Cell

Two Novel GPCR-Type G Proteins Are Abscisic Acid Receptors in *Arabidopsis*

Sona Pandey,[1,2] David C. Nelson,[3,4] and Sarah M. Assmann[1,*]
[1]Biology Department, 208 Mueller Laboratory, Penn State University, University Park, PA 16802, USA
[2]Donald Danforth Plant Science Center, St. Louis, MO 63132, USA
[3]Biotechnology Center and Biochemistry Department, University of Wisconsin, 425 Henry Mall, Madison, WI 53706, USA
[4]Present address: Plant Energy Biology, University of Western Australia, 35 Stirling Highway, Crawley 6009 WA, Australia
*Correspondence: sma3@psu.edu
DOI 10.1016/j.cell.2008.12.026

SUMMARY

In plants, G proteins modulate signaling by the stress hormone, abscisic acid (ABA). We identify and characterize two novel *Arabidopsis* proteins that show homology to an orphan vertebrate GPCR (GPR89) and interact with the sole *Arabidopsis* G protein α subunit, GPA1, but also have intrinsic GTP-binding and GTPase activity. We have named these proteins *G*PCR-*t*ype *G* proteins (GTG1 and GTG2). *Arabidopsis* mutants lacking both *GTG1* and *GTG2* exhibit ABA hyposensitivity. GTG1 and GTG2 bind ABA specifically. The GDP-bound form of the GTGs exhibits greater ABA binding than the GTP-bound form, the GTPase activity of the GTGs is inhibited by GPA1, and *gpa1* null mutants exhibit ABA-hypersensitive phenotypes. These results predict that, unusually, it is the GDP-bound, not the GTP-bound, form of the GTGs that actively relays the signal. We propose that GTG proteins function both as a new type of G protein and as a class of membrane-localized ABA receptors.

INTRODUCTION

Signal transduction processes mediated by G protein signaling components constitute one of the most elaborate receptor-effector signaling networks (Offermanns, 2003). The central components of this network are heterotrimeric G proteins, comprised of Gα, Gβ, and Gγ subunits, and G protein-coupled receptors (GPCRs). The Gα subunit has both GTP-binding and GTPase activity and acts as a bimodal molecular switch, typically with a GDP-bound "off" mode and a GTP-bound "on" mode. GPCRs classically act as *g*uanine nucleotide *e*xchange *f*actors (GEFs), and a change in GPCR conformation upon signal perception leads to exchange of GDP for GTP at the Gα subunit. This promotes dissociation of the heterotrimer into free GTP-Gα and Gβγ dimers, both of which can interact with an array of downstream signaling elements. The intrinsic GTPase activity of Gα regenerates its GDP-bound form, permitting reassociation with the Gβγ dimer and completing the cycle (Cabrera-Vera et al., 2003). Accessory proteins also regulate the G protein cycle, most prominently the *G*TPase-*a*ccelerating *p*roteins (GAPs) exemplified by RGS (*r*egulators of *G* protein *s*ignaling) proteins (Ross, 2008) and the *g*uanine nucleotide *d*issociation *i*nhibitor (GDI) proteins that primarily inhibit dissociation of GDP from Gα. Diversity in mammalian G protein signaling is achieved by a large combinatorial repertoire of G protein signaling components and the range of effectors with which they interact. In human, there are 23 Gα, 5 Gβ, and 12 Gγ subunit proteins and >800 predicted GPCRs, generating a multitude of receptor-G protein combinations (Offermanns, 2003). Fungi and plants, however, have limited numbers of G protein components. The genome of the model plant *Arabidopsis thaliana* contains one prototypical Gα (GPA1), one Gβ (AGB1), and two identified Gγ (AGG1 and AGG2) subunits (Jones and Assmann, 2004) and one RGS protein, AtRGS1 (Chen et al., 2003). GCR1 is the best characterized GPCR-like protein in *Arabidopsis* (Pandey and Assmann, 2004), although no ligand has yet been identified for it.

Despite the paucity of components, G proteins are involved in numerous fundamental growth and developmental processes in plants (Assmann, 2004). There is also evidence suggesting that plants have evolved a scheme with added temporal and spatial aspects that allows a limited number of G protein components to act as nodes for integration and amplification of a host of abiotic, biotic, and hormonal signals (Assmann, 2004).

Based on phenotypic analyses of null mutants, G proteins modulate almost all aspects of ABA signaling in plants (Wang et al., 2001; Coursol et al., 2003; Pandey and Assmann, 2004; Pandey et al., 2006). While biochemical evidence supports the presence of both cell-surface and intracellular receptor(s) for ABA (reviewed in Assmann, 1994), a direct receptor-effector signaling module regulated by G proteins during ABA signaling has remained elusive. The intracellular ABA-binding proteins, FCA and CHLH (Razem et al., 2006; Shen et al., 2006), bear no hallmarks supporting G protein coupling, and the recent report of GCR2 as a G protein-coupled ABA receptor (Liu et al., 2007) seems to be incorrect in its conclusion that GCR2 harbors transmembrane domains (Illingworth et al., 2008; Johnston et al., 2007b). In addition, ABA-related phenotypes are mild to absent in *gcr2* mutants (Gao et al., 2007; Guo et al., 2008). Such data suggest that important ABA receptors in plants remain to be identified.

Additional GPCR-like proteins exist in plants (Gookin et al., 2008 and references therein). Our in silico analyses identified

two new GPCR-like proteins in *Arabidopsis*, At1g64990 and At4g27630, that show extensive sequence homology to a human orphan receptor, GPR89, but also have some unique features. In addition to a predicted GPCR-like topology and sequence similarity to GPR89, both proteins also have a predicted ATP-/GTP-binding domain and a degenerate GTPase-activating protein domain. We have named these proteins *G*PCR-*t*ype *G* proteins 1 and 2 (GTG1 and GTG2). We find that the GTG proteins exemplify a novel class of proteins with topology similar to GPCRs but with classic GTP-binding/GTPase activity. We provide biochemical and phenotypic evidence that GTG1 and GTG2 proteins are redundantly involved in G protein-coupled ABA signaling and are, or are parts of, ABA receptor complexes.

RESULTS

GTG1 and GTG2 Are *Arabidopsis* Homologs of Orphan GPCR, GPR89

GTG1 (At1g64990) and GTG2 (At4g27630) show 90% amino acid sequence identity with each other and 45% identity and 68% similarity with GPR89. The similarity extends throughout the length of the proteins (Figure 1A). BLAST analysis identifies close homologs of these proteins in monocot and dicot plants, vertebrates, invertebrates, fungi, and unicellular organisms. The *Arabidopsis* GTG proteins show about 80% identity and 90% similarity at the amino acid level with their plant homologs, whereas 40%–45% sequence identity and 60%–70% sequence similarity are observed with vertebrate and nonvertebrate animals. Lower homology is observed with fungi and unicellular organisms (about 20% identity and 40% similarity). To assess the evolutionary relationship between these proteins we performed a phylogenetic analysis (Figure 1B). Plants form a separate clade from other organisms and the unicellular green alga *Chlamydomonas* groups with the other lower organisms, suggesting a possible sequence divergence when unicellular and multicellular organisms diverged.

PROSITE motif analysis (http://www.expasy.ch/prosite/) identified a conserved ATP-/GTP-binding region in GTG1 and GTG2 (Figure 1A, region 382–411 for GTG1). This motif is highly conserved in all the plant proteins; however the similarity with non-plant proteins is relatively low with little conservation of the first nine amino acids at the junction of the predicted 4th intracellular loop. The polar charged glutamic acid at position 396 of GTG1 is conserved in all plant species but is replaced by a neutral, uncharged amide (glutamine) in the non-plant proteins. The large third intracellular loop of GTG1 and GTG2 also has a region showing similarity to the degenerate Ras GTPase-activating protein domain ([GSNA]-x-[LIVMF]-[FYCI]-[LIVMFY]-R-[LIVMFY](2)-[GACNS]-[PAV]-[AV]-[LIV]-[LIVM]-[SGANT]-P) with 68% and 62% similarity, respectively (amino acids 230–243 in GTG1). This region also shows high sequence conservation in all the plant proteins analyzed but is divergent elsewhere.

Both GTG1 and GTG2 have nine predicted transmembrane domains, similar to human GPR89 (Figure 1C; Table S3 available online). As anticipated, the FLAG epitope-tagged versions of both GTG1 and GTG2 proteins are detected mostly in the total microsomal fractions isolated from transgenic *Arabidopsis* plants (Figure 1D). Transient expression in *Arabidopsis* mesophyll protoplasts shows localization of GFP-tagged GTG1 and GTG2 at the cell periphery (Figure 1E).

GTG1 and GTG2 Have Specific GTP-Binding and GTPase Activity

Purified recombinant GTG1, GTG2 (Figure S1), and GPR89 proteins were analyzed for GTP-binding and GTPase activity using a real-time assay based on BODIPY-GTPγS or BODIPY-GTP, in which fluorescence increases upon binding of the fluorescently labeled nucleotide and decreases upon GTP hydrolysis (Willard et al., 2005). We first validated this method using commercially available bovine G protein (Figures 2A and 2B). As expected, the bovine protein showed specific binding of a nonhydrolyzable GTP, BODIPY-GTPγS, that could be competed with unlabeled GTP or GDP but not with unlabeled ADP (Figure 2A). The bovine G protein also showed GTPase activity against BODIPY-GTP that was competed by unlabeled GDP or GTP but not by unlabeled ADP (Figure 2B).

We then analyzed the *Arabidopsis* GTG proteins and human GPR89 for GTP binding and hydrolysis. GTG1 and GTG2 both show specific GTP binding that could be efficiently competed by nonfluorescent GDP or GTP but not by ADP (Figures 2C and 2E). Confirming binding specificity, both GTG proteins showed efficient binding with BODIPY-GDP, which could be competed with nonfluorescent GTP but not with nonfluorescent ATP. No binding with BODIPY-ATP or effect of ATP on BODIPY-GTP binding were observed (Figures S2 and S3). GTP binding was also independently confirmed by [^{35}S]GTPγS-binding assays (Figure S6C).

The GTG proteins also show GTPase activity (Figures 2D and 2F). We independently confirmed their GTPase activity by assaying the production of free Pi using the ENZchek phosphate assay kit (Figure S4) and by quantification of [^{32}P]GTP hydrolysis using thin-layer chromatography (Figure S6B). The GTPase activity of the two GTG proteins is Mg^{2+} dependent as the presence of 2 mM EDTA (free Mg^{2+} concentration 0.8 mM) abolished GTPase activity (Figures 2D, 2F, and S5), and it has a broad pH optimum (Figure S6). Recombinant human GPR89 did not exhibit GTP binding or GTPase activity under our assay conditions (Figures 2D and S4). Together, these assays firmly establish that the newly identified GTG1 and GTG2 proteins have specific GTP-binding and GTPase activity, hallmarks of signaling G proteins.

GTG1 and *GTG2* Are Widely Expressed

To evaluate the in planta functions of the *GTGs*, we first analyzed their expression patterns. Quantitative PCR analysis showed widespread expression of both *GTG* genes (Figure 3A). These results were corroborated by GUS reporter gene analysis. GUS staining was observed in all plant organs analyzed: cotyledons, leaves, stems, roots, flowers, and guard cells (Figures 3B and 3C). *GTG1* and *GTG2* transcript levels did not change after treatment with ABA or different abiotic stresses (Figure 3D; analysis of AtGenExpress microarray data [not shown]).

Isolation of T-DNA Insertional Mutants and Complementation

We pursued a reverse genetic approach to decipher GTG1 and GTG2 protein functions in planta. Single T-DNA insertional