Signaling and Communication in Plants

Xuemin Wang *Editor*

Phospholipases in Plant Signaling

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D-53115 Bonn, Germany

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Editor

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Editor
Xuemin Wang
Department of Biology
University of Missouri-St. Louis and Donald
Danforth Plant Science Center
St. Louis
Missouri
USA

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Preface

Phospholipases hydrolyze phospholipids. The activities of phospholipases affect not only the structure and stability of cellular membranes but also the production of cellular mediators. The past decades have brought rapid growth in knowledge about the role of phospholipases in signaling processes. This volume reviews and highlights exciting developments in biochemical, molecular, and functional aspects of various phospholipases in plants.

The first half of the book summarizes our current knowledge of six different types of phospholipases, including phospholipase D (PLD; Chap. 1), phosphoinositide-hydrolyzing phospholipase C (PI-PLC; Chap. 2), nonspecific PLC (NPC. Chap. 3), patatin-related phopholipase A (pPLA; Chap. 5), and secretory PLA_2 and PLA_1 (sPLA; Chap. 6). The activity of PLD, PI-PLC, and NPC contribute to the production of phosphatidic acid (PA), which has been identified as a class of lipid mediators (Chap. 4). The second half of the book describes the progress made investigating the role of various phospholipases on plant stress responses, including response to hyperosmotic stresses (Chap. 7), nitrogen and phosphate availability (Chap. 8), NO and oxidative stress (Chap. 9), and plant–pathogen interactions (Chaps. 10 and 11).

From information presented in these chapters, it becomes evident that each family of phospholipases is comprised of multigene-encoding enzymes with overlapping, yet unique functions. Knowledge on the biochemical and functional heterogeneities of these enzymes will be important to understanding the multifaceted functions of phospholipases including cellular regulation, lipid metabolism, and membrane remodeling. Phospholipase-based signaling in plants differs in many aspects from mammalian cells, and considerable gaps in knowledge exist concerning what lipid mediators are produced by a specific phospholipase and how they function in plants. In addition, activation of more than one phospholipase is often involved in a given stress response, and information on the interplay among different phospholipases will help greatly the understanding of phospholipase signaling in plant processes, such as stress responses, cell size, shape, growth, apoptosis, proliferation, and reproduction.

The publication of this book would not have been possible without the efforts of many people to whom I am deeply indebted to. The authors of the individual chapters generously devoted their time and wisdom to ensure the high quality, up to date information presented in this book. My former and current students, postdoctoral associates, and visiting scientists with whom I have had the privilege to work have made numerous contributions to the field and made my editing of the book possible. Brian Fanella read all the chapters and provided valuable editorial suggestions. Also I thank editorial staff of Springer for their professional guidance in the production of this book.

St Louis, MO Xuemin Wang

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Part I
Plant Phospholipase Families and Derived Messengers

PLD: Phospholipase Ds in Plant Signaling

Xuemin Wang, Liang Guo, Geliang Wang, and Maoyin Li

Abstract Membrane lipids are rich sources for generating intracellular messengers, and the activation of phospholipases is often an early step in the messenger production. Phospholipase D (PLD) is a major family of membrane lipid-hydrolyzing enzymes in plants, and PLD activity increases under a wide range of stress conditions. Recent studies have revealed extensive biochemical and functional heterogeneities of PLDs. Cellular effectors, including Ca^{2+}, phosphoinositides, and oleic acid, bind to specific PLDs and differentially modulate their activities. The differential activation of specific PLDs plays crucial roles in the temporal and spatial production of phosphatidic acid, a class of potent lipid mediators involved in plant growth and stress responses. PLDs also interact directly with proteins involved in various processes, including cell signaling, central metabolism, and cytoskeleton reorganization. Different PLDs have unique and overlapping functions in plant growth, development, and stress responses.

Keywords Phosphatidic acid • Phospholipase D • Lipid signaling • Stress response

1 Introduction

The activity of phospholipase D (PLD) was first described in plants in 1940s (Hanahan and Chaikoff 1947). Some distinctive, perplexing properties of PLD activity were soon noted (Heller 1978). For example, the PLD activity originally analyzed in plants required high millimolar Ca^{2+} for activity in vitro. PLD in plant tissues was readily activated under some conditions, such as tissue homogenization which led to the loss of most nitrogenous phospholipids, such as

X. Wang (✉) • L. Guo • G. Wang • M. Li
Department of Biology, University of Missouri, St. Louis, MO 63121, USA

Donald Danforth Plant Science Center, St. Louis, MO 63132, USA
e-mail: swang@danforthcenter.org

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phosphatidylcholine (PC) and phopshatidylethanolamine (PE) (Quarles and Dawson 1969). When leaves were sprayed with primary alcohols, most PC was converted to phosphatidylalcohol due to PLD's transphosphatidylation activity (Roughan and Slack 1976). The physiological relevance of the PLD activity was questioned, which had remained elusive for some time.

PLD gained renewed attention since 1990s because of its role in cell signaling. PLD was first cloned from castor bean (Wang et al. 1994), which has propelled the understanding of PLD and its functions at the molecular level. It is now known that higher plants have multiple types of PLDs; besides the high millimolar Ca^{2+}-requiring activity, many PLDs require micromolar Ca^{2+}, and others are independent of Ca^{2+} (Pappan et al. 1997a, b; Qin and Wang 2002). Rapid increases in PLD activity upon perturbations have been investigated in the context of stress-induced activation of PLDs. Other cellular effectors, besides Ca^{2+}, have been identified to modulate PLD activities. In addition, PLDs have been found to interact with different proteins. Genetic manipulation of different PLDs has resulted in alterations of plant growth, development, and response to abiotic and biotic stresses. The study of PLD lipid product phosphatidic acid (PA) has provided further insights into the mechanism of action.

2 The PLD Family and Catalysis

PLD was purified to apparent homogeneity from several plant species such as peanut seeds (Heller et al. 1974), cabbage leaves (Allgyer and Wells 1979), rice bran (Lee 1989), and castor bean endosperm (Wang et al. 1993). During the purification and subsequent immunoblotting analyses, the presence of multiple PLDs was observed (Dyer et al. 1994, 1996). The gene family of PLD has been reported in several higher plant species, and all the plants examined contain more than ten PLD genes. For example, there are 12 in Arabidopsis, 18 in soybean (Zhao et al. 2012), 17 in rice (McGee et al. 2003), 18 in poplar (Liu et al. 2010), and 11 in grape (Elias et al. 2002; Liu et al. 2010). The PLD family in *Arabidopsis thaliana* is most extensively characterized and thus will be used to highlight the current understanding of plant PLDs.

2.1 Identification of Different PLDs in Plants

One of the signature properties in vitro noted for the "conventional" PLD is its requirement for high millimolar concentrations of Ca^{2+} for activity and the great stimulation of its activity by detergents such as sodium dodecyl sulfate. The purification of the conventional PLD from castor bean and subsequent N-terminal amino acid sequencing of it led to the first cloning of PLD (Wang et al. 1993, 1994). The availability of the PLD sequence led to the cloning of the Arabidopsis *PLDα1*

and also *PLD*s from yeast and humans (Hammond et al. 1995; Waksman et al. 1996). PLDα1 requires high Ca^{2+} for activity, and antisense suppression of the common plant PLD activity led to the discovery of phosphatidylinositol 4,5-bisphosphate (PIP_2)-dependent PLD activity in Arabidopsis (Pappan et al. 1997b). A PIP_2-requiring PLD, named PLDβ, was soon cloned (Pappan et al. 1997a). At the same time, another PIP_2-dependent PLD, *PLDγ*, was cloned and characterized (Qin et al. 1997). Later, the oleate stimulated PLDδ was identified and analyzed (Wang and Wang 2001). The availability of the Arabidopsis genome sequence facilitated the identification of PLDζs. PLDζ1 requires no Ca^{2+} for activity and appears to be specific to PC as substrate (Qin and Wang 2002). PLDε, which was originally designated PLDα4, is the most permissive of all the characterized PLDs in terms of reaction requirements, and it is active under PLD*α*1, *β*, and *δ* reaction conditions (Hong et al. 2009). These results show that PLDα, β, γ, δ, and ζ display different requirements for Ca^{2+}, PIP_2, and free fatty acids (Table 1).

Analysis of the Arabidopsis genome led to the identification of 12 PLD genes named as *PLDα*(*1,2,3*), *PLDβ*(*1,2*), *PLDγ*(*1,2,3*), *PLDδ*, *PLDε*, and *PLDζ*(*1,2*) based on gene architecture, sequence similarity, domain structure, and biochemical properties (Fig. 1; Table 1). Two *PLDδ* cDNA variants and two *PLDγ*2 variants, which are likely derived from alternative splicing, have been reported (Wang and Wang 2001; Qin et al. 2006). Thus, the total number of PLD enzymes in *Arabidopsis* is greater than 12s. In rice, in addition to the C2-PLDs and PX/PH-PLDs, one unique, putative PLD, PLDφ, which does not contain the C2 or PX/PH domains, was identified (Li and Xue 2007), but the enzymatic identity of PLDφ as PLD is yet to be confirmed. In mammals, a unique mitochondrial PLD (MitoPLD) was identified (Choi et al. 2006). MitoPLD, with only one single HKD catalytic motif, is a divergent and ancestral family member most similar to bacterial cardiolipin synthase (Choi et al. 2006; Wang et al. 2006). MitoPLD hydrolyzes cardiolipin to PA and promotes transmitochondrial membrane adherence (Choi et al. 2006).

2.2 Catalytic Mechanism

The PLD superfamily is characterized by the presence of the catalytic motif HxKxxxxD (HKD) in a single or double copy in the primary structure (Fig. 1; Waite 1999). Besides PLDs, this super family includes cardiolipin synthases, phosphatidylserine synthases, tyrosyl-DNA phosphodiesterase, and nucleases. In higher plants, multiple PLDs characterized so far all contain duplicated HKD motifs. PLD catalyzes the hydrolysis of phospholipids at the terminal phosphodiester bond, leading to the production of PA and a water-soluble head group such as choline or ethanolamine. Two HKD motifs are required for PLD catalysis, with one His residue acting as a nucleophile and the other as a general acid/base (Stuckey and Dixon 1999). The PLD hydrolysis proceeds via a two-step reaction. PLD first forms a phosphatidyl-enzyme intermediate, and the

Table 1 Distinguishable catalytic and regulatory properties of Arabidopsis PLDs

PLD type	Signature property				
	Ca^{2+}	PIP_2	Oleate	Substrate	Subcellular location and others
PLDα1	mM/μM	–	–	PC > PE	Translocation between cytosol and IM, PM, most PM
α2	mM	–		PC = PE	Cytosol = IM and PM
α3	mM	–		PC > PE, PG	Mostly PM
ε	mM/μM	–	–/+	PC = PE > PG	PM; lost Ca^{2+}-binding residues in C2
PLDβ1	μM	+	–	PC = PE	Ca^{2+}-binding at C2 and catalytic region, actin binding
PLDγ1	μM	+	–	PE > PC	Mostly IM; differ from γ2 in PIP_2 and triton effect
γ2	μM	+	–		AA changes in DRY motif
PLDδ	μM-mM	+	+	PE > PC	PM, tubulin binding
PLDζ1	No	+	–	PC	PM
ζ2	Not determined				IM, induced most by Pi deficiency

– indicates no requirement of effectors for PLD activity. + indicates effectors promote PLD activity. *PC*, phosphatidylcholine, *PE*, phosphatidylethanolamine, *PG*, phosphatidylglycerol, *PM*, plasma membrane, *IM*, intracellular membrane

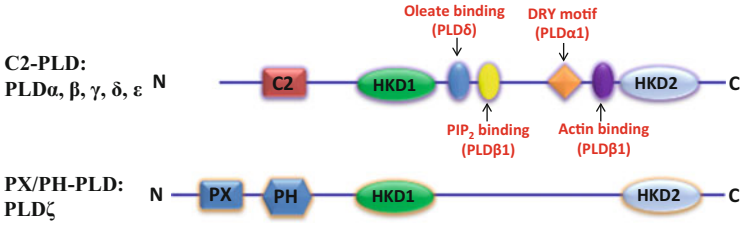

Fig. 1 Two PLD subfamilies and their domain structures. C2, Ca^{2+}-dependent phospholipid binding domain; PH, pleckstrin homology domain; PX, phox homology domain; HKD, HxKxxxxD motif involved in catalysis; DRY motif, interacts with Gα. The different ligand binding motifs have been experimentally determined for specific PLDs, as indicated

phosphatidyl group is then transferred to the −OH moiety in the presence of water (H–OH) to produce PA (Fig. 2).

In the presence of primary alcohols (H–OR), PLD transfers the phosphatidyl to the −OR moiety to produce phosphatidylalcohol (PtdOR; Fig. 2). This activity is referred to transphosphatidylation and has been explored in various industrial applications for the enzymatic synthesis of various natural and tailor-made phospholipids with functional head groups (Sarri et al. 1996). The PtdOR formation is used as an indicator of PLD activity in the cell because this reaction is unique to PLD.

By comparison, PA can be produced by PLD and other reactions such as PLC coupled with diacylglycerol kinase (PLC/DGK). In addition, PtdOR is metabolically stable, unlike PA that can be removed by lipid phosphate phosphatases, kinases, and acyl hydrolases. However, it is important to note that primary alcohols are potent activators of PLDs in plants (Roughan and Slack 1976). The cellular and

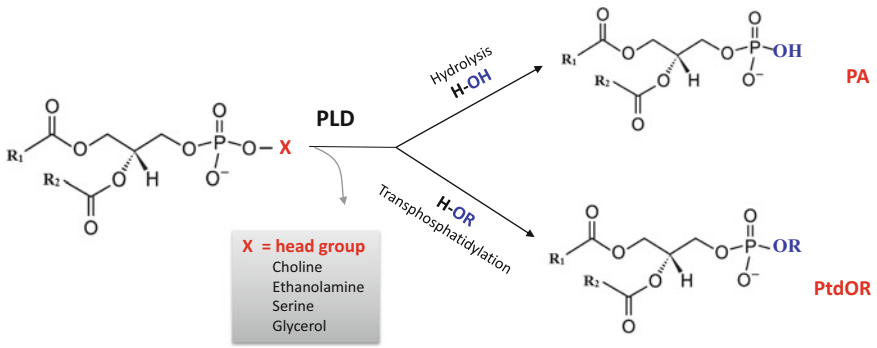

Fig. 2 PLD hydrolysis and transphosphatidylation activities. X represents head group of phospholipids. PLD hydrolyzes phospholipids to produce PA and head group, and in the presence of primary alcohols (H–OR), PLD transfers part of phosphatidyl moieties to –OR to form phosphatidylalcohol, also referred to as transphosphatidylation

physiological effects of increased membrane lipid hydrolysis and the formation of PtdOR remain undetermined. Thus, the alcohol treatments are used often as a supplementary approach and interpreting data involving such treatments requires caution.

2.3 Different Substrate Preferences of PLDs

When assayed in vitro, C2-PLDs use common membrane phospholipids such as PC, PE, phosphatidylglycerol (PG), and phosphatidylserine (PS), but substrate preferences vary among the C2-PLDs (Pappan et al. 1997b; Qin et al. 2002) (Table 1). PLDα1 prefers PC, and such preference has also been shown by measuring phospholipid hydrolysis in plants (Welti et al. 2002). On the other hand, PLDδ prefers PE to PC as substrate (Qin et al. 2002). The surface dilution kinetic analysis indicates that PLDδ displays a sevenfold higher specific catalytic constant (V_{max}/K_m) for PE than PC. PE has an approximate fivefold lower interfacial Michaelis constant, K_m, than PC, indicating that PE has a higher affinity than PC (Qin et al. 2002). PX-PH-PLDζ1 selectively hydrolyzes PC (Qin and Wang 2002). None of the cloned PLDs to date use phosphatidylinositol (PI) as substrate. Thus, the activation of different PLDs may result in the differential hydrolysis of phospholipids and the production of different PA molecular species.

3 Regulation and Activation of PLDs

The activity of PLD in plants increases under a wide spectrum of conditions, as described below. However, over-expression of PLD did not result in increased membrane lipid hydrolysis under normal growth conditions (Hong et al. 2008b), suggesting that PLD activity in cells is highly regulated. Moreover, results from the genetic manipulation of different PLDs clearly indicate that different PLDs are activated under specific stresses. The regulatory domains of the PLD family and the distinguishable biochemical properties of individual PLDs provide insights into the cellular regulation of PLDs.

3.1 Regulatory Domains and Motifs of PLDs

PLDs in plants consist of two divergent subfamilies: C2-PLDs and PX/PH-PLDs (Elias et al. 2002; Qin and Wang 2002). C2 is a Ca^{2+}/phospholipid-binding domain consisting of approximately 130 amino acid residues that form antiparallel eight-stranded β-sandwich structures. C2 domains have been identified in many proteins, most of which are involved in lipid metabolism, signal transduction, or membrane trafficking. PX and PH refer to the Phox homology (PX) and pleckstrin homology (PX) domains, respectively. The PX domain can bind phosphoinositides and SH3 domain (Cheever et al. 2001; Hiroaki et al. 2001; Kanai et al. 2001). Therefore, it may play a critical role in coordinating membrane localization and protein complex assembly during cell signaling. PH domains are composed of approximately 120 amino acids found in more than 100 proteins involved in cell signaling, cytoskeletal rearrangement, and other processes.

Most plant PLDs are C2-PLDs and the C2-PLD subfamily appears to be unique to plants. Ten of the 12 PLDs in Arabidopsis are C2-PLDs but PLDαs miss some of the key acidic residues required for Ca^{2+} binding (Zheng et al. 2000). Two PLDζs are PX/PH-PLDs. The sequences of PLDζ1 and PLDζ2 are more similar to mammalian PLD2 and PLD1, respectively, than to plant C2-PLDs (Elias et al. 2002; Qin and Wang 2002).

Two polybasic motifs (K/RxxxxK/RxK/RK/R) have been identified in the PIP_2-dependent PLDβ, and they have been shown to interact with PIP_2 (Zheng et al. 2000). In addition, PLDβ has other motifs in the catalytic regions that are also involved in PIP_2 binding (Zheng et al. 2002). In contrast, PLDα and PLDδ do not contain these two motifs, and PIP_2 is not required for their activity (Qin et al. 1997, 2002). The region involved in the oleate binding of PLDδ is located approximately 30 amino acid residues after the first HKD motif. Arg-399 of PLDδ was found to be involved in oleate stimulated activity of PLDδ. The presence of oleate stimulates PLDδ's binding to PC. PLDα1 has been shown to interact with the heterotrimeric G protein subunit Gα (Zhao and Wang 2004). PLDβ1 contains a

specific actin-binding region. Whereas monomeric (G-) G-actin inhibits PLDβ activity, filamentous (F-) F-actin stimulates it (Kusner et al. 2002).

3.2 *Differential Activation of Different PLDs by Ca^{2+}, PIP_2, and Oleate*

Analyses of the domain structures of PLD proteins provide a structural basis for the distinguishable biochemical properties. For example, the C2-PLDs need Ca^{2+} for activity whereas PX/PH-PLDs do not (Qin and Wang 2002). In addition, individual PLDs can differ in key amino acid residues in the various domains and motifs. PLDβ C2 has all the conserved Ca^{2+}-binding residues, whereas PLDα1 C2 lacks at least two of these potential Ca^{2+} ligands due to substitution. PLDβ1 and PLDα1 have been shown to bind to Ca^{2+} with different binding affinities, with PLDβ1 requiring micromolar whereas PLDα1 needing millimolar Ca^{2+} for activity (Zheng et al. 2000). Under acidic pH conditions, however, PLDα1 is active at micromolar Ca^{2+} (Pappan and Wang 1999). In addition to direct binding, Ca^{2+} may alter membrane microdomains to facilitate PLD interaction with the phospholipid surface and activate PLD activity.

Phosphoinositides, particularly PIP_2, is another key regulator of PLD activity. PLDβ, PLDγ, and PLDζ require PIP_2 for activity (Pappan et al. 1997a; Qin and Wang 2002). The binding of PIP_2 to PLDβ1 enhances the PLD interaction with membrane lipids and thus its substrate affinity (Qin et al. 2002; Zheng et al. 2002). PLDβ1 requires PIP_2 and PE for its activity, whereas PLDδ is stimulated by PIP_2 but does not require PIP_2 or PE for activity. The interactions and differential affinities are consistent with the domain structures of the plant PLD family. Surface-dilution kinetics analysis indicates that PIP_2 stimulates PLDδ activity by promoting substrate binding to the enzyme, without altering the bulk binding of the enzyme to the micelle surface. Ca^{2+} decreases significantly the interfacial Michaelis constant K_m, indicating that Ca^{2+} activates PLD by promoting the binding of phospholipid substrate to the catalytic site of the enzyme.

PLDδ was activated by free oleic acid in a dose-dependent manner, with the optimal concentration being 0.5 mM (Wang and Wang 2001). Other unsaturated fatty acids, linoleic and linolenic acids, were less effective than oleic acid, whereas the saturated fatty acids, stearic and palmitic acids, were totally ineffective. PIP_2 stimulated PLDδ to a lesser extent than oleate. In addition, *N*-acylethanolamines (NAEs), which are produced from *N*-acyl-PE by PLD, acts as potent inhibitors of PLDα1 (Austin-Brown and Chapman 2002).

3.3 Subcellular Association and Expression of PLDs

PLD activities are associated with soluble and membranous fractions, but individual PLDs vary in their subcellular associations (Table 1). PLDα1 is present in both the cytosol and membranes and undergoes intracellular translocation from the soluble to membrane-associated form in response to stresses (Wang et al. 2000). PLDβ1 is membrane bound and binds to actin (Pappan et al. 1997a; Kusner et al. 2002). PLDδ is associated with the plasma membrane and binds to microtubulin (Gardiner et al. 2001). PLDζ2 is associated with the tonoplast membrane (Yamaryo et al. 2008). Whereas PLDδ, PLDε, and PLDα3 are mostly associated with the plasma membrane (Wang et al. 2000; Hong et al. 2008a, 2009), PLDγ is mostly associated with intracellular membranes (Fan et al. 1999). The subcellular association of PLDs is expected to play an important role in the spatial regulation of membrane lipid hydrolysis and PA production.

PLDs are expressed in all plant tissues examined, but the extent and patterns differ greatly among different PLDs in tissues and timing during development and in response to stresses (Qin et al. 2006; Zhang et al. 2010; Zhao et al. 2013). In Arabidopsis PLDα1 is the most abundant PLD in most tissues. The transcript level of *PLDα1* is highest in all tissues except pollen. The expression of *PLDζ2* is highly induced by phosphorus deficiency (Li et al. 2006), and *PLDδ* is induced highly by extreme hyperosmotic stress (Katagiri et al. 2001). After ABA treatment of leaves, the transcript of *PLDα1* displayed little change but that of *PLDδ* increased (Distefano et al. 2012). The transcription of *PLDζ1* is regulated by the homeobox transcriptional regulator GLABRA2 (GL2) which binds to the *PLDζ1* promoter and inhibits its transcription (Ohashi et al. 2003). However, little is known about the transcriptional regulation of the other PLDs or the detailed cellular pattern of PLD expression. The differences in gene expression, together with those in subcellular association and effector regulation, play important roles in regulating the temporal and spatial activation of individual PLDs.

4 PLD Involvements in Diverse Physiological Processes

PLD has been implicated in a wide range of physiological processes. Analysis of plants deficient in or overexpressing specific PLDs in Arabidopsis and other plants have provided evidence for the involvement of specific PLDs in specific physiological responses (Table 2). The following discussion will focus on the effect of genetic manipulation of PLDs on physiological alterations.

Table 2 PLD family members and functions as indicated by genetic manipulation in plants

Plant water loss and drought tolerance
Water loss (PLDα1-knockdown, Sang et al. 2001; PLDα1-overexpression, Hong et al. 2008b; Zhang et al. 2009; Lu et al. 2013)
H_2O_2 and/or NO response in stomata (PLDδ-knockout, Guo et al. 2012a, b; Distefano et al. 2012)
Drought response (PLDα3-knockout and overexpression, Hong et al. 2008a)
Salt response
Altered sensitivity to salt stress (PLDα3-knockout and overexpression, Hong et al. 2008b)
Decreased salt tolerance (PLDα1PLDδ-double knockout, Bargmann et al. 2009)
Increased aluminum resistance (PLDγ1-knockdown, Zhao et al. 2011)
Nutrient response
Response to phosphorous deprivation (PLDζ1-knockout and PLDζ2-knockout, Li et al. 2006; Cruz-Ramírez et al. 2006)
Response to nitrogen deprivation (PLDε-knockout and overexpression, Hong et al. 2009)
Pathogen resistance
Response to bacterium and fungi (PLDβ1-knockdown, Bargmann et al. 2006; Yamaguchi et al. 2009; PLDβ1-knockdown and knockout, Zhao et al. 2013)
Response to fungi (PLDδ-knockout, Pinosa et al. 2013)
Hormonal responses
ABA response (PLDα1-knockdown and knockout, Zhang et al. 2004; PLDα1PLDδ-double knockout, Uraji et al. 2012; PLDδ-knockout, Jia et al. 2013)
Auxin response (PLDζ2-knockout, Li and Xue 2007)
Pollen tube and root hairs
Actin dynamics and pollen tube growth (PLDβ-knockdown, Pleskot et al. 2010)
Root hair patterning (Ohashi et al. 2003)
Root hair deformation under Pi deprivation (PLDζ1-knockout and PLDζ2-knockout, Li et al. 2006; Cruz-Ramírez et al. 2006)
Root hair elongation under N deficiency (PLDε-knockout and overexpression, Hong et al. 2009)
Seed aging and freezing damages
Delayed seed deterioration and aging (PLDα1-knockdown, Devaiah et al. 2007; Lee et al. 2012).
Freezing tolerance (PLDα1-knockdown, Welti et al. 2002; PLDδ-knockout and overexpression, Li et al. 2004)

4.1 Water Loss, Drought Tolerance, and High Salinity

Genetic abrogation of *PLDα1* or *PLDδ* results in increased water loss (Sang et al. 2001; Guo et al. 2012a; Distefano et al. 2012). One mechanism by which *PLDα1* and *PLDδ* decrease water loss is through their role in the ABA signaling that promotes stomatal closure. When epidermal peels of Arabidopsis leaves were assayed, antisense suppression or knockout of *PLDα1* compromises the effect of ABA-promoted stomatal closure, and the same effect was also observed with *PLDδ*-KO plants (Distefano et al. 2012; Guo et al. 2012a). Increased transpirational water loss is also detected in detached leaves and *PLDα1*- or *PLDδ*-deficient whole plants. Application of PA to epidermal peels mimics the ABA effect on stomatal closure, indicating that PLD-produced PA promotes stomatal closure. *PLDα1* and *PLDδ* occupy different steps in the ABA signaling pathway. PLDα1 promotes H_2O_2

production through PA interaction with NADPH oxidase, whereas PLDδ is involved in H_2O_2 response through the interaction of PLDδ with cytosolic glyceraldehyde-3-phosphate dehydrogenases (Zhang et al. 2009; Guo et al. 2012a). On the other hand, *PLDα1* and *PLDδ* have been suggested to act cooperatively as the *PLDα1PLD-δ*-double KO displayed more robust insensitivity to ABA than *PLDα1*- or *PLD-δ*-single KO did (Uraji et al. 2012). It is possible that some redundancy of *PLDα1* and *PLDδ* or cross talk between them exists under certain experimental conditions.

Increased expression of *PLDα1* results in a decreased water loss in tobacco and canola, lending further support to the role of *PLDα1* in promoting stomatal closure (Hong et al. 2008a; Lu et al. 2013). Under prolonged drought, however, PLDα1-overexpressed tobacco plants displayed more damage, which is likely due to the increased lipid degradation and membrane damage (Hong et al. 2008a). Recently, the expression of *PLDα1* was targeted to guard cells in canola to increase the expression specifically in stomata. These plants lost less water and performed better in biomass and seed yield under drought (Lu et al. 2013). In addition, the alterations of *PLDα3* also change plant response to water deficiency (Hong et al. 2008b). *PLDα3*-KO is more sensitive to drought whereas *PLDα3*-OX is less sensitive. However, *PLDα3*-altered plants did not display changes in ABA-promoted stomatal closure, indicating that it mediates plant response to drought via a mechanism different from that of *PLDα*1 and *PLDδ*.

Manipulations of *PLDα1*, *PLDα3*, *PLDδ*, or *PLDε* have resulted in alterations of Arabidopsis response to high salinity (Hong et al. 2008b; Bargmann et al. 2009; Yu et al. 2010; Zhang et al. 2012). *pldα3-1* seeds are more susceptible to salt stress, as indicated by delayed germination, lower germination rate, retarded seedlings, and reduced root growth. In contrast, *PLDα3*-OE seeds displayed more resistance to salt with enhanced germination rates and seedling growth. The loss of *PLDα3* or *PLDε* also renders plants more sensitive to salt, while plants overexpressing PLDα3 or PLDε show salt tolerance (Hong et al. 2008a). High salinity results in ionic toxicity and hyperosmotic stress, the latter of which is shared with drought. The observations that the activity of many of the PLDs was altered both to drought and salinity may indicate that they play a role in plant response to hyperosmotic stress, rather than specifically to salt stress.

4.2 Response to Nitrogen and Phosphorus Availability

Among all the PLDs tested, alterations of *PLDε* result in more apparent changes in plant response to nitrogen (N) availability (Hong et al. 2009). The *PLDε* effects on root growth and morphology differ at different levels of N. At severe N deprivation (0.1 or 0.6 mM), *PLDε* promotes elongation of primary roots and root hairs, whereas no such effect was observed under sufficient N supply (6 or 60 mM). At sufficient N supply, *PLDε* promotes lateral root growth and biomass production. These results suggest that at sufficient N, *PLDε* promotes biomass accumulation and lateral root growth, whereas under N deficiency, *PLDε* promotes primary root elongation and root hair growth (Hong et al. 2009).

Under phosphate deprivation, phopsholipids, particularly PC, decreases whereas nonphosphorus lipids such as digalactosyldiacylglycerol (DGDG) and sulfolipids increase. The expression of *PLDζ2* increases greatly during Pi starvation in *Arabidopsis,* and the accumulation of DGDG in the roots of Pi-limited *PLDζ2*-KO plants is reduced while PC and PE accumulate in Pi-starved *PLDζ2*-KO roots (Cruz-Ramírez et al. 2006; Li et al. 2006). However, under moderate phosphorus limitation, *PLDζ1PLDζ2*-double KO mutants, but not *PLDζ1*- or *PLDζ2*-single KO, display shorter primary roots than wild type. Thus, both PLDζs are involved in plant response to phosphate deprivation and in primary root growth (Li et al. 2006). PLDs hydrolyze phospholipids, particularly PC to PA, which is dephosphorylated to DAG for galactolipid synthesis. In addition, PA inhibits phosphoethanlonamine methyl transferase (PEAMT) involved in PC synthesis and stimulates MGDG synthase (MSD1) (Jost et al. 2009; Dubots et al. 2010). Thus, PA may act as a coordinator that suppresses PC synthesis with increases in DGDG formation. These results indicate that *PLDζs* have both metabolic and regulatory functions in plant response to phosphorus deprivation.

4.3 Root Hair and Pollen Tube Growth

PLD and its derived PA play an important role in polarized plant cell expansion, such as root hair and pollen tube growth. KO of *PLDζ2* resulted in bulging, deformed root hairs under phosphate deprivation (Li et al. 2006). Under N deprivation, root hairs of *PLDε*-KO Arabidopsis were shorter whereas those of *PLDε*-OE plants were longer than those of WT plants. How PLDs promote root hair growth under the stress is unknown. PA interacts with phosphoinositide-dependent protein kinase 1 (PDK1), which activates AGC2-1 kinase to promote root hair growth in *Arabiodpsis* (Anthony et al. 2004). In addition, *PLDζ1* was reported to be the target of the transcriptional regulator GL2 that regulates root hair patterning, and transient suppression of *PLDζ1* alters root hair patterns and morphology (Ohashi et al. 2003). However, the root hair pattern in single or double KOs of *PLDζ1* and *PLDζ2* is normal (Li et al. 2006), and the exact effect of *PLDζs* on root hair patterning requires further investigation.

Suppression of PLD-mediated PA production by the primary alcohol *N*-Butanol in vivo inhibited pollen germination and tube growth whereas application of PA overcame the inhibition (Potocky et al. 2003). Later studies show that PLD interaction with actin cytoskeleton plays a role in pollen tube elongation as shown by antisense suppression of tobacco NtPLDβ1 (Pleskot et al. 2010). PLDβ1 is activated by F-actin whereas PA promotes the F-actin formation, thereby forming a positive feedback loop for the polarized growth by increasing membrane-F-actin dynamics in the cortex of plant cells.

4.4 Low Temperature and Freezing Damage

Many plants during growth encounter frost and/or prolonged freezing. The plant response to freezing temperatures may be divided into three phases: cold acclimation, freezing, and postfreezing recovery. The PA level in plants increases during cold acclimation (Welti et al. 2002). PLDα1- or PLDδ-deficient plants underwent similar alterations in lipid composition as did wild-type plants, indicating that the two PLDs do not play a major role in the alterations of lipid molecular species that occurred in cold acclimation (Welti et al. 2002; Li et al. 2004). However, manipulations of the two PLDs have opposite effects on Arabidopsis freezing tolerance. Antisense suppression of PLDα1 rendered Arabidopsis plants more tolerant to freezing (Welti et al. 2002), whereas KO of PLDδ rendered Arabidopsis plants more sensitive to freezing and OE increased freezing tolerance (Li et al. 2004). The altered freezing tolerance occurred only in cold-acclimated plants, indicating that cold acclimation is required for PLDδ function during freezing. PLDα1 plays a major role in promoting phospholipid hydrolysis in both freezing and postfreezing phases, but the presence of PLDδ reduced lipid hydrolysis during postfreezing recovery (Li et al. 2008). These data suggest a negative role for PLDα1 and a positive role for PLDδ in freezing tolerance.

One way by which PLDα1 promotes freezing damage is its hydrolysis of PC to PA. PLDα1-deficient plants had a higher level of PC and a lower level of PA, indicating that PC is the major in vivo substrate for PLDα during freezing-induced activation (Welti et al. 2002). This preference of PLDα for PC is supported by in vitro data (Pappan et al. 1998). PC is a bilayer-stabilizing lipid, whereas PA has tendency to form a hexagonal II phase in the presence of cations. The propensity of membranes to form the hexagonal phase has been suggested to be a key event in freezing injury. The suppression of *PLDα1* may decrease the propensity of membrane lipids to undergo a transition from lamellar to hexagonal II phase, thus increasing freezing tolerance (Welti et al. 2002). PLDδ prefers PE to PC as substrate (Qin et al. 2002), and its KO had no major impact on freezing-induced decline of membrane lipids; rather, it produces a small increase in selective PA species. Thus, while high PLDα1 activity destabilizes membranes and increases membrane leakage, regulated increase of PLDδ may produce signaling PA species that mitigate stress damage. Specifically, PLDδ and the resulting PA decrease cell death promoted by the reactive oxygen species H_2O_2. The level of H_2O_2 increases in plant cells in response to freezing stress. Thus, the impaired response to oxidative stress in *PLDδ*-null plants may be a basis for the decreased freezing tolerance.

4.5 Plant–Microbial Interactions

Pathogen infection of higher plants often induces a rapid production of PA and changes in lipid profiles. Suppression of tomato *LePLDβ1* resulted in a strong decrease in a fungal elicitor xylanase-induced PLD activity and enhanced oxidative burst in tomato suspension cells (Bargmann et al. 2006). *OsPLDβ1*-knockdown rice

plants displayed the accumulation of reactive oxygen species in the absence of pathogen infection (Yamaguchi et al. 2009). More than 1,400 genes were up- or downregulated in *OsPLDβ1*-suppressed plants, which include the induction of pathogenesis-related protein genes and WRKY/ERF family transcription factor genes. These data suggest that the *OsPLDβ1*-knockdown plants spontaneously activated the defense responses in the absence of pathogen infection. The *OsPLDβ1*-knockdown plants exhibited increased resistance to the infection of the common rice pathogens, *Pyricularia grisea* and *Xanthomonas oryzae* pv oryzae. These results suggest that *OsPLDβ1* functions as a negative regulator of defense responses and disease resistance in rice. In Arabidopsis infected with *Pseudomonas syringae* pv. DC3000, *PLDβ1*-antisense or KO plants had also less bacterial growth than in WT plants (Zhao et al. 2013). This result is consistent to other observations that *PLDβ*1 is a negative effector of disease resistance.

However, *PLDβ1*-deficient plants were more susceptible than WT plants to the fungus *Botrytis cinerea* (Zhao et al. 2013). The expression levels of salicylic acid (SA)-inducible genes were higher, but those inducible by jasmonic acid (JA) were lower in *PLDβ1* mutants than in wild-type plants. The *PLDβ1*-deficient plants had lower levels of PA-, JA-, and JA-related defense gene expression after *B. cinerea* inoculation. *PLDβ1* plays a positive role in pathogen-induced JA production and plant resistance to necrotrophic fungal pathogen *B. cinerea*, but a negative role in the SA-dependent signaling pathway and plant tolerance to the infection of biotrophic *Pst* DC3000 (Zhao et al. 2013). Among the 12 PLD genes in Arabidopsis, PLDδ deficiency resulted in the most severe compromise on resistance against the penetration of spores of barley and pea powdery mildew fungi (Pinosa et al. 2013). PLDδ accumulates on the plasma membrane of the cells surrounding the attaching sites of the fungus spores, implying its function on cell wall reinforcement (Pinosa et al. 2013).

In addition, PLD has been suggested to be involved in beneficial plant–microbial interactions. *Piriformospora indica* is a root endophytic fungus that colonizes many plant species and promotes growth and resistance to certain plant pathogens. However, *PLDα1*- or *PLDδ*-deficient plants lost the ability to respond to enhanced growth and are impaired in PA production after *P. indica* infection. PA was previously shown to interact with PDK1 (3-PHOSPHOINOSITIDE-DEPENDENT PROTEIN KINASE1). PDK1 regulates another kinase OXI1 (Oxidative Signal Inducible1). These results indicate that the pathway consisting of the PLD → PDK1 → OXI1 cascade mediates the *P. indica*-stimulated growth response. In the symbiotic interaction, the amount and activity of PLDα protein increase in response to rhizobium infection (Wan et al. 2005), and the PLD-produced PA is involved in the Nod factor-induced gene expression (den Hartog et al. 2003).

4.6 Seed Viability and Germination

The catabolism of membrane phospholipids has been associated with decreasing seed quality and viability (Devaiah et al. 2007). In seeds of the *PLDα1*–KO mutant plants, levels of PA, lysoPC, and lysoPE were significantly lower than those of

wild-type seeds, suggesting a role for PLDα1 in membrane lipid degradation in seeds (Devaiah et al. 2006). The *PLDα1*-deficient seeds exhibited a smaller loss of unsaturated fatty acids and lower accumulation of lipid peroxides than did wild-type seeds (Devaiah et al. 2007). A similar effect was also observed in soybean (Lee et al. 2012). When soybean seeds were stored for about 3 years, 30–50 % of *PLDα*-knockdown seeds germinated but WT seeds were non-viable (Lee et al. 2012). The results indicate that the presence of PLDα1 promotes seed deterioration and aging. In Arabidopsis, *PLDα1*-antisense knockdown seeds were more tolerant of aging than were *PLDα1*-KO seeds (Devaiah et al. 2007). Because the antisense may not act as specific as KO to *PLDα1* and antisense may also suppress other PLDαs such as PLDα2 and PLDα3. This difference could mean that other PLDs are also involved in lipid deterioration and seed aging.

During seed germination, KO of *PLDα1* or *PLDδ* results in seeds with decreased sensitivity to the ABA inhibition, and the effect was greater with *PLDα1*- and *PLDδ*-double KO (Uraji et al. 2012). These results suggest that *PLDα1* and *PLDδ* are involved in mediating the ABA effect not only in stomatal closure but also in seed germination.

4.7 PLDs in Plant Response to Auxin, Cytokinin, and Ethylene

Auxin is transported from the sites of synthesis to the sites of action through influx and efflux carrier proteins. *PLDζ2*-null Arabidopsis root displays an attenuated cycling of PIN2-containing vesicles. PLDζ2-overexpression results in an enhanced cycling of PIN2-containing vesicles in roots (Li and Xue 2007). PLDα-deficient plants displayed a slower rate of senescence than did wild type, suggesting a role of PLD in ethylene response (Fan et al. 1999). PA has been found to interact with CTR1 (Constitutive Triple Response) (Testerink et al. 2007), a protein kinase negatively regulates ethylene response. PA inhibits the kinase activity of CTR1 and also blocks the interaction of CTR1 with the ethylene receptor ETR1 (Testerink et al. 2007). Cytokinins are plant hormones that have an opposite effect on ethylene in plant senescence. Cytokinin-induced activation of PLD occurs (Romanov et al. 2002). The role of PLDs in the regulation of ethylene and cytokinin cascades remains to be determined.

5 Mechanism of PLD Actions

The broad range of physiological consequences resulting from PLD alterations raises an important question: How do PLDs mediate plant physiological responses? The metabolic and cellular effect of PLD processes can be divided into three general categories: cell regulation, membrane remodeling, and membrane