cause transformation of NIH 3T3 cells [24]. Although it is premature to suggest that GPα1 functions by a mechanism similar to those of the mammalian

Gα proteins, it is not unreasonable to postulate that GPα1 may regulate cell division and/or differentiation in plants.

A shared property of the undifferentiated cells, which have high levels of GPα1, is that they all require high intake of energy and nutrients. It is possible that GPα1 regulates the uptake of nutrients by these cells. It is intriguing that most of the mature tissues that express GPα1 at high levels are involved in nutrient transport. G_s and G_i were first identified as required for the hormonal regulation of sugar metabolism, and G_s has recently been shown to regulate sugar uptake [71]. Furthermore, a fission yeast Gα protein, *GPA2*, has recently been demonstrated to function in nutrient sensing [76]. Therefore, it would not be surprising if GPα1 indeed is shown to be involved in the regulation of nutrient transport or metabolism in plant cells in the near future.

Isolation of genes encoding small G proteins

Many small G protein genes/cDNAs have been isolated using low-stringency hybridizations or PCR (Table 3). The first cloned plant small G protein gene (*ara*) was from *Arabidopsis thaliana*. Its product is related to a number of known small G proteins: 55% to rab11, 44% identical to YPT1, 31% to H-ras and yeast RAS1, 29% to rho, and 26% to ral [110]. Other *Arabidopsis* genes include *ara2* through *ara5* [3], *rab6* [7], and *rha1* [4], all of which encode proteins related to the rab/ypt proteins. Two other *Arabidopsis* genes were isolated which encode an ARF-related protein [138] and a Sar1 homologue (see below) [31].

A total of 13 genes have been identified in pea which encode small G proteins. One of these, *Rho1Ps*, encodes the first plant member of the rho/rac subfamily of small G proteins; the predicted protein is 59% identical to human rac2 [183]. When the pea rho protein was expressed in *Escherichia coli*, it was shown to be able to bind GTP in a filter assay. Other cloned pea small G protein genes are most similar to members of the rab/Ypt subfamily; these include *Psa-rab*, and *Pra1* through *Pra9A*, *Pra9B*, and *Pra9C* [44, 117]. Rab-related genes have also been isolated from other legumes such as soybean [28] (see below), from tomato [53], from tobacco [32, 161], and from monocots maize [128] and rice [88, 147, 187]. Five small G protein genes of the rab/ypt type were also isolated from the green alga *Volvox carteri*; three of these can bind GTP when expressed in *E. coli* [39, 47, 48].

A comparison of the plant small G proteins with some of those from animals and yeasts is shown in Fig. 3, and a summary of the properties of these proteins is shown in Table 3. Most of the isolated plant small G proteins are more similar to rab/Ypt proteins involved in protein and membrane trafficking than to any other small G proteins. The high levels of sequence similarity between the plant small G proteins with their animal and yeast homologues suggest that this group of proteins also perform highly conserved basic cellular functions in protein and membrane trafficking in plants [139]. It is likely that the genes listed here represent an incomplete set. Even with these cloned genes, there are more than one case where two or more genes from a single plant show high levels of similarity with the same mammalian protein. This could either be that some plants have more functionally redundant genes, or that these plants genes have evolved to carry out slightly different functions. Furthermore, one of the plant Ypt gene, *YptV3* from *Volvox*, is not very similar to any known Ypts/Rabs. As it was pointed by Fabry *et al.* [47], *YptV3* may play a role unique to plants (or algae); alternatively, its homologues in animals may be identified in the future. There are now only a few known plant genes in the other classes, Rho/Rac, Arf, and Sar1; this may represent the more recent history of these classes.

It is intriguing that small G proteins more similar to the true ras proteins have not been identified. It is possible that proteins similar to ras in function are present in plants, but they are not highly similar at the sequence level, and are yet to be isolated. It is known that ras proteins are involved in signal transduction [63, 149], as are heterotrimeric G proteins. The failure thus far to identify plant ras proteins, and the scarcity of isolated heterotrimeric G protein α and β sub-

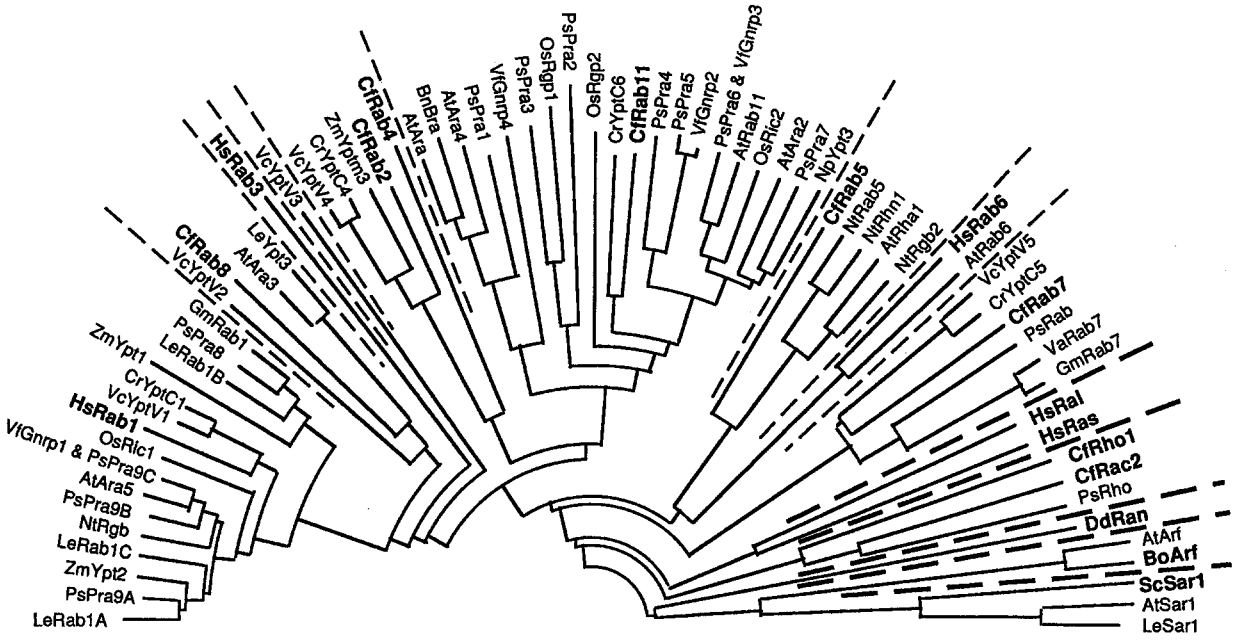

Fig. 3. Comparison of plant small G proteins with each other and with representative ones from animals and microbes. Different major subfamilies are separated by thick dashed lines, and the different type within the Rab/Ypt subfamily are separated by thin dashed lines. The names of each protein are preceded by two letters indicating the species. The species designations are: At, *Arabidopsis thaliana*; Bn, *Brassica napus*; Bo, bovine; Cf, *Canis familaris* (dog); Cr, *Chlamydomonas reinhardtii*; Dd, *Dictyostelium discoideum* (slime mold); Gm, *Glycine max* (soybean); Hs, *Homo sapiens*; Le, *Lycopersicon esculentum* (tomato); Np, *Nicotiana plumbaginifolia*; Nt, *Nicotiana tabacum*; Os, *Oryza sativa* (rice); Ps, *Pisum sativum* (pea); Sc, *Saccharomyces cerevisiae* (baker's yeast); Va, *Vigna aconitifolia*; Vc, *Volvox carteri*; Vf, *Vicia faba* (broad bean); Zm, *Zea mays* (maize). The sequences of VfGnrp1 and VfGnrp3 are identical to those of PsGBP11 and PsGBP6, respectively. References for the non-plant proteins are: bovine Arf [131]; CfRab2, CfRab5, and CfRab7 [26]; CfRab4, CfRab8, CfRab11, CfRho1 and CfRac2 [27]; HsRab1, HsRab3, and HsRab6 [188]; HsRas [23]; HsRalA [25]; DdRan [19]; and ScSar1 [120]; see Table 3 for references of plant proteins. Due to space constraints, most of the known yeast small G proteins are not shown here; the following are the known homologues between mammals and yeasts (Sp, *Schizosaccharomyces pombe*; references given for the yeast proteins): Rab1/ScYpt1 [55]; Rab6/SpRyh1 [69]; Rab7/ScYpt7 [176]; Rab5/SpYpt5 [5]; Rab8/SpYpt2 [68]; Rab11 /SpYpt3 [50]. The tree was drawn using MacDraft and MacDraw programs based on the output from the Molecular Evolutionary Genetics Analysis program (version 1.01) [93], following an alignment using the Pileup program of the GCG Package (version 7) [57].

units, suggest that proteins of these classes are not highly similar to those in animals and simple eukaryotes. This may reflect the fact that plant respond to environmental signals in very different ways than animals and microbes; such differences may account for the likely divergence of proteins involved in signal transduction pathways [85]. Because GTP-binding proteins have been implicated in a variety of plant signalling processes (see previous section), there probably exist additional G proteins in plants. These plant G proteins may be distant relatives of known heterotrimeric or small G proteins, or they may represent new families of GTP-binding proteins. Genetic and biochemical studies of plant signal transduction pathways should reveal the nature of these proteins in the near future.

Functional complementation of yeast mutants by plant small G proteins

One of the ways to test *in vivo* function of a protein encoded by a cloned cDNA is to introduce it into a yeast host which lacks a homologous function. This is feasible for many highly conserved proteins, including G proteins. This approach has been used for some of the isolated

small G protein cDNAs. For example, a soybean homologue (*sRAB1*) of the budding yeast *YPT1* and mammalian *rab1* genes was isolated by PCR. The sRab1 protein is 75% identical to the rab1 protein. Furthermore, when the soybean *sRAB1* gene was fused to a yeast *GAL1* promoter and introduced into a yeast *ypt1* mutant, it complemented the cold-sensitive growth phenotype of the mutant [28]. Other plant Rab1/Ypt1 homologues, including the algal *YptV1* and *YptC1* (S. Fabry, pers. comm.), tomato *Rab1b* and *Rab1c* (A. Loraine and W. Gruissem, pers. comm.), and the maize *Yptm1* and *Yptm2* genes [127], have also been shown to complement a *S. cerevisiae ypt1* mutant. In another case, an *Arabidopsis* homologue (*AtRAB6*) of the fission yeast *Rhy1* and the human *rab6* genes was isolated using PCR, and shown to complement a budding yeast *ypt6* mutation (*YPT6* is a homologue of *Rhy1* and *rab6*) [7]. The predicted AtRab6 protein shares a high degree of similarity with the human and fission yeast homologues (>70% identity). When the *AtRAB6* cDNA was fused to the yeast *GAL10* promoter, and expressed in a temperature-sensitive *ypt6* null mutant of budding yeast, the *ypt6* mutant defective was corrected by the expression of *AtRAB6*. This complementation was specifically due to the production of a functional AtRab6 protein because a mutant *Atrab6* cDNA encoding a protein with a single amino acid change (Asn-122 → Ile) failed to complement.

Because highly conserved proteins can function in heterologous systems, functional complementation of yeast mutants can also be used as a means of isolating plant homologues of yeast genes. This approach has been successfully used to isolate an *Arabidopsis* homologue of the yeast *SAR1* gene, which encodes a distinct type of small G protein [31]. The yeast *SAR1* gene was identified as a clone which, when present at a high copy number, suppresses the temperature-sensitive phenotype of a *sec12* mutation. Since the *sec12* mutant is defective in formation of secretory vesicles from the endoplasmic reticulum, the *SAR1* gene is probably also involved in this process. The *Arabidopsis SAR1* cDNA was isolated by introducing a yeast expression cDNA library containing *Arabidopsis* cDNAs into a *sec12* mutant, and selecting for transformants which can grow at the restrictive temperature. The predicted budding yeast and *Arabidopsis* Sar1 protein sequences are 63% identical.

In these successful cases of functional complementation in yeast, the similarity between the homologues are about 60% or more. For many of the isolated small G proteins from plants, similarly high levels of sequence identity exist with particular animal/yeast homologues (Table 3); it is likely that these plant small G proteins are also able to complement the corresponding yeast mutations. For heterotrimeric G proteins, the similarity between the yeast proteins and the ones from multicellular organisms is much lower, ranging from 35% to 48% between the yeast GPA1 and various mammalian Gα's. It is not surprising, therefore, that only one of the mammalian Gα's (G_s) has been shown to complement the growth defect of the yeast *gpa1* mutant, but not its defect in pheromone response. These results once again suggest that G proteins for the fundamental cellular functions such as secretion are more conserved than G proteins involved in different signalling pathways.

Functional analysis using transgenic plants

In addition to biochemical studies and expression analysis, one important way of characterizing G protein functions is to examine the effects of altering gene expression in transgenic plants, including antisense, over-expression and ectopic expression. Recently, the effect of antisense RNA of two small G proteins from soybean (*sRAB1*) and another legume *Vigna aconitifolia* (*vRAB7*) was studied in transgenic soybean nodules [28]. The growth of nodules expressing the *sRAB1* antisense RNA were severely inhibited at an early stage, with only about 1/10 to 1/5 of the normal weight, and proportional reduction in nitrogenase activity. The yeast Ypt1 protein and its mammalian homologue rab1 are known to be involved in vesicular transport between ER and Golgi. Since the membranes (PBM) surrounding the symbiotic nitrogen-fixing bacteria are derived from the

plasma membrane formed by fusion of vesicles, the phenotypes of *sRAB1* antisense nodules and the fact that *sRAB1* expression increases during nodulation suggest that *sRab1* is involved in the synthesis of the PBMs. A similar analysis was done with the *vRAB7* gene. In the nodules expressing antisense *vRAB7*, the infected cells had reduced number of bacteroids, and many small vesicles. In addition, there are some large vesicular structures which apparently contain degraded bacterial materials. In mammals, rab7 is localized to late endosomes, and the yeast homologue (Ypt7) is involved in the transport of vacuolar proteins. It seems that the legume rab7 homologue is required for the biogenesis of the PBM, which is similar to vacuoles in certain ways.

In another study, a rice gene, *rgp1*, encoding a rab-related protein having a 62% identity with the fission yeast Ypt3 protein, was introduced into tobacco plants by transformation in either the sense or the antisense orientation [83]. Both the transgenic plants carrying sense and the antisense constructs have a decrease in apical dominance and a dwarf phenotype. Since the *rgp1* antisense RNA apparently causes a reduction in the mRNA level of the tobacco homologue (*tgp1*), but *tgp1* is expressed normally in the sense transgenic plants, both a reduction and an increase in the gene function seem to disrupt the same process. Since reduction of apical dominance is also seen when the balance between cytokinin and auxin is shifted towards more cytokinin, the phenotype of the transgenic plants may be due to an alteration in hormonal balance. The transgenic plants also have abnormal floral phenotypes, with some homeotic organ conversion, suggesting either that the *rgp1* gene is involved in regulation of flower development, or that hormonal balance affects flower development. The latter possibility is supported by the recent observation that hormones affect the function of known *Arabidopsis* floral homeotic genes [126].

Future prospects

G proteins have been demonstrated to be important molecular switches in animals and simple eukaryotes. The spectrum of processes regulated by G proteins continues to widen, and the precise mechanisms by which G proteins function are becoming more and more clear. In plants, the presence of both heterotrimeric and small G proteins has be demonstrated. However, many questions remain to be answered. An immediate problem is the nature of the putative G proteins suggested by biochemical studies. Since pertussis toxin-sensitive G proteins seem to exist in plants, and the only known Gα protein, encoded by the *Arabidopsis GPA1* gene and homologues, does not have the conserved cysteine residue near the C terminus, plants are likely to have other heterotrimeric G proteins that are quite different from any known ones. Another puzzle is that known light receptors in plant are not like the classic G protein-coupled receptors that are characterized by seven transmembrane segments. In fact, no such membrane receptor has been identified in plants. An equally intriguing question is whether plants have true ras homologues. Certainly plants do not have genes that are highly similar to *ras*, as they do other types of small G proteins. It is still possible that plants have small G proteins which carry out signalling functions similar to ras proteins, but these plant proteins would be structurally divergent from the known ras proteins. As genetic, molecular, biochemical and physiological approaches continue to be employed for the analysis of plant signal transduction and G protein function, it is likely that new G proteins will be identified in plants, that the nature of G proteins implicated by biochemical studies will be determined, and that interaction between plant G proteins and receptors/effectors will be characterized. We are at the beginning of an exciting era of new discoveries and new insights about plant signal transduction and G protein functions.

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References

1. Ahmad M, Cashmore AR: *HY4* gene of *A. thaliana* encodes a protein with characteristics of a blue-light receptor. Nature 366: 162–166 (1993).
2. Amatruda TT III, Steele DA, Slepak VZ, Simon MI: Gα16, a G protein α subunit specifically expressed in hematopoietic cells. Proc Natl Acad Sci USA 88: 5587–5591 (1991).
3. Anai T, Hasegawa K, Watanabe Y, Uchimiya H, Ishizaki R, Matsui M: Isolation and analysis of cDNAs encoding small GTP-binding proteins of *Arabidopsis thaliana*. Gene 108: 259–264 (1991).
4. Anuntalabhochai S, Terryn N, Van Montagu M, Inzé D: Molecular characterization of an *Arabidopsis thaliana* cDNA encoding a small GTP-binding protein, Rha1. Plant J 1: 167–174 (1991).
5. Armstrong J, Craighead MW, Watson R, Ponnambalam S, Bowden S: *Schizosaccharomyces pombe* ypt5: a homologue of the rab5 endosome fusion regulator. Mol Biol Cell 4: 583–592 (1993).
6. Bakalyar HA, Reed RR: Identification of a specialized adenylyl cyclase that may mediate odorant detection. Science 250: 1403–1406 (1990).
7. Bennarek SY, Reynolds TL, Schroeder M, Grabowski R, Hengst L, Gallwitz D, Raikhel NV: A small GTP-binding protein from *Arabidopsis thaliana* functionally complements the yeast *ypt6* null mutant. Plant Physiol 104: 591–596 (1994).
8. Bilushi SV, Shebunin AG, Babakov AV: Purification and subunit composition of a GTP-binding protein from maize root plasma membranes. FEBS Lett 291: 219–221 (1991).
9. Birnbaumer L: Receptor-to-effector signalling through G proteins: roles for βγ dimers as well as α subunits. Cell 71: 1069–1072 (1992).
10. Blum W, Hinsch K-D, Schultz G, Weiler EW: Identification of GTP-binding proteins in the plasma membrane of higher plants. Biochem Biophys Res Commun 156: 954–959 (1988).
11. Boguski MS, McCormick F: Proteins regulating ras and its relatives. Nature 366: 643–654 (1993).
12. Bolwell GP, Coulson V, Rodgers MW, Murphy DL, Jones D: Modulation of the elicitation response in cultured French bean cells and its implication for the mechanism of signal transduction. Phytochemistry 30: 397–405 (1991).
13. Bossen ME, Kendrick RE, Vredenberg WJ: The involvement of a G-protein in phytochrome-regulated, Ca^{2+}-dependent swelling of etiolated wheat protoplasts. Physiol Plant 80: 55–62 (1990).
14. Botstein D, Segev N, Stearns T, Hoyt MA, Holden J, Kahn RA: Diverse biological functions of small GTP-binding proteins in yeast. Cold Spring Harbor Symp Quant Biol 53: 629–636 (1988).
15. Bourne HR: Do GTPases direct membrane traffic in secretion? Cell 53: 669–671 (1988).
16. Bourne HR, Sanders DA, McCormick F: The GTPase superfamily: a conserved switch for diverse cell functions. Nature 348: 125–132 (1990).
17. Bourne HR, Sanders DA, McCormick F: The GTPase superfamily: conserved structure and molecular mechanism. Nature 349: 117–127 (1991).
18. Brown AM, Birnbaumer L: Ionic channels and their regulation by G protein subunits. Annu Rev Physiol 52: 197–213 (1990).
19. Bush J, Cardelli JA: Molecular cloning and DNA sequence of a *Dictyostelium* cDNA encoding a ran/TC4 related GTP binding protein. GenBank (1993).
20. Buss JE, Munby SM, Casey PJ, Gilman AG, Sefton BM: Myristoylated α subunits of guanine nucleotide-binding regulatory proteins. Proc Natl Acad Sci USA 84: 7493–7497 (1987).
21. Buss JE, Sefton BM: Direct identification of palmitic acid as the lipid attached to p21 ras. Mol Cell Biol 6: 116–122 (1986).
22. Camps M, Carozzi A, Schnabel P, Scheer A, Parker PJ, Gierschik P: Isozyme-selective stimulation of phospholipase C-β2 by G protein βγ-subunits. Nature 360: 684–686 (1992).
23. Capon DJ, Chen EY, Levinson AD, Seeburg PH, Goeddel DV: The complete nucleotide sequences of the T24 human bladder carcinoma oncogene and its normal homologue. Nature 302: 33–37 (1983).
24. Chan AM-L, Fleming TP, McGovern ES, Chedid M, Miki T, Aaronson SA: Expression cDNA cloning of a transforming gene encoding the wild-type Gα12 gene product. Mol Cell Biol 13: 762–768 (1993).
25. Chardin P, Tavitian A: Coding sequences of human ralA and ralB cDNAs. Nucl Acids Res 17: 4380 (1989).
26. Chavrier P, Parton RG, Hauri HP, Simons K, Zerial M: Localization of low molecular weight GTP binding proteins to exocytic and endocytic compartments. Cell 62: 317–329 (1990).
27. Chavrier P, Vingron M, Sander C, Simons K, Zerial M: Molecular cloning of YPT1/sec4-related cDNAs from an epithelial cell line. Mol Cell Biol 10: 6578–6585 (1990).
28. Cheon C-I, Lee N.-G., Siddique A-BM, Bal AK, Verma

DPS: Roles of plant homologs of Rab1p and Rab7p in the biogenesis of the peribacteroid membrane, a subcellular compartment formed *de novo* during root nodule symbiosis. EMBO J 12: 4125–4135 (1993).
29. Clark GB, Memon AR, Tong C-G, Thompson GA Jr, Roux SJ: Phytochrome regulates GTP-binding protein activity in the envelope of pea nuclei. Plant J 4: 399–402 (1993).
30. Conklin BR, Bourne HR: Structural elements of Gα subunits that interact with Gβγ, receptors, and effectors. Cell 73: 631–641 (1993).
31. d'Enfert C, Gensse M, Gaillardin C: Fission yeast and a plant have functional homologues of the Sar1 and Sec12 proteins involved in ER to Golgi traffic in budding yeast. EMBO J 11: 4205–4211 (1992).
32. Dallmann G, Sticher L, Marshallsay C, Nagy F: Molecular characterization of tobacco cDNAs encoding two small GTP-binding proteins. Plant Mol Biol 19: 847–857 (1992).
33. Dalrymple MA, Peterson-Bjorn S, Friessen JD, Beggs JD: The product of the *PRP4* gene of S. cerevisiae shows homology to β subunits of G proteins. Cell 58: 811–812 (1989).
34. Davies C: Cloning and characterization of a tomato GTPase-like gene related to yeast and *Arabidopsis* genes involved in vesicular transport. Plant Mol Biol 24: 523–531 (1994).
35. de Hostos EL, Bradtke B, Lottspeich F, Guggenheim R, Gerish G: Coronin, an actin binding protein of *Dictyostelium discoideum* localized to cell surface projections, has sequence similarities to g protein β subunits. EMBO J 10: 4097–4104 (1991).
36. de Sousa SM, Hoveland LL, Yarfitz S, Hurley JB: The *Drosophila* Go alpha-like G protein gene produces multiple transcripts and is expressed in the nervous system and in ovaries. J Biol Chem 264: 18544–18551 (1989).
37. Deng XW, Caspar T, Quail PH: *cop1*: a regulatory locus involved in light-controlled development and gene expression in *Arabidopsis*. Genes Devel 5: 1172–1182 (1991).
38. Deng XW, Matsui M, Wei N, Wagner D, Chu AM, Feldmann KA, Quail PH: *COP1*: an Arabidopsis regulatory gene, encodes a protein with both a zinc-binding motif and a Gβ homologous domain. Cell 71: 791–801 (1992).
39. Dietmaier W, Fabry S: Analysis of the introns in genes encoding small G proteins. Curr Genet 26: 497–505 (1994).
40. Dietzel C, Kurjan J: The yeast *SCG1* gene: a Gα-like protein implicated in the a- and α-factor response pathway. Cell 50: 1001–1010 (1987).
41. Dillenschneider M, Hetherington A, Graziana A, Alibert G, Berta P, Haiech J, Ranjeva R: The formation of inositol phosphate derivatives by isolated membranes from *Acer psudoplatanus* is stimulated by guanine nucleotides. FEBS Lett 208: 413–417 (1986).
42. Dohlman HG, Thorner J, Caron MG, Lefkowitz RJ: Model systems for the study of seven-transmembrane-segment receptors. Annu Rev Biochem 60: 653–688 (1991).
43. Downward J: The ras superfamily of small GTP-binding proteins. Trends Biochem Sci 15: 469–472 (1990).
44. Drew JE, Bown D, Gatehouse JA: Sequence of a novel plant *ras*-related cDNA from *Pisum sativum*. Plant Mol Biol 21: 1195–1199 (1993).
45. Drobak BK, Allan EF, Comerford JG, Roberts R, Dawson AP: Presence of guanine nucleotide-binding proteins in a plant hypoctyl fraction. Biochem Biophys Res Commun 150: 899–903 (1988).
46. Ephritikhine G, Pradier J-M, Guern J: Complexity of GTPγS binding to tobacco plasma membranes. Plant Physiol Biochem 31: 573–584 (1993).
47. Fabry S, Jacobsen A, Huber H, Palme K, Schmitt R: Structure, expression, and phylogenetic relationships of a family of *ypt* genes encoding small G-proteins in the green alga *Volvox carteri*. Curr Genet 24: 229–240 (1993).
48. Fabry S, Na N, Huber H, Palme K, Jaenicke L, Schmitt R: The *yptV1* gene encodes a small G-protein in the green alga *Volvox carteri*: gene structure and properties of the gene product. Gene 118: 153–162 (1992).
49. Fairley-Grenot K, Assmann SM: Evidence for G-protein regulation of inward K^+ channnel current in guard cells of fava bean. Plant Cell 3: 1037–1044 (1991).
50. Fawell E, Hook S, Sweet D, Armstrong J: Novel YPT1-related genes from *Schizosaccharomyces pombe*. Nucl Acids Res 18: 4264 (1990).
51. Fino SI, Plasterk RH: Characterization of a G-protein α-subunit gene from the nematode *Caenorhabditis elegans*. J Mol Biol 215: 483–487 (1990).
52. Firtel RA, van Haastert PJM, Kimmel AR, Devreotes PN: G protein linked signal transduction pathways in development: Dictyostelium as an experimental system. Cell 58: 235–239 (1989).
53. Fleming AJ, Mandel T, Roth I, Kuhlemeier C: The patterns of gene expression in tomato shoot apical meristem. Plant Cell 5: 297–309 (1993).
54. Fong HW, Hurley JB, Hopkins RS, Miake-Lye R, Johnson MS, Doolittle RF, Simon MI: Repetitive segmental structure of the transducin β subunit: homology with the *CDC4* gene and identification of related mRNAs. Proc Natl Acad Sci USA 83: 2162–2166 (1986).
55. Gallwitz D, Donath C, Sander C: A yeast gene encoding a protein homologous to the human *c-has/bas* proto-oncogene product. Nature 306: 704–707 (1983).
56. Galvin-Parton PA, Watkins DC, Malbon CC: Retinoic acid modulation of transmembrane signalling: analysis in F9 teratocarcinoma cells. J Biol Chem 265: 17771–17779 (1990).
57. Genetics Computer Group I: University Research Park, 575 Science Dr., Suite B, Madison, WI 53711, USA.
58. Gill DM, Woolkalis MJ: Cholera toxin-catalyzed

[^{32}P]ADP-ribosylation of proteins. Meth Enzymol 195: 267–280 (1991).
59. Gilman AG: G proteins: transducers of receptor-generated signals. Annu Rev Biochem 56: 615–649 (1987).
60. Guillemot F, Billault A, Auffray C: Physical linkage of a guanine nucleotide-binding protein-related gene to the chicken major histocompatibility complex. Proc Natl Acad Sci USA 86: 4594–4598 (1989).
61. Gupta SK, Gallego C, Johnson GL: Mitogenic pathways regulated by G protein oncogenes. Mol Biol Cell 3: 123–128 (1992).
62. Hadwiger JA, Wilkie TM, Strathmann M, Firtel RA: Identification of *Dictyostelium* G_α genes expressed during multicellular development. Proc Natl Acad Sci USA 88: 8213–8217 (1991).
63. Hall A: The cellular functions of small GTP-binding proteins. Science 249: 634–640 (1990).
64. Hall A: Ras-related proteins. Curr Opin Cell Biol 5: 265–268 (1993).
65. Hasunuma K, Funadera K: GTP-binding protein(s) in green plant, *Lemna paucicostata*. Biochem Biophys Res Commun 143: 908–912 (1987).
66. Hasunuma K, Furukawa K, Funadera K, Kubota M, Watanabe M: Partial characterization and light-induced regulation of GTP-binding proteins in *Lemna paucicostata*. Photochem Photobiol 46: 531–535 (1987).
67. Hasunuma K, Furukawa K, Tomita K, Mukai C, Nakamura T: GTP-binding proteins in etiolated epicotyls of *Pisum sativum* (Alaska) seedlings. Biochem Biophys Res Commun 148: 133–139 (1987).
68. Haubruck H, Engelke U, Mertins P, Gallwitz D: Structural and functional analysis of *ypt2*, an essential ras-related gene in the fission yeast *Schizosaccharomyces pombe* encoding a sec4 protein homologue. EMBO J 9: 1957–1962 (1990).
69. Hengst D, Lehmeier T, Gallwitz D: The *ryh1* gene in the fission yeast *Schizosaccharomyces pombe* encoding a GTP-binding protein related to ras, rho and ypt: structure, expression and identification of its human homologue. EMBO J 9: 1949–1955 (1990).
70. Herskowitz I: A regulatory hierarchy for cell specialization in yeast. Nature 342: 749–757 (1990).
71. Honnor RC, Naghshineh S, Cushman SW, Wolff J, Simpson IA, Londos C: Cholera and pertussis toxins modify regulation of glucose transport activity in rat adipose cell: evidence for mediation of a cAMP-independent process by G-proteins. Cell Signalling 4: 87–98 (1992).
72. Huang H, Weiss CA, Ma H: Regulated expression of the *Arabidopsis* Gα gene *GPA1*. Int J Plant Sci 155: 3–14 (1994).
73. Im M-J, Graham RM: A novel guanine nucleotide-binding protein coupled to the α_1-adrenergic receptor. J Biol Chem 265: 18944–18951 (1990).
74. Iñiguez-Lluhi J, Kleuss C, Gilman AG: The importance of G-protein $\beta\gamma$ subunits. Trends Cell Biol 3: 230–236 (1993).
75. Ishida S, Takahashi Y, Nagata T: Isolation of cDNA of an auxin-regulated gene encoding a G protein β subunit-like protein from tobacco BY-2 cells. Proc Natl Acad Sci USA 90: 11152–11156 (1993).
76. Isshiki T, Mochizuki N, Maeda T, Yamamoto M: Characterization of a fission yeast gene, *gpa2*, that encodes a Gα subunit involved in the monitoring of nutrition. Genes Devel 6: 2455–2462 (1992).
77. Jacobs M, Thelen MP, Farndale RW, Astle MC, Rubery PH: Specific guanine nucleotide binding by membranes from *Cucurbita pepo* seedlings. Biochem Biophys Res Commun 155: 1478–1484 (1988).
78. Jelsema CL, Axelrod J: Stimulation of phospholipase A2 activity in bovine rod outer segments by the $\beta\gamma$ subunits of transducin and its inhibition by the α subunit. Proc Natl Acad Sci USA 84: 3623–3627 (1987).
79. Jo H, Cha BY, Davis HW, McDonald JM: Identification, partial purification, and characterization of two guanosine triphosphate-binding proteins associated with insulin receptors. Endocrinology 131: 2855–2862 (1992).
80. Jo H, Radding W, Anantharamaiah GM, McDonald JM: An insulin receptor peptide (1135–1156) stimulates guanosine 5′-[γ-thio]triphosphate binding to the 67 kDa G protein associated with the insulin receptor. Biochem J 294: 19–24 (1993).
81. Jones DT, Reed RR: G_{olf}: an olfactory neuron specific-G protein involved in odorant signal transduction. Science 244: 790–795 (1989).
82. Kahn RA, Goddard C, Newkirk M: Chemical and immunological characterization of the 21 kDa ADP-ribosylation factor of adenylate cyclase. J Biol Chem 263: 8282–8287 (1988).
83. Kamada I, Yamauchi S, Toussefian S, Sano H: Transgenic tobacco plants expressing *rgp1*, a gene encoding a *ras*-related GTP-binding protein from rice, show distinct morphological characteristics. Plant J 2: 799–807 (1992).
84. Katz A, Wu D, Simon MI: Subunits $\beta\gamma$ of heterotrimeric G protein activate β2 isoform of phospholipase C. Nature 360: 686–689 (1992).
85. Kaufman LS: G proteins, paradigms, and plants. Int J Plant Sci 155: 1–2 (1994).
86. Kaufman LS: GTP-binding signalling proteins in higher plants. J Photochem Photobiol 22: 3–7 (1994).
87. Kaziro Y, Itoh H, Kozasa T, Nakafuku M, Satoh T: Structure and function of signal-transducing GTP-binding proteins. Annu Rev Biochem 60: 349–400 (1991).
88. Kidou S-I, Anai T, Umeda M, Aotsuka S, Tsuge T, Kato A, Uchimiya H: Molecular structure of ras-related small GTP-binding protein genes of rice plants and GTPase activities of gene products in *Escherichia coli*. FEBS Lett 332: 282–286 (1993).
89. Kleuss C, Scherübl H, Hescheler J, Schultz G, Wittig B:

Different β-subunits determine G-protein interaction with transmembrane receptors. Nature 358: 424–426 (1992).
90. Kleuss C, Scherübl H, Hescheler J, Schultz G, Wittig B: Selectivity in signal transduction determined by γ subunits of heterotrimeric G proteins. Science 259: 832–834 (1993).
91. Korolkov SN, Garnovskaya MN, Basov AS, Chunaev AS, Dumler IL: The detection and characterization of G-proteins in the eyespot of *Chlamydomonas reinhardtii*. FEBS Lett 270: 132–134 (1990).
92. Kumagai A, Pupillo M, Gundersen R, Miake-Lye R, Devreotes PN, Firtel RA: Regulation and function of Gα protein subunits in Dictyostelium. Cell 57: 265–275 (1989).
93. Kumar S, Tamura K, Nei M: Molecular Evolutionary Genetics Analysis (version 1.01). Institute for Molecular and Evolutionary Genetics, Pennsylvania State University, University Park, PA 16802, USA.
94. Lam E: Heterotrimeric guanine nucleotide-binding proteins and light responses in higher plants. In: Raskin I, Schultz J (eds) Current Topics in Plant Physiology, vol. 11: Plant Signals in Interactions with Other Organisms, pp. 7–13. American Society for Plant Physiology, Rockville, TN (1993).
95. LaMorte VJ, Harootunian AT, Spiegel AM, Tsien RY, Feramisco JR: Mediation of growth factor induced DNA synthesis and calcium mobilization by G_q and G_{i2}. J Cell Biol 121: 91–99 (1993).
96. Landis CA, Masters SB, Spada A, Pace AM, Bourne HR, Vallar L: GTPase inhibiting mutations activate the α chain of G_s and stimulate adenylyl cyclase in human pituitary tumours. Nature 340: 692–696 (1989).
97. Lee HJ, Tucker EB, Crain RC, Lee Y: Stomatal opening is induced in epidermal peels of *Commelina communis* L. by GTP analogs or pertussis toxin. Plant Physiol 102: 95–100 (1993).
98. Lefkowitz RJ: The subunit story thickens. Nature 358: 372 (1992).
99. Legendre L, Heinstein PF, Low PS: Evidence for participation of GTP-binding proteins in elicitation of the rapid oxidative burst in cultured soybean cells. J Biol Chem 267: 20140–20147 (1992).
100. Li W, Assmann S: Characterization of a G-protein-regulated outward K^+ current in mesophyll cells of *Vicia faba* L. Proc Natl Acad Sci USA 90: 262–266 (1993).
101. Lilly P, Wu L, Welker DL, Devreotes PN: A G-protein β subunit is essential for *Dictyostelium* development. Genes Devel 7: 986–995 (1993).
102. Lochrie MA, Mendel JE, Sternberg PW, Simon MI: Homologous and unique G protein α subunits in the nematode *Caenorhabditis elegans*. Cell Regul 2: 135–54 (1991).
103. Lochrie MA, Simon MI: G protein multiplicity in eukaryotic signal transduction systems. Biochemistry 27: 4957–4965 (1988).
104. Logothetis DE, Kurachi Y, Galper J, Neer EJ, Clapham DE: The βγ subunits of GTP-binding proteins activate the muscarinic K^+ channel in heart. Nature 325: 321–326 (1987).
105. Lyons J, Landis CA, Harsh G, Vallar L, Grunewald K, Feichtinger H, Duh Q-H, Clark OH, Kawasaki E, Bourne HR, McCormick F: Two G protein oncogenes in human endocrine tumors. Science 249: 655–659 (1990).
106. Ma H, Yanofsky MF, Huang H: Isolation and sequence analysis of *TGA1* cDNAs encoding a tomato G protein α subunit. Gene 107: 189–195 (1991).
107. Ma H, Yanofsky MF, Meyerowitz EM: Molecular cloning and characterization of *GPA1*, a G protein α subunit gene from *Arabidopsis thaliana*. Proc Natl Acad Sci USA 87: 3821–3825 (1990).
108. Markby DW, Onrust R, Bourne HR: Separate GTP binding and GTPase activating domains of a Gα subunit. Science 262: 1895–1901 (1993).
109. Marshall MS: The effector interactions of $p21^{ras}$. Trends Biochem Sci 18: 250–254 (1993).
110. Matsui M, Sasamoto S, Kunieda T, Nomura N, Ryotaro I: Cloning of *ara*, a putative *Arabidopsis thaliana* gene homologous to the *ras*-related gene family. Gene 76: 313–319 (1989).
111. McLaughlin SK, McKinnon PJ, Margolskee RF: Gustducin is a taste-cell-specific G protein closely related to the transducins. Nature 357: 563–569 (1992).
112. Memon AR, Clark GB, Thompson GA Jr: Identification of an ARF type low molecular mass GTP-binding protein in pea (*Pisum sativum*). Biochem Biophys Res Comm 193: 809–813 (1993).
113. Memon AR, Herrin DL, Thompson GA Jr: Intracellular translocation of a 28 kDa GTP-binding protein during osmotic shock-induced cell volume regulation in *Dunaliella salina*. Biochim Biophys Acta 1179: 11–22 (1993).
114. Millner PA: Are guanine nucleotide-binding proteins involved in regulation of thylakoid protein kinase activity? FEBS Lett 226: 155–160 (1987).
115. Miyajima I, Nakafuku M, Nakayama N, Brenner C, Miyajima A, Kaibuchi K, Arai K, Kaziro Y, Matsumoto K: *GPA1*, a haploid-specific essential gene, encodes a yeast homolog of mammalian G protein which may be involved in mating factor signal transduction. Cell 50: 1011–1019 (1987).
116. Moore MS, Blobel G: The GTP-binding protein Ran/TC4 is required for protein import into the nucleus. Nature 365: 661–663 (1993).
117. Nagano Y, Murai N, Matsuno R, Sasaki Y: Isolation and characterization of cDNAs that encode eleven small GTP-binding protein from *Pisum sativum*. Plant Cell Physiol 34: 447–455 (1993).
118. Nakafuku M, Itoh H, Nakamura S, Kaziro Y: Occurrence in *Saccharomyces cerevisiae* of a gene homologous to the cDNA coding for the α subunit of mammalian G

proteins. Proc Natl Acad Sci USA 84: 2140–2144 (1987).
119. Nakafuku M, Obara T, Kaibuchi K, Miyajima I, Miyajima A, Itoh H, Nakamura S, Arai K-I, Matsumoto K, Kaziro Y: Isolation of a second yeast *Saccharomyces cerevisiae* gene (*GPA2*) coding for guanine nucleotide-binding regulatory protein: studies on its structure and possible function. Proc Natl Acad Sci USA 85: 1374–1378 (1988).
120. Nakano A, Muramatsu M: A novel GTP-binding protein, Sar1p, is involved in transport from the endoplasmic reticulum to the Golgi apparatus. J Cell Biol 109: 2677–2691 (1989).
121. Neuhaus G, Bowler C, Kern R, Chua N-H: Calcium/calmodulin-dependent and -indepenent phytochrome signal transduction pathways. Cell 73: 937–952 (1993).
122. Nishida E, Gotoh Y: The MAP kinase cascade is essential for diverse signal transduction pathways. Trends Biochem Sci 18: 128–131 (1993).
123. Noel JP, Hamm HE, Sigler PB: The 2.2 A crystal structure of transducin-α complexed with GTPγS. Nature 366: 654–663 (1993).
124. Obara T, Nakafuku M, Yamamoto M, Kaziro Y: Isolation and characterization of a gene encoding a G-protein α subunit from *Schizosaccharomyces pombi*: involvement in mating and sporulation pathways. Proc Natl Acad Sci USA 88: 5877–5881 (1991).
125. Ohmura T, Sakata A, Onoue K: A 68-kD GTP-binding protein associated with the T cell receptor complex. J Exp Med 176: 887–891 (1992).
126. Okamuro JK, den Boer BGW, Jofuku KD: Regulation of Arabidopsis flower development. Plant Cell 5: 1183–1193 (1993).
127. Palme K, Diefenthal T, Moore I: The *ypt* gene family from maize and Arabidopsis: structural and functional analysis. J Exp Bot 44: 183–195 (1993).
128. Palme K, Diefenthal T, Vingron M, Sander C, Schell J: Molecular cloning and structural analysis of genes from *Zea mays* (L.) coding for members of the *ras*-related *ypt* gene family. Proc Natl Acad Sci USA 89: 787–791 (1992).
129. Parks S, Wieschaus E: The *Drosophila* gastrulation gene concertina encodes a G_{α}-like protein. Cell 64: 447–458 (1991).
130. Pfeuffer E, Mollner S, Lancer D, Pfeuffer T: Olfactory adenylyl cyclase: identification and purification of a novel enzyme form. J Biol Chem 264: 18803–18807 (1989).
131. Price SR, Nightingale M, Tsai S-C, Williamson KC, Adamik R, Chen H-CC, Moss J, Vaughan M: Guanine nucleotide-binding proteins that enhance choleragen ADP-ribosyltransferase activity: nulceotide sequence and deduced amino acid sequence of an ADP-ribosylation factor cDNA. Proc Natl Acad Sci USA 85: 5488–5491 (1988).
132. Provost NM, Somers DE, Hurley JB: A *Drosophila melanogaster* G protein α subunit gene is expressed primarily in embryos and pupae. J Biol Chem 263: 12070–12076 (1988).
133. Pupillo M, Kumagai A, Pitt GS, Firtel RA, Devreotes PN: Multiple α subunits of guanine nucleotide-binding proteins in *Dictyostelium*. Proc Natl Acad Sci USA 86: 4892–4896 (1989).
134. Quail PH: Phytochrome: a light-activated moleculer switch that regulates plant gene expression. Annu Rev Genet 25: 389–409 (1991).
135. Quan F, Forte MA: Two forms of *Drosophila melanogaster* Gs α are produced by alternate splicing involving an unusual splice site. Mol Cell Biol 10: 910–917 (1990).
136. Quan F, Thomas L, Forte M: *Drosophila* stimulatory G protein α subunit activates mammalian adenylyl cyclase but interacts poorly with mammalian receptors: implications for receptor-G protein interaction. Proc Natl Acad Sci USA 88: 1898–1902 (1991).
137. Quan F, Wolfgang WJ, Forte MA: The *Drosophila* gene coding for the α subunit of a stimulatory G protein is preferentially expressed in the nervous system. Proc Natl Acad Sci USA 86: 4321–4325 (1989).
138. Regad F, Bardet C, Tremousaygue D, Moisan A, Lescure B, Axelos M: cDNA cloning and expression of an *Arabidopsis* GTP-binding protein of the ARF family. FEBS Lett 316: 133–136 (1993).
139. Reynolds TL, Raikhel NV: Targeting and trafficking of vacuolar proteins. In Tartakoff A (ed) Membranes: Specialized Functions in Plants. JAI Press, Greenwich, CT, in press (1994).
140. Roberge M: Checkpoint controls that couple mitosis to completion of DNA replication. Trends Cell Biol 2: 277–281 (1992).
141. Rodríguez-Rosales M, Herrin DL, Thompson GA Jr: Identification of low molecular mass GTP-binding proteins in membranes of the halotolerant alga *Dunaliella salina*. Plant Physiol 98: 446–451 (1992).
142. Romero LC, Lam E: Guanine nucleotide binding protein involvement in early steps of phytochrome-regulated gene expression. Proc Natl Acad Sci USA 90: 1465–1469 (1993).
143. Romero LC, Sommer D, Gotor C, Song P-S: G-protein in etiolated *Avena* seedlings: possible phytochrome regulation. FEBS Lett 282: 341–346 (1991).
144. Ross EM: Signal sorting and amplification through G protein-coupled receptors. Neuron 3: 141–152 (1989).
145. Rubin GM: Signal transduction and the fate of the R7 photoreceptor in *Drosophila*. Trends Genet 7: 372–377 (1991).
146. Ryba NJP, Pottinger JDD, Keen JN, Findlay JBC: Sequence of the β-subunit of the phosphatidylinositol-specific phospholipase C-directed GTP-binding protein from squid (*Loligo forbesi*) photoreceptors. Biochem J 273: 225–228 (1991).
147. Sano H, Youssefian S: A novel *ras*-related *rgp1* gene encoding a GTP-binding protein has reduced expression

in 5-azacytidine induced dwarf rice. Mol Gen Genet 228: 227–232 (1991).
148. Sasaki Y, Sekiguchi K, Nagano Y, Matsuno R: Detection of small GTP-binding proteins in the outer envelop membrane of pea chloroplasts. FEBS Lett 293: 124–126 (1991).
149. Schlessinger J: How receptor tyrosine kinases activate Ras. Trends Biochem Sci 18: 273–275 (1993).
150. Schloss JA: A chlamydomonas gene encodes a G protein β subunit-like polypeptide. Mol Gen Genet 221: 443–452 (1990).
151. Schultz DG, Carlson M: Molecular analysis of *SSN6*, a gene functionally related to the SNF1 protein kinase of *Saccharomyces cerevisiae*. Mol Cell Biol 7: 3637–3645 (1987).
152. Schwaninger R, Plutner H, Bokoch GM, Balch WE: Multiple GTP-binding proteins regulate vesicular transport from the ER to Golgi membranes. J Cell Biol 119: 1077–1096 (1992).
153. Seuwen K, Pouysségur J: G protein-controlled signal transduction pathways and the regulation of cell proliferation. Adv Cancer Res 58: 75–94 (1992).
154. Sharrock RA, Quail PH: Novel phytochrome sequences in *Arabidopsis thaliana*: structure, evolution, and differential expression of a plant regulatory photoreceptor family. Genes Devel 3: 1745–1757 (1989).
155. Simon MI, Strathmann MP, Gautam N: Diversity of G proteins in signal transduction. Science 252: 802–808 (1991).
156. Srivastava SK, Singh US: Insulin activates guanosine 5′-[γ-thio] triphosphate (GTPγS) binding to a novel GTP-binding protein, G_{IR}, from human placenta. Biochem Biophys Res Comm 173: 501–506 (1990).
157. Sternberg PW, Horvitz HR: Signal transduction during *C. elegans* vulval induction. Trends Genet 7: 366–371 (1991).
158. Strathmann M, Simon MI: G protein diversity: a distinct class of α subunits is present in vertebrates and invertebrates. Proc Natl Acad Sci USA 87: 9113–9117 (1990).
159. Strathmann MP, Simon MI: Gα12 and Gα13 subunits define a fourth class of G protein α subunits. Proc Natl Acad Sci USA 88: 5582–5586 (1991).
160. Stryer L: Cyclic GMP cascade of vision. Annu Rev Neurosci 9: 87–119 (1986).
161. Terryn N, Anuntalabhochai S, Van Montagu M, Inzé D: Analysis of a *Nicotiana plumbaginifolia* cDNA encoding a novel small GTP-binding protein. FEBS Lett 299: 287–290 (1992).
162. Terryn N, Arias MB, Engler G, Tiré C, Villarroel R, Van Montagu M, Inzé D: *rha1*, a gene encoding a small GTP binding protein from Arabidopsis, is expressed primarily in developing guard cells. Plant Cell 5: 1761–1769 (1993).
163. Terryn N, Van Montagu M, Inzé D: GTP-binding proteins in plants. Plant Mol Biol 22: 143–152 (1993).
164. Ullrich A, Schlessinger J: Signal transduction by receptors with tyrosine kinase activity. Cell 61: 203–212 (1990).
165. van der Voorn L, Gebbink M, Plasterk RH, Ploegh HL: Characterization of a G-protein β-subunit gene from the nematode *Caenorhabditis elegans*. J Mol Biol 213: 17–26 (1990).
166. Walworth NC, Goud B, Kabcenell AK, Novick PJ: Mutational analysis of *SEC4* suggests a cyclical mechanism for the regulation of vesicular traffic. EMBO J 8: 1685–1693 (1989).
167. Wang H, Watkins DC, Malbon CC: Antisense oligodeoxynucleotides to G_s protein α-subunit sequence accelerate differentiation of fibroblasts to adipocytes. Nature 358: 334–337 (1992).
168. Wang M, Sedee NJA, Heidekamp F, Snaar-Jagalska BE: Detection of GTP-binding proteins in barley aleurone protoplasts. FEBS Lett 329: 245–248 (1993).
169. Warpeha KMF, Hamm HE, Rasenick MM, Kaufman LS: A blue-light-activated GTP-binding protein in the plasma membranes of etiolated peas. Proc Natl Acad Sci USA 88: 8925–8929 (1991).
170. Watkins DC, Johnson GL, Malbon CC: Regulation of the differentiation of teratocarcinoma cell into primitive endoderm by $G\alpha_{i2}$. Science 258: 1373–1375 (1992).
171. Weiss CA, Garnaat CW, Mukai K, Hu Y, Ma H: Isolation of cDNAs encoding G protein β subunit homologues from maize *(ZGB1)* and *Arabidopsis* (*AGB1*). Proc Natl Acad Sci USA 91: 9554–9558 (1994).
172. Weiss CA, Huang H, Ma H: Immunolocalization of the G protein α subunit encoded by the *GPA1* gene in Arabidopsis. Plant Cell 5: 1513–1528 (1993).
173. White IR, Wise A, Finan PM, Clarkson J, Millner PA: GTP-binding proteins in higher plant cells. In: Cooke DT, Clarkson DT (eds) Transport and Receptor Proteins of Plant Membranes, pp. 185–192. Plenum Press, New York (1992).
174. Whiteway M, Hougan L, Dignard D, Thomas DY, Bell L, Saari GC, Grant FJ, O'Hara P, Mackay VL: The *STE4* and *STE18* genes of yeast encode potential β and γ subunits of the mating factor receptor-coupled G protein. Cell 56: 467–477 (1989).
175. Whiteway M, Hougan L, Thomas DY: Overexpression of the STE4 gene leads to mating response in haploid *Saccharomyces cerevisiae*. Mol Cell Biol 10: 217–222 (1990).
176. Wichmann H, Hengst L, Gallwitz D: Endocytosis in yeast: evidence for the involvement of a small GTP-binding protein (Ypt7p). Cell 71: 1131–1142 (1992).
177. Wigler M, Field J, Powers S, Broek D, Toda T, Cameron S, Nikawa J, Michaeli T, Colicelli J, Ferguson K: Studies of *RAS* function in the yeast *Saccharomyces cerevisiae*. Cold Spring Harbor Symp Quant Biol 53: 649–655 (1988).
178. Wigler MH: The RAS system in yeasts. In: Lacal JC,

McCormick F (eds) The *ras* Superfamily of GTPases, pp. 155–172. CRC Press, Boca Raton, FL (1993).
179. Wilkie TM, Scherle PA, Strathmann MP, Slepak VZ, Simon MI: Characterization of G-protein α subunits in the G_q class: expression in murine tissues and stromal and hematopoietic cell lines. Proc Natl Acad Sci USA 88: 10049–10053 (1991).
180. Williams FE, Trumbly RJ: Characterization of *TUP1*, a mediator of glucose repression in *Saccharomyces cerevisiae*. Mol Cell Biol 10: 6500–6511 (1990).
181. Williams FE, Varanasi U, Trumbly RJ: The CYC8 and TUP1 proteins involved in glucose repression in *Saccharomyces cerevisiae* are associated in a protein complex. Mol Cell Biol 11: 3307–3316 (1991).
182. Wu LJ, Devreotes PN: *Dictyostelium* transiently expresses eight distinct G-protein α-subunits during its developmental program. Biochem Biophys Res Commun 179: 1141–1147 (1991).
183. Yang Z, Watson JC: Molecular cloning and characterization of rho, a ras-related small GTP-binding protein from the garden pea. Proc Natl Acad Sci USA 90: 8732–8736 (1993).
184. Yarfitz S, Niemi GA, McConnell JL, Fitch CL, Hurley JB: A G β protein in the Drosophila compound eye is different from that in the brain. Neuron 7: 429–438 (1991).
185. Yarfitz S, Provost NM, Hurley JB: Cloning of a *Drosophila melanogaster* guanine nucleotide regulatory protein β-subunit gene and characterization of its expression during development. Proc Natl Acad Sci USA 85: 7134–7138 (1988).
186. Yi Y, Guerinot M: A new member of the small GTP-binding protein family in *Arabidopsis thaliana*. Plant Physiol 104: 295–296 (1994).
187. Youssefian S, Nakamura M, Sano H: Molecular characterization of *rgp2*, a gene encoding a small GTP-binding protein from rice. Mol Gen Genet 237: 187–192 (1993).
188. Zahraoui A, Touchot N, Chardin P, Tavitian A: The human rab genes encode a family of GTP-binding proteins related to yeast ypt1 and sec4 products involved in secretion. J Biol Chem 264: 12394–12401 (1989).
189. Zaina S, Breviario D, Mapelli S, Bertani A, Reggiani R: Two putative G-protein α subunits dissociate from rice coleoptile membranes after GTP stimulation. J Plant Physiol 143: 293–297 (1994).
190. Zaina S, Reggiani R, Bertani A: Preliminary evidence for involvement of GTP-binding protein(s) in auxin signal transduction in rice (*Oryza sativa* L.). J Plant Physiol 136: 653–658 (1990).