Arabidopsis Phospholipase Da1 Interacts with the Heterotrimeric G-protein α-Subunit through a Motif Analogous to the DRY Motif in G-protein-coupled Receptors*

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Phospholipase D (PLD) and heterotrimeric G-protein both play important, diverse roles in cellular regulation and signal transduction. Here we have determined the physical interaction between plant PLD and the only canonical α-subunit (Ga) of the G-protein in Arabidopsis thaliana and the molecular basis for the interaction. PLDα1 expressed in either Escherichia coli or Arabidopsis was co-precipitated with Ga. PLDα1 contains a sequence motif analogous to the Ga-interacting DRY motif normally conserved in G-protein-coupled receptors. Mutation of the central Lys residue PLD K564A of this motif abolished the PLDα1-Ga binding, whereas mutation of the two flanking residues PLD E563A and PLD F565A decreased the binding. Addition of Ga to PLDα1 inhibited PLDα1 activity, whereas the PLD E563A mutation that disrupted the Ga-PLDα1 binding abolished the inhibition. GDP relieved the Ga inhibition of PLDα1 activity and also inhibited the binding between PLDα1 and Ga. Meanwhile, the PLDα1-Ga interaction stimulated the intrinsic GTPase activity of Ga. Therefore, these results have demonstrated the direct binding between Ga and PLDα1, identified the DRY motif on PLDα1 as the site for the interaction, and indicated that the interaction modulates reciprocally the activities of PLDα1 and Ga.

Phospholipase D (PLD), which hydrolyzes phospholipids to phosphatidic acid and a head group, plays diverse roles in cellular metabolism and regulation. Plant PLD comprises a family of enzymes with different regulatory properties (1). Several Arabidopsis PLDs have been shown to display different requirements for Ca2+, polyphosphoinositides, and free fatty acids as well as varied substrate selectivity. Arabidopsis has at least 12 PLDs, of which PLDα1 is most prevalent and responsible for the common plant PLD activity (1, 2). PLDα1 produces a majority of the phosphatic acid under several stress conditions, such as freezing and wounding (3, 4). Suppression of PLDα1 delayed abscisic acid (ABA)-promoted senescence (5), decreased wound-induced accumulation of jasmonic acid (4) and reactive oxygen generation (6), and increased freezing tolerance and water loss (3, 7). These results show that the common plant PLD has multifaceted functions, including roles in metabolism and cell signaling, dependent on the nature and severity of the stress conditions.

The Ga subunit of heterotrimeric G-proteins plays an important role in signal transduction. In animal systems, Ga interacts with the upstream transmembrane G-protein-coupled receptors (GPCRs) and with the β-subunit. The binding of a ligand to a cognate receptor promotes the exchange of GDP for GTP on Ga, and the GTP-bound Ga activates the downstream effector proteins (8). In addition, Ga may interact with non-receptor proteins to mediate signaling (9). Mammalian cells contain a number of Gαs that mediate many distinctive cellular functions (8). In contrast, the number of Gαs is very limited in plants; Arabidopsis has only a single canonical Ga gene, GPA1 (10, 11). Arabidopsis Gα-null mutants are impaired in several cellular processes, including cell division, certain ABA-signaling steps in guard cells, and germination behaviors in response to glucose and hormones (12–14). These changes indicate that Ga is involved in multiple cellular processes in plants and thus may interact with multiple effector proteins for different functions. The only protein reported to interact with Ga in Arabidopsis is the cupin-domain protein AtPirin, which interacts with a CCAAT box-binding transcriptional factor (15). The mechanism by which plant Ga interacts with its targets and the function of the interaction are unknown.

A role of G-proteins in regulating plant PLD has been proposed recently. Most of the studies have involved the use of potential G-protein activators and inhibitors, such as toxins, nonhydrolyzable guanine nucleotide analogues, and alcohols (16, 17). In barley aleurone cells, a PLDα-like activity has been suggested to be associated with a G-protein on the plasma membrane to mediate ABA signaling (17). Co-incubation of bacterially expressed tobacco PLDs with Ga decreased the PLD activity (18). It has also been shown that ablation of either Gα or PLDα1 in Arabidopsis affects ABA-mediated stomatal movement and increases plant water loss (7, 12). These observations raise intriguing questions of whether PLDα1 and Ga directly interact with one another and, if so, what the molecular bases are for the interaction. Here we show that PLDα1 binds to Ga through a motif analogous to the DRY motif present in many GPCRs and that the binding modulates the activity of PLDα1 and Ga.

EXPERIMENTAL PROCEDURES

Expression and Purification of Active Plant PLDα in Escherichia coli—Arabidopsis PLDα1 cDNA was cloned previously in pBlue SK (19). The 2.4-kb PLDα1 cDNA was amplified by polymerase chain reaction and cloned into the pGEM T-easy vector. The forward and reverse primers were 5’-GCGGATCCATGGCCAGCATGTTTGACGC and...
5′-CCGAGGTCCTAGGTTAGAACCTAGGAGGACC, respectively, and the bold letters mark the inserted BamHI and SacI sites. The PLDΔ1 cDNA insert was digested with BamHI and SacI and ligated into the pET28(+) vector to produce PLDΔ1 with 6 histidine residues fused at the N terminus. The recombinant plasmid was transformed into *E. coli* BL21(DE3). Expression of PLDΔ1 was induced by 0.1 mM isopropyl-1-thio-β-D-galactopyranoside at room temperature for 12 h. After induction, bacteria were precipitated and lysed by sonication in phosphate-buffered saline plus 2 mM phenylmethylsulfonyl fluoride. Bacterial lysate was centrifuged at 12,000 × g for 10 min. The resulting supernatant was incubated with Ni-affinity agarose beads for 1 h. The beads were pelleted by centrifugation at 500 × g at 4 °C and washed three times with a washing buffer containing 20 mM Tris-HCl, 0.5 M NaCl, and 20 mM imidazole at pH 8.0. PLDΔ1 bound to the resin was eluted with an elution buffer with 1× imidazole, Tris-HCl, and 0.5 M NaCl. In some cases, PLDΔ1 bound to resin was used directly for assaying PLDΔ1 activity. Concentration of the purified PLDΔ1 was measured by the Bradford method using a Bio-Rad kit with bovine serum albumin (BSA) as a standard. The protein was stored in 20% glycerol at −20 °C until use.

Expression and Purification of Active Plant *G. hirsutum* cDNA in *E. coli*—An Arabidopsis *G. hirsutum* cDNA clone was generously provided by Dr. Hong Ma (Pennsylvania State University, University Park, PA) (20). The 1.3-kb cDNA was amplified by PCR and cloned into the pGEM T-easy vector. The forward and reverse primers were 5′-GAAATTCAGGGCTTACTCTGCAGTAGAA and 5′-CTCGAGTAATAAAGGCCCAGGCTC-CTCGAG, respectively, and the bold letters mark the inserted EcoRI and XhoI sites. The cDNA insert was digested with XhoI and EcoRI and ligated into pGEX-4T to produce *G. hirsutum* glutathione S-transferase (GST) fused at the N terminus. Junction of the GST-GPA1 fusion and full-length cDNA of *G. hirsutum* was confirmed by sequencing. The recombinant plasmid was transformed into *E. coli* BL21(DE3) to express the GST-fused *G. hirsutum* according to the procedure described previously (21).

Site-directed Mutagenesis of the DRY Motif in PLDΔ1—Mutagenesis of the three codons in the putative DRY motif of PLDΔ1 was performed using the QuikChange XL site-directed mutagenesis kit according to the manufacturer's instructions (Stratagene, La Jolla, CA). The three complementary reverse primers for mutating E563A, K564A, and F565A were as follows: 5′-GAATTGAAAGAGGGAAGAAGGTTGAGTCTAGGTG-CTAGTTGTTGG, 5′-GAATTGAAAAGGAGAAGGCGGTTCAGGTCCTGATTGTGG, and 5′-GAGAAGGAGAGAGGGCCAGGTCACTATGTGG-TGG, respectively. The wild-type PLDΔ1 cDNA in pET28(+) served as the PCR template. The mutant clones were verified by DNA sequencing and were then transformed into BL21(DE3) for protein expression. Expression and purification of the mutated PLDΔ1 proteins in *E. coli* were performed with the same procedure as that for the original PLDΔ1.

Protein Extraction from Arabidopsis—Fully expanded leaves of *Arabidopsis thaliana* (Columbia) plants were frozen with liquid N2 and homogenized with a buffer containing 50 mM Tris-HCl (pH 7.5), 80 mM KCl, 2 mM EDTA, 5 mM dithiothreitol, and 2 mM phenylmethylsulfonyl fluoride. The homogenate was centrifuged at 10,000 × g at 4 °C for 15 min, and the resultant supernatant was used as protein extracts for co-precipitation. Protein concentration in the supernatant was measured by the Bradford method using a Bio-Rad kit with BSA as a standard.

A.

**Fig. 1. Expression and purification of Arabidopsis *G. hirsutum* and PLDΔ1 in *E. coli***. A, purified GST-Ga, His-PLDΔ1, and GST resolved by a 10% SDS-PAGE gel and stained with Coomassie Blue. GST and His tag were fused to the N terminus of Arabidopsis *G. hirsutum* and PLDΔ1, respectively. B, GTPase activity of expressed Ga. Hydrolysis of GTP was assayed by measuring P1 release as a function of time and amounts of purified GST-Ga.

**Fig. 2. Binding of Ga with PLDΔ1 expressed in *E. coli***. A, co-precipitation of PLDΔ1 with GST-Ga. Top, immunoblotting of PLDΔ1 that was co-precipitated with GST-Ga. GST-Ga (0.05 μmol) was used to precipitate bacterially expressed His-PLDΔ1 (0.15 μmol). GST+PLDΔ1, PLDΔ1 precipitated with GST-bound beads (molar equivalent to the GST-Ga-bound beads). Total PLDΔ1, the starting PLDΔ1 used for precipitation. Ga+pET, bacterial lysate harboring an empty pET vector precipitated by Ga. Bottom, PLDΔ1 activity in the co-precipitates corresponding to those in the immunoblot above. PLDΔ1 activity was expressed as nmol of choline released/min of starting proteins. B, effect of different molar ratios of Ga to PLDΔ1 on the binding. Purified GST-Ga (0.05 μmol) was mixed with 0, 0.025, 0.05, and 0.1 μmol His-PLDΔ1 in a binding buffer followed by pulldown with glutathione beads. Top, immunoblotting of PLDΔ1 that was co-precipitated with GST-Ga. Middle, Coomassie Blue staining of GST-Ga in the precipitates resolved by 10% SDS-PAGE. Bottom, PLDΔ1 activity in the Ga precipitates, expressed as nmol of choline released/min from the same amount of precipitates.
involved the use of phosphatidylcholine (PC), 25 mM Ca\(^{2+}\), and 0.3 mM SDS (22). This assay is economical and specific for PLD\(\alpha\)1 and thus was used for most PLD\(\alpha\)1 assays unless otherwise stated. When the effect of

![Image](101x559 to 264x695)

**FIG. 3.** Binding of G\(\alpha\) with PLD\(\alpha\)1 from Arabidopsis leaf extracts. A, immunoblotting of PLD\(\alpha\)1 co-precipitated with G\(\alpha\). Increasing amounts of purified GST-G\(\alpha\) were added to plant extracts (PE; 200 \(\mu\)g/reaction) to precipitate PLD\(\alpha\)1. As a control, GST-bound beads (GST) were added to plant extracts and precipitated in the same manner. Precipitates pulled down with GST-G\(\alpha\) were subjected to 8% SDS-PAGE followed by blotting with PLD\(\alpha\)1 antibody. The PLD\(\alpha\)1 band was made visible by staining alkaline phosphatase activity conjugated to a second antibody. B, PLD\(\alpha\)1 activity in the GST-G\(\alpha\) precipitates corresponding to those in panel A. PLD activity was expressed as nmol of choline released/min/mg of starting plant proteins.

**RESULTS**

**Ga Binds to PLD\(\alpha\)1 Expressed in E. coli and Arabidopsis—**

Ga and PLD\(\alpha\)1 were expressed in E. coli as GST-fused and His-tagged proteins, respectively (Fig. 1A). Both PLD\(\alpha\)1 and Ga are catalytically active as indicated by the presence of their respective activities (Figs. 1B and 2A). When bacterial lysate harboring His-PLD\(\alpha\)1 was incubated with the lysate containing GST-Ga, PLD\(\alpha\)1 was pulled down together with GST-Ga by

![Image](272x39 to 563x440)

**FIG. 4.** The DRY motif of PLD\(\alpha\)1 and the amino acid residues involved in the PLD\(\alpha\)1 binding to Ga. A, schematic presentation of various domain structures of PLD\(\alpha\)1. C2 is a Ca\(^{2+}\)-dependent phospholipids-binding fold, HKD1 and HKD2 are the duplicated catalytic motifs. B, alignment of the DRY motif sequences between PLD\(\alpha\)1 and the chicken GPCR rhodopsin. C, PLD\(\alpha\)1 activity in wild type (WT) and the DRY motif mutants E563A, K564A, and F565A. Proteins expressed and purified from E. coli were assayed for PLD activity. D, immunoblotting of wild-type and mutated PLD\(\alpha\)s with PLD\(\alpha\)1 antibodies before Ga precipitation with glutathione beads. E, immunoblotting of wild-type and mutated PLD\(\alpha\)s in Ga precipitates from the same amounts of starting PLD\(\alpha\) proteins (0.2 \(\mu\)mol; see panel D). F, PLD activity in the Ga precipitates, expressed as nmol of choline released/min/mg of starting PLD\(\alpha\) proteins.
glutathione beads (Fig. 2A). The presence of PLDα1 in the precipitates was measured by immunoblotting with PLDα1-specific antibodies and assaying PLDα1 activity (Fig. 2A). PLDα1 was not pulled down with GST, indicating that the co-precipitation resulted from the presence of Go but not GST or the beads. To estimate the stoichiometry of the interaction, Go and PLDα1 were purified and co-incubated at different molar ratios (Fig. 2B). Increasing the ratio of PLDα1 to Go from 0.5 to 1 led to a doubling of the PLD co-precipitated with Go, whereas an increase in the molar ratio to 2:1 did not lead to a further increase in PLDα1 complexed with Go. These results indicate that PLDα1 binds to Go in a 1:1 ratio.

To demonstrate the association of Go with native PLDα1, the purified Go was incubated with Arabidopsis leaf protein extracts followed by precipitation with glutathione beads. Native PLDα1 was co-precipitated with Go as measured by immunoblotting and PLDα1-specific activity assays (Fig. 3). As a control, GST beads gave negligible binding to PLDα1 from plant extracts. The amount of PLDα1 co-precipitated with Go increased as the amount of Go was increased (Fig. 3), indicating that the Go and PLDα1 binding is dose-dependent.

**PLDα1 Contains a DRY-like Motif That Is Responsible for Its Binding to Go—**To determine the molecular basis for the PLDα1 and Go interaction, the PLDα1 sequence was analyzed for the presence of sequences with similarity to known G-protein-interacting motifs. At amino acid residues 562–586, the sequence of PLDα1 is highly similar to the DRY motif that has been found in more than 200 GPCRs (Fig. 4, A and B). This motif in GPCRs is located at the cytosolic juncture of the third transmembrane domain and is thought to associate with Go of heterotrimeric G-proteins (24, 25). The DRY motif consists of a core triplet of amino acids, D-R-Y, and also a highly conserved hydrophobic region, VYVV, located immediately downstream. The hydrophobic region is completely conserved in PLDα1 (Fig. 4B) and is conservatively modified in eight other Arabidopsis PLDs. The corresponding triplet amino acids in PLDα1 are conservatively substituted to EKF, which lies between the two duplicated, catalytic HKD motifs (Fig. 4A). Conservative substitutions of the DRY amino acid residues are allowable. For example, receptors in chicken and bullfrog both exhibit E-R-F (Fig. 4B).

The EKF residues were mutated individually to Ala to determine the involvement of each of the residues in Go binding. All of the three mutated PLDα1s displayed the PLD activity comparable with that of wild-type PLD (Fig. 4C), indicating that changing any of the residues to Ala results in no major alteration of PLD catalytic activity. GST-Go was then co-incubated with the same amounts of wild-type and mutated PLDα1s (Fig. 4D), followed by precipitation of Go with glutathione beads. Virtually no PLDα1K564A was co-precipitated as measured by the lack of PLDα1 protein (Fig. 4E) and activity (Fig. 4F) in the Go precipitates. The amount of mutated PLDα1s co-precipitated with Go was more than 3-fold lower than that of wild-type PLDα1 (Fig. 4, E and F). These results indicate that the residue Lys564 is essential for PLDα1 to interact with Go, whereas the flanking Glu563 and Phe565 are important for enhancing this interaction.

**Association of Go with PLDα1 Decreases PLDα1 Activity—**To test the effect of the Go binding on PLDα1 activity, purified Go

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**Fig. 5. Effect of Go on PLDα1 activity.** A, effect of Go on the PLD activity of wild-type (WT) PLDα1 and mutated PLDα1s expressed in E. coli. Purified PLDα1s were co-incubated with GST-Go or BSA (0.05 μmol each) for 30 min. As controls, equivalent molar amounts of GST-bound beads (GST) and His tag-bound beads (pET) were assayed for PLDα1 activity. B, effect of Go on PLDα1 activity in plant extracts. GST-Go beads (0.05 μmol of GST-Go) or the same molar amounts of GST beads and BSA were added to plant protein extracts (200 μg). PLD activity was assayed using the PC-PIP2 method.

**Fig. 6. Effects of guanine nucleotide analogues on PLDα1 activity.** A, dose-dependent increase and decrease of PLDα1 activity by GTPγS and GDPβS, respectively, in the presence of Go. B, GTPγS (5 μM) stimulation of PLD activity of bacterially expressed PLDα1 and its mutants. WT, wild-type. C, GDPβS (5 μM) inhibition of PLD activity of PLDα1 and its DRY motif mutants. Equal molar amounts of bacterially expressed, purified PLDα1 and Go (0.17 μmol each) were co-incubitated in a binding buffer for 30 min before the assays. PLD activity was assayed using the PC-PIP2 method.
was co-incubated with wild-type and mutated PLDα1s at approximately a 1:1 molar ratio, followed by assaying PLDα1 activity (Fig. 5). Addition of Go decreased the wild-type PLDα1 activity by more than 3-fold, whereas addition of the same amount of an unrelated protein, BSA, had no effect, indicating that the Go inhibition is not a nonspecific protein effect (Fig. 5A). The Go inhibition of PLDα1K566A or PLDα1K566E was not as severe as that of wild-type PLDα1, and the lower degree of inhibition is consistent with the decreased Go binding exhibited by these mutated PLDs. Go had the least inhibitory effect on PLDα1K566A (Fig. 5A), which exhibited very little binding to Go (Fig. 4, E and F). The extent of Go inhibition of PLDα1 is conversely correlated with the binding ability of the various PLDα1s to Go, indicating that the binding is required for the Go inhibition of PLDα1 activity.

The Go inhibition of PLD activity was also observed with proteins from Arabidopsis (Fig. 5B). When purified GST-Go was co-incubated with Arabidopsis leaf protein extracts, Go decreased ~60% of PLDα1 activity, whereas co-incubation with the same molar amount of GST or BSA had no effect on PLD activity. This result indicates that the inhibition is caused by Go, not by GST or agarose beads present in the solution. The extent of inhibition for the leaf PLD was smaller than that of the bacterially expressed PLDα1, which could be caused by having other PLDs in plant extracts, as all Arabidopsis PLDs are active under the PC-PIP2 assay conditions (1, 2). In addition, because the molar amount of PLDα1 in plant extracts was difficult to determine, the presence of excessive PLDα1 could also contribute to less inhibition of PLD by Go.

**Guanine Nucleotides Affect the Ga-PLDα1 Binding and PLDα1 Activity**—Ga can bind GDP and GTP; it is GDP-bound in the resting state, whereas an exchange of GDP for GTP is associated with the activation of Ga. To determine whether guanine nucleotides affect the Ga-PLDα1 interaction, purified PLDα1 and Go were co-incubated at a 1:1 molar ratio in the presence of different concentrations of the hydrolysis-resistant analogues GDPβS and GTPγS. GDPβS gave a dose-dependent inhibition, whereas GTPγS yielded a dose-dependent stimulation of PLDα1 activity (Fig. 6A). When GDPβS or GTPγS was added to the DRY motif mutants and Go complexes, the activity of PLDα1K566A was not affected significantly (Fig. 6, B and C; GST versus K564A). Compared with that of wild-type PLDα1 and PLDα1K566A, GDPβS and GTPγS had an intermediate inhibitory and stimulatory effect, respectively, on the activity of PLDα1K566A and PLDα1F565A (Fig. 6, B and C; wild-type (WT) versus E563A and F565A). Binding results showed that PLDα1K566A was unable to bind to Go and that PLDα1K566A and PLDα1F565A had decreased binding (Fig. 4E). Thus, these nucleotide effects on PLD activity are in agreement with the Go binding abilities of these PLDs, indicating that guanine nucleotides modulate PLD activity through their effects on the Go and PLDα1 binding.

The effect of guanine nucleotides on the Ga-PLDα1 binding was further determined using Arabidopsis leaf extracts (Fig. 7). Consistent with the results shown in Figs. 1–6, Go was able to bind to PLDα1 without any added guanine nucleotide. Adding GDPβS to plant protein extracts increased the amounts of PLDα1 co-purified with Ga as measured by immunoblotting and assaying PLDα1 activity in the co-precipitates (Fig. 7). Conversely, GTPγS decreased the Ga-PLDα1 binding in a dose-dependent manner. The inhibition of Go-PLDα1 binding by Gpp(NH)p, another guanosine triphosphate nucleotide analogue, was even greater than that by GTPγS; almost no PLDα1 was co-precipitated with Ga at 20 μM Gpp(NH)p. This difference could be attributed to the possibility that the Gpp(NH)p is more stable than GTPγS (Fig. 7). These results indicate that the binding of GTP to Ga, thus the activation of Ga, decreases the interaction between Ga and PLDα1, whereas Ga in the resting, presumably GDP-bound state binds to PLD.

**PLDα1 Binding Stimulates GTPase Activity**—To determine potential reciprocal effects of the Ga-PLDα1 interaction, the intrinsic GTPase activity of Ga was determined in the presence of wild-type PLDα1 or the non-Ga-binding mutant PLDα1K564A. Wild-type PLDα1 increased the GTPase activity by about 35%, whereas PLDα1K564A had no stimulatory effect on the GTPase activity (Fig. 8A). This result indicates that binding of PLDα1 to Go is required for the PLDα1 stimulation of Ga-mediated hydrolysis of GTP. The kinetics of the GTPase activity was assessed by varying the substrate GTP concentrations in the presence or absence of PLDα1 (Fig. 8B).
that G domains. Rather, PLD stimulates the GTPase activity of G also determines the PLD/H9251.

pressed tobacco G bind GTP-bound G/H9251. The results in a variety of modified phenotypes, including constitutive, moderate, and null receptor activities (24, 25). This raises the question of whether the DRY motif on plant PLDs competes with plant GPCRs from the receptor-Ga interaction. However, almost nothing is known about the coupling of Ga to GPCR in plants (10, 11). The only reported putative Arabidopsis GPCR is GCR1 (27), but its direct interaction with Ga has yet to be demonstrated. In addition, our sequence analysis indicates that Arabidopsis GCR1 does not contain the DRY motif. It has also been stated that the loss-of-function mutant of GCR1 (gcr1) showed no shared phenotypes with that of Ga (gpa1) and that Ga might not be coupled to GCR1 in the cell (11). Thus, the study on the role of the PLD–Ga interaction in the receptor-Ga interaction awaits identification of a receptor interacting with Ga in plants.

It is unlikely that PLDα1 itself serves as a canonical Ga-coupled receptor protein because of its lack of transmembrane domains. Rather, PLDα1 may function as an intracellular regulator of G-proteins. A number of regulator proteins have been identified in other organisms, which include GTPase-activating protein (GAP) and guanine nucleotide exchange factors (8, 9). Identified in other organisms, which include GTPase-activating proteins have been noted for some PLDs (32–34), which could be important determinants for the Ga interaction with different PLDs in different cells and timing. In addition, individual PLDs exhibit different requirements for Ca2+, PIP2, free fatty acids, and lipid environments for activity (1, 33, 35, 36). Thus, the other cellular factors might also modulate the PLD–Ga interaction and specificity. It should be noted that analyses of PLDα1-depleted (7) and Ga-null (12) Arabidopsis have shown some shared phenotypic alterations, such as retardation of stomatal movement and increasing plant water loss. It is likely that the PLDα1-Ga interaction documented in this study underlies a structural and molecular basis for their involvement in specific plant signaling pathways.

One intriguing question raised from the PLDα1-Ga interaction is whether the PLDα1-Gα interaction modulates the association of Ga with Gβγ. According to the mammalian heterotrimeric G-protein paradigm, Ga in the GDP-bound resting state associates with Gβγ, whereas Ga in the GTP-bound activated state dissociates from Gβγ (8). Some activators of G-protein signaling proteins identified in other organisms have been shown to bind to the resting or GDP-bound form of Ga to release Gβγ (28, 29). The present results demonstrate that PLDα1-Ga interaction occurs with or without added GDP, and thus, PLDα1 binds to the resting state, GDP-bound Ga. One canonical Gβ gene and two Gγ genes have been identified in Arabidopsis (30, 31). The association has been demonstrated for Gβ and Gγ subunits (31), but not for Ga and Gβγ. Further studies will be needed to identify the condition under which Ga interacts with Gβγ and then to determine whether the PLDα1 binding regulates the association and/or dissociation between Ga with Gβγ. Possibilities remain also that the association of Ga with Gβγ may affect the Ga–PLDα1 interaction.

Another question relevant to the understanding of the PLD–Ga interaction is the specificity of the Ga interaction with various PLDs. Except for two PLDαs and PLDβ2, none of the 12 PLDs in Arabidopsis have the DRY motif-like sequences (data not shown) and thus can potentially interact with Ga. Different patterns of temporal and spatial expression and distribution have been noted for some PLDs (32–34), which could be important determinants for the Ga interaction with different PLDs in various plants. In addition, individual PLDs exhibit different requirements for Ca2+, PIP2, free fatty acids, and lipid environments for activity (1, 33, 35, 36). Thus, the other cellular factors might also modulate the PLD–Ga interaction and specificity. It should be noted that analyses of PLDα1-depleted (7) and Ga-null (12) Arabidopsis have shown some shared phenotypic alterations, such as retardation of stomatal movement and increasing plant water loss. It is likely that the PLDα1-Ga interaction documented in this study underlies a structural and molecular basis for their involvement in specific plant signaling pathways.

Based on “Results” and “Discussion,” a working model is proposed for the role and further investigation of the PLDα1-Gα interaction (Fig. 9). PLDα1 binds to the resting state or GDP-bound Ga through the DRY motif. The PLDα1-Gα binding hinders PLDα1 activity and may also promote dissociation of Gβγ from Ga. In addition, the PLDα1-Ga binding
Thus, the association and dissociation between PLD and G/H9251 and G/H9251 would form a positive loop to stimulate the activity of PLD/H9251 to G change of GDP for GTP. At the same time, the binding of GTP stimulates GTPase activity, possibly via promoting the extransduction. PLDs and heterotrimeric G-protein in plant signal transduction will facilitate the understanding of the cellular roles of G-proteins. Further studies on elucidating this novel signaling process and identifying upstream regulators and downstream effectors of the interaction will facilitate the understanding of the cellular roles of PLDs and heterotrimeric G-protein in plant signal transduction.

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Note Added in Proof—A Go-interacting regulator of G-protein signaling (RGS) protein, designated AtRGS1, has been identified recently in Arabidopsis. AtRGS1 has a predicted structure similar to a GPCR and an RGS box with GTPase-accelerating activity (37).

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