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Plant hormone perception and action: a role for G-protein signal transduction?

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Plants perceive and respond to a profusion of environmental and endogenous signals that influence their growth and development. The G-protein signalling pathway is a mechanism for transducing extracellular signals that is highly conserved in a range of eukaryotes and prokaryotes. Evidence for the existence of G-protein signalling pathways in higher plants is reviewed, and their potential involvement in plant hormone signal transduction evaluated. A range of biochemical and molecular studies have identified potential components of G-protein signalling in plants, most notably a homologue of the G-protein coupled receptor superfamily (*GCR1*) and the G_α and G_β subunits of heterotrimeric G-proteins. G-protein agonists and antagonists are known to influence a variety of signalling events in plants and have been used to implicate heterotrimeric G-proteins in gibberellin and possibly auxin signalling. Antisense suppression of *GCR1* in *Arabidopsis* leads to a phenotype which supports a role for this receptor in cytokinin signalling. These observations suggest that higher plants have at least some of the components of G-protein signalling pathways and that these might be involved in the action of certain plant hormones.

Keywords: cytokinin; gibberellin; heterotrimeric G-protein; plant hormones; receptor

1. PLANT HORMONES AND MEMBRANE-BASED SIGNALLING MECHANISMS

Currently, six principal classes of plant hormones, ethylene, cytokinin, auxin, gibberellin (GA), abscisic acid (ABA), and brassinosteroids (BRs) are recognized. Each is necessary for normal growth and development and influences a range of events during the life cycle of plants. Evidence is accumulating that each of these classes of plant hormone may be perceived by membrane-located receptors. The ethylene receptor is a transmembrane protein with good homology to histidine-kinase two-component response-regulators (Bleecker & Schaller 1996). It has been suggested that cytokinins may also be perceived by a receptor of this class, CKI1 (Kakimoto 1996). The putative BR receptor is a transmembrane receptor kinase with leucine-rich repeats on one side of the membrane (probably the plasma membrane) and a serine–threonine protein kinase domain on the other (Li & Chory 1997). GA appears to be perceived at the plasma membrane of aleurone cells (Hooley *et al.* 1991; Gilroy & Jones 1994) although a candidate receptor has yet to be identified. In the case of ABA, there is evidence for both cell surface and intracellular receptors (Gilroy & Jones 1994; Allan *et al.* 1994; Anderson *et al.* 1994). Finally, auxin is thought to act at the cell surface (Venis & Napier 1995). These observations suggest that membrane-located signalling systems are involved in the perception and transduction of plant hormones.

2. G-PROTEIN SIGNALLING PATHWAY COMPONENTS IN PLANTS

(a) *Heterotrimeric G-proteins*

One of the most highly conserved membrane-located signalling mechanisms is the G-protein signalling pathway (Strader *et al.* 1994). Heterotrimeric G-proteins are an essential component of this pathway and are associated with the cytoplasmic face of the plasma membrane of a variety of eukaryotic cells where they transduce information from cell surface G-protein coupled receptors (GPCRs) to downstream effector proteins (Neer 1995). A range of biochemical, molecular and cell biological evidence suggests that higher plants employ heterotrimeric G-proteins as signalling components. However, the extent to which these might be functional counterparts of mammalian and other eukaryotic heterotrimeric G-proteins is, for the moment, unclear (Ma 1994).

The first evidence for plant heterotrimeric G-proteins came from biochemical and immunological studies. Microsomal and plasma membranes from a number of plant species have been shown to contain high affinity (α^{32}P)GTP- and (^{35}S)GTP-γ-S-binding activity along with polypeptides which can be ADP-ribosylated or cross-react with antisera against G_α subunits or G_β subunit peptides (reviewed by Ma (1994)). These observations suggest that polypeptides similar to the G_α subunit of heterotrimeric G-proteins might be present in plants. This was confirmed by the isolation of genomic and cDNA

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clones encoding a single class of G_α subunit (*GPA1*), and a G_β subunit, from several plant species (Ma *et al.* 1990, 1991; Poulsen *et al.* 1994; Weiss *et al.* 1994; Kim *et al.* 1995; Ishikawa *et al.* 1995; Seo *et al.* 1995). To date no gene encoding a G_γ subunit has been isolated from plants.

The high sequence conservation of *GPA1* between different plant species suggests that it might have an important function. In an attempt to gain insight into the possible roles of GPα1, Weiss *et al.* (1993) studied its expression in *Arabidopsis* by immunolocalization using antiserum raised against a synthetic peptide corresponding to the GPα1 C terminus, and by monitoring expression of a *GPA1* promoter–GUS fusion. This revealed that GPα1 has a complex expression pattern. It is present in roots, rosette leaves, floral stems, cauline leaves, flowers and seed pods, though it is not present in mature seeds. Highest levels of GPα1 occur in meristems and immature organs. In mature tissues it is found in various cell types but particularly in the vascular system. This pattern of expression does not suggest a specific function for the protein and if anything indicates that it might be involved either in responses to a range of different signals or to a stimulus that has pleiotropic effects possibly depending on the developmental stage and/or tissue.

At a subcellular level GPα1 appears to be present in the ER and plasma membrane. This may also be consistent with a multi-functional role. GPα1 might be involved in transducing signals at either, or both, of these locations. It might also be involved in endomembrane trafficking (Weiss *et al.* 1997).

The involvement of plant heterotrimeric G-proteins in signalling pathways has also been investigated by using pharmacological agonists and antagonists. There is a wealth of evidence indicating a role for G-proteins in the regulation of K^+ influx channels of stomatal guard cells (Assman 1996). The non-hydrolysable GTP analogues, GTP-γ-S and GDP-β-S, the bacterial cholera and pertussis toxins, and the G-protein agonist Mas 7, have been shown to modulate guard cell inward K^+ channel activity in a complex manner. This has been interpreted as indicating that several G-protein signalling pathways may operate in guard cells (Fairley-Grenot & Assmann 1991; Li & Assmann 1993; Lee *et al.* 1993; Wu & Assman 1994; Armstrong & Blatt 1995; Kelly *et al.* 1995).

Similar studies have implicated G-proteins in responses to blue and red light (Warpeha *et al.* 1991; Romero & Lam 1993; Neuhaus *et al.* 1993; Bowler *et al.* 1994), pathogen resistance and pathogen-related gene expression (Beffa *et al.* 1995), fungal elicitors (Legendre *et al.* 1992) and plant hormones (Bossen *et al.* 1991; Zaina *et al.* 1990). In addition, it is possible that one effect of G-protein activation in plants is the stimulation of a phospholipase D signalling pathway (Munnik *et al.* 1995, 1998).

(b) *G-protein coupled receptors*

A primary component of the G-protein signalling pathway is the receptor. The superfamily of GPCRs are involved in the transduction of a wide range of ligands that include excitatory amino acids, pheromones, polypeptide hormones and odorants. GPCRs have been identified in vertebrates, invertebrates, arthropods, insects, nematodes, fungi, yeast and viruses. The deduced protein sequence of more than 700 GPCRs is known and these all have seven stretches of hydrophobic amino acids each capable of forming a transmembrane α-helix. Thus, GPCRs are thought to traverse the membrane seven times forming a cluster of seven α-helices connected by alternating intracellular and extracellular loops (Strader *et al.* 1994). When activated by a ligand it is thought that GPCRs undergo a conformational change, such that the cytoplasmic loop regions are able to interact with the Gα subunit of a heterotrimeric G-protein and stimulate a GDP–GTP exchange reaction thus initiating a signalling cascade.

The fact that higher plants appear to have functional heterotrimeric G-proteins led to the suggestion that they might also have GPCRs (Millner & Causier 1996; Armstrong & Blatt 1995). In an attempt to determine if plant heterotrimeric G-proteins are regulated by GPCRs, Mu and co-workers (1997) introduced five human muscarinic acetylcholine receptors (MAChRs) into stably transformed tobacco plants and BY2 callus. Of the constructs, two were wild-type m1 and m2 MAChRs, the others were MAChR:β-adrenergic receptor chimeras that are thought to be less selective for their cognate G-proteins and therefore more likely to interact with heterologous G-proteins. All of the constructs were expressed in calli; four were expressed in plants. Expression levels were comparable with other heterologous systems and the receptors displayed the expected ligand-binding specificity and kinetics indicating that functional MAChRs and MAChR chimeras had been produced in tobacco. However, there was no obvious phenotype in tobacco plants expressing the receptors, and no reproducible effects of the agonist on the expression of genes thought to be regulated by plant G-proteins. Thus, although tobacco cells are able to express functional human GPCRs these were unable to stimulate plant G-proteins. This might be because the human GPCRs were unable to mimic their plant counterparts or because plant G-proteins are regulated by a different mechanism that does not involve this class of receptors.

Recently however, a plant homologue of the GPCR superfamily has been identified (Josefson & Rask 1997; Plakidou-Dymock *et al.* 1998). The database of expressed sequence tags (dbEST) (Boguski *et al.* 1993) contains several *Arabidopsis* sequences, and one each from pine and rice, that predicted products with sequence similarity to GPCRs. One of the *Arabidopsis* ESTs was used to isolate the *GCR1* gene and cDNA (Plakidou-Dymock *et al.* 1998). The *GCR1* cDNA encodes a 326-amino-acid polypeptide that has up to 23% amino-acid identity (53% similarity) to known GPCRs. Hydropathy analysis indicates that GCR1 has seven potential transmembrane-spanning domains and membrane topology prediction algorithms support a structure characteristic of GPCRs.

Arabidopsis therefore has homologues of three essential components of a G-protein signalling pathway, GCR1, a putative GPCR, GPα1, a putative Gα subunit and AGB1 a putative Gβ subunit. Although further investigation is required, the possibility exists that these components, or polypeptides related to them, may interact with one another as elements of a plant G-protein signal transduction chain.

3. ARE HETEROTRIMERIC G-PROTEINS AND GCR1 INVOLVED IN PLANT HORMONE SIGNALLING?

(a) *Auxin*

Preliminary evidence for the possible involvement of G-proteins in plant hormone signalling has come from the work of Zania and co-workers (1990) who found that indole-3-acetic acid (IAA) increased binding of [^{35}S] GTPγS to rice coleoptile membrane vesicles. Naphthyl-2-acetic acid, which has lower biological activity than IAA, did not affect [^{35}S] GTPγS binding. These observations have been interpreted as suggesting that IAA might stimulate GDP–GTP exchange by a G-protein. In addition, Zania and co-workers (1990) observed that GTPγS reduced [^{3}H]IAA binding to rice coleoptile membrane vesicles and suggested that this might indicate that G-protein activation somehow inhibits IAA binding by a receptor. These observations have not been followed up in further investigations, although there has been a report of a possible involvement of G-proteins in swelling responses of protoplasts isolated from etiolated wheat leaves to auxin and other hormones (Bossen *et al.* 1991).

(b) *Gibberellin*

More substantive evidence of a role for heterotrimeric G-proteins in plant hormone signalling has come from studies with wild oat aleurone protoplasts. Aleurone cells respond to GA by expressing genes encoding a variety of hydrolases, including α-amylase, and secreting these enzymes into the endosperm. This response is one of a number of plant responses to GAs, and it has been used extensively to study GA signal transduction (Hooley 1994). Evidence from two quite different experimental approaches suggests that GAs are perceived at the aleurone plasma membrane. First, GA_4 coupled to Sepharose beads is membrane-impermeant but nevertheless stimulates high-level α-amylase gene expression and protein secretion when presented to wild oat aleurone protoplasts (Hooley *et al.* 1991). Second, microinjection of GA into barley aleurone protoplasts does not stimulate α-amylase gene expression and it is only when GA is present in the medium bathing the protoplasts that they respond (Gilroy & Jones 1994). These data provide indirect evidence for a plasma membrane-based GA perception mechanism in aleurone although it should be emphasized that identification of a GA receptor has not yet been accomplished.

Following GA treatment of aleurone cells a number of events occur which may be elements of GA signalling that are downstream of a plasma membrane-located GA receptor (reviewed by Bethke *et al.* (1997)). These include an increase in cytoplasmic Ca^{2+} concentration which appears to result from calcium influx from the apoplast (Gilroy & Jones 1992; Bush 1996). Following this, and before the increase in transcription of α-amylase genes, there is a transient increase in cGMP (Penson *et al.* 1996) which appears to be essential for GA-regulated gene expression.

These types of signalling events are compatible with a G-protein signalling pathway, and recent research has examined the possible role of heterotrimeric G-proteins in signal transduction in wild oat aleurone (Jones *et al.* 1998). The mastoparan analogue Mas7 stimulates GDP–GTP exchange by heterotrimeric G-proteins and is thought to mimic an activated GPCR (Higashijima *et al.* 1988, 1990). Jones *et al.* (1998) found that when wild oat aleurone protoplasts are incubated with Mas7 they produce and secrete α-amylase in a dose-dependent manner. Concentrations as low as 0.1 mM Mas7 produce a significant response compared with untreated controls. Mas7 induces α-amylase mRNA and drives expression of an α-*Amy2/54:GUS* promoter:reporter construct. It stimulates α-amylase enzyme production and secretion with a virtually identical time course to GA and, similar to GA, its effect is largely overcome by ABA. The inactive mastoparan analogues, MasCP (control peptide), differing from Mas7 by a single amino-acid substitution, and Mas7-COOH, a peptide with the same amino-acid sequence as Mas7, but with a free acid replacing the amine group at the C terminus, do not induce α-amylase. Mas7 therefore appears to be an effective GA mimic, thus raising the possibility that it is activating a heterotrimeric G-protein in the GA signalling pathway.

Further evidence that GA signalling may involve a heterotrimeric G-protein has come from studying the effects of hydrolysis-resistant guanine nucleotides on GA-induction of α-*Amy2/54:GUS* expression (Jones *et al.* 1998). The hydrolysis-resistant guanine nucleotide analogues GTP-γ-S and GDP-β-S bind to G_α subunits and hold them in either the activated (GTP-γ-S-bound) or inactivated (GDP-β-S-bound) form. GDP-β-S introduced into aleurone protoplasts during transfection with reporter gene constructs completely prevented GA_1 induction of α-*Amy2/54:GUS* expression, whereas GTP-γ-S stimulated expression slightly. Jones and co-workers (1998) also used PCR to clone a partial G_α subunit cDNA ($AfG_\alpha 1$) and two related G_β cDNAs ($AfG_\beta 1$ and $AfG_\beta 2$) from wild oat aleurone. Northern blot analysis confirmed that these are expressed in aleurone cells. The deduced amino-acid sequence of $AfG_\alpha 1$ is 40% identical to GPα1 (Ma *et al.* 1990). The amino-acid sequence of one of the Gβ subunits, $AfG_\beta 1$, is 91% identical to the maize G_β subunit $ZG_\beta 1$ (Weiss *et al.* 1994). $AfG_\beta 2$ has been partially sequenced and appears to be related to, but distinct from, the G_β subunit, with 51% identity to $AfG_\beta 1$ over a 250-amino-acid region.

The effects of Mas7 and guanine nucleotide analogues on GA-induction of α-amylase and α-Amy2/54:GUS expression in wild oat aleurone protoplasts, combined with the expression of transcripts encoding G_α and G_β subunits suggest that a heterotrimeric G-protein or proteins are involved in GA signal transduction in this tissue.

(c) *Cytokinin*

The plant GPCR homologue GCR1 has the highest sequence similarity to the *Dictyostelium discoideum* cAMP receptors and this raised the possibility that it might be involved in the action of a purine or purine-related signalling molecule in plants. Antisense suppression of *GCR1* expression in transgenic *Arabidopsis* gave rise to a specific reduction in sensitivity to the cytokinin benzyl adenine in both root and shoot tissues, suggesting a role for GCR1 in the perception or transduction of this plant hormone (Plakidou-Dymock *et al.* 1998).

At present it is not known if GCR1 is a cytokinin receptor. It might equally be a downstream component in cytokinin signalling, or a receptor for another ligand, the signalling pathway for which interacts with cytokinin signalling. In fact, another membrane protein, CKI1, that has sequence similarity to histidine-kinase two-component response-regulators, is also a candidate cytokinin receptor (Kakimoto 1996). Further research should help elaborate the function of, and relation between, these molecules.

4. CYTOKININS AND G-PROTEIN SIGNALLING: NEW LIGHT ON ESTABLISHED DATA?

Cytokinin action might, directly or indirectly, involve a G-protein signalling pathway initiated by GCR1. This possibility prompts a re-examination of some published data that are based on the use of G-protein agonists and antagonists, to question whether or not certain G-protein mediated responses might actually involve cytokinins.

(a) *Stomata*

Stomatal aperture is regulated by a range of environmental and endogenous signals including light, carbon dioxide, abscisic acid and auxin. The effect of cytokinins on stomata has been overlooked in recent years although there is clear evidence that they can open stomata (Blackman & Davies 1984). Could some of the known effects of G-protein agonists and antagonists on guard cell K^+ influx channel activity (Assmann 1996) be the result of perturbations of a cytokinin signalling pathway?

Lee and co-workers (1993) demonstrated that release of caged GTPγS that had been microinjected into *Commelina communis* guard cells stimulated stomatal opening. Opening of stomata involves stimulation of K^+ influx channels and it is has been shown that the G-protein antagonist GDPβS can inhibit guard cell K^+ influx channel activity (Kelly *et al.* 1995). Both these observations are consistent with the GTPγS activating and the GDPβS inhibiting a heterotrimeric G-protein that could possibly be involved in transducing a cytokinin signal that causes stomata to open. Clearly this theory will require testing experimentally. However, it does present an alternative interpretation of some of the data obtained with G-protein agonists and antagonists in stomata.

From other observations it is clear that regulation of guard cell K^+ influx channel activity by heterotrimeric G-proteins is more complex (Assmann 1996; Thiel & Wolf 1997). Measurements made under different experimental conditions suggest that cytoplasmic Ca^{2+} concentration may influence G-protein regulation of inward K^+ channel activity, and that there may also be a Ca^{2+}-independent mechanism (Thiel & Wolf 1997). Thus, in other reports there is clear inhibition of K^+ influx channels by the G-protein agonists Mas 7 and GTPγS (Fairley-Grenot & Assmann 1991; Armstrong & Blatt 1995) which suggest that a heterotrimeric G-protein may also be involved in stomatal closure. These data are not irreconcilable and could be explained by a dual regulation of K^+ influx channels by opposing heterotrimeric G-proteins in which cytoplasmic Ca^{2+} might be an important regulator. It is possible that cytokinins might influence one or more of these pathways.

(b) *Phytochrome*

A number of investigations have suggested that phytochrome signalling involves activation of one or more heterotrimeric G-proteins. For example, treatment of dark-adapted soybean cells with cholera or pertussis toxins uncouples phytochrome-dependent expression of chlorophyll *a*/*b*-binding protein (*cab*) (Romero & Lam 1993). In the *aurea* mutant of tomato G-protein agonists and antagonists are able to reproduce the effects of phyA on three light-regulated events: (i) anthocyanin production; (ii) chloroplast development; and (iii) the expression of a *cab* promoter–GUS reporter construct (Neuhaus *et al.* 1993; Bowler *et al.* 1994). These studies also led to the proposal that phytochrome stimulation of heterotrimeric G-proteins activated three different signalling pathways: (i) cGMP-dependent; (ii) Ca^{2+}-dependent; and (iii) cGMP and Ca^{2+}-dependent (Neuhaus *et al.* 1993; Bowler *et al.* 1994).

The suggestion that a heterotrimeric G-protein or proteins may be very close to the initial photoperception event in phyA signalling (Neuhaus *et al.* 1993; Bowler *et al.* 1994) raises some intriguing questions about how phyA might activate a G-protein. Phytochrome is a cytoplasmic protein that does not associate with membranes (Quail 1991). Therefore, if a direct interaction occurs between phyA and a heterotrimeric G-protein(s) this will involve a mechanism quite unlike that in which these signalling molecules are stimulated by photoreceptors in other eukaryotic cells.

Cytokinin and light signals interact in a complex way that is not understood (Thomas *et al.* 1997). Photomorphogenesis, and some light-regulated genes such as *cab* and chalcone synthase, are also regulated by cytokinins (Chory *et al.* 1994; Thomas *et al.* 1997). The discovery of a plant homologue of the GPCR superfamily (*GCR1*) identifies a receptor which could potentially directly stimulate plant heterotrimeric G-proteins, although this has yet to be proven experimentally. However, because GCR1 appears to be involved in cytokinin signal transduction (Plakidou-Dymock *et al.* 1998) it is possible that one or more of the heterotrimeric G-protein(s) implicated in phyA signal transduction might also be components of a cytokinin signalling pathway. This theory will need to be tested experimentally. However, it does illustrate that recent insight gained into potential G-protein signalling mechanisms in plants might help elaborate the molecular basis of plant signalling systems and their interactions.

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