Regulatory Functions of Phospholipase D and Phosphatidic Acid in Plant Growth, Development, and Stress Responses[1]

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Phospholipase D (PLD) hydrolyzes membrane lipids to generate phosphatidic acid (PA) and a free-head group (Fig. 1), and this activity is widespread in plants. Recent results indicate that PLD plays multiple regulatory roles in diverse plant processes, including abscisic acid (ABA) signaling, programmed cell death, root hair patterning, root growth, freezing tolerance, and other stress responses (Fig. 1). In some cases, direct molecular targets of PLD and PA have been identified, providing insights into the mechanism by which the phospholipase and lipid messenger mediate plant functions.

PLD is composed of a family of heterogeneous enzymes with distinguishable biochemical, regulatory, and structural properties. The *PLD* gene family in plants is more complex than that in other organisms: 12 *PLD* genes are in Arabidopsis (*Arabidopsis thaliana*), whereas two *PLD* genes are in mammals and one in baking yeast (*Saccharomyces cerevisiae*; Wang, 2002, 2004, and refs. therein). The 12 Arabidopsis *PLDs* can be classified into six types, *PLD*α(3), β(2), γ(3), δ, ε, and ζ(2) (Fig. 2). Based on the overall protein domain structures, PLDs can be divided into two subfamilies, C2-PLD and PX/PH-PLDs (Fig. 2). C2 is a Ca^{2+} and phospholipid-binding domain, and the PX and PH domains refer to two distinct phosphoinositide-interacting structural folds, phox homology and pleckstrin homology, respectively. Ten of the 12 Arabidopsis PLDs (α, β, γ, δ, and ε) contain the C2 domain. The PLDζs contain the PX and PH domains, and this domain structure is present in mammalian PLDs (Elias et al., 2002; Qin and Wang, 2002). The overall sequences of *PLD*ζs are more similar to mammalian *PLD*s than to other Arabidopsis *PLD*s.

In addition, new structural motifs have been identified in PLDs that interact with G proteins, Ca^{2+}, and phosphoinositides (Fig. 2; Zheng et al., 2002; Pappan et al., 2004; Zhao and Wang, 2004). Individual PLDs differ in key amino acid residues in these regulatory motifs. These differences underlie a structural basis for the distinguishable biochemical and regulatory properties in different PLDs. The information on the molecular, structural, and biochemical heterogeneity of PLDs provides clues as to the regulation and diverse functions of *PLD*s in plants. This *Update* focuses on the recent developments toward understanding the function of specific *PLD*s and PA that they produce.

PLDα1: A CONNECTION BETWEEN G PROTEIN AND PROTEIN PHOSPHATASE 2C

The most abundant plant PLD requires high millimolar concentrations of Ca^{2+} for activity in vitro. PLDα1 possesses the common PLD activity (Wang, 2002). A gene knockout of *PLD*α*1* abolishes the millimolar Ca^{2+}-requiring PLD activity in Arabidopsis (Zhang et al., 2004), demonstrating that PLDα1 is the predominant one responsible for the common PLD activity in the plant. Arabidopsis deficient in PLDα1 displays alterations in various plant processes, such as reactive oxygen production, wound-induced accumulation of jasmonic acid, freezing tolerance, water loss, and abscisic acid signaling (for review, see Wang, 2002).

Recent results indicate that PLDα1 and its lipid product PA are intermediary links between important cellular regulators in plant cells (Fig. 3). PLDα1 interacts directly with Gα, the only canonical α-subunit of the heterotrimeric G protein in Arabidopsis (Zhao and Wang, 2004). PLDα1 binds to Gα through a sequence motif analogous to the DRY motif normally conserved in animal G-protein-coupled receptors. Mutation of amino acid residues in this motif abolishes the PLDα1-Gα binding and the Gα inhibition of PLDα1 activity. Meanwhile, the PLDα1-Gα interaction stimulates the intrinsic GTPase activity of Gα. These results indicate that the interaction reciprocally modulates the activities of PLDα1 and Gα.

Both *PLD*α*1* and *G*α are involved in ABA signaling. Gene suppression and overexpression results show that that *PLD*α*1* plays a role in the ABA response (Sang et al., 2001). A recent study has unveiled a mechanism by which *PLD*α*1* mediates such a response (Zhang et al., 2004). PLDα1-derived PA binds to ABI1 (ABA insensitive), a protein phosphatase 2C (PP2C) that is a negative regulator of ABA responses in Arabidopsis. The PA binding decreases PP2C activity and also appears to reduce its translocation to nuclei in re-

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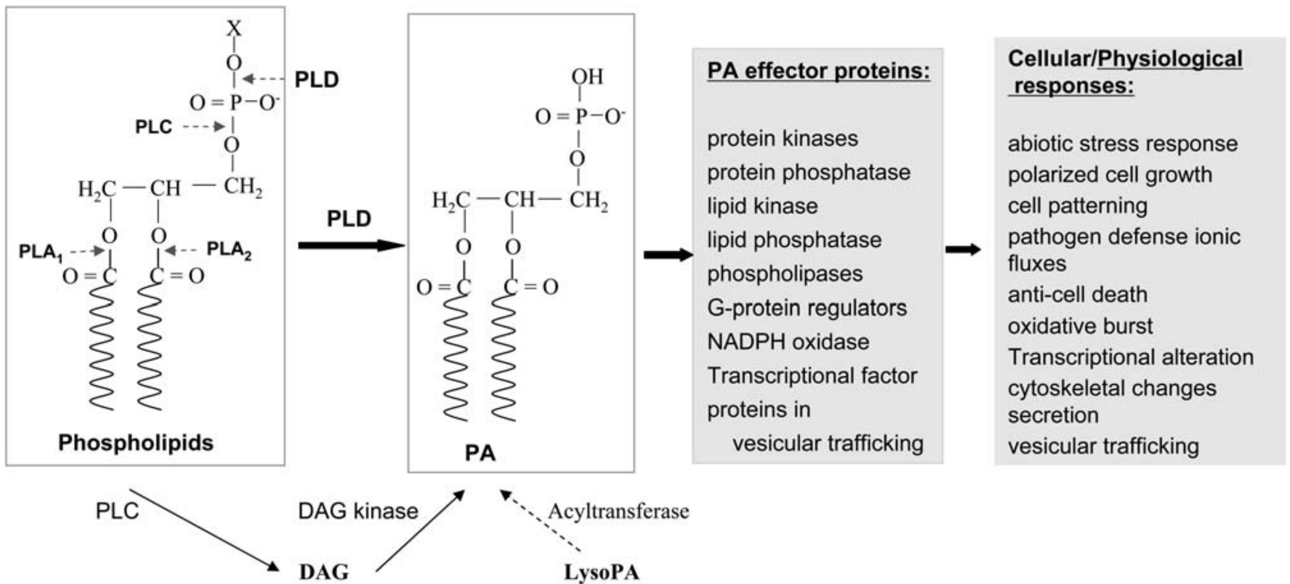

Figure 1. PLD-catalyzed hydrolysis of phospholipids, downstream targets, and cellular functions. The activity of PLD produces PA and free-head group (X). PA target proteins have been identified in animals, plants, and yeast (see also Table I). Multiple cellular effects of PLD and PA have been documented or implicated. Note that in addition to PLD, signaling PA can be generated from DAG kinase coupled to the activation of PLC and potentially from acylation reactions.

sponse to ABA by tethering ABI1 to the plasma membrane. These results show that activation of PLDα1 inhibits the function of the negative regulator ABI1, thus promoting ABA signaling (Fig. 3).

PLDδ POSITIVELY REGULATES STRESS TOLERANCE

PLDδ exhibits several properties that distinguish it from other PLDs. It is activated by oleic acid and is tightly associated with the plasma membrane and microtubule cytoskeleton. The expression of *PLD*δ increases in severe dehydration, high salt, and during cold acclimation. Analyses of *PLD*δ-altered Arabidopsis suggest that *PLD*δ positively regulates plant tolerance to stresses such as freezing, oxidative assault, and ultra-violet irradiation (Zhang et al., 2003; Li et al., 2004). *PLD*δ-null Arabidopsis is less tolerant to freezing, whereas overexpression of *PLD*δ increases freezing tolerance (Li et al., 2004). Lipid profiling indicates that PLDδ activity produces selective PA species but does not result in substantial lipid hydrolysis during freezing stress.

By comparison, antisense suppression of *PLD*α*1* increases freezing tolerance, and lipid profiling shows that PLDα1 activity results in a large decrease in phosphatidylcholine (PC) and an increase in PA (Welti et al., 2002). These results indicate that *PLD*α*1* and δ are involved in the response to freezing via different mechanisms and also demonstrate that manipulation of different *PLD*s can have different phenotypic alter-

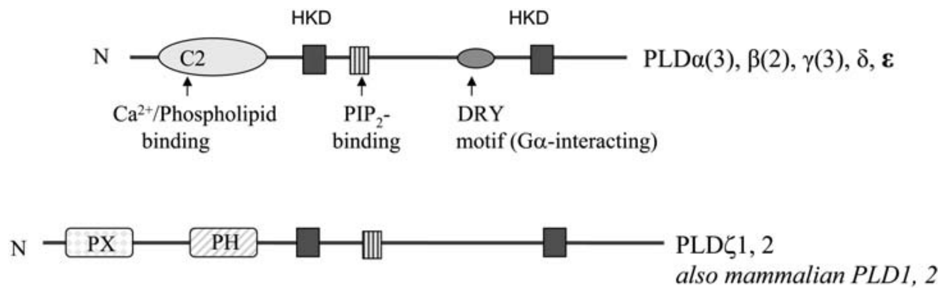

Figure 2. Domain structures of Arabidopsis PLDs. Plant PLDs consist of two distinctive subfamilies: the C2-PLDs and the PX/PH-PLDs. Individual PLDs can differ in key amino acid residues in regulatory motifs, such as C2, PIP_2 binding, and DRY. The duplicated HKD motifs are involved in catalysis. Note that the 12 Arabidopsis PLDs have now been classified into six types instead of five types; the only modification is that PLDα4 has been reclassified to PLDε because this PLD is quite distant from all the other PLDs.

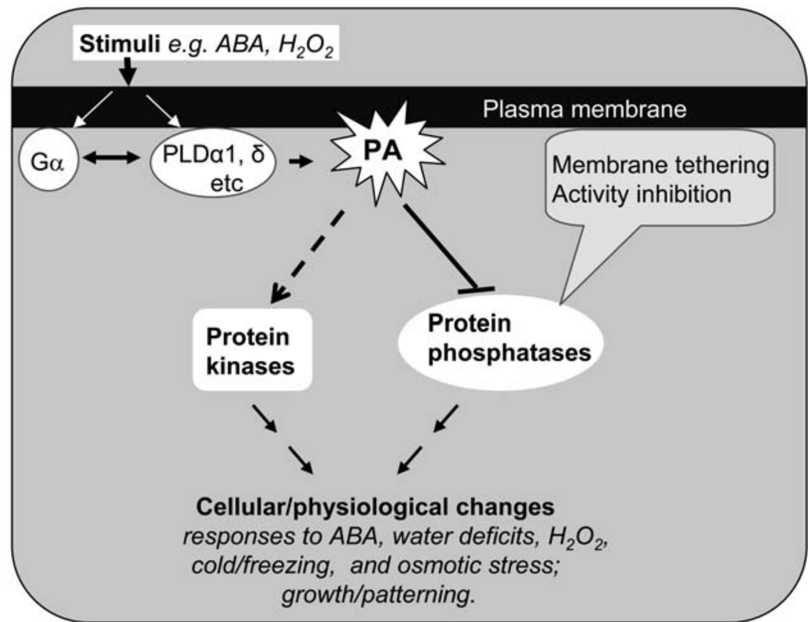

Figure 3. A working model depicting the activation of PLDs and the role of PA in regulating kinase and phosphatase activities in cell's response to ABA, H_2O_2, or other stimuli. Potential mediators in the PLD activation include changes in G protein function and membrane conformations and/or increases in cytosolic Ca^{2+}, phopshoinositides, and oleic acid. PLD-produced PA may stimulate protein kinase cascades and also inhibit protein phosphatases. PA may do so by directly affecting the enzymatic activities and/or by tethering the signaling enzymes to specific membranes or regions of a membrane. These interactions modulate the cell's ability to respond to stresses and inhibit cell death.

ations. In addition, the *PLDδ* alterations do not result in changes in the expression of the cold-regulated genes *COR47* or *COR78*, nor in any change in cold-induced increases in the levels of compatible osmolytes, Pro, or soluble sugars, which are known to play a role in plant freezing tolerance. These results suggest that PLDs and associated membrane lipid hydrolysis present a novel signaling pathway involved in mediating plant freezing tolerance.

PLD/PA IN REACTIVE OXYGEN RESPONSE, PRODUCTION, AND CELL DEATH

One mechanism by which *PLDδ* positively mediates plant stress tolerance is through its role in signaling resistance to damages inflicted by reactive oxygen species (ROS; Li et al., 2004). PLDδ is activated by hydrogen peroxide (H_2O_2), and the resulting PA functions to decrease H_2O_2-promoted programmed cell death (Zhang et al., 2003). Examining mitogen-activated protein (MAP) kinase activity suggests that PLDδ and PA play a role in H_2O_2-induced activation of MAP kinase cascades (Zhang et al., 2003). The ROS H_2O_2 is an important cellular mediator, and its concentration increases under various stress conditions, including freezing. Activation of MAP kinase cascades is involved in plant response to H_2O_2 and various stress responses. The involvement of PLDδ and its derived PA in these processes may present a mechanism by which *PLDδ* plays a positive role in stress response.

PLDδ is activated by H_2O_2, but it is not involved in H_2O_2 production under the conditions tested (Zhang et al., 2003). By comparison, PLDα1 and PA are involved in the production of reactive oxygen species (Sang et al., 2001). These results indicate that specific PLDs function in different steps in plant oxidative stress pathways; whereas PLDα1 promotes the ROS production, PLDδ mediates plant responses to ROS. The role of PA in ROS production is supported by another study: PA treatment of whole leaves and single cells increased the levels of ROS and promoted leaf cell death (Park et al., 2004). This study also suggests that PA promotes the ROS production via activating the Rho-related small G protein GTPase-mediated pathway. The PA-induced cell death appears contradictory to the role of PLDδ-derived PA in mitigating H_2O_2-induced cell death. However, the location and timing of PA production and appropriate cellular concentrations and molecular species of PA are important determinants of PA function.

PLDζ1 AND PA IN ROOT HAIR GROWTH AND PATTERNING

Unlike C2-containing PLDs, the PX/PH-containing PLDζ1 is Ca^{2+} independent and PC specific (Qin and Wang, 2002). A study of the molecular target of the homeobox gene *GLABRA2* (*GL2*) has provided insights into one physiological function of *PLDζ1* (Ohashi et al., 2003). *PLDζ1* is a direct target of *GL2*, a key component of a regulatory circuit composed of transcription factor genes that regulate the root hair pattern of Arabidopsis. *GL2* is thought to be a negative regulator of root hair development. Inducible expression of *PLDζ1* promoted ectopic root hair initiation, whereas inducible suppression inhibits root hair initiation (Ohashi et al., 2003). The results suggest that GL2 regulates root hair development through modulation of *PLDζ1*. However, loss of *PLDζ1* does not cause obvious changes in root hair patterning (M. Li, C. Qin, and X. Wang, unpub-

lished data). These results may indicate that more than one *PLD* could be involved in the root hair growth and development.

How *PLD* regulates the root hair patterning and initiation is not known. A recent study indicates that PA regulates a protein kinase pathway involved in root growth and initiation (Anthony et al., 2004). PA binds to the 3′-phosphoinositide-dependent kinase-1 (AtPDK1) and stimulates the activity of AGC2-1 in an AtPDK1-dependent manner. AGC refers to a kinase superfamily comprising the prototypes of the cAMP-dependent protein kinases A (PKA), cGMP-dependent protein kinases G (PKG), and protein kinases C (PKC). AGC2-1 is a target protein kinase of AtPDK1, and the AGC2-1 knockout plants exhibit a decrease in root hair length. It remains to be determined whether the AtPDK1-binding PA results from the activation of PLDs because signaling PA can be derived from diacylglycerol (DAG) kinase coupled to the activation of phospholipase C (PLC; den Hartog et al., 2003; De Jong et al., 2004) and potentially from acylation reactions (Fig. 1).

PLD INTERACTIONS WITH CYTOSKELETON

PLD and PA have been implicated in affecting both actin and tubulin cytoskeletons. Arabidopsis PLDβ1 binds to α-actin (Kusner et al., 2003). The effects of actin on the activities of the PLD are polymerization dependent; monomeric G-actin inhibits PLD activity, whereas polymerized F-actin augments PLD activity. Actin modulation of PLDβ1 has kinetic characteristics, efficacies, and potencies similar to those of human PLD1 (Kusner et al., 2003). This conservation of PLD-actin interaction between PLDs from plants and mammals indicates that other plant PLDs, such as PLDζs, may also bind to actin because of their close similarity to mammalian PLDs.

PA is reported to promote actin polymerization in soybean (*Glycine max*) cells (Lee et al., 2003). PA is found to bind a heterodimeric capping protein from Arabidopsis (AtCP; S. Huang and C.J. Staiger, personal communication). The PA-binding inhibits the activity of AtCP; in the presence of PA, AtCP is unable to cap the barbed or rapidly growing and shrinking end of actin filaments and fails to nucleate filament formation. The PA inhibition of capping-protein activity in plant cells results in stimulation of actin polymerization from a large pool of profilin-actin.

Following the finding that PLDδ binds to tubulin, two studies explored the effect of PLD on microtubule cytoskeleton organization. In one study, seeds and young seedlings of Arabidopsis were incubated with 1-butanol, and this treatment disrupted the organization of interphase cortical microtubules and also inhibited seed germination and seedling growth (Gardiner et al., 2003). In another study using tobacco (*Nicotiana tabacum*) Bright Yellow-2 cells expressing the microtubule reporter green fluorescent protein-MAP4, 1-butanol treatment promoted a partial depolymerization of cortical microtubules and the release of them from the plasma membrane (Dhonukshe et al., 2003). It has been postulated that PLD links microtubules via the PLD catalytic intermediate phosphatidyl moiety. Results from both of the studies suggest that activation of PLD is critical to triggering microtubule reorganization. It is likely that, besides PLDδ, other PLDs are involved in microtubule reorganization because the *PLDδ*-knockout plants grow and develop normally under regular growth conditions. However, *PLDδ*-null plants are less tolerant to stresses (Zhang et al., 2003; Li et al., 2004), and their role in cytoskeletal reorganization may contribute to the weakening of plant stress responses.

It is worth noting that butanol treatment takes advantage of the unique, transphosphatidylation activity of PLD, which uses primary alcohols as substrates to form phosphatidylalcohol at the expense of PA. Because there is no specific, effective inhibitor for PLD, this approach has been useful in helping to determine the cellular functions of the PLD activity. However, primary alcohols stimulate rather than inhibit PLD activity. Also, the diversion of PA formation by alcohol is incomplete. Thus, an effect associated with an alcohol treatment needs to be interpreted with caution because it may result from other alcoholic effects, such as increases in lipid hydrolysis, changes in lipid composition, and/or release of lipid head groups that have regulatory functions (Chapman, 2004).

PLD IN POLLEN TUBE GROWTH AND PRO ACCUMULATION

Tobacco pollen germination and tube growth are stopped in the presence 0.5% 1-butanol, and this inhibition could be overcome by addition of exogenous PA, suggesting that PLD-derived PA plays a role in stimulating pollen germination and tube elongation (Potocky et al., 2003). In another study, the expansion of the pollen tube apical region is associated with a severalfold increase in PA, which is generated by PLD (Zonia and Munnik, 2004). Different phospholipid signals are differentially stimulated or attenuated in pollen tube volume changes in response to the osmotic perturbations. Hypo-osmotic stress induces rapid increases in PA, but hyperosmotic stress and cell shrinkage inhibit PLD activity and induce increases in the levels of phosphatidylinositides.

In other cells or tissues, however, PLD is activated under hyperosmotic stresses, such as high salinity and dehydration (Testerink and Munnik, 2005). Under these stresses, many plants accumulate Pro, which has been suggested to play a role in plant stress adaptation. A recent study probed the role of PLD activation in the regulation of Pro metabolism in Arabidopsis (Thiery et al., 2004). Application of 1-butanol stimulated Pro biosynthesis without hyperosmotic constraints and enhanced the Pro responsiveness of seedlings to mild

hyperosmotic stress. Gene expression results suggest that the activation of PLD plays both positive and negative roles in hyperosmotic stress signaling in plants. These seemingly conflicting effects of PLDs are consistent with the hypothesis that different PLDs have unique functions during the stress responses (Fig. 4), as shown for the role of PLDα1 and PLDδ in the ROS stress and freezing tolerance (Sang et al., 2001; Welti et al., 2002; Zhang et al., 2003; Li et al., 2004).

NEW INSIGHTS ON CA^{2+} REGULATION OF PLD

All C2-PLDs require Ca^{2+} for activity, but how Ca^{2+} affects PLD activity is not well understood. It was previously reported that Ca^{2+} binds to the regulatory C2 domain that occurs in the N terminus of PLDs (Zheng et al., 2000). Using a C2-deleted PLDβ1 (PLDβcat) and other PLDβ1 domain constructs, a recent study has shown that Ca^{2+} also interacts with the catalytic regions of PLD (Pappan et al., 2004). The Ca^{2+}-PLDβcat interaction increases the affinity of the protein for the activator, phosphatidylinositol 4,5-bisphosphate (PIP_2), but not for the substrate, PC. This is in contrast to the effect of Ca^{2+} binding to the C2 domain, which stimulates PC binding but inhibits PIP_2 binding of the domain. The PIP_2-bound catalytic domain increases the enzyme's affinity for its substrate PC. These results demonstrate the contrasting and complementary effects of the Ca^{2+}- and lipid-binding properties of the C2 and catalytic domains of plant PLD, and provide insight into the mechanism by which Ca^{2+} regulates PLD activity. A membrane-scooting model has been proposed to explain the regulation of PLD activity by changing cellular Ca^{2+} concentrations. These findings also account in part for why Ca^{2+} is required for the activity of the C2-PLDs but not the PX/PH-PLDs (Pappan et al., 2004).

UNIQUE AND MULTIFACETED FUNCTIONS OF DIFFERENT PLDS

The distinguishable phenotypes resulting from loss of different PLDs indicate that the loss of one PLD is not compensated for, at least completely, by the other 11 PLDs in Arabidopsis (Zhang et al., 2003, 2004; Li et al., 2004). Unique functions of different PLDs may occur via one or a combination of the following (Fig. 4): (1) Individual PLDs are regulated and activated differently in the cell; (2) they have different substrate preferences and have the potential to generate different PAs or other derivatives; (3) they are associated

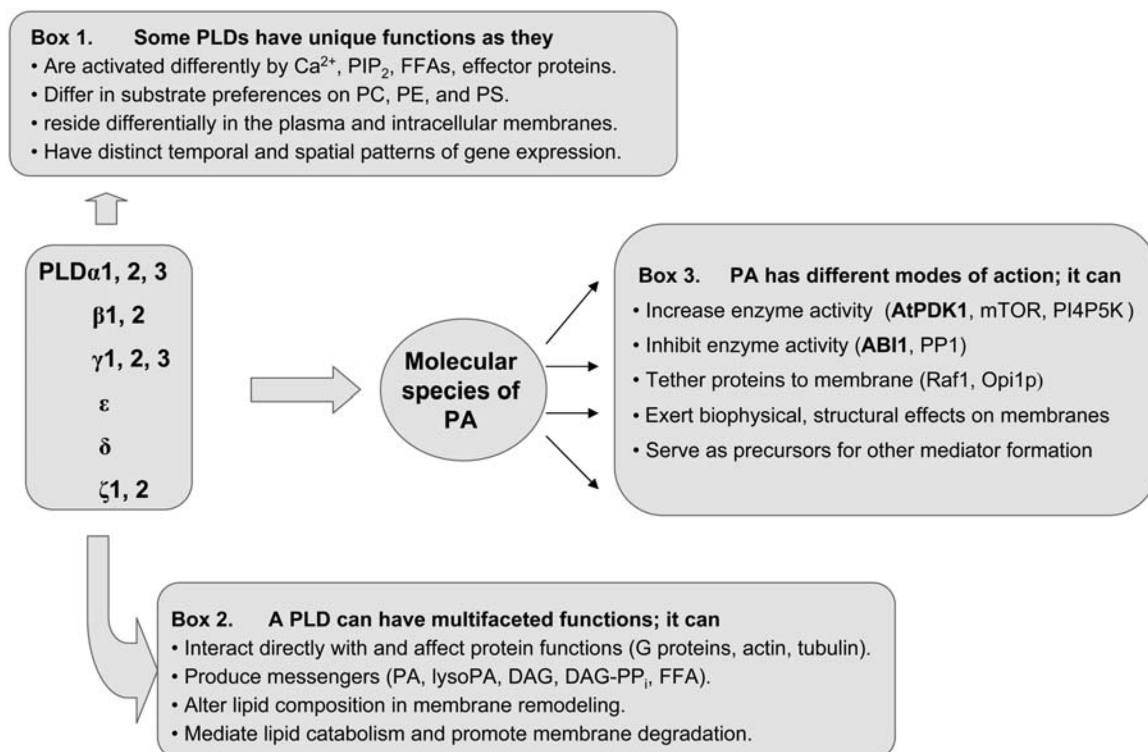

Figure 4. Unique roles of different PLDs, multifaceted functions of a PLD, and multiple modes of action of PA. Unique functions have been shown for PLDα1 and PLDδ. Box 1 lists some of the available findings that support different functions of PLDs. Box 2 indicates that a given PLD can be involved in different cellular processes, depending upon the nature of biotic and abiotic cues, such as the severity of stresses. Box 3 lists several effects that PA has on cell regulation. Documented examples are given in parentheses, and those in bold denote PA target proteins identified in plants. The symbol and function of the target proteins are explained in Table I and text. FFA, Free fatty acid; $DAG\text{-}PP_i$, DAG-pyrophosphate.

Table I. *Examples of PA-binding proteins identified in plants, animals, and yeast*

PA-Binding Protein	Protein Function	Reference
From plants:		
ABI1	Protein phosphatase 2C like	Zhang et al. (2004)
AtPDK1	3′-Phopshoinositide-dependent kinase 1	Anthony et al. (2004)
PEPC	Phospho*enol*pyruvate carboxylase	Testerink et al. (2004)[a]
From yeast:		
Opi1p	Soluble transcriptional repressor	Loewen et al. (2004)
From animals:		
mTOR	Mammalian target of rapamycin	Fang et al. (2001)
Raf1	MAP kinase kinase kinase	Ghosh et al. (2003)
Fgr	Tyr kinases expressed in neutrophils	Sergeant et al. (2001)
PKCz	Protein kinase Cζ	Cummings et al. (2002)
SHP-1	SH2-containing protein-tyrosine phosphatase	Frank et al. (1999)
PP1	Protein phosphatase-1	Jones and Hannun (2002)
Type1 PI4P5K	Phosphatidylinositol 4P-5 kinase	Jenkins et al. (1994)
RGS4	Regulators of G-protein signaling protein	Ouyang et al. (2003)
SPHK	Sphingosine kinase	Delon et al. (2004)
ARF	ADP-ribosylation factor	Manifava et al. (2001)
NSF	*N*-ethylmaleimide-sensitive factor	
Kinesin	Vesicular trafficking	
Phox 47	A cytosolic component of NADPH oxidase	Karathanassis et al. (2002)
PDE4D3	cAMP-specific phosphodiesterase	Grange et al. (2000)

[a]Several other proteins were also found to be associated with PA-affinity beads.

with different membranes; and (4) they have different temporal and spatial patterns of expression. The differences in regulation, activation, substrate preference, intracellular association, and expression patterns have been demonstrated for several PLDs (Wang 2002, 2004, and refs. therein). These differences ultimately regulate the location and timing of the PLD activities and PA produced by the enzymes. Spatial and temporal regulation is important to all signaling events, but it is particularly critical to intracellular lipid messengers because of their limited mobility.

In addition, one PLD can mediate cell function via different modes of action, depending upon the nature of stimuli and severity of stresses (Fig. 4). These include (1) production of lipid mediators, such as PA, *N*-acyletanolamine, and other lipid derivatives; (2) direct interaction with other proteins, such as Gα, actin, and tubulin; (3) altering membrane lipid composition; and (4) membrane degradation. The role in changes of membrane lipid composition has been documented for PLDα1 in response to freezing (Welti et al., 2002). PLDα1 selectively hydrolyzes PC and alters the ratios of membrane lipids and contributes to membrane lipid degradation. Historically, PLD activity was associated with lipid degradation and membrane deterioration. This is perhaps best reflected by or attributed to a large increase of PA under conditions such as wounding, tissue handling, or uncontrolled lipid extraction. Such high PLD activity is likely to result from deregulation of PLD, such as disruption of cellular compartments. This deregulation may occur in plant tissues under severe stress conditions such as freezing, aging, or storage and thus is physiologically relevant. However, under normal plant growth, PLD activity is tightly regulated; for example, it is activated in response to specific stimuli, such as ABA and H_2O_2. These results indicate multifaceted functions of a PLD.

MOLECULAR TARGETS OF PLD-DERIVED PA

PLD is activated under various growth conditions, and PA is the direct lipid product of such activation (Welti et al., 2002; den Hartog et al., 2003; Potocky et al., 2003; Zhang et al., 2003, 2004; Li et al., 2004; Zonia and Munnik, 2004). Genetic and pharmacological manipulations of PLD-mediated PA formation suggest that PA mediates various signaling processes, as described here and reviewed earlier (Wang, 2002, 2004; Testerink and Munnik, 2005). It should be noted that signaling PA can be produced by another route (Fig. 1); the activation of the PLC/DAG kinase pathway can occur under certain stress conditions, such as hyperosmotic stress and pathogen elicitation (De Jong et al., 2004; Testerink and Munnik, 2005).

Major progress has been made recently in understanding how PA functions as a signaling messenger. In particular, direct molecular targets of PA have been identified in two specific plant processes. In ABA responses, PLDα1-derived PA has been shown to bind to ABI1 PP2C (Zhang et al., 2004). The amino acid residue required for PA-ABI1 interaction has been

identified and resides in the N-terminal region of ABI1. The binding of PA to ABI1 decreases the PP2C activity and also tethers ABI1 to the plasma membrane. These changes reduce the translocation of ABI1 to the nucleus and thus inhibit the ABI1 function (Zhang et al., 2004). In mediating root growth and initiation, PA binds to AtPDK1 and stimulates the activity of a downstream kinase AGC2-1 (Anthony et al., 2004). In addition, a SnRK2 Ser/Thr protein kinase and the PP2A regulatory subunit RCN1 are potential PA targets, as they were identified through PA-affinity chromatography (Testerink et al., 2004; Table I).

The PA targets in plants go beyond protein phophatases and kinases. Several additional proteins were associated with a PA-affinity column; these include phospho*enol*pyruvate carboxylase, Hsp90, and 14-3-3 (Testerink et al., 2004; Table I). The binding to phospho*enol*pyruvate carboxylase has been shown to be specific to PA. Several PA target proteins have been identified in other systems (Table I). In the budding yeast *S. cerevisiae*, PA on the endoplasmic reticulum directly bound to the soluble transcriptional repressor Opi1p to maintain it as inactive outside the nucleus (Loewen et al., 2004). A decrease in PA due to lipid biosynthesis releases Opi1p from the endoplasmic reticulum, allowing its nuclear translocation and repression of target genes. These results show that PA functions as both an essential metabolic intermediate and a signaling lipid. In animal cells, PLD-derived PA regulates mammalian target of rapamycin (mTOR), which belongs to the family of phosphatidylinositol kinase-like kinases and regulates translation initiation in mammals, and transcription, translation, and cytoskeletal rearrangement in yeast (Chen, 2004). PA directly interacts with the domain in the mTOR, and this binding is critical for mTOR's ability to activate downstream effectors (Fang et al., 2001).

It is important to note that PA may mediate cellular processes via different ways of action (Fig. 4). First, as a signaling messenger, PA can bind to its effector proteins, and this binding may activate or inhibit the function of its target proteins, as described above. Second, as a lipid in membranes, PA can tether its effector proteins to membranes, and the tethering may help to direct proteins to a specific type or region of a membrane. Third, PA may function via its structural effect on membranes to promote membrane fusion and trafficking. The space of PA's head group is smaller than that of its acyl chains, and this geometry renders PA a negative curvature and fusigenic lipid (Kooijman et al., 2005). PLD-derived PA is required for cell plate formation during meiosis and sporulation in yeast (Xie et al., 1998) and is a key facilitator of membrane vesicle trafficking events in different mammalian cells (Huang et al., 2005). In addition, PA serves as a substrate and/or an activator for enzymes that promote the formation of other lipid regulators, such as lysoPA, free fatty acids, DAG, DAG-pyrophosphate, and oxylipins (Wang 2002, 2004; Testerink and Munnik, 2005).

PERSPECTIVES

Substantial progress has been made recently toward understanding the regulation and function of PLDs. These advances present new opportunities to investigate the signaling mechanisms in plants. The finding of the novel signaling interaction between PLDα1 and Gα raises several intriguing questions. For instance, would PLDs function as GTPase-activating proteins or guanine nucleotide exchange factors? How would the PLD-Gα interaction affect the interactions of Gα with G$\beta\gamma$ subunits and with G-protein receptors? What would be the function and specificity of the PLD-G protein interaction in the cell? The observations that PA interacts with protein phosphatases and kinases warrant further investigation. PLD and PA may play an important role in the homeostasis of protein phosphorylation by concerted regulation of the two groups of signaling enzymes with opposite biochemical functions in a specific signaling response (Fig. 3). In the case of the PA-ABI1 interaction, it is conceivable that that PLD and PA may also affect the functions of other PP2Cs because Arabidopsis contains 69 PP2C-like genes.

A combination of the multiple routes of PA production, different PA species, diverse protein targets, and varied modes of action makes PA versatile mediators in cell regulation. Elucidation of the structural determinants that are required for PA-protein interaction will help to determine the specificity of the lipid-protein interaction and to understand how that interaction modulates structure, dynamics, and function in the ensuing lipid-protein complex. A better understanding of the regulation and function of PLDs and their derived messengers will advance not only the understanding of the major phospholipase family, but also the field of cell signaling and signaling networks in plant growth and stress responses.

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LITERATURE CITED

Anthony RG, Henriques R, Helfer A, Meszaros T, Rios G, Testerink C, Munnik T, Deak M, Koncz C, Bogre L (2004) A protein kinase target of a PDK1 signalling pathway is involved in root hair growth in Arabidopsis. EMBO J **23:** 572–581

Chapman KD (2004) Occurrence, metabolism, and prospective functions of N-acylethanolamines in plants. Prog Lipid Res **43:** 302–327

Chen J (2004) Novel regulatory mechanisms of mTOR signaling. Curr Top Microbiol Immunol **279:** 245–257

Cummings R, Parinandi N, Wang L, Usatyuk P, Natarajan V (2002) Phospholipase D/phosphatidic acid signal transduction: role and physiological significance in lung. Mol Cell Biochem **234:** 99–109

De Jong CF, Laxalt AM, Bargmann BO, De Wit PJ, Joosten MH, Munnik T (2004) Phosphatidic acid accumulation is an early response in the Cf-4/Avr4 interaction. Plant J **39:** 1–12

Delon C, Manifava M, Wood E, Thompson D, Krugmann S, Pyne S, Ktistakis NT (2004) Sphingosine kinase 1 is an intracellular effector of phosphatidic acid. J Biol Chem **279:** 44763–44774

den Hartog M, Verhoef N, Munnik T (2003) Nod factor and elicitors activate different phospholipid signaling pathways in suspension-cultured alfalfa cells. Plant Physiol **132:** 311–317

Dhonukshe P, Laxalt AM, Goedhart J, Gadella TW, Munnik T (2003) Phospholipase D activation correlates with microtubule reorganization in living plant cells. Plant Cell **15:** 2666–2679

Elias M, Potocky M, Cvrckova F, Zarsky V (2002) Molecular diversity of phospholipase D in angiosperms. BMC Genomics **3:** 2

Fang Y, Vilella-Bach M, Bachmann R, Flanigan A, Chen J (2001) Phosphatidic acid-mediated mitogenic activation of mTOR signaling. Science **294:** 1942–1945

Frank C, Keilhack H, Opitz F, Zschornig O, Boomer FD (1999) Binding of phosphatidic acid to the protein-tyrosine phosphatase SHP-1 as a basis for activity modulation. Biochemistry **38:** 11993–12002

Gardiner J, Collings DA, Harper JD, Marc J (2003) The effects of the phospholipase D-antagonist 1-butanol on seedling development and microtubule organisation in Arabidopsis. Plant Cell Physiol **44:** 687–696

Ghosh S, Moore S, Bell RM, Dush M (2003) Functional analysis of a phosphatidic acid binding domain in human Raf-1 kinase: mutations in the phosphatidate binding domain lead to tail and trunk abnormalities in developing zebrafish embryos. J Biol Chem **278:** 45690–45696

Grange M, Sette C, Cuomo M, Conti M, Lagarde M, Prigent AF, Nemoz G (2000) The cAMP-specific phosphodiesterase PDE4D3 is regulated by phosphatidic acid binding: consequences for cAMP signaling pathway and characterization of a phosphatidic acid binding site. J Biol Chem **275:** 33379–33387

Huang P, Altshuller YM, Hou JC, Pessin JE, Frohman MA (2005) Insulin-stimulated plasma membrane fusion of Glut4 glucose transporter-containing vesicles is regulated by phospholipase D1. Mol Biol Cell **16:** 2614–2623

Jenkins GH, Fisette PL, Anderson RA (1994) Type I phosphatidylinositol 4-phosphate 5-kinase isoforms are specifically stimulated by phosphatidic acid. J Biol Chem **269:** 11547–11554

Jones JA, Hannun YA (2002) Tight binding inhibition of protein phosphatase-1 by phosphatidic acid: specificity of inhibition by the phospholipid. J Biol Chem **277:** 15530–15538

Karathanassis D, Stahelin RV, Bravo J, Perisic O, Pacold CM, Cho W, Williams RL (2002) Binding of the PX domain of p47phox to phosphatidylinositol 3,4-bisphosphate and phosphatidic acid is masked by an intramolecular interaction. EMBO J **21:** 5057–5068

Kooijman EE, Chupin V, Fuller NL, Kozlov MM, de Kruijff B, Burger KN, Rand PR (2005) Spontaneous curvature of phosphatidic acid and lysophosphatidic acid. Biochemistry **44:** 2097–2102

Kusner DJ, Barton JA, Qin C, Wang X, Iyer SS (2003) Evolutionary conservation of physical and functional interactions between phospholipase D and actin. Arch Biochem Biophys **412:** 231–241

Lee S, Park J, Lee Y (2003) Phosphatidic acid induces actin polymerization by activating protein kinases in soybean cells. Mol Cells **15:** 313–319

Li W, Li M, Zhang W, Welti R, Wang X (2004) The plasma membrane-bound phospholipase Ddelta enhances freezing tolerance in Arabidopsis thaliana. Nat Biotechnol **22:** 427–433

Loewen CJ, Gaspar ML, Jesch SA, Delon C, Ktistakis NT, Henry SA, Levine TP (2004) Phospholipid metabolism regulated by a transcription factor sensing phosphatidic acid. Science **304:** 1644–1647

Manifava M, Thuring JW, Lim ZY, Packman L, Holmes AB, Ktistakis NT (2001) Differential binding of traffic-related proteins to phosphatidic acid- or phosphatidylinositol (4,5)-bisphosphate-coupled affinity reagents. J Biol Chem **276:** 8987–8994

Ohashi Y, Oka A, Rodrigues-Pousada R, Possenti M, Ruberti I, Morelli G, Aoyama T (2003) Modulation of phospholipid signaling by *GLABRA2* in root-hair pattern formation. Science **300:** 1427–1430

Ouyang YS, Tu Y, Barker SA, Yang F (2003) RGS4: insertion into model membranes and inhibition of activity by phosphatidic acid. J Biol Chem **278:** 11115–11122

Pappan K, Zheng L, Krishnamoorthi R, Wang X (2004) Evidence for and characterization of Ca^{2+} binding to the catalytic region of Arabidopsis thaliana phospholipase Dβ. J Biol Chem **279:** 47833–47839

Park J, Gu Y, Lee Y, Yang Z, Lee Y (2004) Phosphatidic acid induces leaf cell death in Arabidopsis by activating the Rho-related small G protein GTPase-mediated pathway of reactive oxygen species generation. Plant Physiol **134:** 129–136

Potocky M, Elias M, Profotova B, Novotna Z, Valentova O, Zarsky V (2003) Phosphatidic acid produced by phospholipase D is required for tobacco pollen tube growth. Planta **217:** 122–130

Qin C, Wang X (2002) The Arabidopsis phospholipase D family: characterization of a Ca^{2+}-independent and phosphatidylcholine-selective PLDζ1 with distinct regulatory domains. Plant Physiol **128:** 1057–1068

Sang Y, Cui D, Wang X (2001) Phospholipase D- and phosphatidic acid-mediated generation of superoxide in Arabidopsis. Plant Physiol **126:** 1449–1458

Sergeant S, Waite KA, Heravi J, McPhail LC (2001) Phosphatidic acid regulates tyrosine phosphorylating activity in human neutrophils: enhancement of Fgr activity. J Biol Chem **276:** 4737–4746

Testerink C, Dekker HL, Lim ZY, Johns MK, Holmes AB, Koster CG, Ktistakis NT, Munnik T (2004) Isolation and identification of phosphatidic acid targets from plants. Plant J **39:** 527–536

Testerink C, Munnik T (2005) Phosphatidic acid: a multifunctional stress signaling lipid in plants. Trends Plant Sci **10:** 368–375

Thiery L, Leprince AS, Lefebvre D, Ghars MA, Debarbieux E, Savoure A (2004) Phospholipase D is a negative regulator of proline biosynthesis in Arabidopsis thaliana. J Biol Chem **279:** 14812–14818

Wang X (2002) Phospholipase D in hormonal and stress signaling. Curr Opin Plant Biol **5:** 408–414

Wang X (2004) Lipid signaling. Curr Opin Plant Biol **7:** 329–336

Welti R, Li W, Li M, Sang Y, Biesiada H, Zhou HE, Rajashekar CB, Williams TD, Wang X (2002) Profiling membrane lipids in plant stress responses: role of phospholipase D alpha in freezing-induced lipid changes in Arabidopsis. J Biol Chem **277:** 31994–32002

Xie Z, Fang M, Rivas MP, Faulkner AJ, Sternweis PC, Engebrecht JA, Bankaitis VA (1998) Phospholipase D activity is required for suppression of yeast phosphatidylinositol transfer protein defects. Proc Natl Acad Sci USA **95:** 12346–12351

Zhang W, Qin C, Zhao J, Wang X (2004) Phospholipase Dalpha1-derived phosphatidic acid interacts with ABI1 phosphatase 2C and regulates abscisic acid signaling. Proc Natl Acad Sci USA **101:** 9508–9513

Zhang W, Wang C, Qin C, Wood T, Olafsdottir G, Welti R, Wang X (2003) The oleate-stimulated phospholipase D, PLDδ, and phosphatidic acid decrease H_2O_2-induced cell death in Arabidopsis. Plant Cell **15:** 2285–2295

Zhao J, Wang X (2004) Arabidopsis phospholipase Dα1 interacts with the heterotrimeric G-protein a subunit through a motif analogous to the DRY motif in G-protein-coupled receptors. J Biol Chem **279:** 1794–1800

Zheng L, Krishnamoorthi R, Zolkiewski M, Wang X (2000) Distinct calcium binding properties of novel C2 domains of plant phospholipase Dα and β. J. Biol Chem **275:** 19700–19706

Zheng L, Shan J, Krishnamoorthi R, Wang X (2002) Activation of plant phospholipase Dβ by phosphatidylinositol 4,5-bisphosphate: characterization of binding site and mode of action. Biochemistry **41:** 4546–4553

Zonia L, Munnik T (2004) Osmotically induced cell swelling versus cell shrinking elicits specific changes in phospholipid signals in tobacco pollen tubes. Plant Physiol **134:** 813–823