A role for G proteins in plant hormone signalling?

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(Received January 20, 1999; accepted February 2, 1999)

Abstract — The G protein signalling pathway is one of the most highly conserved mechanisms that enables cells to sense and respond to changes in their environment. Essential components of this are cell surface G protein-coupled receptors (GPCRs) that perceive extracellular ligands, and heterotrimeric G proteins (G proteins) that transduce information from activated GPCRs to downstream effectors such as enzymes or ion channels. It is now clear from a range of biochemical and molecular studies that some potential G protein signalling components exist in plants. The best examples of these are the seven transmembrane receptor homologue GCR1 and the Go (GPA1) and Gβγ (Gβ1) subunit homologues 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 G proteins in a range of signalling pathways that include the plant hormones gibberellin and auxin. Furthermore, antisense suppression of GCR1 expression in Arabidopsis leads to a phenotype that supports a role for this receptor in cytokinin signalling. This review considers the current evidence for and against functional G protein signalling pathways in higher plants and questions whether or not these might be involved in the action of certain plant hormones. © Elsevier, Paris

Auxin / cytokinin / gibberellin / heterotrimeric G protein / G protein-coupled receptor

BA, benzyladenine / G protein, heterotrimeric G protein / GA, gibberellin / GPCR, G protein-coupled receptor / IAA, indole-3-acetic acid

1. INTRODUCTION

A long-standing objective of plant hormone biology has been to elucidate the molecular basis of signalling systems that transduce hormone messages into the wide range of responses that they regulate. The last few years have witnessed substantial progress in our understanding of plant intracellular signalling mechanisms and some of these have a direct bearing on hormone signal transduction. One emerging signalling pathway in plants is the G protein signalling cascade. This article reviews the evidence that higher plants have some of the components of the well-characterised G protein signalling pathway and considers to what extent these components may or may not define a plant G protein signalling pathway that is involved in hormone signal transduction.

2. THE G PROTEIN SIGNALLING PATHWAY

The G protein signalling pathway is one of the most highly conserved membrane-located signalling mecha-
GPCRs, these receptors share considerable structural similarity. This probably reflects conservation in their mechanism of action that has been retained during evolution.

When GPCRs bind their cognate ligand they undergo a conformational change that enables them to interact with heterotrimeric GTP-binding proteins (G proteins) [31, 42]. These G proteins consist of α, β and γ subunits which, due to lipid modification of the α and β subunits, are associated with the cytoplasmic face of the plasma membrane. G protein activation by GPCRs stimulates the exchange of GDP for GTP in the guanine nucleotide binding site of the Gα subunit. This causes conformational change in the Gα subunit that promotes dissociation from the Gβγ complex. Both the GTP-Gα subunit and the Gβγ complex are capable of signalling to effectors that include adenyl cyclase, phospholipases, K+ and Ca2+ channels, and cyclic-GMP phosphodiesterase. The inherent GTPase activity of the Gα subunit restores the inactive heterotrimer with bound GDP ready for another cycle of stimulation by the GPCR (figure 1).

3. PLANT HETEROTrIMERIC G PROTEINS

At the present time, there is no conclusive evidence that a G protein signalling pathway operates in higher plants. However, it is becoming clear that higher plants do have some proteins that are similar to G protein signalling pathway components in other organisms. The first of these to be discovered were proteins similar to Gα subunits of G proteins and these were rapidly followed by Gβ subunits. The extent to which these might be functional counterparts of mammalian and other eukaryotic Gα and Gβ subunits, or for that matter components of a heterotrimer, is for the moment unresolved [23]. Nevertheless, their occurrence in plants has spurred investigation of this question.

The first evidence that plants have Gα subunits came from biochemical and immunological studies (reviewed by Ma [23]). Microsomal and plasma membranes from a range of species have been shown to contain high affinity [α32P]-GTP- and [35S]-GTPγS-binding activity along with polypeptides that could be ADP-ribosylated or which cross-reacted with antisera against G protein Gα subunits, or Gβ subunit peptides. However, as pointed out by Millner et al. [26], some reports should be treated with caution because they lack rigorous controls. Nevertheless, these observations were later borne out by molecular studies that identified genomic and cDNA clones that encode for proteins with many of the features characteristic of Gα subunits (figure 2) from a number of plant species. The first, GPA1, was identified in Arabidopsis [25] and is a single copy gene [23] on chromosome 2, now known to be about 10 kbp from Erecta (GenBank accession AC004484). Homologues of GPA1 have been isolated from tomato (TGA1) [24], Lotus japonicus (LjGPA1) [37], yellow lupin (LLGPROA) [20], spinach (SOG-PROALP) (GenBank accession Y16383), potato (STGPA1GEN) (GenBank accession X87836), Nicotiana tabacum (NTHTGPA) (GenBank accession Y08154) and Nicotiana plumbaginifolia (NPG-PROTSU) (GenBank accession Z72389). In other species, two or more closely related GPA1-like sequences have been identified. These include two sequences in soybean (SGA1) [19], (SGA2) [9], four sequences in rice (RGA1) [13, 40] and two sequences in pea (GenBank accessions U97043 and U97044).

A multiple alignment of the open reading frames encoded by these sequences illustrates that they are closely related and probably represent a single class of plant Gα subunits (figure 3). Gotor et al. [9] suggested that they should be placed in a new Gp family. The sequences are 60 to 100% identical at the amino acid level. Differences between them are minor, the most obvious being a 30-amino acid deletion in one of the classes of rice sequence, a 20-amino acid deletion in the N. plumbaginifolia clone, a potential alternative initiating methionine in the N. tabacum sequence and a different C-terminal motif in the rice sequences. These differences may be the basis of some functional diversity, certainly the differences in the C-terminal receptor interaction region could be significant, although at present, there is no biochemical or physiological evidence to support this. The G1, G2, G3 and G4 regions of the aligned plant sequences show good homology to consensus motifs for Gα subunits [42]. However, the G5 consensus motif of the plant sequences differs from the majority of other Gα subunits [42] perhaps reflecting differences in GDP/GTP binding or GTPase activity between the plant and other eukaryotic Gα subunits. Pertussis toxin is a well characterised G protein antagonist that ADP-ribosylates a C-terminal cysteine residue found in many Gα subunits [31]. A number of investigators have found that pertussis toxin will interfere with plant signalling [23] yet none of the plant Gα subunits have the C-terminal pertussis-sensitive cysteine residue (figure 3). This suggests that other plant Gα subunits may exist but have not yet been cloned.

A second type of Gα subunit has recently been identified in Arabidopsis (GenBank accessions
cloned from maize, Arabidopsis and wild oat [15, 47]. They have the seven WD-40 repeat regions characteristic of this class of signalling proteins and good sequence similarity to a range of \( \alpha \) subunits from eukaryotes. No \( \gamma \) subunit has been identified in plants, and, although there is a certain amount of confidence that one or more will turn up in the plant genome databases [23], until this happens it cannot be certain that the \( \alpha \) and \( \beta \) subunits are part of a heterotrimer.

What is the function of these plant \( G \) protein subunits? The \( \alpha \) subunits have between 36 and 42% amino acid sequence identity to other eukaryotic \( \alpha \) subunits. This suggests a similar but by no means identical function. The differences in the G5 region raised questions about the ability of the plant proteins to bind and hydrolyse guanine nucleotides. However, these have recently been dispelled by the discoveries that recombinant Arabidopsis GPA1 binds GTP\( \gamma \)S [49], recombinant rice GPA1 binds GTP, GTP\( \gamma \)S and the photoaffinity label 8-N\( _3 \)GTP [14, 41] and that recombinant tomato TGA1 binds GTP [1]. In addition, GTPase activity of the recombinant rice and tomato proteins [1, 14, 41] suggests that plant GPA1 proteins are functionally related to \( \alpha \) subunits. Nevertheless, the \( K_{\text{cat}} \) of GTP hydrolysis appears to be lower than that of other eukaryotic \( \alpha \) subunits [1]. The low sequence similarity between plant and other \( \alpha \) subunits in receptor, effector and \( \beta/\gamma \) interaction regions may simply reflect a requirement for correct secondary structure conformation rather than primary amino acid sequence identity. Nevertheless, identifying proteins that interact with plant GPA1 is an important question that needs to be addressed experimentally.

\( \alpha \) subunit expression has been studied in Arabidopsis and rice [41, 46]. Immunolocalisation of GPA1 in Arabidopsis shows that it is present in roots, rosette leaves, floral stems, cauline leaves, flowers and seed pods, though not in mature seed. The highest levels of GPA1 occur in meristems, vascular tissue and immature organs [46]. Immunolocalisation of the rice \( \alpha \) subunit RGA1 shows that it is more abundant in young seedlings than mature plants. The highest levels were found in seedling leaves and immature embryos while the lowest levels were in mature roots [41]. RGA1 is clearly present in rice plasma membrane [14], and both Arabidopsis GPA1 and tomato TGA1 have been detected predominantly in plasma membrane and endoplasmic reticulum [1, 48]. This could indicate a potential role of these plant \( \alpha \) subunits in signal transduction events at the plasma membrane as well as in endomembrane trafficking.
4. PLANT G PROTEIN-COUPL ED RECEPTORS

Although a number of outstanding questions remain, there is a good likelihood that higher plants have functional heterotrimeric G proteins that might be regulated by GPCRs. An attempt to couple human GPCRs to plant G proteins was not successful although this might be a consequence of subtle differences between the signalling mechanisms of these particular proteins [27]. Identification of the plant homologue of the GPCR superfamily GCR1 [16, 36] provided the first substantive evidence that this class of receptors predate the divergence of plants and animals and identified a receptor that might potentially activate GPA1. The GCR1 cDNA encodes a 326-amino acid polypeptide that has up to 23% amino acid identity (53% similarity) to known GPCRs and a number of amino acid motifs that are conserved in some classes of GPCRs. Hydrophathy analysis indicates that GCR1 has seven potential transmembrane spanning domains and membrane topology prediction algorithms support a structure characteristic of GPCRs (figure 4).

5. PLANT G PROTEINS AND SIGNAL TRANSDUCTION

Most efforts that have sought to define the function of plant G proteins have relied on the use of pharmacological agonists and antagonists. These include (a) the hydrolysis resistant GTP analogues, GTP-γ-S and GDP-β-S that respectively hold the Gα subunit in active and inactive conformations, (b) the G protein agonist Mas7 that mimics the third cytoplasmic loop of activated GPCRs [10, 11], and (c) the bacterial cholera and pertussis toxins that ADP-ribosylate specific amino acids of certain classes of Gα subunit and either activate (CTX) or inactivate (PTX) them. There
is conclusive evidence that some of these agents affect the activity of inward K⁺ channels implying that this type of channel may be subject to G protein regulation. In guard cells, inward K⁺ channel activity is modulated by G protein agonists and antagonists in a complex manner. Current interpretations of the data are that multiple G protein signalling pathways probably regulate inward K⁺ channels in guard cells [2]. Guard cells are not the only cell types in which inward K⁺ channels are G protein regulated. The activity of two classes of inward K⁺ channels in plasma membrane patches isolated from barley xylem parenchyma protoplasts can be specifically stimulated by the hydrolysis resistant G protein agonist guanosine 5'-[β,γ-imido]triphosphate [45]. In a more direct approach with patch clamped plasma membrane, Aharon et al. [1] have demonstrated a stimulation in Ca²⁺ channel activity by recombinant tomato TGA1 suggesting that plasma membrane Ca²⁺ channels may also be regulated by G proteins in plants. Another molecular target of plant G proteins appears to be phospholipase D [28, 29].

Similar types of investigations have suggested G protein involvement in blue and red light signalling [6, 32, 38, 44], responses to fungal elicitors [21], pathogen resistance and pathogen-related gene expression [3], nodulation factor induction of MiENOD12 gene expression [35] and the transduction of plant hormones [5, 15, 50].

5.1. G proteins and auxin signalling

The molecular basis of auxin signal transduction is largely unsolved although a range of evidence points to a plasma membrane-based signalling system for certain auxin responses [30]. Some of the earliest evidence suggesting a possible involvement of G proteins in plant hormone signalling came from studies with auxin [50]. They observed that indole-3-acetic acid (IAA) increased the binding of [³⁵S]-GTPγS to rice coleoptile membrane vesicles while naphthyl-2-acetic acid, which has a lower biological activity than IAA, did not affect [³⁵S]-GTPγS binding. Using a vesicles and suggested that this might indicate that G protein activation inhibits IAA binding by a receptor. There has also 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 [5] though this has not been developed further.

An early event in auxin signal transduction appears to be the activation of phospholipase A₂ [33], although it is not yet clear how auxin activates this enzyme. The fact that mastoparan and melittin can partially mimic auxin-stimulated elongation growth and activate phospholipase A₂ suggests that a G protein may be involved; however, the possibility that these peptides are acting directly on the phospholipase A₂ cannot be discounted [39]. In summary, there is a possibility that G proteins may be involved in auxin signalling, although at present the evidence is not entirely compelling.

5.2. G proteins and gibberellin signalling

Efforts to understand GA signal transduction have concentrated on the isolation and characterisation of GA-response mutants and the GA induction of hydro-lase gene expression in aleurone cells [12]. In aleu-

rone, evidence from two quite different experimental approaches suggests that GAs are perceived at the plasma membrane [12] and although identification of a GA receptor has not yet been accomplished, plasma membrane located GA-binding proteins have been identified by GA photoaffinity labelling [22]. In GA treated aleurone, events occur that may be components of GA signalling downstream of a plasma membrane located GA receptor (reviewed by Bethke et al. [4]). These include an increase in cytoplasmic Ca²⁺ concentration that appears to result from Ca²⁺ influx from the apoplast [7, 8]. This is followed by a transient increase in cGMP [34] that appears to be necessary for GA-regulated gene expression. This leads to stimulation of the level of transcript for GAMyb, a transcription factor capable of transactivating gene expression from the GA response element present in α-amylase
drive the expression of an α-Amy2/54:GUS promoter: reporter construct. It stimulates α-amyrase enzyme production and secretion with a virtually identical time course to GA and, like GA, the effect of Mas7 is overcome by ABA. The inactive mastoparan analogues, MasCP (control peptide), differing from Mas7 by a single amino acid substitution, and Mas7-COOH, in which a free acid replaces the amine group at the C terminus, do not induce α-amyrase. Mas7 is therefore an effective GA mimic. Does it mimic GA by activating a G protein or is it interacting with some other component of GA signalling?

Jones et al. [15] addressed this question by studying the effects of the hydrolysis-resistant guanine nucleotides GTP-γ-S and GDP-β-S on GA-induction of α-Amy2/54:GUS expression. These nucleotide analogues bind to Gα subunits and hold them in either the active (GTP-γ-S-bound) or inactive (GDP-β-S-bound) form. GDP-β-S introduced into aleurone protoplasts during transfection with reporter gene constructs completely prevented GA1 induction of α-Amy2/54:GUS expression, whereas GTP-γ-S slightly stimulated expression. Taken together the effects of Mas7 and guanine nucleotide analogues on GA-induction of α-amyrase and α-Amy2/54:GUS expression in wild oat aleurone protoplasts suggest that a G protein or G proteins are involved in GA signal transduction in this tissue.

Because GPA1 is not thought to be expressed in mature seeds [14, 46], Jones et al. [15] used PCR to clone a partial Gα subunit cDNA (AfGα1) and two related Gβ cDNAs (AfGβ1 and AfGβ2) from aleurone of mature wild oat seed. Northern blot analysis confirmed that these are expressed in aleurone cells. The deduced amino acid sequence of AfGα1 is 40% identical to GPα1 [25]. The amino acid sequence of one of the Gβ subunits, AfGβ1, is 91% identical to the maize Gβ subunit ZGβ1 [47].

6. GCR1 AND CYTOKININ SIGNALLING

Antisense suppression of the expression of the plant GPCR homologue GCR1 in transgenic Arabidopsis gave rise to a specific reduction in the sensitivity to the cytokinin benzyladenine (BA) [36]. Reduced sensitivity was observed with a range of concentrations of BA in the inhibition of both root growth and hypocotyl etiolation [36]. Responses to other plant hormones were unaffected by antisense suppression of GCR1. This suggests a role for GCR1 in either the perception or transduction of this plant hormone [36].

It is not known if GCR1 is a cytokinin receptor. It might instead be a downstream component in cytokinin signalling, or a receptor for a different ligand, whose signalling pathway interacts with cytokinin signalling. Another candidate cytokinin receptor is CKI1 that has sequence similarity to histidine-kinase two-component response-regulators [17]. Further research is needed to establish the function of, and relationship between, GCR1 and CKI1 although a number of possible models can be envisaged (figure 5).

Figure 5. Diagram depicting some of the possible relationships between GCR1 and CKI1. A, GCR1 and CKI1 may be receptors, or downstream signalling components, in the transduction of different cytokinin ligands; B, either GCR1 or CKI1 may be a cytokinin receptor in a pathway subject to crosstalk from a signalling pathway involving the other protein; C, either GCR1 or CKI1 may be a cytokinin receptor with the other protein as a downstream signalling component.
Although GCR1 has some of the characteristics of a GPCR, there is as yet no proof that it functions in a manner analogous to GPCRs. Therefore, it is important to determine not only the ligand that it binds but also proteins with which it interacts. One candidate is of course the Arabidopsis Gα subunit homologue GPA1 but this needs to be tested experimentally.

7. SUMMARY

In summary, a range of biochemical and molecular evidence suggests that plants may have a functional G protein signalling pathway. Pharmacological and molecular evidence argues that the action of three classes of plant hormones may be mediated, or strongly affected, by G protein signalling. Although persuasive, the present data is far from conclusive. Many further questions need to be answered, not least of which why G proteins are implicated in so many signalling events in plants, yet only a single GPCR homologue and at most two Gα subunits have so far been identified in plants.

Acknowledgments

IACR receives grant-aided support from the Biotechnology and Biological Sciences Research Council (BBSRC) of the United Kingdom. This work was supported by the BBSRC Intracellular Signalling programme, the BBSRC Integration in Cellular Responses Programme and European Union Framework IV Programme.

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