Signaling and Communication in Plants

Xuemin Wang *Editor*

Phospholipases in Plant Signaling

Springer

Signaling and Communication in Plants

Series Editor

František Baluška
Department of Plant Cell Biology, IZMB, University of Bonn, Kirschallee 1,
D-53115 Bonn, Germany

For further volumes:
http://www.springer.com/series/8094

Xuemin Wang
Editor

Phospholipases in Plant Signaling

Springer

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

ISSN 1867-9048 ISSN 1867-9056 (electronic)
ISBN 978-3-642-42010-8 ISBN 978-3-642-42011-5 (eBook)
DOI 10.1007/978-3-642-42011-5
Springer Heidelberg New York Dordrecht London

Library of Congress Control Number: 2013958085

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

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

Contents

Part III Phospholipases in Plant Biotic Interactions

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

X. Wang (ed.), *Phospholipases in Plant Signaling*, Signaling and Communication in Plants 20, DOI 10.1007/978-3-642-42011-5_1, © Springer-Verlag Berlin Heidelberg 2014

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

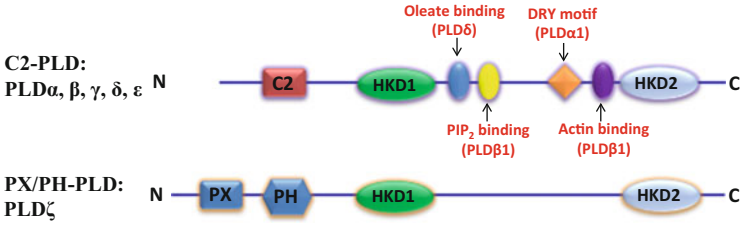

Fig. 1 Two PLD subfamilies and their domain structures. C2, Ca^{2+}-dependent phospholipid binding domain; PH, pleckstrin homology domain; PX, phox homology domain; HKD, HxKxxxxD motif involved in catalysis; DRY motif, interacts with Gα. The different ligand binding motifs have been experimentally determined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

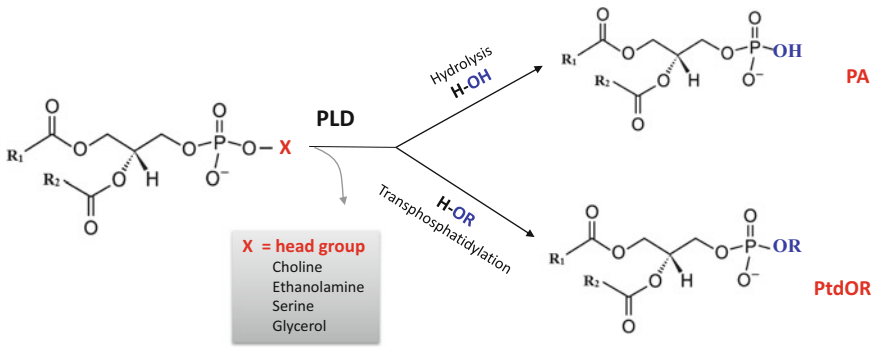

Fig. 2 PLD hydrolysis and transphosphatidylation activities. X represents head group of phospholipids. PLD hydrolyzes phospholipids to produce PA and head group, and in the presence of primary alcohols (H–OR), PLD transfers part of phosphatidyl moieties to –OR to form phosphatidylalcohol, also referred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

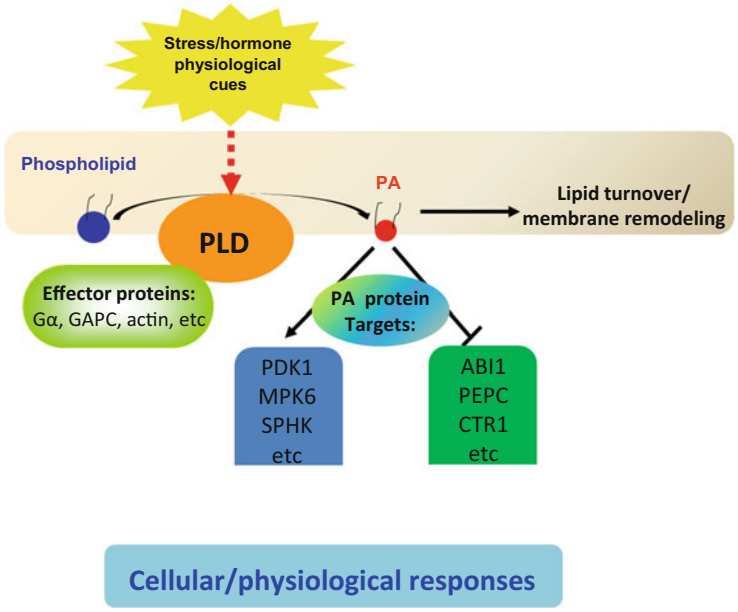

Fig. 3 PLD in stimulus-activated cell signaling. Stimulus-activated PLD hydrolyzes phospholipids to produces lipid messenger PA that interacts and regulates target protein functions. PLD hydrolysis can also be involved in lipid turnover and membrane remodeling. In addition, PLD interacts with effector proteins. *GAPC* cytosolic glyceraldehyde dehydrogenase, *PDK1* phosphoinositide-dependent protein kinase 1, *MPK6* mitogen-activated protein kinase 6, *SPHK* sphingosine kinase, *ABI1* abscisic acid-insensitive 1, *PEPC* phosphoenolpyruvate carboxylase, *CTR1* constitutive triple response 1

degradation (Fig. 3). The degradation refers to excessive hydrolysis of phospholipids, such as PC, that leads to the loss of membrane integrity and functions. Examples of these catabolic actions include the PLDα1 effect on freezing damages and seed aging. Membrane remodeling refers to the involvement of PLD activities in the changing of membrane lipid composition. An excellent example is the role of PLD on decreasing PC and increasing DGDG under phosphate deprivation. Cell regulation includes PLD roles in cell signaling, vesicular trafficking, and cytoskeletal rearrangements. The three functions of PLDs are not mutually exclusive and the same PLD can be involved in signaling, membrane remodeling, and degradation depending on the nature of stimuli and severity of stresses. The following discussion will focus on the role of PLD in cell regulation.

5.1 PLD Regulated Production of the Lipid Mediator PA

One major mode of PLD action is the production of PA (Fig. 3). PA is a potent lipid mediator that directly interacts with proteins and regulates their activity and intracellular location, as described in chapter "Phosphatidic Acid as Lipid Messenger and Growth Regulators in Plants." PA is present in small amounts in biological membranes, but the cellular level of PA changes rapidly under various conditions in plants. PLD plays a key role in the stimulus-induced production of PA. Moreover, genetic manipulations of individual PLDs have demonstrated that different PLDs often mediate the generation of signaling PA in plant responses to different stresses. PLDα1 is responsible for most of the PA produced in response to ABA (Zhang et al. 2004; Guo et al. 2012a), whereas PLDδ produces PA under H_2O_2-treated cells (Zhang et al. 2003). PLDζ2 plays a major role in the PA production under phosphate deprivation. PLDα1, PLDα3, and PLDδ each have been shown to contribute to hyperosmotic stress-induced PA production (Hong et al. 2009).

The activity of different PLDs is expected to play a crucial role in regulating the location, timing, and amount of PA production. Spatial and temporal regulation is critical in events that depend on intracellular lipid mediators because of their limited mobility in the cell. Lipid messengers can be compartmentalized not only to different membranes, such as the plasma, endoplasmic reticulum, and nuclear membranes, but also to microdomains within a specific membrane. The timing of lipid messenger production is important in differentiating among potential pathways of messenger production. In addition, PA has many molecular species as the acyl groups differ in the number of carbons and double bonds. PA species display different abilities to interact with target proteins and affect differently enzyme activities. PLDs, such as PLDζ, PLDδ, and PLDα1, display different substrate preferences and produce different PA species. The differences of individual PLDs in activation, intracellular association, expression patterns, and substrate preferences provide a basis for the distinguishable cellular and physiological roles of different PLDs in stress-induced PA production.

5.2 PLD Interaction with Proteins

Another mode of PLD actions is through PLD interactions with proteins. The interaction affects the activity of PLDs and reciprocally the PLD-interacting proteins (Fig. 3). Plant PLDs have been shown to interact with different proteins, including a heterotrimeric Gα, cytosolic glycolytic enzymes glyceraldehyde-3-phosphate dehydrogenases (GAPC), actin filaments (Guo et al. 2012a), microtubule cytoskeleton (Gardiner et al. 2001), and aspartic proteinase cardosin A (Simoes et al. 2005). In the PLDα1-Gα interaction, PLDα1 binds to the GDP-bound Gα and the interaction decreases PLDα1 activity. In response to ABA, activation of Gα may disrupt the binding of $G\alpha\text{-}_{GDP}$ to PLDα1, and thus activates PLDα1.

PLDα1-derived PA then mediates ABA-induced promotion of stomatal closure (Guo et al. 2012b). On the other hand, PLDα1 stimulates the intrinsic GTPase activity that converts active $G\alpha\text{-}_{GTP}$ to inactive $G\alpha\text{-}_{GDP}$ (Zhao and Wang 2004).

PLDδ interacts with GAPC, which converts glyceraldhyde-3-Pi (G3P) to 1,3-bisphosphoglycerate during glycolysis. H_2O_2 promoted the GAPC–PLDδ interaction and PLDδ activity (Guo et al. 2012a). Knockout of *GAPC*s decreased ABA- and H_2O_2-induced activation of PLD and stomatal sensitivity to ABA. The loss of *GAPC*s or *PLDδ* rendered plants less responsive to water deficits than wild type. The results indicate that the H_2O_2-promoted interaction of GAPC and PLDδ may provide a direct connection between membrane lipid-based signaling, energy metabolism, and growth control in the plant response to ROS and water stress.

Arabidopsis PLDβ1 was found to bind to actin. Whereas monomeric (G-) G-actin inhibits PLDβ activity, filamentous (F-) F-actin stimulates it (Kusner et al. 2002). A later study using a tobacco PLDβ has found that the F-actin activation of PLD and PA promotion of F-actin forms a positive feedback loop to increase locally membrane-F-actin dynamics in the cortex of plant cells, including promoting pollen tube growth (Pleskot et al. 2010, 2013). In addition, PLDδ was shown to bind to microtubules at membrane-cytoskeleton interface (Gardiner et al. 2001). PLDα1 promotes microtubule polymerization and bundling and is involved in plant response to salt stress (Zhang et al. 2012). The results indicate that PA and multiple PLDs are involved in both actin and microtubule cytoskeletal dynamics and rearrangements in plants.

5.3 Cross Talk of PLDs with Other Signaling Pathways

The activation of PLD is an integral part of signaling cascades. Cross talk between PLD-mediated pathway and other lipid-mediated signaling pathways, such as PI-PLC, PLC/DGK, and PLA, have been proposed to generate several classes of lipid mediators, such as PA, lysophospholipids, DAG, and free polyunsaturated fatty acids (Wang et al. 2000, 2006). In addition, PLD-derived PA is a potent stimulator of PIP-5 kinase that synthesizes PIP_2 which is a potent activator of PLDs. It has been proposed that activation of PLD and PIP-5 kinase in mammalian cells forms a positive feedback loop that leads to rapid generation of PA and PIP_2.

Recent studies show that the PLD-mediated signaling interplays with sphingosine kinase (SPHK) to mediate plant responses to ABA and stomatal closure. PA interacts directly with two *Arabidopsis* SPHKs (Guo et al. 2011). PA binds to both *Arabidopsis* SPHKs, and the interaction stimulates SPHK activity, as shown in vitro and also in plants. In response to ABA, the level of the SPHK products, long-chain base phosphate (LCBP) is lower in *pldα1*-knockout, and PA increased the LCBP production. These results suggest that PA mediates the SPHK activation in response to ABA (Guo et al. 2012b). On the other hand, in response to ABA, the PA production in *sphk1-1* and *sphk2-1* was significantly lower than WT while overexpression of *SPHK* increased PA production, suggesting that PLDα1

activation depends on SPHK (Guo et al. 2012b). These results indicate a codependence of PLD/PA and SPHK/phyto-S1P in the production of PA and phyto-S1P lipid messengers.

In addition, the interplay between PLDα1 and SPHK provides a mechanism by which stress signaling events are communicated between the plasma and vacuolar membranes. The subcellular localization of membrane-based lipid signaling is expected to play an important role in the regulation of enzyme activation, generation of lipid messengers, and mediation of downstream events (Li et al. 2009). It is not well understood how signaling events between different subcellular compartments are coordinated. PLDα1 is present in both the soluble and membrane fractions, and it translocates from the cytosol to membranes in response to stress (Ryu and Wang 1998; Fan et al. 1999). In response to ABA, SPHK is activated to produce phyto-S1P (possibly along with other LCBPs) on the vacuolar membrane. Phyto-S1P does not activate PLDα1 directly in vitro (Guo et al. 2012b). It was shown that S1P caused an increase in Ca^{2+} in response to ABA (Ng et al. 2001), and thus phyto-S1P may increase cytoplasmic Ca^{2+} to promote PLDα1 translocation to the plasma membranes and tonoplasts. Ca^{2+} is a key factor required for PLDα1 activity (Qin et al. 1997). Ca^{2+} promotes PLD translocation and its binding to the C2 domain increases the protein association with membrane lipids such as PC. This membrane association activates PLD to generate PA that binds to SPHK to promote its activity, thus, forming a positive feedback loop. The binding to phyto-S1P may also promote the translocation to the plasma membrane.

5.4 Unique and Overlapping Functions of Different PLDs

The genetic manipulation of different PLDs has resulted in specific stress-inducible phenotypes, demonstrating that individual PLDs have unique functions. The unique functions may result from one or a combination of the following properties of PLDs, such as (1) their different mechanism(s) of regulation that lead to differential activation under different stress conditions, as described in Sect. 3, (2) its substrate preferences, leading to formation of a particular PA molecular species and free head group products, (3) its propensity to associate with specific proteins and/or particular subcellular membranes, which may define substrate availability, and (4) its temporal and spatial (tissue and cell type-specific) pattern of expression. On the other hand, under some conditions, such as response to Pi deprivation, ABA-promoted stomatal closure, freezing, and high salinity, more overt inhibition of PA production and phenotypic changes were reported when two PLDs were ablated (Li et al. 2006; Bargmann et al. 2009). These results suggest that different PLDs have overlapping functions in specific stress response.

In addition, one PLD may have different functions, depending on the nature of the stress. For example, increased expression of PLDα1 promotes stomatal closure at earlier stages of drought but accelerates membrane deterioration in prolonged drought stress (Hong et al. 2008b). PLDζ1 and ζ2 produce PA that promotes

primary root elongation under a moderate Pi deprivation, whereas under severe P starvation, PLDζ1- and ζ2-produced PA is rapidly dephosphorylated presumably for DGDG synthesis (Li et al. 2006).

6 Perspectives

Cell membranes are an initial and focal point of signal perception and transduction involved in various biological processes, and the activation of phospholipases often occupies an early step in the messenger production. PLD is the most active family of phospholipases in plants. The plant PLD family has more members and more diverse domain structures than other organisms, which is in contrast to other types of phospholipases. Results increasingly indicate that PLDs play important roles in mediating various physiological processes in plants. The molecular heterogeneities of multiple PLDs play important roles in the diverse cellular functions of PLDs. The function of different PLDs can be unique and overlapping, depending on the specific type of stress and severity and stages of the stress. PLD and PA may provide key linkages among cellular regulators such as hormones, oxidative stress, G proteins, and protein phosphatase and kinases in plant growth and stress responses. Further investigations are needed to understand the complex interplays and functions of specific PLDs in the cell and their mode of actions. Specifically, how are different PLDs activated by specific types of hyperosmotic stresses? What are the molecular targets of PA and how the PLD and PA interactions with the effectors affect cell functions? In addition, what are the functions of lipid head groups released by PLD? How would the changes in lipid composition and membrane structure resulting from PLD activation influence plant stress responses. A better understanding of PLD and PA-based membrane lipid signaling has the potential to connect the stimulus perception at the cell membrane to intracellular actions and physiological responses.

Acknowledgments The work resulting from Wang laboratory was supported by grants from the National Science Foundation Grant IOS-0818740, the US Department of Agriculture Grant 2007-35318-18393, and the US Department of Energy Grant DE-SC0001295. We thank Brian Fanella for critical reading of the manuscript.

References

Allgyer TT, Wells MA (1979) Phospholipase D from savoy cabbage: purification and preliminary kinetic characterization. Biochemistry 18:5348–5353

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

Austin-Brown SL, Chapman KD (2002) Inhibition of phospholipase D alpha by N-acylethanolamines. Plant Physiol 129:1892–1898

Bargmann BO, Laxalt AM, Riet BT, Schouten E, van Leeuwen W, Dekker HL et al (2006) LePLDbeta1 activation and relocalization in suspension-cultured tomato cells treated with xylanase. Plant J 45:358–368

Bargmann BO, Laxalt AM, ter Riet B, van Schooten B, Merquiol E, Testerink C et al (2009) Multiple PLDs required for high salinity and water deficit tolerance in plants. Plant Cell Physiol 50:78–89

Cheever ML, Sato TK, de Beer T, Kutateladze TG, Emr SD, Overduin M (2001) Phox domain interaction with PtdIns(3)P targets the Vam7 t-SNARE to vacuole membranes. Nat Cell Biol 3:613–618

Choi S-Y, Huang P, Jenkins GM, Chan DC, Schiller J, Frohman MA (2006) A common lipid links Mfn-mediated mitochondrial fusion and SNARE-regulated exocytosis. Nat Cell Biol 8:1255–1262

Cruz-Ramírez A, Oropeza-Aburto A, Razo-Hernández F, Ramírez-Chávez E, Herrera-Estrella L (2006) Phospholipase Dζ2 plays an important role in extraplastidic galactolipid biosynthesis and phosphate recycling in Arabidopsis roots. Proc Natl Acad Sci USA 103:6765–6770

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

Devaiah SP, Roth MR, Baughman E, Li M, Tamura P, Jeannotte R et al (2006) Quantitative profiling of polar glycerolipid species from organs of wild-type Arabidopsis and a phospholipase Dalpha1 knockout mutant. Phytochemistry 67:1907–1924

Devaiah SP, Pan X, Hong Y, Roth M, Welti R, Wang X (2007) Enhancing seed quality and viability by suppressing phospholipase D in Arabidopsis. Plant J 50:950–957

Distefano AM, Scuffi D, Garcia-Mata C, Lamattina L, Laxalt AM (2012) Phospholipase Ddelta is involved in nitric oxide-induced stomatal closure. Planta 236:1899–1907

Dubots E, Audry M, Yamaryo Y, Bastien O, Ohta H, Breton C et al (2010) Activation of the chloroplast monogalactosyldiacylglycerol synthase MGD1 by phosphatidic acid and phosphatidylglycerol. J Biol Chem 285:6003–6011

Dyer JH, Ryu SB, Wang X (1994) Multiple forms of phospholipase D following germination and during leaf development of castor bean. Plant Physiol 105:715–724

Dyer JH, Zheng S, Wang X (1996) Structural heterogeneity of phospholipase D in 10 dicots. Biochem Biophys Res Commun 221:31–36

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

Fan L, Zheng S, Cui D, Wang X (1999) Subcellular distribution and tissue expression of phospholipase Dalpha, Dbeta, and Dgamma in Arabidopsis. Plant Physiol 119:1371–1378

Gardiner JC, Harper JD, Weerakoon ND, Collings DA, Ritchie S, Gilroy S et al (2001) A 90-kD phospholipase D from tobacco binds to microtubules and the plasma membrane. Plant Cell 13:2143–2158

Guo L, Mishra G, Taylor K, Wang X (2011) Phosphatidic acid binds and stimulates Arabidopsis sphingosine kinases. J Biol Chem 286:13336–13345

Guo L, Devaiah SP, Narasimhan R, Pan X, Zhang Y, Zhang W et al (2012a) Cytosolic glyceraldehyde-3-phosphate dehydrogenases interact with phospholipase Ddelta to transduce hydrogen peroxide signals in the Arabidopsis response to stress. Plant Cell 24:2200–2212

Guo L, Mishra G, Markham JE, Li M, Tawfall A, Welti R et al (2012b) Connections between sphingosine kinase and phospholipase D in the abscisic acid signaling pathway in Arabidopsis. J Biol Chem 287:8286–8296

Hammond SM, Altshuller YM, Sung TC, Rudge SA, Rose K, Engebrecht J et al (1995) Human ADP-ribosylation factor-activated phosphatidylcholine-specific phospholipase D defines a new and highly conserved gene family. J Biol Chem 270:29640–29643

Hanahan DJ, Chaikoff IL (1947) A new phospholipide-splitting enzyme specific for the ester linkage between the nitrogenous base and the phosphoric acid grouping. J Biol Chem 169:699–705

Heller M (1978) Phospholipase D. Adv Lipid Res 16:267–326

Heller M, Mozes N, Peri I, Maes E (1974) Phospholipase D from peanut seeds: IV. Final purification and some properties of the enzyme. Biochim Biophys Acta 369:397–410

Hiroaki H, Ago T, Ito T, Sumimoto H, Kohda D (2001) Solution structure of the PX domain, a target of the SH3 domain. Nat Struct Biol 8:526–530

Hong Y, Pan X, Welti R, Wang X (2008a) The effect of phospholipase Dalpha3 on Arabidopsis response to hyperosmotic stress and glucose. Plant Signal Behav 3:1099–1100

Hong Y, Zheng S, Wang X (2008b) Dual functions of phospholipase Dalpha1 in plant response to drought. Mol Plant 1:262–269

Hong Y, Devaiah SP, Bahn SC, Thamasandra BN, Li M, Welti R et al (2009) Phospholipase D epsilon and phosphatidic acid enhance Arabidopsis nitrogen signaling and growth. Plant J 58:376–387

Jia Y, Tao F, Li W (2013) Lipid profiling demonstrates that suppressing Arabidopsis phospholipase Dδ retards ABA-promoted leaf senescence by attenuating lipid degradation. PLoS One 8: e65687

Jost R, Berkowitz O, Shaw J, Masle J (2009) Biochemical characterization of two wheat phosphoethanolamine N-methyltransferase isoforms with different sensitivities to inhibition by phosphatidic acid. J Biol Chem 284:31962–31971

Kanai F, Liu H, Field SJ, Akbary H, Matsuo T, Brown GE et al (2001) The PX domains of p47phox and p40phox bind to lipid products of PI(3)K. Nat Cell Biol 3:675–678

Katagiri T, Takahashi S, Shinozaki K (2001) Involvement of a novel Arabidopsis phospholipase D, AtPLDdelta, in dehydration-inducible accumulation of phosphatidic acid in stress signalling. Plant J 26:595–605

Kusner DJ, Barton JA, Wen KK, Wang X, Rubenstein PA, Iyer SS (2002) Regulation of phospholipase D activity by actin. Actin exerts bidirectional modulation of Mammalian phospholipase D activity in a polymerization-dependent, isoform-specific manner. J Biol Chem 277:50683–50692

Lee MH (1989) Phospholipase D of rice bran. I. Purification and characterization. Plant Sci 59:25–33

Lee J, Welti R, Roth M, Schapaugh WT, Li J, Trick HN (2012) Enhanced seed viability and lipid compositional changes during natural ageing by suppressing phospholipase Dalpha in soybean seed. Plant Biotechnol J 10:164–173

Li G, Xue HW (2007) Arabidopsis PLDzeta2 regulates vesicle trafficking and is required for auxin response. Plant Cell 19:281–295

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

Li M, Qin C, Welti R, Wang X (2006) Double knockouts of phospholipases Dzeta1 and Dzeta2 in Arabidopsis affect root elongation during phosphate-limited growth but do not affect root hair patterning. Plant Physiol 140:761–770

Li W, Wang R, Li M, Li L, Wang C, Welti R et al (2008) Differential degradation of extraplastidic and plastidic lipids during freezing and post-freezing recovery in Arabidopsis thaliana. J Biol Chem 283:461–468

Li M, Hong Y, Wang X (2009) Phospholipase D- and phosphatidic acid-mediated signaling in plants. Biochim Biophys Acta 1791:927–935

Liu Q, Zhang C, Yang Y, Hu X (2010) Genome-wide and molecular evolution analyses of the phospholipase D gene family in Poplar and Grape. BMC Plant Biol 10:117

Lu S, Bahn SC, Qu G, Qin H, Hong Y, Xu Q et al (2013) Increased expression of phospholipase Dalpha1 in guard cells decreases water loss with improved seed production under drought in Brassica napus. Plant Biotechnol J 11:380–389

McGee JD, Roe JL, Sweat TA, Wang X, Guikema JA, Leach JE (2003) Rice phospholipase D isoforms show differential cellular location and gene induction. Plant Cell Physiol 44:1013–1026

Ng CK, Carr K, McAinsh MR, Powell B, Hetherington AM (2001) Drought-induced guard cell signal transduction involves sphingosine-1-phosphate. Nature 410:596–599

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

Pappan K, Wang X (1999) Plant phospholipase Dalpha is an acidic phospholipase active at near-physiological Ca(2+) concentrations. Arch Biochem Biophys 368:347–353

Pappan K, Qin W, Dyer JH, Zheng L, Wang X (1997a) Molecular cloning and functional analysis of polyphosphoinositide-dependent phospholipase D, PLDbeta, from Arabidopsis. J Biol Chem 272:7055–7061

Pappan K, Zheng S, Wang X (1997b) Identification and characterization of a novel plant phospholipase D that requires polyphosphoinositides and submicromolar calcium for activity in Arabidopsis. J Biol Chem 272:7048–7054

Pappan K, Austin-Brown S, Chapman KD, Wang X (1998) Substrate selectivities and lipid modulation of plant phospholipase D alpha, -beta, and -gamma. Arch Biochem Biophys 353:131–140

Pleskot R, Potocky M, Pejchar P, Linek J, Bezvoda R, Martinec J et al (2010) Mutual regulation of plant phospholipase D and the actin cytoskeleton. Plant J 62:494–507

Pleskot R, Li J, Zárský V, Potocký M, Staiger CJ (2013) Regulation of cytoskeletal dynamics by phospholipase D and phosphatidic acid. Trends Plant Sci 18:496–504

Pinosa F, Buhot N, Kwaaitaal M, Fahlberg P, Thordal-Christensen H, Ellerström M, Andersson MX (2013) Arabidopsis phospholipase Dδ is involved in basal defense and nonhost resistance to powdery mildew fungi. Plant Physiol 163:896–906

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 calcium-independent and phosphatidylcholine-selective PLD zeta 1 with distinct regulatory domains. Plant Physiol 128:1057–1068

Qin W, Pappan K, Wang X (1997) Molecular heterogeneity of phospholipase D (PLD). Cloning of PLDgamma and regulation of plant PLDgamma, -beta, and -alpha by polyphosphoinositides and calcium. J Biol Chem 272:28267–28273

Qin C, Wang C, Wang X (2002) Kinetic analysis of Arabidopsis phospholipase Ddelta. Substrate preference and mechanism of activation by Ca^{2+} and phosphatidylinositol 4,5-biphosphate. J Biol Chem 277:49685–49690

Qin C, Li M, Qin W, Bahn SC, Wang C, Wang X (2006) Expression and characterization of Arabidopsis phospholipase Dgamma2. Biochim Biophys Acta 1761:1450–1458

Quarles RH, Dawson RM (1969) The distribution of phospholipase D in developing and mature plants. Biochem J 112:787–794

Romanov GA, Kieber JJ, Schmulling T (2002) A rapid cytokinin response assay in Arabidopsis indicates a role for phospholipase D in cytokinin signalling. FEBS Lett 515:39–43

Roughan PG, Slack CR (1976) Is phospholipase D really an enzyme? A comparison of in situ and in vitro activities. Biochim Biophys Acta 431:86–95

Ryu SB, Wang X (1998) Increase in free linolenic and linoleic acids associated with phospholipase D-mediated hydrolysis of phospholipids in wounded castor bean leaves. Biochim Biophys Acta 1393:193–202

Sang Y, Zheng S, Li W, Huang B, Wang X (2001) Regulation of plant water loss by manipulating the expression of phospholipase Dα. Plant J 28:135–144

Sarri E, Servitja J-M, Picatoste F, Claro E (1996) Two phosphatidylethanol classes separated by thin layer chromatography are produced by phospholipase D in rat brain hippocampal slices. FEBS Lett 393:303–306

Simoes I, Mueller EC, Otto A, Bur D, Cheung AY, Faro C et al (2005) Molecular analysis of the interaction between cardosin A and phospholipase D(alpha). Identification of RGD/KGE sequences as binding motifs for C2 domains. FEBS J 272:5786–5798

Stuckey JA, Dixon JE (1999) Crystal structure of a phospholipase D family member. Nat Struct Biol 6:278–284

Testerink C, Larsen PB, van der Does D, van Himbergen JA, Munnik T (2007) Phosphatidic acid binds to and inhibits the activity of Arabidopsis CTR1. J Exp Bot 58:3905–3914

Uraji M, Katagiri T, Okuma E, Ye W, Hossain MA, Masuda C et al (2012) Cooperative function of PLDdelta and PLDalpha1 in abscisic acid-induced stomatal closure in Arabidopsis. Plant Physiol 159:450–460

Waite M (1999) The PLD superfamily: insights into catalysis. Biochim Biophys Acta 1439:187–197

Waksman M, Eli Y, Liscovitch M, Gerst JE (1996) Identification and characterization of a gene encoding phospholipase D activity in yeast. J Biol Chem 271:2361–2364

Wan J, Torres M, Ganapathy A, Thelen J, DaGue BB, Mooney B et al (2005) Proteomic analysis of soybean root hairs after infection by Bradyrhizobium japonicum. Mol Plant Microbe Interact 18:458–467

Wang C, Wang X (2001) A novel phospholipase D of Arabidopsis that is activated by oleic acid and associated with the plasma membrane. Plant Physiol 127:1102–1112

Wang X, Dyer JH, Zheng L (1993) Purification and immunological analysis of phospholipase D from castor bean endosperm. Arch Biochem Biophys 306:486–494

Wang X, Xu L, Zheng L (1994) Cloning and expression of phosphatidylcholine-hydrolyzing phospholipase D from Ricinus communis L. J Biol Chem 269:20312–20317

Wang X, Wang C, Sang Y, Zheng L, Qin C (2000) Determining functions of multiple phospholipase Ds in stress response of Arabidopsis. Biochem Soc Trans 28:813–816

Wang X, Devaiah SP, Zhang W, Welti R (2006) Signaling functions of phosphatidic acid. Prog Lipid Res 45:250–278

Welti R, Li W, Li M, Sang Y, Biesiada H, Zhou HE et al (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

Yamaguchi T, Kuroda M, Yamakawa H, Ashizawa T, Hirayae K, Kurimoto L et al (2009) Suppression of a phospholipase D gene, OsPLDbeta1, activates defense responses and increases disease resistance in rice. Plant Physiol 150:308–319

Yamaryo Y, Dubots E, Albrieux C, Baldan B, Block MA (2008) Phosphate availability affects the tonoplast localization of PLDzeta2, an Arabidopsis thaliana phospholipase D. FEBS Lett 582:685–690

Yu L, Nie J, Cao C, Jin Y, Yan M, Wang F et al (2010) Phosphatidic acid mediates salt stress response by regulation of MPK6 in Arabidopsis thaliana. New Phytol 188:762–773

Zhang W, Wang C, Qin C, Wood T, Olafsdottir G, Welti R et al (2003) The oleate-stimulated phospholipase D, PLDdelta, and phosphatidic acid decrease H2O2-induced cell death in Arabidopsis. Plant Cell 15:2285–2295

Zhang W, Qin C, Zhao J, Wang X (2004) Phospholipase D alpha 1-derived phosphatidic acid interacts with ABI1 phosphatase 2C and regulates abscisic acid signaling. Proc Natl Acad Sci USA 101:9508–9513

Zhang Y, Zhu H, Zhang Q, Li M, Yan M, Wang R et al (2009) Phospholipase dalpha1 and phosphatidic acid regulate NADPH oxidase activity and production of reactive oxygen species in ABA-mediated stomatal closure in Arabidopsis. Plant Cell 21:2357–2377

Zhang W, Wen B, Li W, Hong Y, Wang X (2010) Plant phospholipase D. In: Munnik T (ed) Lipid signaling in plants. Springer, Berlin, pp 39–62

Zhang Q, Lin F, Mao T, Nie J, Yan M, Yuan M et al (2012) Phosphatidic acid regulates microtubule organization by interacting with MAP65-1 in response to salt stress in Arabidopsis. Plant Cell 24:4555–4576

Zhao J, Wang X (2004) Arabidopsis phospholipase Dalpha1 interacts with the heterotrimeric G-protein alpha-subunit through a motif analogous to the DRY motif in G-protein-coupled receptors. J Biol Chem 279:1794–1800

Zhao J, Wang C, Bedair M, Welti R, Sumner LW, Baxter I, Wang X (2011) Suppression of phospholipase Dγs confers increased aluminum resistance in Arabidopsis thaliana. PLoS One 6:e28086

Zhao J, Zhou D, Zhang Q, Zhang W (2012) Genomic analysis of phospholipase D family and characterization of GmPLDalphas in soybean (Glycine max). J Plant Res 125:569–578

Zhao J, Devaiah SP, Wang C, Li M, Welti R, Wang X (2013) Arabidopsis phospholipase Dbeta1 modulates defense responses to bacterial and fungal pathogens. New Phytol 199:228–240

Zheng L, Krishnamoorthi R, Zolkiewski M, Wang X (2000) Distinct Ca^{2+} binding properties of novel C2 domains of plant phospholipase dalpha and beta. J Biol Chem 275:19700–19706

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

PI-PLC: Phosphoinositide-Phospholipase C in Plant Signaling

Teun Munnik

Abstract Historically, phosphoinositide-specific phospholipase C (PI-PLC) catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) to generate the second messengers, inositol 1,4,5-trisphosphate ($InsP_3$) and diacylglycerol (DAG), which release Ca^{2+} from intracellular stores and activate members of the protein kinase C (PKC) family, respectively. While this signaling system is well understood in animal cells, for plants this is still far from clear, as they lack homologs for the $InsP_3$ receptor and PKC, and display extremely low PIP_2 levels in their membranes under normal conditions. Plant genomes do contain numerous genes coding for phosphatidylinositol kinase (PIK) to make phosphatidylinositol 4-phosphate (PIP), PIP kinase (PIPK) to synthesize PIP_2, and PI-PLC to hydrolyze these lipids. Data is also emerging that not $InsP_3$ or DAG but their phosphorylated products, i.e., inositolpolyphosphates (IPPs) such as $InsP_5$ and $InsP_6$, and phosphatidic acid (PA), are functioning as plant signaling molecules. The goal of this chapter is to provide a critical overview of what is currently known about plant PI-PLC signaling and to indicate directions for future research.

Keywords Plant stress • Plant signaling • Signal transduction • Lipid signaling • Phosphoinositides • Inositolphosphates • Inositol lipids • Phospholipase C • Lipid kinases • Phosphatidic acid

1 Introduction: History of Plant PI-PLC Signaling

Since the discovery of the PI-PLC signaling system in animals in the late 1980s, the plant field traditionally followed closely, and perhaps too closely. From animal systems it is clear that receptor stimulation by an extracellular stimulus causes

T. Munnik (✉)
Section Plant Physiology, Swammerdam Institute for Life Sciences (SILS), University of Amsterdam, Science Park904, 1098XH Amsterdam, The Netherlands
e-mail: t.munnik@uva.nl

X. Wang (ed.), *Phospholipases in Plant Signaling*, Signaling and Communication in Plants 20, DOI 10.1007/978-3-642-42011-5_2, © Springer-Verlag Berlin Heidelberg 2014

activation of a PI-PLC to produce $InsP_3$ and diacylglycerol (DAG). While the water-soluble $InsP_3$ diffuses into the cytosol where it triggers the release of Ca^{2+} from an intracellular store through binding and activation of a ligand-gated calcium channel (the $InsP_3$ receptor), the other second messenger, the lipid DAG, remains in the membrane where it recruits and activates members of the PKC family via a highly conserved C1 domain. The subsequent increase in Ca^{2+} together with the change in phosphorylation status causes various protein targets to become activated, inactivated, and/or relocalized, leading to a massive reprogramming of the cell, allowing it to respond appropriately to the initial extracellular stimulus.

Since plants exhibited many components of the PI-PLC system with structural or functional equivalents, it was reasonable to assume a similar function. They contained the minor lipids PIP and PIP_2, the kinases that make them (i.e., PIK and PIPK), and PI-PLC activity to break the PIP_2 down into $InsP_3$ and DAG (Cote et al. 1996; Hetherington and Drøbak 1992; Drøbak 1992; Drøbak et al. 1999; Munnik et al. 1998a; Einspahr and Thompson 1990). When then in the early 1990s, microinjected $InsP_3$ (or photoactivation of a caged variant) was shown to trigger an increase in intracellular Ca^{2+} (Blatt et al. 1990; Gilroy et al. 1990), the plant PI-PLC signaling system was thought to be another eukaryotic fact (Hunt and Gray 2001; Krinke et al. 2007).

Recent advances, however, suggest that the plant PI-PLC signaling system is quite different from the "canonical" pathway described for mammalians. For example, homologs of both the primary targets of the mammalian PI-PLC system, i.e., the $InsP_3$ receptor and PKC, are lacking from higher plant genomes (Munnik and Testerink 2009; Munnik and Nielsen 2011; Munnik and Vermeer 2010; Wheeler and Brownlee 2008). Moreover, not $InsP_3$, but its phosphorylated product $InsP_6$ was shown to be responsible for the release of intracellular Ca^{2+} (Lemtiri-Chlieh et al. 2000, 2003). Similarly, evidence has been accumulating that not DAG but its phosphorylated product phosphatidic acid (PA) functions as the plant lipid second messenger in this pathway (Arisz et al. 2009; Testerink and Munnik 2005, 2011; Munnik 2001). Still, what the precise role of plant PI-PLC and its reaction products is, remains mostly enigmatic. A summary of what we currently know and what remains confusing is given below.

2 Plant PI-PLCs Belong to the PLCζ Class

Eukaryotic PI-PLCs have been classified into six subfamilies, i.e., β, γ, δ, ε, η and ζ, based on domain structure and organization [Fig. 1; (Munnik and Testerink 2009)]. Mammalian cells contain all six isoforms (13 in total) whereas plants only exhibit one, i.e., PLCζ. This class lacks the Pleckstrin Homology (PH) domain that is present in all other PI-PLCs and only contains the catalytic X- and Y-domains, along with a C2 lipid-binding domain (Fig. 1). Most, but not all of the plant PI-PLCs, also contain one EF-hand lobe. Besides the aforementioned domains, the other PI-PLC subfamilies contain various conserved sequences that allow them to be regulated by, e.g., heterotrimeric G-proteins (PLCβ), tyrosine kinases (PLCγ), or Ras (PLCε). How animal PLCδ, -η and -ζ isoforms are regulated is still not clear

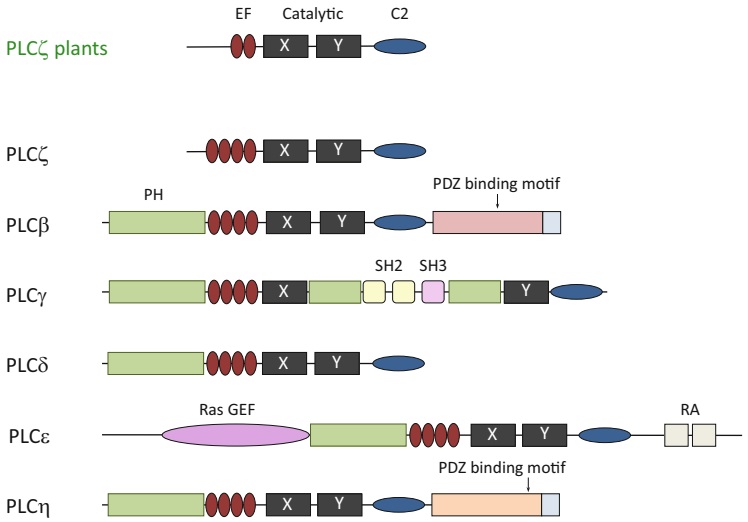

Fig. 1 Domain structure and organization of PI-PLC isozymes. There are six different eukaryotic PI-PLCs: β, γ, δ, ε, η and ζ. Plant PI-PLCs are most closely related to the PLCζ subfamily, which lack the PH domain and only consist of the minimal core structure, the catalytic X- and Y boxes, a C2 domain, and the first EF-hand motif (even though the latter is not always present). Other subfamilies contain additional domains (see Sect. 4). PLCη undergoes alternative splicing, generating variable C termini with the PDZ-binding motif only being present in the longer forms. *EF* EF-hand domain, *PH* Pleckstrin homology domain, *RA* Ras-binding domain, *RasGEF* guanine nucleotide-exchange factor for Ras, *SH2*/*SH3* Src homology domain 2 or 3, *X* and *Y* catalytic domains

but may involve Ca^{2+} (Cockcroft 2006; Balla et al. 2009). Interestingly, mammalian PLCζ is only expressed in sperm cells and is involved in generating an $InsP_3$/Ca^{2+} signal in egg cells upon fertilization (Swann and Lai 2013).

Plants contain multiple gene families, with the Arabidopsis genome encoding 9 *PLC* genes, tomato 6, and rice 4 (Munnik and Testerink 2009; Tasma et al. 2008; Vossen et al. 2010). Genes are differentially expressed in various cells and tissues, during different stages of plant development, and in response to various abiotic and biotic stresses (Tasma et al. 2008; Vossen et al. 2010; Wang et al. 2008; Lin et al. 2004; Singh et al. 2013; Hirayama et al. 1995, 1997; Das et al. 2005; Song et al. 2008; Pan et al. 2005).

3 Enzymatic Activity: PI-PLCs Can Hydrolyze PI4P and PI(4,5)P_2

Mammalian PI-PLCs have an absolute requirement for Ca^{2+}. At physiological concentrations (low μM level), the preferred substrates are PI4P and PI(4,5)P_2 but at mM Ca^{2+} concentrations, most enzymes also hydrolyze PI. Polyphosphoinositides

(PPIs) that are phosphorylated at the D3-position of the inositol ring (i.e., PI3P, PI(3,4)P_2, PI(3,5)P_2, and PI(3,4,5)P_3) are not used as substrates (Munnik et al. 1998b). Bacteria also exhibit a PI-PLC activity, but this type only hydrolyzes PI and does not require Ca^{2+}. The coding genes are also totally different from the eukaryotic *PI-PLCs*, lacking any sequence homology (Munnik et al. 1998b; Meijer and Munnik 2003; Mueller-Roeber and Pical 2002). Plants do contain bacterial-type of PLCs but these enzymes hydrolyze structural phospholipids, like PE and PC, and are involved in lipid metabolism rather than signaling. Arabidopsis contains six of them, called NPCs, for nonspecific PLC (*see* Chap. 3 by Yuki Nakamura—NPC: Nonspecific phospholipase Cs in plant functions).

In vitro, plant PLC activity has been measured in various tissues and species (reviewed in Munnik et al. 1998b). Most enzymes are optimally active between pH 6 and 7 and stimulated by detergents. Roughly, PLC activity can be segregated into two types: one being present in the soluble fraction, preferring PI over PI4P or PI(4,5)P_2 and requiring mM Ca^{2+} concentrations, and the other type being predominantly bound to membranes, in particular the plasma membrane, preferring PI4P and PI(4,5)P_2 as a substrate, and being fully active at low μM Ca^{2+} concentrations (Munnik et al. 1998b). This classification is, however, very oversimplified since all data comes from crude protein extracts: none of the enzyme activities have ever been linked to any of the *PLC* genes cloned. Nonetheless, in vitro PLC activity has been confirmed for recombinant AtPLC1-5 (Hunt et al. 2004).

In vivo, PLCs are traditionally considered to hydrolyze PI(4,5)P_2 because this yields the InsP_3 to release the intracellular Ca^{2+} via the ligand-gated receptor. However, plants lack this receptor and, compared to mammalian cells, exhibit extremely low PIP_2 levels. Plant PI-PLCs also lack the PH domain that would potentially be able to find PIP_2. Mammalian PLCζ was recently found to bind PIP_2 via the linker between the X- and Y-domain (Nomikos et al. 2007, 2011) but this sequence is not conserved in Arabidopsis (Munnik, unpublished). On the other hand, plant cells contain relatively large amounts of PI4P in the plasma membrane (Vermeer et al. 2009), and this lipid is hydrolyzed in vitro equally well (munnik et al. 1998b), so there is no basis to stay focused only on PI(4,5)P_2 as the sole substrate for PLC. Moreover, if the role of plant PLC is to generate PA and InsP_6 (or other IPPs or metabolites of inositol; see below), then PI4P hydrolysis would fulfill this role equally well, if not better since there is enough PI4P in the plasma membrane (Munnik and Vermeer 2010). Of course, this may be different under conditions where PI(4,5)P_2 synthesis is triggered, i.e., during salt and osmotic stress (Darwish et al. 2009; Konig et al. 2008a; Beno-Moualem et al. 1995; DeWald et al. 2001; van Leeuwen et al. 2007; Kaye et al. 2011; Drøbak and Watkins 2000; Takahashi et al. 2001), in response to heat (Liu et al. 2006a, b; Zheng et al. 2012; Mishkind et al. 2009), or during development (Pan et al. 2005; van Leeuwen et al. 2007; Ischebeck et al. 2010a, b; Stenzel et al. 2008; Kusano et al. 2008; Zhao et al. 2010; Sousa et al. 2008; Dowd et al. 2006; Xu et al. 2005; Monteiro et al. 2005; Zonia and Munnik 2004; Lee et al. 2007). Note, that at high Ca^{2+} concentrations, PI might also function as a substrate (e.g., during wounding).

4 Mode of Regulation

In animals, PI-PLC activity is classically activated by receptor tyrosine kinases and via small- or heterotrimeric-G proteins. To fall under such control, however, highly specific domains are required which plant PLCζs typically lack [Fig. 1; (Munnik and Testerink 2009)].

How plant PLC activity is controlled is still completely unknown. In vitro assays indicate that most activity is located at the plasma membrane (Munnik et al. 1998b; Pical et al. 1992; Kopka et al. 1998a; Melin et al. 1987; Kim et al. 2004; Otterhag et al. 2001). Theoretically, there is enough PI4P there, but it does not appear that this pool is continuously hydrolyzed as witnessed by ^{32}P-PPI labeling and lipid biosensor studies (*see* Sect. 5, van Leeuwen et al. 2007; Vermeer et al. 2009); Vermeer and Munnik, unpublished). This implies that the PI-PLC in the plasma membrane of plants is *inactive* and that it requires some form of activation to start the hydrolysis. Below, is a summary of potential regulators, both old and new.

4.1 Calcium

Ca^{2+} is obviously one of the candidates to regulate plant PLC. The enzyme is catalytically dependent on it and putatively contains an EF-hand- and/or a C2 domain to control this (Kopka et al. 1998a, b; Otterhag et al. 2001; Rupwate and Rajasekharan 2012). Whether this is the sole form of regulation is, however, unlikely. Almost everything triggers an increase in intracellular Ca^{2+}, yet only a few conditions are known to activate PLC. The fact that Ca^{2+} often oscillates, e.g., during the growth of pollen tubes or root hairs, could provide a nice model system to test for simultaneous changes in Ca^{2+}, $InsP_3$, DAG, PI4P, and $PI(4,5)P_2$ using genetically encoded biosensors (van Leeuwen et al. 2007; Mishkind et al. 2009; Vermeer et al. 2006, 2009; Swanson and Gilroy 2013; Choi et al. 2012; Vermeer and Munnik 2013; Thole et al. 2008; Remus et al. 2006).

4.2 Phosphorylation

Phosphorylation is an attractive and potential form to control enzymatic activity (and localization), because phosphate additions induce strong/large conformational changes. Like many proteins, PLCs contain multiple putative phosphorylation sites (Munnik, unpublished). Interestingly, in a proteomic analysis of plasma membrane proteins phosphorylated in response to the microbial elicitor flagellin (flg22), AtPLC2 was identified as one the proteins (Nuhse et al. 2003). Consequences for PLC activity, however, remain unknown. Nonetheless, various elicitors, including flg22, have been shown to trigger relatively fast PPI responses and to generate PA

via DGK in ^{32}P-labeled cell suspensions (van der Luit et al. 2000; den Hartog et al. 2001, 2003; de Jong et al. 2004; Raho et al. 2010, 2011; Bargmann et al. 2006; Chen et al. 2007).

4.3 G-Protein Regulation

G proteins are guanosine nucleotide-binding proteins that are typically involved in signal transduction and function as molecular switches. Their activity is regulated by factors that control their ability to bind and hydrolyze GTP to GDP. In their GTP-bound form they are "on" and when bound to GDP, they are "off". G proteins belong to the larger group of enzymes called GTPases. There are two classes of G proteins: the monomeric small GTPases (e.g., Rho, Rac, Ras, Ran) and heterotrimeric-G proteins, which are composed of G_{α} (the GTPase) and a $G_{\beta\gamma}$ subunit that dissociate from each other in a GTP-dependent manner.

Earlier plant studies, mostly performed in the green algae *Chlamydomonas*, which do have an $InsP_3$ receptor, showed that mastoparan [a 14-aa peptide isolated from wasp venom that is a potent G-protein activator (Munnik et al. 1998a)] stimulated the turnover of PPIs and/or increased the formation of $InsP_3$ (Munnik et al. 1998a; Quarmby et al. 1992; Legendre et al. 1993; Yueh and Crain 1993; Drøbak and Watkins 1994; Cho et al. 1995; Kuin et al. 2000; Musgrave et al. 1992; Van Himbergen et al. 1999), and hence suggested that plant PLCs might be regulated via a heterotrimeric G-protein coupled pathway. However, we now know that higher plant PI-PLCs belong to the PLCζ class, which lack the conserved sequences found in G-protein regulated PLCβ- or ε-types (Fig. 1). An alternative explanation for the ability of mastoparan to stimulate the PLC pathway is that these peptides can induce small pores, allowing small molecules like Ca^{2+} to leak into the cell (Suh et al. 1996; Tucker and Boss 1996) and thus stimulate PLC activity directly.

PLC has been claimed to function downstream of the Arabidopsis GRC1 (G protein-coupled receptor 1 Apone et al. 2003); however, this conclusion was predominantly based on the use of U73122, an unreliable PLC inhibitor (see Sect. 5.4), and GRC1's role itself upstream of G-protein signaling remains controversial (Urano and Jones 2013). Remarkably, Tuteja's lab reported a PLC in pea that interacted with both Gα and Gβ (Misra et al. 2007). Despite these publications, however, it is unlikely that plant PI-PLCs are regulated by trimeric G-proteins.

Some of the plant PIPKs are regulated by small G-proteins and, in this way, could control plant PLC activity indirectly by supplying substrate. For example, during tip growth of root hairs or pollen tubes there is clear evidence for the involvement of PLC, PIPK, and ROPs (Rho-like G proteins for plants) (Ischebeck et al. 2008, 2010a, b, 2011; Kusano et al. 2008; Sousa et al. 2008; Dowd et al. 2006; Kost et al. 1999; Kost 2008; Saavedra et al. 2011). Since mastoparan can also activate small G proteins (Munnik et al. 1998a; Van Himbergen et al. 1999), this may represent a third explanation for the potential increase in PLC activity reported above.

4.4 The Unknown Tobacco Linker

The C2 domain seems to be responsible for keeping PLC in the membrane (Kim et al. 2004; Otterhag et al. 2001; Rupwate and Rajasekharan 2012; Rupwate et al. 2012). Normally, this is considered to be a Ca^{2+}- and lipid-dependent interaction where Ca^{2+} drives the conformational change for the domain to bind the phospholipid bilayer (Kopka et al. 1998b). Another potential form of linkage was recently found by coincidence. Screening tobacco for genes that were transcriptionally activated during the early stages of wounding resulted in the isolation of *NtC7*, encoding an unknown membrane-localized protein. Overexpression of *NtC7* in tobacco plants conferred a marked tolerance to salt stress, suggesting a role in osmoregulation (Tamura et al. 2003). To search for proteins that interact with NtC7, a yeast two-hybrid screen was used and this resulted in the identification of a *Nicotiana tabacum PI-PLC* (Nakamura and Sano 2009). Physical interaction between NtC7 and NtPI-PLC was confirmed by pull-down assays and this required the C2 domain. Transient expression studies of GFP-fusion proteins in onion epidermal cells indicated that both proteins predominantly localized to the plasma membrane while bimolecular fluorescence complementation suggests that the proteins interact *in planta* too. The role of this novel protein and its effect on PLC activity was unfortunately not addressed, nor was the effect of salt or wounding analyzed.

4.5 Gene Expression

Gene expression analysis in rice, pea, tobacco, potato, tomato, and Arabidopsis revealed that several *PLCs* are responsive to biotic and/or abiotic stresses (Tasma et al. 2008; Vossen et al. 2010; Lin et al. 2004; Singh et al. 2013; Hirayama et al. 1995, 1997; Das et al. 2005; Kim et al. 2004; Sanchez-Cach et al. 2008; Suzuki et al. 2007; Repp et al. 2004; Venkataraman et al. 2003; Coursol et al. 2002). Whether this reflects truly "signaling" or is already part of the plant's response to acclimate is a matter of discussion. Nonetheless, cell-specific or stress/developmentally induced gene expression is a way to control PLC activity indirectly.

In silico promoter analysis of Arabidopis (Tasma et al. 2008) or rice PLC genes (Singh et al. 2013) have identified several putative cis-acting regulatory elements, which may reflect control by stress and/or hormones. Recently, *AtPLC1* was identified as one of the downstream targets of ETHYLENE RESPONSE FACTOR 53 (AtERF53), which belongs to the AP2/ERF family of transcription factors that is induced in the early hours of dehydration and salt stress and is involved in abiotic stress tolerance in Arabidopsis (Hsieh et al. 2013). These results are in agreement with the data of Hirayama et al. (Hirayama et al. 1995), showing that *AtPLC1* is strongly upregulated in response to salt-and drought stress.

5 Measuring PLC Activity In Vivo: Complexities and Matter to Consider

PI-PLC signaling has been implicated in many different stress responses, both biotic and abiotic, yet whether all these observations are significant remains to be seen. How would one know *if* and *when* PLC signaling is involved? Many claims in the literature come from studies using the "quick and dirty" approach, based on a "specific" PLC inhibitor. These inhibitors are not specific though (*see* Sect. 5.4), so claims solely based on that should be treated with great caution in general.

Probably the most reliable observations come from studies measuring the level of PPIs and/or IPPs in vivo. A number of complexities, however, restrict such analyses. First of all, the concentrations of these molecules are extremely low and hardly ever pop-up in lipidomic- or metabolomic screens. At most, phosphatidylinositol for the lipids, or inositol- and inositolphosphate for the IPPs, is reported, since the MS detection cannot discriminate between lower and higher IPPs, let alone the isomers. Similarly, different PPI isomers exist and the PLC pathway only involves PI4P and PI(4,5)P_2. Measuring product formation, i.e. Ins(1,4)P_2/Ins(1,4,5)P_3 and DAG, has the same limitations: quantity and complexity of isomers. Below, a more detailed overview of their measurement is given.

5.1 Polyphosphoinositides

The concentration of PPIs in plant cells is extremely low, i.e., less than a percent of the total phospholipids and even 3–5 times lower when all glycerolipids are considered. In animal cells, but also in green algae like *Chlamydomonas* and *Dunaliella*, the ratio of PIP:PIP_2 is approximately 1:1. In higher plant cells, however, PIP_2 levels are 20- to 100-fold lower (Munnik et al. 1994, 1998b; Einspahr et al. 1988; Arisz et al. 2000).

5.1.1 Radioactive Labeling In Vivo

Quantity problems can be overcome by using radioactive tracer molecules such as $^{32}P_i$ (or $^{33}P_i$) and ^{3}H- (or ^{14}C-) inositol. Plant tissues and cells take up these tracers relatively easy and PPIs tend to incorporate them rather quickly due to their rapid turnover (Munnik et al. 1998a, b). Lipids can then be extracted and separated by thin-layer chromatography (TLC) (Munnik and Zarza 2013). PPI isomers can be distinguished by analyzing their radioactive headgroups through HPLC analysis after removal of the fatty acids (Munnik 2013). In this way, five of the seven natural occurring PPIs have been detected in plants, i.e., PI3P, PI4P, PI5P, PI(3,5)P_2, and PI(4,5)P_2 (Munnik et al. 1994; Munnik 2013; Brearley and Hanke 1992, 1993, 1994; Parmar and Brearley 1993, 1995; Meijer et al. 1999, 2001a). Plants do not

make PI(3,4,5)P_3 or its breakdown product PI(3,4)P_2, although the latter has been reported (Brearley and Hanke 1995), but this may have been PI(3,5)P_2, an isomer that was still unknown at that time (Munnik and Testerink 2009; Meijer et al. 1999).

In higher plants, PPI levels are usually measured in multicellular tissues, and it not clear whether all cell types express the complete PI system. Therefore, it remains to be seen whether certain specialized cells contain amounts equivalent to those in algae. In the latter, short-term labeling studies reveal most of the radioactivity in PIP and PIP_2, belying their low concentrations (Munnik et al. 1998a; Einspahr et al. 1988). In higher plants, only PIP is labeled like that, indicating that most of the PIP is not converted to PIP_2 and likely fulfills signaling roles on its own, including regulating polarized membrane trafficking (Munnik and Nielsen 2011; Ischebeck et al. 2010b; Heilmann and Heilmann 2013).

5.1.2 Determining PPI-Mass Levels Through Fatty Acid Measurements

Recently, a method was published in which PPI levels were determined on the basis of their fatty acid content using TLC and gas chromatography (Konig et al. 2008b). Strangely however, much higher PIP_2 levels are found than with isotope labeling. On the basis of the fatty-acids method, PIP/PIP_2 ratios range from 3:1 in Arabidopsis leaves to ~2:1 in Arabidopsis roots and tobacco cells suspensions (Konig et al. 2008a, b), which is in huge contrast to numerous $^{32}P_i$- and ^{3}H-Ins labeling studies of different plants and tissues, where ratios range from 20:1 to 100:1 (reviewed in Munnik et al. 1998b). Small differences may be explained by the fact that PIP_2 contains more ^{32}P-phosphates than PIP, or that the PIP_2 is more easily lost from extracts due to its polarity, but this is unlikely to explain the differences seen in so many studies. Another possibility is that the fatty acid method used by Konig et al. (2008a, b) slightly overestimates the PIP_2 (and PIP) levels due to contamination of fatty acids from other lipids. For example, their tissue-extracted lipids were separated by TLC without pre-purification, and PIP- and PIP_2 locations were only scraped-off approximately, on the basis of a stained marker lane that ran on the side of the TLC (the chemical abundance of PPIs in plant extracts is too low to be stained). Obviously, such a method is prone to contamination since many lipids stick to each other, even during TLC, or leave the origin of application at different rates due to dissolving problems of other components present in the extract (pigments, waxes, etc). For example, when the fatty-acid composition of PPIs in *Chlamydomas* was determined, pre-purification on a silica column and elution with various different solvents was required before pure PIP and PIP_2 could be eluted (Arisz et al. 2000, 2003). Similarly, when the fatty acid composition of PPIs in carnation flowers was analyzed, only PIP could be detected after pre-purification, not PIP_2 (Munnik et al. 1994). $^{32}P_i$- and ^{3}H-Inositol labeling of the same tissue estimated the molar ratio for PI:PIP:PIP_2 to be 385:35:1 (Munnik et al. 1994).

The take-home lesson here is that fatty acids can be used to measure PPI mass levels but that care should be taken when assuming their purity.

5.1.3 Visualizing PI4P and PI(4,5)P_2 in Living Plant Cells Using Lipid Biosensors

Lipid biosensors have proven themselves as being very valuable new tools (Vermeer and Munnik 2013). Not always quantitatively, but certainly qualitatively. For the first time, we are able to see *where* certain lipids reside! The lipid biosensors for PIP and PIP_2 are composed of a specific lipid-binding domain, i.e., the PH domain of human PLCδ1 for PI(4,5)P_2, or the PH domain of the human FAPP1 protein for PI4P, fused to a fluorescent protein (FP) of any color, which is stably expressed in cell suspensions or plants behind a constitutive promoter (35S, UBI10). Specificity for these probes in vitro and in vivo is remarkably good (van Leeuwen et al. 2007; Mishkind et al. 2009; Vermeer et al. 2006, 2009; Thole et al. 2008; Balla 2009; Varnai and Balla 2008; Hammond et al. 2012).

Confocal imaging of tobacco BY-2 cells or Arabidopsis seedlings expressing FP-PH_{FAPP1} revealed that most of the PI4P is localized in the plasma membrane, with a small, second pool residing at the early endosomal/*trans*-Golgi network (Vermeer et al. 2009). The PIP_2 biosensor, however, mostly resides in the cytosol (van Leeuwen et al. 2007). This is not because most of the PIP_2 is there, but because the concentration of PIP_2 in plant membranes is too low to keep the biosensor there (van Leeuwen et al. 2007). This is in huge contrast to mammalian cells where PIP_2 is predominantly localized at the inner leaflets of the plasma membrane (Balla 2009; Varnai and Balla 2008). Nonetheless, in response to salt stress, the PIP_2 biosensor clearly relocalizes to the plasma membrane and this coincides with an unmistakable increase in PIP_2 synthesis as observed with ^{32}P-labelling (van Leeuwen et al. 2007). Importantly, the quantitative ^{32}P-PIP_2 response in the biosensor line was the same as in control cell lines. Phenotypically, cell suspensions or Arabidopsis plants stably expressing the biosensor, are indistinguishable from non-transgenic lines too (van Leeuwen et al. 2007; Vermeer et al. 2009).

Heat stress also triggers a rapid ^{32}P-PIP_2 response (Mishkind et al. 2009). Using the biosensor, first an increase of PIP_2 at the plasma membrane was revealed. Later, small unknown intracellular compartments became labeled and after 30-45 min, even the nuclear envelope (Mishkind et al. 2009). While in the animal field, nuclear PPI- and PLC signaling is well known (Fiume et al. 2012; Keune et al. 2011; Irvine 2006); for plants this is still an unexplored area of research (Dieck et al. 2012a, b).

In growing root hairs and pollen tubes, there is a clear plasma membrane gradient of PIP_2 at the tip which quickly disappears when the cells stop growing (van Leeuwen et al. 2007; Ischebeck et al. 2008, 2010a, b, 2011; Kusano et al. 2008; Sousa et al. 2008; Dowd et al. 2006; Vermeer et al. 2009; Vermeer and Munnik 2013; Kost et al. 1999; Kost 2008; Saavedra et al. 2011).

5.2 *Inositolpolyphosphates*

IPPs can be measured nonradioactively but then predominantly $InsP_6$ is detected, mostly reflecting phytate research in storage tissues (Munnik and Vermeer 2010; Raboy 2003). In Arabidopsis seedlings, besides $InsP_6$, a small amount of $InsP_5$ can be seen (Stevenson-Paulik et al. 2005). Using in vivo labeling with ^{3}H-Inositol, however, changes in pool size and synthesis of other InsPPs ($InsP_{1–6}$) can be witnessed (Lemtiri-Chlieh et al. 2000; Stevenson-Paulik et al. 2005; Perera et al. 2008). Clear from these studies is that $InsP_3$ levels are extremely low; so low, that not even a positive ID of the isomer can be made (Stevenson-Paulik et al. 2005). Overnight labeling of potato guard cell preparations revealed predominant labeling of $InsP_1$, $InsP_2$, and $InsP_6$ (Lemtiri-Chlieh et al. 2000).

5.2.1 $InsP_3$ Responses: Fact or Flux?

In the plant field, many $InsP_3$ responses have been reported, e.g., in response to gravitropism, ABA, heat stress, salt stress, or drought (reviewed in Munnik and Vermeer 2010). But if $InsP_3$ concentrations are so low (Sect. 5.2), and there is hardly any PIP_2 in the membrane to produce it (Sect. 5.1), then what are people measuring? Fact is that most (if not all) of the reported $InsP_3$ responses were measured using a commercial $InsP_3$-mass assay. This kit uses an $InsP_3$-binding protein that can be pelleted, together with a known amount of radioactive Ins(1,4,5)P_3. The idea is that if there is any $InsP_3$ present in a plant extract, then it will compete with the binding of the radioactive probe, whose displacement can be subsequently quantified via a calibration curve. While this works well in mammalian cells where $InsP_3$ levels via PLC mediated-PIP_2 hydrolysis is relatively high, in plants it is a completely different story. Both PIP_2- and $InsP_3$ levels are extremely low and, meanwhile, plants contain huge amounts of other IPPs (Lemtiri-Chlieh et al. 2000; Stevenson-Paulik et al. 2005; Flores and Smart 2000; Brearley and Hanke 1996a, b). Obviously, the specificity of the $InsP_3$-binding protein is limited and we don't even know what all of the plant-IPP isomers are, let alone that they have been tested for interference with the commercial displacement assay. For example, overnight labeling of potato guard cell preparations with ^{3}H-Inositol revealed predominant labeling of $InsP_1$, $InsP_2$, and $InsP_6$. For $InsP_3$, three tiny peaks were detected of which one co-migrated (which does not necessarily mean that it is the same compound) with Ins(1,4,5)P_3 (16). Also three major peaks of $InsP_5$ were detected. In the commercial $InsP_3$-detection kit, $InsP_5$ (no isomer given) competes for 5 % with the binding of Ins(1,4,5)P_3. Obviously, if the concentration of $InsP_5$ is 100-fold higher and changing, then changes in "$InsP_3$" will be documented.

An alternative explanation for the reported $InsP_3$ responses is that they reflect changes in certain IPP levels, due to an induced change in the flux of $InsP_6$, which can be synthesis as well as degradation (see Munnik and Vermeer 2010). This flux

is becoming very relevant since several roles for non-$InsP_3$ IPPs are emerging in eukaryotic cell signaling and also in plants (see below).

5.2.2 Not $InsP_3$ But $InsP_6$ Releases Ca^{2+}

In guard cells, ABA inhibits stomatal opening and stimulates its closure through coordinated regulation of ion transport. Initially, photorelease of microinjected caged-Ins(1,4,5)P_3 was shown to trigger an increase in cytosolic Ca^{2+},and to inhibit a K^+-influx channel in a Ca^{2+}-dependent manner (Blatt et al. 1990; Gilroy et al. 1990). Data from Brearley's lab, however, provides compelling evidence that this was caused through its metabolism into $InsP_6$ (Lemtiri-Chlieh et al. 2000, 2003). They showed that when $InsP_3$ was microinjected, it was rapidly converted into $InsP_6$, and that the ability of $InsP_6$ to release Ca^{2+} was ~100 times more potent than $InsP_3$. This was not a simple charge effect since only *myo*-$InsP_6$ was able to induce K^+ influx-channel inhibition and not *neo*-$InsP_6$ or *scyllo*-$InsP_6$. In vivo, ^{3}H-$InsP_6$ responses were found within 30 s to 5 min after ABA treatment of ^{3}H-inositol-prelabeled guard-cell preparations from potato (Lemtiri-Chlieh et al. 2000). The latter would be a nice system to simultaneously monitor PPIs, Ca^{2+}, $InsP_3$, and DAG using genetically encoded, FP-based, biosensors (Munnik and Nielsen 2011; Swanson and Gilroy 2013; Vermeer and Munnik 2013; Remus et al. 2006).

5.3 *Diacylglycerol*

Besides being a product of PLC activity, DAG is also a precursor for de novo synthesis of all glycerolipids, including phospholipids, glycolipids, and the neutral storage lipid, triacylglycerol (TAG). Free DAG levels are difficult to measure, however (Wewer et al. 2013; Vom Dorp et al. 2013). Probably because most DAG is only present as a short-lived intermediate: either in the pipeline of glycerolipid synthesis or because the PLC-generated DAG is rapidly converted by DGK into PA (Munnik et al. 1998a; Arisz et al. 2009, 2013; Arisz and Munnik 2013). The PLC-generated DAG pool would not be very large anyway, because PI4P and PI(4,5)P_2 are only minor lipids, representing less than a percent of the total phospholipid pool, and even >3-5 times lower when all glycerolipids are considered (i.e. MGDG, DGDG, SQDG,TAG) (Arisz et al. 2000).

One of the ideas of the extremely low PIP_2 levels in plants was that plant PI-PLCs would be constitutively active, hydrolyzing the pool continuously. However, using a genetically encoded DAG biosensor, a C1-domain from human PKCγ that specifically binds DAG, fused to a fluorescent protein (GFP or mRFP) expressed in tobacco BY-2 cells or in *Arabidopsis* plants, revealed that there was no measurable amount of DAG in the cytosolic leaflet of the plasma membrane (Vermeer and Munnik, unpublished). Interestingly, a small new pool was

discovered at *trans*-Golgi membranes. DAG pools involved in general lipid metabolism were not picked up, because these processes reside at the inner leaflets of intracellular compartments, (ER, plastid, mitochondrion), whereas the biosensor is only expressed in the cytosol (Vermeer and Munnik, unpublished) (Vermeer and Munnik 2013).

In animal cells, the observation that DAG was phosphorylated to PA was initially seen as signal attenuation. Nowadays, however, PA is considered to be an important lipid second messenger. In plant cells, there is no evidence that DAG plays a role in signaling. They lack homologs of PKC and also the TRP channels that are activated by DAG. The Arabidopsis genome encodes some genes with a C1-like domain, but testing DAG binding to one of them failed (Reznik et al. unpublished). In contrast, there is a huge amount of evidence that PA plays a signaling role in plants (Testerink and Munnik 2011; Hong et al. 2010; Li et al. 2009; McLoughlin et al. 2012, 2013). Evidence that DAG is converted to PA via DGK in vivo, can be obtained by means of a differential-labeling protocol (Arisz et al. 2009; Arisz and Munnik 2013). As such, DGK-generated PA has been shown in response to salt- and cold stress and to pathogenic elicitors (Arisz et al. 2003, 2009, 2013; de Jong et al. 2004; Arisz and Munnik 2013; Delage et al. 2012; Laxalt and Munnik 2002; Ruelland et al. 2002). The discovery of the novel NPCs, however, adds another layer of complexity to the DAG-PLC link. PA can also be produced via phospholipase D (PLD) hydrolysis of structural lipids (Wang 2005; Qin and Wang 2002). To distinguish DGK- and PLD-generated PA, the capacity of PLD to transphosphatidylate primary alcohols in vivo can be used (Munnik and Laxalt 2013; Munnik et al. 1995).

5.4 *U73122 and Neomycin are* Not *Specific PLC Inhibitors!*

Many plant studies have claimed a role for PI-PLC in a particular pathway on the basis of using "specific" PLC inhibitors. These inhibitors are, however, far from specific and hence, their results should be ignored completely. Neomycin is an aminoglycoside, which, like gentamycin, is often used as an antibiotic in plant molecular biology. It binds negatively-charged molecules and therefore can affect the hydrolysis of PIP_2 in vitro but the compound does not act on the enzyme itself (Balla 2001). Since most of the phospholipid bilayer consists of negatively-charged phospholipids, with various enzymes crucially being dependent on that, it is of no surprise that in the end, one will find a concentration of neomycin that will affect a plant's response. This has likely nothing to do with PLC signaling, however.

Similarly, this holds for the aminosteroid, U-73122. This "PLC-specific inhibitor" was originally found during a search for phospholipase-A_2 inhibitors (Balla 2001), a totally different enzyme. While there are several reports that claim PLC inhibition, U-73122 has been shown to affect several cellular processes independent of PLC (Balla 2001; Mogami et al. 1997), including inhibition of 5-lipoxygenase and PLD (Burgdorf et al. 2010; Hornig et al. 2012). A recent study even claims that

PLC is activated by U-73122 (Klein et al. 2011). Fact is that we have no idea what U73122 does in plant cells; hence, all PLC-signaling claims based on its use should be ignored.

6 Role in Plant Stress and Development

6.1 New PLC Signaling Scenarios

While PLC signaling in plant is unlikely to fulfill the mammalian paradigm, several other scenarios and ideas are emerging and we should keep an open eye to everything. In a first scenario, PIP and PIP_2 are functioning as lipid signals themselves, and PLC would be the attenuator of this process (Munnik and Nielsen 2011). As such, it could, e.g., play a dual role in vesicle trafficking, where PLC removes the PPIs functioning as molecular beacons for protein targets involved in membrane fusion/fission and simultaneously generates neutral DAG to facilitate the process biophysically. In a second scenario, PLC follows the classical view, except that $InsP_3$ is phosphorylated to $InsP_6$ to release Ca^{2+}, and the DAG is converted to PA to recruit protein kinases or other targets (Testerink and Munnik 2011). In a third scenario, IPPs function as signaling molecules independent of Ca^{2+}, e.g., regulating ion channels and affecting gene transcription and mRNA export (Perera et al. 2004; York et al. 1999; Zonia and Munnik 2006; Zonia et al. 2002). Initially, this PLC-dependent pathway was discovered in yeast (which also lacks an $InsP_3$/Ca^{2+} and DAG/PKC pathway) but meanwhile has been confirmed in mammalian signalling (York et al. 2001; Tsui and York 2010; Alcazar-Roman et al. 2006, 2010; Alcazar-Roman and Wente 2008; Noble et al. 2011; Hodge et al. 2011). These include roles for $InsP_4$, $InsP_5$, and $InsP_6$ but also on the inositol pyrophosphates, $InsP_7$ and $InsP_8$, which are also present in plants (Munnik and Nielsen 2011; Munnik and Vermeer 2010). Whether the recent identification of $InsP_6$ in the auxin receptor, TIR1 (Tan et al. 2007) and that of $InsP_5$ in the jasmonate receptor COI1 (Sheard et al. 2010) represent such signalling events remains to be established (Munnik and Nielsen 2011; Munnik and Vermeer 2010). Nonetheless, it is interesting that auxin previously was shown to induce rapid changes in PPI- and IPP levels [19]. Wounding also affects PPI and IPP levels (Mosblech et al. 2008), and *ipk1* mutants, which cannot make $InsP_6$, and hence accumulate high levels of its precursor, $InsP_5$, display increased COI1-dependent sensitivity to wounding and root-growth inhibition by jasmonate (Mosblech et al. 2011). The recent discovery that $InsP_6$ (Murphy et al. 2008) and PLC (Vossen et al. 2010) have roles in pathogen resistance, and the fact that both TIR1 and COI1 belong to large, related families of F-box proteins, raises the exciting possibility that IPPs may act similarly in a broad array of signaling pathways in plants. A last and forth scenario involves the role of PLC in generating non-phosphorylated inositol metabolites, i.e., via oxidation, methylation, or via conversion into RFOs (Fig. 2; Munnik and Vermeer 2010).

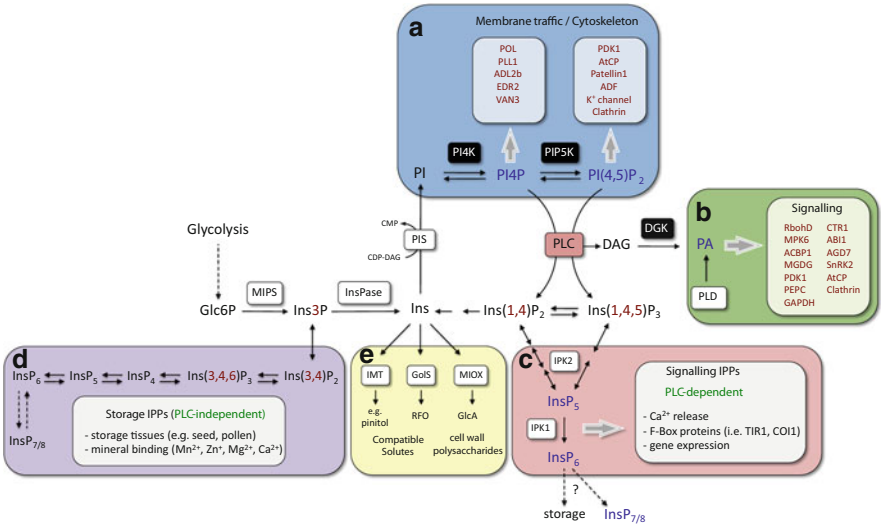

Fig. 2 Potential roles of PLC in plant signalling and metabolism. PIP and PIP_2 are synthesized by sequential phosphorylation of the structural phopholipid PI at the D4- and 5-position by PI 4kinase (PIK) and PI4P 5-kinase (PIPK), respectively (**a**). Both lipids exhibit unique identities in structure and function for signaling and membrane trafficking. PLC can hydrolyze both PPIs, which generates membrane-bound DAG and the water-soluble headgroups, Ins(1,4)P_2 or Ins(1,4,5)P_3. While DAG is rapidly phosphorylated by DGK to form the signaling lipid PA (**b**), the IPPs can be stepwise phosphorylated by the inositolpolyphosphate multikinases, IPK2 and IPK1, to produce $InsP_5$ and $InsP_6$, for which several new signaling functions are emerging (**c**), e.g., releasing Ca^{2+} ($InsP_6$) and activating F-box controlled plant hormone signaling pathways, such as TIR1 ($InsP_6$, auxin) and COI1 ($InsP_5$, jasmonic acid). "*Signaling IPPs*" (**c**) should not be confused with those involved in P_i storage occurring in seeds and other storage tissues (named here as: "*Storage IPPs*" (**d**). The latter is made through a "lipid-independent" pathway, starting from glycolytic glucose 6-phosphate (Glc6P), which is first cyclized to Ins3*P* by *myo*-inositol-3-phosphate synthase (MIPS) and then further phosphorylated by IPKs (*Note*: isomer difference between PLC- and storage-generated $InsP_2$/$InsP_3$; highlighted in *red*). Free *myo*-inositol (Ins) is predominantly generated through the glycolytic pathway, through dephosphorylating of Ins3*P* via inositol monophosphatase (InsPase); however, it can also be produced through dephosphorylation of IPPs from the PLC- and/or "storage" pathway. Inositol not only is used for the PPI pathway to synthesize PI via PI synthase (PIS) but also functions as precursor for three distinct sugar-metabolite pathways (**e**), including: (1) Myo-inositol oxygenase (MIOX), which catalyzes the oxidative cleavage of Ins into glucuronic acid (GlcA) that is subsequently activated to UDP-GlcA to serve as precursor for cell wall polysaccharides, (2) Galactinol synthase (GolS) for the production of Raffinose Family Oligosaccharides (RFO) for sugar transport and compatible solute synthesis, and (3) Inositol methyl transferase (IMT) to produce compatible solutes like pinitol, xylitol, etc. Figure is adapted from Munnik and Nielsen (2011). For further details, *see* text. Described protein targets for individual lipids are indicated in *dark red*. Lipid (-derived) signals are indicated in *blue*

While this may seem unlikely, as there is a more direct way to generate inositol from Glc6P, locally or intracellularly, this may have its advantage. Whatever scenario, it may be worthwhile to take each into account when considering observations from the past or below.

6.2 Plant Stress and Development

While it remains debatable whether plants contain a canonical "*PLC-signaling pathway*," PLC's role in plant signaling is certainly emerging. Until now, no Arabidopsis T-DNA insertion mutants with phenotypes have been reported, but this may reflect functional redundancy within the gene family. Nevertheless, there is genetic evidence from knockdown experiments with Arabidopsis and tobacco *PLC* genes that indicate a role for PLC in ABA signaling, while *PLC* overexpression studies in maize, tobacco, and rape seed revealed a substantial increase in their tolerance to drought and salinity (Wang et al. 2008; Georges et al. 2009; Tripathy et al. 2012). Similarly, genetic evidence has been obtained for PLC (and DGK) in fungal disease resistance (Vossen et al. 2010; Zhang et al. 2008).

Upon salt- and drought stress (the latter mimicked by, e.g., mannitol, sorbitol, or PEG), various plant systems have been shown to respond with a relatively rapid (<15 min) increase in the synthesis of PIP_2 (Darwish et al. 2009; DeWald et al. 2001; van Leeuwen et al. 2007; Takahashi et al. 2001; Zonia and Munnik 2004; Pical et al. 1999). $InsP_3$ responses have also been reported (Takahashi et al. 2001), but as discussed above and earlier (Munnik and Vermeer 2010), it is not clear whether this reflects PIP_2 hydrolysis by PLC or changes in the flux of $InsP_6$. Another possibility is that PI4P is hydrolyzed. Using ^{3}H-inositol labeled carrot cells, Cho et al. (1993) reported a rapid (30 min) decrease in PIP (PIP_2 levels were undetectable). If a PLC is indeed activated, then the decrease in PIP makes more sense than the increase of PIP_2, even though the latter can already be a secondary response of the initial decrease (which may be too low to be witnessed). This could make sense in seedlings where not every cell will respond and where differentiated cells express distinct pathways of the PPI/IPP system. In cell suspensions, however, every cell more or less responds. PA formation due to DAG phosphorylation has also been shown (Arisz et al. 2003; Munnik et al. 2000; Meijer et al. 2001b).

A link between ABA and PPI turnover has been claimed but this data is still controversial (Munnik and Vermeer 2010). What is clear, however, is that ABA can trigger IPP responses, in particular $InsP_6$, which was generated within minutes in ABA-responsive tissue, i.e., in a guard-cell preparation of potato (Lemtiri-Chlieh et al. 2000, 2003) or duck weed turions (a storage tissue) (Flores and Smart 2000). What is more, in the former system, $InsP_6$ was shown to trigger the release of intracellular Ca^{2+} (Lemtiri-Chlieh et al. 2000, 2003).

Whether $InsP_6$-released Ca^{2+} is indeed important for ABA induced-stomatal closure remains to be seen. In this response, guard cell-anion channels play a central role because they mediate anion efflux and, in turn, cause a depolarization-induced K^+ release. Recent studies in Hedrich's lab (Levchenko et al. 2005) revealed that cytosolic ABA activates these guard cell-anion channels without preceding Ca^{2+} signals. What is more, they showed that none of the previously proposed ABA-signaling intermediates, including $Ins(1,4,5)P_3$ and $InsP_6$ (but also nicotinic acid adenine dinucleotide phosphate (NAADP) or cyclic ADP-ribose), were able to mimic ABA as anion channel activator. Clearly, more research is required to put all these individual observations into perspective. Especially, we need to find out what is general and what is guard-cell specific, and whether the ABA- and salt/drought-stress responses are related with respect to inositol metabolism.

To dampen the so-called $InsP_3$ signal in response to drought stress in Arabidopsis plants, overexpression of a human type I inositol polyphosphate 5-phosphatase (InsP 5-ptase) has been used (Perera et al. 2008). However, rather than the plants becoming more sensitive to drought, the opposite occurred: plants became more resistant. Using ^{3}H-Inositol labeling for 4 days, $InsP_3$ levels were only slightly reduced in both seedlings and guard-cell preparations (Note: with the commercial $InsP_3$-assay kit, a 16.6-fold reduction was measured, 5 % remaining of basal levels), but this was nothing compared to the huge decrease in $InsP_6$. Whether the effects are related to altered Ca^{2+} signaling remain to be seen. Compared to the minor changes in Ca^{2+} levels (measured by aequorin) found in the transgenic plants, huge differences in gene expression were observed that are more realistic to explain the observed phenotype, including the constitutive overexpression of the stress regulated transcription factor, DREB2A. Interestingly, these transgenic plants exhibited increased expression of *PLC7* and two galactinol syntases. The latter encode enzymes that use inositol to produce galactinol, which is a precursor for Raffinose Family Oligosaccharides (RFOs) that are involved in various abiotic stress responses (Valluru and Van den Ende 2011), which may represent another explanation of how IPPs and PLC may be involved in salt and drought stress [Fig. 2e; (Munnik and Vermeer 2010)]. Alternatively, oxidation of inositol by myo-inositol oxygenase (MIOX) to produce sugar precursors for cell wall assembly or its methylation by inositol methyl transferases (IMT) to produce compatible solutes such as pinitol (Fig. 2e) may explain water stress-related phenotypes (Munnik and Vermeer 2010).

Interestingly, a number of genes coding for enzymes involved in inositol metabolism have been reported to affect vascular patterning. These include CVP2, CVL1, and At5PTAse13 which are all inositolpolyphosphate 5-phosphatases (although not always clear whether the lipid or the water-soluble headgroup is used as a substrate) and an ARF-GEF which can bind PI4P via its PH domain (Carland and Nelson 2004, 2009; Lin et al. 2005; Zhong and Ye 2004; Sieburth et al. 2006). It is unknown whether this reflects functions of PPI- or IPPs signaling or metabolism of inositol into other compounds. Similarly, 5-phosphatases have been picked up as

fragile fiber (*fra*) mutants that are disturbed in cell wall assembly (Zhong et al. 2004, 2005).

Heat stress also rapidly triggers a PIP_2 response (Mishkind et al. 2009; Horvath et al. 2012). Concomitantly, PA levels were found to increase, but these were not generated via DGK, but via a PLD pathway (Zheng et al. 2012; Mishkind et al. 2009; Horvath et al. 2012). Heat stress-induced $InsP_3$ responses have been reported (Liu et al. 2006a, b) and the same lab recently claimed to have identified a PLC involved, as KO mutants of two independent T-DNA insertion lines lacked the $InsP_3$ response and exhibited decreased thermotolerance (Zheng et al. 2012). Problem with this scenario, however, is that *AtPLC9* was implicated, and this PLC is predicted to lack some of the conserved amino acids in the X-Y domain that are required for the enzyme's catalytic activity; hence, it is expected to generate an inactive enzyme (Hunt et al. 2004). $InsP_3$ was measured with the displacement assay, so this may reflect changes in the flux of other IPPs (Sect. 5.2.1). The increase in PIP_2 found by Mishkind et al. (2009; Horvath et al. 2012) was more likely to be due to activation of a PIPK rather than through inhibition of its breakdown (Mishkind et al. 2009). If, however, the inactive AtPLC9 is able to bind PIP_2, than one of its functions may be to sustain PIP_2's role as messenger through competition with catalytically active PLCs or through microdomain formation.

For a long time, gravitropism has been claimed to reflect a PLC/$InsP_3$/Ca^{2+} pathway (Perera et al. 1999, 2006; Stevenson et al. 2000; Boss et al. 2006, 2010). Most evidence, however, is based on $InsP_3$ measurements using the commercial $InsP_3$-displacement kit (Sect. 5.2.1). While this may reflect changes in $InsP_6$ flux, which would be equally interesting and exciting, it is a pity to keep this strict "tunnel vision" (Salinas-Mondragon et al. 2010; Khodakovskaya et al. 2010) while there is so much data out. Maybe we have to get back to where it all started in 1988: Auxin (Ettlinger and Lehle 1988).

Acknowledgments I thank Tamas Balla for his discussions on U73122 and sharing unpublished information on this. I thank my colleagues in the field for general discussions, in particular my close collaborators and (previous) members from my lab, especially Joop Vermeer who also critically read this manuscript.

References

Alcazar-Roman AR, Wente SR (2008) Inositol polyphosphates: a new frontier for regulating gene expression. Chromosoma 117:1–13

Alcazar-Roman AR, Tran EJ, Guo S, Wente SR (2006) Inositol hexakisphosphate and Gle1 activate the DEAD-box protein Dbp5 for nuclear mRNA export. Nat Cell Biol 8:711–716

Alcazar-Roman AR, Bolger TA, Wente SR (2010) Control of mRNA export and translation termination by inositol hexakisphosphate requires specific interaction with Gle1. J Biol Chem 285:16683–16692

Apone F, Alyeshmerni N, Wiens K, Chalmers D, Chrispeels MJ, Colucci G (2003) The G-protein-coupled receptor GCR1 regulates DNA synthesis through activation of phosphatidylinositol-specific phospholipase C. Plant Physiol 133:571–579

Arisz SA, Munnik T (2013) Distinguishing phosphatidic acid pools from de novo synthesis, PLD, and DGK. Methods Mol Biol 1009:55–62

Arisz SA, van Himbergen JA, Musgrave A, van den Ende H, Munnik T (2000) Polar glycerolipids of *Chlamydomonas moewusii*. Phytochemistry 53:265–270

Arisz SA, Valianpour F, van Gennip AH, Munnik T (2003) Substrate preference of stress-activated phospholipase D in Chlamydomonas and its contribution to PA formation. Plant J 34:595–604

Arisz SA, Testerink C, Munnik T (2009) Plant PA signaling via diacylglycerol kinase. Biochim Biophys Acta 1791:869–875

Arisz SA, van Wijk R, Roels W, Zhu JK, Haring MA, Munnik T (2013) Rapid phosphatidic acid accumulation in response to low temperature stress in Arabidopsis is generated through diacylglycerol kinase. Front Plant Sci 4:1

Balla T (2001) Pharmacology of phosphoinositides, regulators of multiple cellular functions. Curr Pharm Des 7:475–507

Balla T (2009) Green light to illuminate signal transduction events. Trends Cell Biol 19:575–586

Balla T, Szentpetery Z, Kim YJ (2009) Phosphoinositide signaling: new tools and insights. Physiology 24:231–244

Bargmann BO, Laxalt AM, Riet BT, Schouten E, van Leeuwen W, Dekker HL, de Koster CG, Haring MA, Munnik T (2006) LePLDβ1 activation and relocalization in suspension-cultured tomato cells treated with xylanase. Plant J 45:358–368

Beno-Moualem D, Naveh L, Jacob B (1995) Responses of red beet tissue to hypertonic salt-shock: inositol 1,4,5-trisphosphate, ATPase activation and protein phosphorylation. Plant Physiol Biochem 33:311–318

Blatt MR, Thiel G, Trentham DR (1990) Reversible inactivation of K^+ channels of Vicia stomatal guard cells following the photolysis of caged inositol 1,4,5-trisphosphate. Nature 346:766–769

Boss WF, Davis AJ, Im YJ, Galvao RM, Perera IY (2006) Phosphoinositide metabolism: towards an understanding of subcellular signaling. Subcell Biochem 39:181–205

Boss WF, Sederoff HW, Im YJ, Moran N, Grunden AM, Perera IY (2010) Basal signaling regulates plant growth and development. Plant Physiol 154:439–443

Brearley CA, Hanke DE (1992) 3- and 4-phosphorylated phosphatidylinositols in the aquatic plant *Spirodela polyrhiza* L. Biochem J 283:255–260

Brearley CA, Hanke DE (1993) Pathway of synthesis of 3,4- and 4,5-phosphorylated phosphatidylinositols in the duckweed *Spirodela polyrhiza* L. Biochem J 290:145–150

Brearley CA, Hanke DE (1994) Phosphoinositides in Barley (*Hordeum vulgare* L.) Aleurone Tissue. Plant Physiol 104:1381–1384

Brearley CA, Hanke DE (1995) Evidence for substrate-cycling of 3-, 3,4-, 4-, and 4,5-phosphorylated phosphatidylinositols in plants. Biochem J 311:1001–1007

Brearley CA, Hanke DE (1996a) Inositol phosphates in the duckweed *Spirodela polyrhiza* L. Biochem J 314:215–225

Brearley CA, Hanke DE (1996b) Metabolic evidence for the order of addition of individual phosphate esters in the myo-inositol moiety of inositol hexakisphosphate in the duckweed *Spirodela polyrhiza* L. Biochem J 314:227–233

Burgdorf C, Schafer U, Richardt G, Kurz T (2010) U73122, an aminosteroid phospholipase C inhibitor, is a potent inhibitor of cardiac phospholipase D by a PIP_2-dependent mechanism. J Cardiovasc Pharmacol 55:555–559

Carland FM, Nelson T (2004) Cotyledon vascular pattern2-mediated inositol (1,4,5) triphosphate signal transduction is essential for closed venation patterns of Arabidopsis foliar organs. Plant Cell 16:1263–1275

Carland F, Nelson T (2009) CVP2- and CVL1-mediated phosphoinositide signaling as a regulator of the ARF GAP SFC/VAN3 in establishment of foliar vein patterns. Plant J 59:895–907

Chen J, Zhang W, Song F, Zheng Z (2007) Phospholipase C/diacylglycerol kinase-mediated signalling is required for benzothiadiazole-induced oxidative burst and hypersensitive cell death in rice suspension-cultured cells. Protoplasma 230:13–21

Cho MH, Shears SB, Boss WF (1993) Changes in phosphatidylinositol metabolism in response to hyperosmotic stress in *Daucus carota* L. cells grown in suspension culture. Plant Physiol 103:637–647

Cho MH, Tan Z, Erneux C, Shears SB, Boss WF (1995) The effects of mastoparan on the carrot cell plasma membrane polyphosphoinositide phospholipase C. Plant Physiol 107:845–856

Choi WG, Swanson SJ, Gilroy S (2012) High-resolution imaging of Ca^{2+}, redox status, ROS and pH using GFP biosensors. Plant J 70:118–128

Cockcroft S (2006) The latest phospholipase C, PLCeta, is implicated in neuronal function. Trends Biochem Sci 31:4–7

Cote GG, Yueh YG, Crain RC (1996) Phosphoinositide turnover and its role in plant signal transduction. Subcell Biochem 26:317–343

Coursol S, Pierre JN, Vidal J, Grisvard J (2002) Cloning and characterization of a phospholipase C from the C4 plant *Digitaria sanguinalis*. J Exp Bot 53:1521–1524

Darwish E, Testerink C, Khalil M, El-Shihy O, Munnik T (2009) Phospholipid signaling responses in salt-stressed rice leaves. Plant Cell Physiol 50:986–997

Das S, Hussain A, Bock C, Keller WA, Georges F (2005) Cloning of *Brassica napus* phospholipase C2 (*BnPLC2*), phosphatidylinositol 3-kinase (*BnVPS34*) and phosphatidylinositol synthase1 (*BnPtdInsS1*) comparative analysis of the effect of abiotic stresses on the expression of phosphatidylinositol signal transduction-related genes in *B. napus*. Planta 220:777–784

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

Delage E, Ruelland E, Guillas I, Zachowski A, Puyaubert J (2012) Arabidopsis type-III phosphatidylinositol 4-kinases β1 and β2 are upstream of the phospholipase C pathway triggered by cold exposure. Plant Cell Physiol 53:565–576

den Hartog M, Musgrave A, Munnik T (2001) Nod factor-induced phosphatidic acid and diacylglycerol pyrophosphate formation: a role for phospholipase C and D in root hair deformation. Plant J 25:55–65

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

DeWald DB, Torabinejad J, Jones CA, Shope JC, Cangelosi AR, Thompson JE, Prestwich GD, Hama H (2001) Rapid accumulation of phosphatidylinositol 4,5-bisphosphate and inositol 1,4,5-trisphosphate correlates with calcium mobilization in salt-stressed *Arabidopsis*. Plant Physiol 126:759–769

Dieck CB, Boss WF, Perera IY (2012a) A role for phosphoinositides in regulating plant nuclear functions. Front Plant Sci 3:50

Dieck CB, Wood A, Brglez I, Rojas-Pierce M, Boss WF (2012b) Increasing phosphatidylinositol (4,5) bisphosphate biosynthesis affects plant nuclear lipids and nuclear functions. Plant Physiol Biochem 57:32–44

Dowd PE, Coursol S, Skirpan AL, Kao TH, Gilroy S (2006) Petunia phospholipase C1 is involved in pollen tube growth. Plant Cell 18:1438–1453

Drøbak BK (1992) The plant phosphoinositide system. Biochem J 288:697–712

Drøbak BK, Watkins PA (1994) Inositol(1,4,5)trisphosphate production in plant cells: stimulation by the venom peptides, melittin and mastoparan. Biochem Biophys Res Commun 205:739–745

Drøbak BK, Watkins PA (2000) Inositol(1,4,5)trisphosphate production in plant cells: an early response to salinity and hyperosmotic stress. FEBS Lett 481:240–244

Drøbak BK, Dewey RE, Boss WF (1999) Phosphoinositide kinases and the synthesis of polyphosphoinositides in higher plant cells. Int Rev Cytol 189:95–130

Einspahr KJ, Thompson GA (1990) Transmembrane signaling via phosphatidylinositol 4,5-bisphosphate hydrolysis in plants. Plant Physiol 93:361–366

Einspahr KJ, Peeler TC, Thompson GA Jr (1988) Rapid changes in polyphosphoinositide metabolism associated with the response of *Dunaliella salina* to hypoosmotic shock. J Biol Chem 263:5775–5779

Ettlinger C, Lehle L (1988) Auxin induces rapid changes in phosphatidylinositol metabolites. Nature 331:176–178

Fiume R, Keune WJ, Faenza I, Bultsma Y, Ramazzotti G, Jones DR, Martelli AM, Somner L, Follo MY, Divecha N, Cocco L (2012) Nuclear phosphoinositides: location, regulation and function. Subcell Biochem 59:335–361

Flores S, Smart CC (2000) Abscisic acid-induced changes in inositol metabolism in *Spirodela polyrrhiza*. Planta 211:823–832

Georges F, Das S, Ray H, Bock C, Nokhrina K, Kolla VA, Keller W (2009) Over-expression of Brassica napus phosphatidylinositol-phospholipase C2 in canola induces significant changes in gene expression and phytohormone distribution patterns, enhances drought tolerance and promotes early flowering and maturation. Plant Cell Environ 32:1664–1681

Gilroy S, Read ND, Trewavas AJ (1990) Elevation of cytoplasmic calcium by caged calcium or caged inositol triphosphate initiates stomatal closure. Nature 346:769–771

Hammond GR, Fischer MJ, Anderson KE, Holdich J, Koteci A, Balla T, Irvine RF (2012) PI4P and PI(4,5)P2 are essential but independent lipid determinants of membrane identity. Science 337:727–730

Heilmann M, Heilmann I (2013) Arranged marriage in lipid signalling? The limited choices of PtdIns(4,5)P_2 in finding the right partner. Plant Biol 15(5):789–797

Hetherington AM, Drøbak BK (1992) Inositol-containing lipids in higher plants. Prog Lipid Res 31:53–63

Hirayama T, Ohto C, Mizoguchi T, Shinozaki K (1995) A gene encoding a phosphatidylinositol-specific phospholipase C is induced by dehydration and salt stress in *Arabidopsis thaliana*. Proc Natl Acad Sci U S A 92:3903–3907

Hirayama T, Mitsukawa N, Shibata D, Shinozaki K (1997) AtPLC2, a gene encoding phosphoinositide-specific phospholipase C, is constitutively expressed in vegetative and floral tissues in *Arabidopsis thaliana*. Plant Mol Biol 34:175–180

Hodge CA, Tran EJ, Noble KN, Alcazar-Roman AR, Ben-Yishay R, Scarcelli JJ, Folkmann AW, Shav-Tal Y, Wente SR, Cole CN (2011) The Dbp5 cycle at the nuclear pore complex during mRNA export I: dbp5 mutants with defects in RNA binding and ATP hydrolysis define key steps for Nup159 and Gle1. Genes Dev 25:1052–1064

Hong Y, Zhang W, Wang X (2010) Phospholipase D and phosphatidic acid signalling in plant response to drought and salinity. Plant Cell Environ 33:627–635

Hornig M, Markoutsa S, Hafner AK, George S, Wisniewska JM, Rodl CB, Hofmann B, Maier T, Karas M, Werz O, Steinhilber D (2012) Inhibition of 5-lipoxygenase by U73122 is due to covalent binding to cysteine 416. Biochim Biophys Acta 1821:279–286

Horvath I, Glatz A, Nakamoto H, Mishkind ML, Munnik T, Saidi Y, Goloubinoff P, Harwood JL, Vigh L (2012) Heat shock response in photosynthetic organisms: membrane and lipid connections. Prog Lipid Res 51:208–220

Hsieh EJ, Cheng MC, Lin TP (2013) Functional characterization of an abiotic stress-inducible transcription factor AtERF53 in *Arabidopsis thaliana*. Plant Mol Biol 82:223–237

Hunt L, Gray JE (2001) ABA signalling: a messenger's FIERY fate. Curr Biol 11:R968–R970

Hunt L, Otterhag L, Lee JC, Lasheen T, Hunt J, Seki M, Shinozaki K, Sommarin M, Gilmour DJ, Pical C, Gray JE (2004) Gene-specific expression and calcium activation of *Arabidopis thaliana* phospholipase C isoforms. New Phytol 162:643–654

Irvine RF (2006) Nuclear inositide signalling – expansion, structures and clarification. Biochim Biophys Acta 1761:505–508

Ischebeck T, Stenzel I, Heilmann I (2008) Type B phosphatidylinositol-4-phosphate 5-kinases mediate Arabidopsis and Nicotiana tabacum pollen tube growth by regulating apical pectin secretion. Plant Cell 20:3312–3330

Ischebeck T, Vu LH, Jin X, Stenzel I, Lofke C, Heilmann I (2010a) Functional cooperativity of enzymes of phosphoinositide conversion according to synergistic effects on pectin secretion in tobacco pollen tubes. Mol Plant 3:870–881

Ischebeck T, Seiler S, Heilmann I (2010b) At the poles across kingdoms: phosphoinositides and polar tip growth. Protoplasma 240:13–31

Ischebeck T, Stenzel I, Hempel F, Jin X, Mosblech A, Heilmann I (2011) Phosphatidylinositol-4,5-bisphosphate influences Nt-Rac5-mediated cell expansion in pollen tubes of *Nicotiana tabacum*. Plant J 65:453–468

Kaye Y, Golani Y, Singer Y, Leshem Y, Cohen G, Ercetin M, Gillaspy G, Levine A (2011) Inositol polyphosphate 5-phosphatase7 regulates the production of reactive oxygen species and salt tolerance in Arabidopsis. Plant Physiol 157:229–241

Keune W, Bultsma Y, Sommer L, Jones D, Divecha N (2011) Phosphoinositide signalling in the nucleus. Adv Enzyme Regul 51:91–99

Khodakovskaya M, Sword C, Wu Q, Perera IY, Boss WF, Brown CS, Winter Sederoff H (2010) Increasing inositol (1,4,5)-trisphosphate metabolism affects drought tolerance, carbohydrate metabolism and phosphate-sensitive biomass increases in tomato. Plant Biotechnol J 8:170–183

Kim YJ, Kim JE, Lee JH, Lee MH, Jung HW, Bahk YY, Hwang BK, Hwang I, Kim WT (2004) The *Vr-PLC3* gene encodes a putative plasma membrane-localized phosphoinositide-specific phospholipase C whose expression is induced by abiotic stress in mung bean (*Vigna radiata* L.). FEBS Lett 556:127–136

Klein RR, Bourdon DM, Costales CL, Wagner CD, White WL, Williams JD, Hicks SN, Sondek J, Thakker DR (2011) Direct activation of human phospholipase C by its well known inhibitor U73122. J Biol Chem 286:12407–12416

Konig S, Ischebeck T, Lerche J, Stenzel I, Heilmann I (2008a) Salt-stress-induced association of phosphatidylinositol 4,5-bisphosphate with clathrin-coated vesicles in plants. Biochem J 415:387–399

Konig S, Hoffmann M, Mosblech A, Heilmann I (2008b) Determination of content and fatty acid composition of unlabeled phosphoinositide species by thin-layer chromatography and gas chromatography. Anal Biochem 378:197–201

Kopka J, Pical C, Gray JE, Muller-Rober B (1998a) Molecular and enzymatic characterization of three phosphoinositide-specific phospholipase C isoforms from potato. Plant Physiol 116:239–250

Kopka J, Pical C, Hetherington AM, Muller-Rober B (1998b) Ca^{2+}/phospholipid-binding (C2) domain in multiple plant proteins: novel components of the calcium-sensing apparatus. Plant Mol Biol 36:627–637

Kost B (2008) Spatial control of Rho (Rac-Rop) signaling in tip-growing plant cells. Trends Cell Biol 18:119–127

Kost B, Lemichez E, Spielhofer P, Hong Y, Tolias K, Carpenter C, Chua NH (1999) Rac homologues and compartmentalized phosphatidylinositol 4,5-bisphosphate act in a common pathway to regulate polar pollen tube growth. J Cell Biol 145:317–330

Krinke O, Novotna Z, Valentova O, Martinec J (2007) Inositol trisphosphate receptor in higher plants: is it real? J Exp Bot 58:361–376

Kuin H, Koerten H, Ghijsen WE, Munnik T, van den Ende H, Musgrave A (2000) Chlamydomonas contains calcium stores that are mobilized when phospholipase C is activated. Planta 210:286–294

Kusano H, Testerink C, Vermeer JE, Tsuge T, Shimada H, Oka A, Munnik T, Aoyama T (2008) The Arabidopsis phosphatidylinositol phosphate 5-kinase PIP5K3 is a key regulator of root hair tip growth. Plant Cell 20:367–380

Laxalt AM, Munnik T (2002) Phospholipid signalling in plant defence. Curr Opin Plant Biol 5:332–338

Lee Y, Kim YW, Jeon BW, Park KY, Suh SJ, Seo J, Kwak JM, Martinoia E, Hwang I (2007) Phosphatidylinositol 4,5-bisphosphate is important for stomatal opening. Plant J 52:803–816

Legendre L, Yueh YG, Crain R, Haddock N, Heinstein PF, Low PS (1993) Phospholipase C activation during elicitation of the oxidative burst in cultured plant cells. J Biol Chem 268:24559–24563

Lemtiri-Chlieh F, MacRobbie EA, Brearley CA (2000) Inositol hexakisphosphate is a physiological signal regulating the K^+-inward rectifying conductance in guard cells. Proc Natl Acad Sci U S A 97:8687–8692

Lemtiri-Chlieh F, MacRobbie EA, Webb AA, Manison NF, Brownlee C, Skepper JN, Chen J, Prestwich GD, Brearley CA (2003) Inositol hexakisphosphate mobilizes an endomembrane store of calcium in guard cells. Proc Natl Acad Sci U S A 100:10091–10095

Levchenko V, Konrad KR, Dietrich P, Roelfsema MR, Hedrich R (2005) Cytosolic abscisic acid activates guard cell anion channels without preceding Ca^{2+} signals. Proc Natl Acad Sci U S A 102:4203–4208

Li M, Hong Y, Wang X (2009) Phospholipase D- and phosphatidic acid-mediated signaling in plants. Biochim Biophys Acta 1791:927–935

Lin WH, Ye R, Ma H, Xu ZH, Xue HW (2004) DNA chip-based expression profile analysis indicates involvement of the phosphatidylinositol signaling pathway in multiple plant responses to hormone and abiotic treatments. Cell Res 14:34–45

Lin WH, Wang Y, Mueller-Roeber B, Brearley CA, Xu ZH, Xue HW (2005) At5PTase13 modulates cotyledon vein development through regulating auxin homeostasis. Plant Physiol 139:1677–1691

Liu HT, Liu YY, Pan QH, Yang HR, Zhan JC, Huang WD (2006a) Novel interrelationship between salicylic acid, abscisic acid, and PIP2-specific phospholipase C in heat acclimation-induced thermotolerance in pea leaves. J Exp Bot 57:3337–3347

Liu HT, Huang WD, Pan QH, Weng FH, Zhan JC, Liu Y, Wan SB, Liu YY (2006b) Contributions of PIP_2-specific-phospholipase C and free salicylic acid to heat acclimation-induced thermotolerance in pea leaves. J Plant Physiol 163:405–416

McLoughlin F, Galvan-Ampudia CS, Julkowska MM, Caarls L, van der Does D, Lauriere C, Munnik T, Haring MA, Testerink C (2012) The Snf1-related protein kinases SnRK2.4 and SnRK2.10 are involved in maintenance of root system architecture during salt stress. Plant J 72:436–449

McLoughlin F, Arisz SA, Dekker HL, Kramer G, de Koster CG, Haring MA, Munnik T, Testerink C (2013) Identification of novel candidate phosphatidic acid-binding proteins involved in the salt-stress response of Arabidopsis thaliana roots. Biochem J 450:573–581

Meijer HJG, Munnik T (2003) Phospholipid-based signaling in plants. Annu Rev Plant Biol 54:265–306

Meijer HJG, Divecha N, van den Ende H, Musgrave A, Munnik T (1999) Hyperosmotic stress induces rapid synthesis of phosphatidyl-D-inositol 3,5-bisphosphate in plant cells. Planta 208:294–298

Meijer HJG, Berrie CP, Iurisci C, Divecha N, Musgrave A, Munnik T (2001a) Identification of a new polyphosphoinositide in plants, phosphatidylinositol 5-monophosphate (PtdIns5P), and its accumulation upon osmotic stress. Biochem J 360:491–498

Meijer HJG, Arisz SA, van Himbergen JAJ, Musgrave A, Munnik T (2001b) Hyperosmotic stress rapidly generates lyso-phosphatidic acid in *Chlamydomonas*. Plant J 25:541–548

Melin PM, Sommarin M, Sandelius AS, Jergil B (1987) Identification of Ca^{2+}-stimulated polyphosphoinositide phospholipase C in isolated plant plasma membranes. FEBS Lett 223:87–91

Mishkind M, Vermeer JE, Darwish E, Munnik T (2009) Heat stress activates phospholipase D and triggers PIP_2 accumulation at the plasma membrane and nucleus. Plant J 60:10–21

Misra S, Wu Y, Venkataraman G, Sopory SK, Tuteja N (2007) Heterotrimeric G-protein complex and G-protein-coupled receptor from a legume (*Pisum sativum*): role in salinity and heat stress and cross-talk with phospholipase C. Plant J 51:656–669

Mogami H, Lloyd Mills C, Gallacher DV (1997) Phospholipase C inhibitor, U73122, releases intracellular Ca^{2+}, potentiates Ins(1,4,5)P_3-mediated Ca^{2+} release and directly activates ion channels in mouse pancreatic acinar cells. Biochem J 324:645–651

Monteiro D, Liu Q, Lisboa S, Scherer GE, Quader H, Malho R (2005) Phosphoinositides and phosphatidic acid regulate pollen tube growth and reorientation through modulation of $[Ca^{2+}]c$ and membrane secretion. J Exp Bot 56:1665–1674

Mosblech A, Konig S, Stenzel I, Grzeganek P, Feussner I, Heilmann I (2008) Phosphoinositide and inositolpolyphosphate signalling in defense responses of *Arabidopsis thaliana* challenged by mechanical wounding. Mol Plant 1:249–261

Mosblech A, Thurow C, Gatz C, Feussner I, Heilmann I (2011) Jasmonic acid perception by COI1 involves inositol polyphosphates in *Arabidopsis thaliana*. Plant J 65:949–957

Mueller-Roeber B, Pical C (2002) Inositol phospholipid metabolism in Arabidopsis. Characterized and putative isoforms of inositol phospholipid kinase and phosphoinositide-specific phospholipase C. Plant Physiol 130:22–46

Munnik T (2001) Phosphatidic acid: an emerging plant lipid second messenger. Trends Plant Sci 6:227–233

Munnik T (2013) Analysis of D3-,4-,5-phosphorylated phosphoinositides using HPLC. Methods Mol Biol 1009:17–24

Munnik T, Laxalt AM (2013) Measuring PLD activity in vivo. Methods Mol Biol 1009:219–231

Munnik T, Nielsen E (2011) Green light for polyphosphoinositide signals in plants. Curr Opin Plant Biol 14:489–497

Munnik T, Testerink C (2009) Plant phospholipid signaling: "in a nutshell". J Lipid Res 50: S260–S265

Munnik T, Vermeer JE (2010) Osmotic stress-induced phosphoinositide and inositol phosphate signalling in plants. Plant Cell Environ 33:655–669

Munnik T, Zarza X (2013) Analyzing plant signaling phospholipids through $^{32}P_i$-labeling and TLC. Methods Mol Biol 1009:3–15

Munnik T, Musgrave A, de Vrije T (1994) Rapid turnover of polyphosphoinositides in carnation flower petals. Planta 193:89–98

Munnik T, Arisz SA, De Vrije T, Musgrave A (1995) G protein activation stimulates phospholipase D signaling in plants. Plant Cell 7:2197–2210

Munnik T, van Himbergen JAJ, ter Riet B, Braun FJ, Irvine RF, van den Ende H, Musgrave A (1998a) Detailed analysis of the turnover of polyphosphoinositides and phosphatidic acid upon activation of phospholipases C and D in Chlamydomonas cells treated with non-permeabilizing concentrations of mastoparan. Planta 207:133–145

Munnik T, Irvine RF, Musgrave A (1998b) Phospholipid signalling in plants. Biochim Biophys Acta 1389:222–272

Munnik T, Meijer HJ, Ter Riet B, Hirt H, Frank W, Bartels D, Musgrave A (2000) Hyperosmotic stress stimulates phospholipase D activity and elevates the levels of phosphatidic acid and diacylglycerol pyrophosphate. Plant J 22:147–154

Murphy AM, Otto B, Brearley CA, Carr JP, Hanke DE (2008) A role for inositol hexakisphosphate in the maintenance of basal resistance to plant pathogens. Plant J 56:638–652

Musgrave A, Kuin H, Jonen M, de Wildt P, Schuring F, Klerk H, van den Ende H (1992) Ethanol stimulates phospholipid turnover and inositol 1,4,5-trisphosphate production in *Chlamydomonas eugametos* gametes. Planta 186:442–449

Nakamura K, Sano H (2009) A plasma-membrane linker for the phosphoinositide-specific phospholipase C in tobacco plants. Plant Signal Behav 4:26–29

Noble KN, Tran EJ, Alcazar-Roman AR, Hodge CA, Cole CN, Wente SR (2011) The Dbp5 cycle at the nuclear pore complex during mRNA export II: nucleotide cycling and mRNP remodeling by Dbp5 are controlled by Nup159 and Gle1. Genes Dev 25:1065–1077

Nomikos M, Mulgrew-Nesbitt A, Pallavi P, Mihalyne G, Zaitseva I, Swann K, Lai FA, Murray D, McLaughlin S (2007) Binding of phosphoinositide-specific phospholipase C-zeta (PLCζ) to

phospholipid membranes: potential role of an unstructured cluster of basic residues. J Biol Chem 282:16644–16653

Nomikos M, Elgmati K, Theodoridou M, Calver BL, Nounesis G, Swann K, Lai FA (2011) Phospholipase Cζ binding to PtdIns(4,5)P_2 requires the XY-linker region. J Cell Sci 124:2582–2590

Nuhse TS, Boller T, Peck SC (2003) A plasma membrane syntaxin is phosphorylated in response to the bacterial elicitor flagellin. J Biol Chem 278:45248–45254

Otterhag L, Sommarin M, Pical C (2001) N-terminal EF-hand-like domain is required for phosphoinositide-specific phospholipase C activity in *Arabidopsis thaliana*. FEBS Lett 497:165–170

Pan YY, Wang X, Ma LG, Sun DY (2005) Characterization of phosphatidylinositol-specific phospholipase C (PI-PLC) from *Lilium daviddi* pollen. Plant Cell Physiol 46:1657–1665

Parmar PN, Brearley CN (1993) Identification of 3- and 4-phosphorylated phosphoinositides and inositol phosphates in stomatal guard cells. Plant J 4:255–263

Parmar PN, Brearley CA (1995) Metabolism of 3- and 4-phosphorylated phosphatidylinositols in stomatal guard cells of *Commelina communis* L. Plant J 8:425–433

Perera IY, Heilmann I, Boss WF (1999) Transient and sustained increases in inositol 1,4,5-trisphosphate precede the differential growth response in gravistimulated maize pulvini. Proc Natl Acad Sci U S A 96:5838–5843

Perera NM, Michell RH, Dove SK (2004) Hypo-osmotic stress activates Plc1p-dependent phosphatidylinositol 4,5-bisphosphate hydrolysis and inositol hexakisphosphate accumulation in yeast. J Biol Chem 279:5216–5226

Perera IY, Hung CY, Brady S, Muday GK, Boss WF (2006) A universal role for inositol 1,4,5-trisphosphate-mediated signaling in plant gravitropism. Plant Physiol 140:746–760

Perera IY, Hung CY, Moore CD, Stevenson-Paulik J, Boss WF (2008) Transgenic Arabidopsis plants expressing the type 1 inositol 5-phosphatase exhibit increased drought tolerance and altered abscisic acid signaling. Plant Cell 20:2876–2893

Pical C, Sandelius AS, Melin PM, Sommarin M (1992) Polyphosphoinositide Phospholipase C in Plasma Membranes of Wheat (*Triticum aestivum* L.): orientation of active site and activation by Ca^{2+} and Mg^{2+}. Plant Physiol 100:1296–1303

Pical C, Westergren T, Dove SK, Larsson C, Sommarin M (1999) Salinity and hyperosmotic stress induce rapid increases in phosphatidylinositol 4,5-bisphosphate, diacylglycerol pyrophosphate, and phosphatidylcholine in *Arabidopsis thaliana* cells. J Biol Chem 274:38232–38240

Qin C, Wang X (2002) The Arabidopsis phospholipase D family. Characterization of a calcium-independent and phosphatidylcholine-selective PLD zeta 1 with distinct regulatory domains. Plant Physiol 128:1057–1068

Quarmby LM, Yueh YG, Cheshire JL, Keller LR, Snell WJ, Crain RC (1992) Inositol phospholipid metabolism may trigger flagellar excision in *Chlamydomonas reinhardtii*. J Cell Biol 116:737–744

Raboy V (2003) myo-Inositol-1,2,3,4,5,6-hexakisphosphate. Phytochemistry 64:1033–1043

Raho N, Ramirez L, Lanteri ML, Gonorazky G, Lamattina L, ten Have A, Laxalt AM (2010) Phosphatidic acid production in chitosan-elicited tomato cells, via both phospholipase D and phospholipase C/diacylglycerol kinase, requires nitric oxide. J Plant Physiol 168:534–539

Raho N, Ramirez L, Lanteri ML, Gonorazky G, Lamattina L, ten Have A, Laxalt AM (2011) Phosphatidic acid production in chitosan-elicited tomato cells, via both phospholipase D and phospholipase C/diacylglycerol kinase, requires nitric oxide. J Plant Physiol 168:534–539

Remus TP, Zima AV, Bossuyt J, Bare DJ, Martin JL, Blatter LA, Bers DM, Mignery GA (2006) Biosensors to measure inositol 1,4,5-trisphosphate concentration in living cells with spatio-temporal resolution. J Biol Chem 281:608–616

Repp A, Mikami K, Mittmann F, Hartmann E (2004) Phosphoinositide-specific phospholipase C is involved in cytokinin and gravity responses in the moss *Physcomitrella* patens. Plant J 40:250–259

Ruelland E, Cantrel C, Gawer M, Kader JC, Zachowski A (2002) Activation of phospholipases C and D is an early response to a cold exposure in Arabidopsis suspension cells. Plant Physiol 130:999–1007

Rupwate SD, Rajasekharan R (2012) C2 domain is responsible for targeting rice phosphoinositide specific phospholipase C. Plant Mol Biol 78:247–258

Rupwate SD, Rupwate PS, Rajasekharan R (2012) Regulation of lipid biosynthesis by phosphatidylinositol-specific phospholipase C through the transcriptional repression of upstream activating sequence inositol containing genes. FEBS Lett 586:1555–1560

Saavedra L, Balbi V, Lerche J, Mikami K, Heilmann I, Sommarin M (2011) PIPKs are essential for rhizoid elongation and caulonemal cell development in the moss *Physcomitrella patens*. Plant J 67:635–647

Salinas-Mondragon RE, Kajla JD, Perera IY, Brown CS, Sederoff HW (2010) Role of inositol 1,4,5-triphosphate signalling in gravitropic and phototropic gene expression. Plant Cell Environ 33:2041–2055

Sanchez-Cach LA, Ortiz-Garcia MM, Minero-Garcia Y, Munoz-Sanchez JA, Hernandez-Sotomayor ST, Suarez-Solis VM, De Los Santos-Briones C (2008) Isolation of cDNA encoding the catalytic site of phosphatidylinositol-specific phospholipase C from *Coffea arabica* L.: recombinant expression and peptide purification. Plant Signal Behav 3:913–916

Sheard LB, Tan X, Mao H, Withers J, Ben-Nissan G, Hinds TR, Kobayashi Y, Hsu FF, Sharon M, Browse J, He SY, Rizo J, Howe GA, Zheng N (2010) Jasmonate perception by inositol-phosphate-potentiated COI1-JAZ co-receptor. Nature 468:400–405

Sieburth LE, Muday GK, King EJ, Benton G, Kim S, Metcalf KE, Meyers L, Seamen E, Van Norman JM (2006) SCARFACE encodes an ARF-GAP that is required for normal auxin efflux and vein patterning in Arabidopsis. Plant Cell 18:1396–1411

Singh A, Kanwar P, Pandey A, Tyagi AK, Sopory SK, Kapoor S, Pandey GK (2013) Comprehensive genomic analysis and expression profiling of phospholipase C gene family during abiotic stresses and development in rice. PLoS One 8:e62494

Song MF, Liu SJ, Zhou Z, Han YZ (2008) TfPLC1, a gene encoding phosphoinositide-specific phospholipase C, is predominantly expressed in reproductive organs in *Torenia fournieri*. Sex Plant Reprod 21:259–267

Sousa E, Kost B, Malho R (2008) Arabidopsis phosphatidylinositol-4-monophosphate 5-kinase 4 regulates pollen tube growth and polarity by modulating membrane recycling. Plant Cell 20:3050–3064

Stenzel I, Ischebeck T, Konig S, Holubowska A, Sporysz M, Hause B, Heilmann I (2008) The type B phosphatidylinositol-4-phosphate 5-kinase 3 is essential for root hair formation in Arabidopsis thaliana. Plant Cell 20:124–141

Stevenson JM, Perera IY, Heilmann I, Persson S, Boss WF (2000) Inositol signaling and plant growth. Trends Plant Sci 5:252–258

Stevenson-Paulik J, Bastidas RJ, Chiou ST, Frye RA, York JD (2005) Generation of phytate-free seeds in Arabidopsis through disruption of inositol polyphosphate kinases. Proc Natl Acad Sci U S A 102:12612–12617

Suh BC, Song SK, Kim YK, Kim KT (1996) Induction of cytosolic Ca^{2+} elevation mediated by Mas-7 occurs through membrane pore formation. J Biol Chem 271:32753–32759

Suzuki M, Tanaka K, Kuwano M, Yoshida KT (2007) Expression pattern of inositol phosphate-related enzymes in rice (Oryza sativa L.): implications for the phytic acid biosynthetic pathway. Gene 405:55–64

Swann K, Lai FA (2013) PLCzeta and the initiation of Ca^{2+} oscillations in fertilizing mammalian eggs. Cell Calcium 53:55–62

Swanson SJ, Gilroy S (2013) Imaging changes in cytoplasmic calcium using the yellow cameleon 3.6 biosensor and confocal microscopy. Methods Mol Biol 1009:291–302

Takahashi S, Katagiri T, Hirayama T, Yamaguchi-Shinozaki K, Shinozaki K (2001) Hyperosmotic stress induces a rapid and transient increase in inositol 1,4,5-trisphosphate independent of abscisic acid in Arabidopsis cell culture. Plant Cell Physiol 42:214–222

Tamura T, Hara K, Yamaguchi Y, Koizumi N, Sano H (2003) Osmotic stress tolerance of transgenic tobacco expressing a gene encoding a membrane-located receptor-like protein from tobacco plants. Plant Physiol 131:454–462

Tan X, Calderon-Villalobos LI, Sharon M, Zheng C, Robinson CV, Estelle M, Zheng N (2007) Mechanism of auxin perception by the TIR1 ubiquitin ligase. Nature 446:640–645

Tasma IM, Brendel V, Whitham SA, Bhattacharyya MK (2008) Expression and evolution of the phosphoinositide-specific phospholipase C gene family in *Arabidopsis thaliana*. Plant Physiol Biochem 46:627–637

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

Testerink C, Munnik T (2011) Molecular, cellular, and physiological responses to phosphatidic acid formation in plants. J Exp Bot 62:2349–2361

Thole JM, Vermeer JE, Zhang Y, Gadella TW Jr, Nielsen E (2008) Root hair defective-4 encodes a phosphatidylinositol-4-phosphate phosphatase required for proper root hair development in *Arabidopsis thaliana*. Plant Cell 20:381–395

Tripathy MK, Tyagi W, Goswami M, Kaul T, Singla-Pareek SL, Deswal R, Reddy MK, Sopory SK (2012) Characterization and functional validation of tobacco PLCdelta for abiotic stress tolerance. Plant Mol Biol Rep 30:488–497

Tsui MM, York JD (2010) Roles of inositol phosphates and inositol pyrophosphates in development, cell signaling and nuclear processes. Adv Enzyme Regul 50:324–337

Tucker EB, Boss WF (1996) Mastoparan-induced intracellular Ca^{2+} fluxes may regulate cell-to-cell communication in plants. Plant Physiol 111:459–467

Urano D, Jones AM (2013) "Round up the usual suspects": a comment on nonexistent plant g protein-coupled receptors. Plant Physiol 161:1097–1102

Valluru R, Van den Ende W (2011) Myo-inositol and beyond – emerging networks under stress. Plant Sci 181:387–400

van der Luit AH, Piatti T, van Doorn A, Musgrave A, Felix G, Boller T, Munnik T (2000) Elicitation of suspension-cultured tomato cells triggers the formation of phosphatidic acid and diacylglycerol pyrophosphate. Plant Physiol 123:1507–1516

Van Himbergen JAJ, ter Riet B, Meijer HJG, van den Ende H, Musgrave A, Munnik T (1999) Mastoparan analogues activate phospholipase C- and phospholipase D activity in *Chlamydomonas*: a comparative study. J Exp Bot 50:1735–1742

van Leeuwen W, Vermeer JE, Gadella TW Jr, Munnik T (2007) Visualization of phosphatidylinositol 4,5-bisphosphate in the plasma membrane of suspension-cultured tobacco BY-2 cells and whole Arabidopsis seedlings. Plant J 52:1014–1026

Varnai P, Balla T (2008) Live cell imaging of phosphoinositides with expressed inositide binding protein domains. Methods 46:167–176

Venkataraman G, Goswami M, Tuteja N, Reddy MK, Sopory SK (2003) Isolation and characterization of a phospholipase C delta isoform from pea that is regulated by light in a tissue specific manner. Mol Genet Genomics 270:378–386

Vermeer JEM, Munnik T (2013) Using genetically encoded fluorescent reporters to image lipid signalling in living plants. Methods Mol Biol 1009:283–289

Vermeer JE, van Leeuwen W, Tobena-Santamaria R, Laxalt AM, Jones DR, Divecha N, Gadella TW Jr, Munnik T (2006) Visualization of PtdIns3P dynamics in living plant cells. Plant J 47:687–700

Vermeer JE, Thole JM, Goedhart J, Nielsen E, Munnik T, Gadella TW Jr (2009) Imaging phosphatidylinositol 4-phosphate dynamics in living plant cells. Plant J 57:356–372

Vom Dorp K, Dombrink I, Dormann P (2013) Quantification of diacylglycerol by mass spectrometry. Methods Mol Biol 1009:43–54

Vossen JH, Abd-El-Haliem A, Fradin EF, van den Berg GC, Ekengren SK, Meijer HJ, Seifi A, Bai Y, ten Have A, Munnik T, Thomma BP, Joosten MH (2010) Identification of tomato phosphatidylinositol-specific phospholipase-C (PI-PLC) family members and the role of PLC4 and PLC6 in HR and disease resistance. Plant J 62:224–239

Wang X (2005) Regulatory functions of phospholipase D and phosphatidic acid in plant growth, development, and stress responses. Plant Physiol 139:566–573

Wang CR, Yang AF, Yue GD, Gao Q, Yin HY, Zhang JR (2008) Enhanced expression of phospholipase C 1 (ZmPLC1) improves drought tolerance in transgenic maize. Planta 227:1127–1140

Wewer V, Dormann P, Holzl G (2013) Analysis and quantification of plant membrane lipids by thin-layer chromatography and gas chromatography. Methods Mol Biol 1009:69–78

Wheeler GL, Brownlee C (2008) Ca^{2+} signalling in plants and green algae–changing channels. Trends Plant Sci 13:506–514

Xu J, Brearley CA, Lin WH, Wang Y, Ye R, Mueller-Roeber B, Xu ZH, Xue HW (2005) A role of Arabidopsis inositol polyphosphate kinase, AtIPK, in pollen germination and root growth. Plant Physiol 137:94–103

York JD, Odom AR, Murphy R, Ives EB, Wente SR (1999) A phospholipase C-dependent inositol polyphosphate kinase pathway required for efficient messenger RNA export. Science 285:96–100

York JD, Guo S, Odom AR, Spiegelberg BD, Stolz LE (2001) An expanded view of inositol signaling. Adv Enzyme Regul 41:57–71

Yueh YG, Crain RC (1993) Deflagellation of *Chlamydomonas reinhardtii* follows a rapid transitory accumulation of inositol 1,4,5-trisphosphate and requires Ca^{2+} entry. J Cell Biol 123:869–875

Zhang W, Chen J, Zhang H, Song F (2008) Overexpression of a rice diacylglycerol kinase gene OsBIDK1 enhances disease resistance in transgenic tobacco. Mol Cells 26:258–264

Zhao Y, Yan A, Feijo JA, Furutani M, Takenawa T, Hwang I, Fu Y, Yang Z (2010) Phosphoinositides regulate clathrin-dependent endocytosis at the tip of pollen tubes in Arabidopsis and tobacco. Plant Cell 22:4031–4044

Zheng SZ, Liu YL, Li B, Shang ZL, Zhou RG, Sun DY (2012) Phosphoinositide-specific phospholipase C9 is involved in the thermotolerance of Arabidopsis. Plant J 69:689–700

Zhong R, Ye ZH (2004) Molecular and biochemical characterization of three WD-repeat-domain-containing inositol polyphosphate 5-phosphatases in *Arabidopsis thaliana*. Plant Cell Physiol 45:1720–1728

Zhong R, Burk DH, Morrison WH 3rd, Ye ZH (2004) FRAGILE FIBER3, an Arabidopsis gene encoding a type II inositol polyphosphate 5-phosphatase, is required for secondary wall synthesis and actin organization in fiber cells. Plant Cell 16:3242–3259

Zhong R, Burk DH, Nairn CJ, Wood-Jones A, Morrison WH 3rd, Ye ZH (2005) Mutation of SAC1, an Arabidopsis SAC domain phosphoinositide phosphatase, causes alterations in cell morphogenesis, cell wall synthesis, and actin organization. Plant Cell 17:1449–1466

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

Zonia L, Munnik T (2006) Cracking the green paradigm: functional coding of phosphoinositide signals in plant stress responses. Subcell Biochem 39:207–237

Zonia L, Cordeiro S, Tupy J, Feijo JA (2002) Oscillatory chloride efflux at the pollen tube apex has a role in growth and cell volume regulation and is targeted by inositol 3,4,5,6-tetrakisphosphate. Plant Cell 14:2233–2249

NPC: Nonspecific Phospholipase Cs in Plant Functions

Yuki Nakamura

Abstract Nonspecific phospholipase C (NPC) is an emerging class of phospholipases in plants that hydrolyzes primary membrane phospholipids such as phosphatidylcholine to yield *sn*-1,2-diacylglycerol and a phosphate-containing head group. Unlike the phosphoinositide-specific type of phospholipase C, which is ubiquitous from bacteria to mammals, NPC was known only in bacteria. Analysis of plant genomic sequences has revealed that Arabidopsis and many other plants possess bacterial NPC homologs. Since the first report of an NPC in Arabidopsis in 2005, NPC has been shown to have multiple physiological roles in lipid metabolism or signaling in plants. This chapter summarizes recent advances in NPC studies, focusing on Arabidopsis NPC isoforms and the basic biochemical properties of this enzyme type in different plant species.

Keywords Arabidopsis • Nonspecific phospholipase C • Phospholipase C • Phospholipids • *sn*-1,2-diacylglycerol

Abbreviations

ABA	Abscisic acid
DAG	Diacylglycerol
DGDG	Digalactosyldiacylglycerol
IAA	Indole acetic acid
MGDG	Monogalactosyldiacylglycerol
NPC	Nonspecific phospholipase C

Y. Nakamura (✉)
Institute of Plant and Microbial Biology, Academia Sinica, 128 Section 2 Academia Road, Nankang, Taipei 11529, Taiwan

Japan Sciences and Technology Agency, PRESTO, Saitama, Japan
e-mail: nakamura@gate.sinica.edu.tw

X. Wang (ed.), *Phospholipases in Plant Signaling*, Signaling and Communication in Plants 20, DOI 10.1007/978-3-642-42011-5_3,

PA	Phosphatidic acid
PAP	PA phosphatase
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
$PI(4,5)P_2$	Phosphatidylinositol 4,5-bisphosphate
PI-PLC	Phosphoinositides-specific phospholipase C
PLD	Phospholipase D

1 Introduction

Nonspecific phospholipase C (NPC) is a PLC subtype that hydrolyzes a range of membrane phospholipids such as phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylserine to produce *sn*-1,2-diacylglycerol (DAG) and a corresponding phosphate head group (Fig. 1). By comparison, the phosphoinositides-specific PLC (PI-PLC) specifically hydrolyzes phosphatidylinositol 4,5-bisphosphate [$PI(4,5)P_2$] and its phosphorylated/dephosphorylated derivatives. NPC was first discovered in the pathogenic bacterium *C. welchii*, which exhibits lecithinase C-like activity and causes lysis of the host-cell plasma membrane (Macfarlane and Knight 1941). Since then, NPCs (also called PC-PLCs) have been identified in a variety of bacteria and can be clearly categorized into two groups. The first group, found mostly in Gram-positive bacteria, is cytotoxic with hemolytic characteristics and requires Zn^{2+} for activity. The second group, which is predominantly found in Gram-negative bacteria such as Pseudomonas, Burkholderia, and Legionella, has neither hemolytic properties nor a Zn^{2+} requirement for activity (Titball 1993). PI-PLC has been studied extensively in bacteria, humans, and plants for its vital roles in signal transduction, whereas NPC has only been studied in prokaryotes (Titball 1993).

In higher plants, NPC-like activity and other phospholipase activities were first identified in plant plastid fractions in which lecithin (or PC) hydrolysis was detected (Kates 1955). NPC activity is also found in peanut seeds (Strauss et al. 1976), rice grains (Chrastil and Parrish 1987), tomatoes (Rouet-Mayer et al. 1995), cultured plant cells (Scherer et al. 2002), and petunia flowers (Nakamura and Ohta 2007). Six NPC isoforms have been identified in the Arabidopsis genome (Nakamura et al. 2005), and subsequent studies with T-DNA-tagged mutants of the respective isoforms showed the isoforms to be involved in diverse physiological functions: NPC4 and NPC5 in phosphate starvation (Nakamura et al. 2005; Gaude et al. 2008), NPC4 in salt stress (Peters et al. 2010; Kocourkova et al. 2011), NPC4 in signaling mediated by abscisic acid (ABA) (Peters et al. 2010) and auxin (Wimalasekera et al. 2010), and NPC4 and NPC3 in brassinolide signaling (Wimalasekera et al. 2010). These results clearly indicate that NPCs are an emerging class of enzymes involved in numerous biological processes.

This chapter describes the biochemical evidence for NPCs in various plant tissues and summarizes recent evidence for the physiological roles of the NPC family in the model plant Arabidopsis. For more extensive information on NPC,

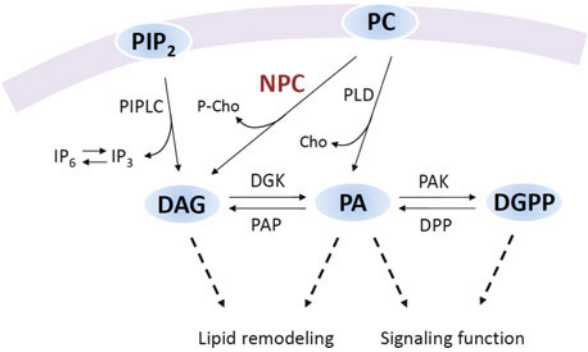

Fig. 1 Schematic illustration of plant lipid signaling mediated by phospholipase C and phospholipase D. Membrane phospholipids are hydrolyzed by different classes of phospholipases such as phosphoinositides-specific phospholipase C (PI-PLC), nonspecific phospholipase C (NPC), or phospholipase D (PLD) according to substrate specificity. Resulting lipid intermediates such as diacylglycerol (DAG) and phosphatidic acid (PA) or its phosphorylated derivative diacylglycerol pyrophosphate (DGPP) are shown, which are interconnected by specific kinases/phosphatases. DAG is utilized to reassemble polar glycerolipids or triglycerides, whereas DGPP serves a signaling function. PA possesses both characteristics. *Cho* choline, *DGK* diacylglycerol kinase, *DPP* DGPP phosphatase, IP_3 inositol 1,4,5-trisphosphate, IP_6 inositol 1,2,3,4,5,6-hexokisphosphate, *PAP* PA phosphatase, *PC* phosphatidylcholine, *P-Cho* phosphorylcholine, *PAK* PA kinase, PIP_2 phosphatidylinositol 4,5-bisphosphate

Pokotylo et al. (2013) provide an excellent review including in silico phylogenetic, structural, and transcriptomic analyses of the plant NPC family.

2 Biochemical Features of Plant NPC Activity

Plant NPC activity was first demonstrated in 1955 (Kates 1955). Analyses of plant extracts rich in plastids revealed that PC can be metabolized via multiple pathways to yield choline and phosphatidic acid (PA; which can be further hydrolyzed to DAG and inorganic phosphate) or yield fatty acids and glycerophosphate (which can be further hydrolyzed to glycerol and inorganic phosphate). In addition, Kates (1955) found that NPC activity hydrolyzes PC to phosphorylcholine (P-choline) and DAG. A PLC-like activity in peanuts was later demonstrated using lysoPC as a substrate, giving P-choline as a product (Strauss et al. 1976). In rice grains, high PLC activity was suggested to hydrolyze PC and other phospholipids as well as inorganic phosphates such as P-choline (Chrastil and Parrish 1987), although the activity of a purified fraction was not studied, leaving uncertain whether the broad substrate specificity was due to a single enzyme. NPC-related signaling activity occurs in parsley and tobacco culture cells (Scherer et al. 2002), primarily producing DAG from exogenously fed labeled PC. Response to an elicitor downregulated NPC activity, suggesting its involvement in defense responses (Scherer et al. 2002). NPC activity also is relatively high in floral organs of *Petunia hybrida*, with the

PC-hydrolyzing activity (forming DAG) being 1.5-fold, 2.5-fold, and 3-fold higher in petals, pistils, and stamens, respectively, compared with leaf tissue (Nakamura and Ohta 2007). The transphosphatidylation assay using 1-butanol facilitates measurement of PLD activity (Yang et al. 1967). This assay showed that PC-hydrolyzing activity to produce DAG in stamens and pistils is attributable to NPC, whereas in petals most of the activity is attributable to PLD (Nakamura and Ohta 2007).

3 The Arabidopsis NPC Family

3.1 Enzymatic Properties

The first in vitro activity assay for recombinant NPC was achieved with Arabidopsis NPC4 (Nakamura et al. 2005). The C-terminally His-tagged full-length protein coding sequence was expressed in *Escherichia coli*. However, induction of protein synthesis by IPTG needed to be carried out at a relatively low temperature (e.g., 23 °C) because NPC easily forms insoluble inclusion bodies if induced at higher temperatures. The purified NPC4 hydrolyzed PC to yield DAG with specific activity of ~120 pmol DAG formed/min/mg (Nakamura et al. 2005). NPC4 can hydrolyze PE, but substrate preference is higher towards PC. Furthermore, phosphatidylserine and phosphatidylglycerol are possible substrates although the enzyme's specific activity in each case is lower than that with PE (Peters et al. 2010). Unlike PI-PLC, NPC4 does not hydrolyze PI(4,5)P_2, suggesting that NPC substrate specificity is distinct from that of PI-PLC (Nakamura et al. 2005). Interestingly, addition of EGTA to chelate Ca^{2+} does not affect NPC activity, indicating that NPC does not require Ca^{2+} for its activity, unlike most PI-PLCs. NPC4 exhibits phosphatase activity when dipalmitoyl PA is used as substrate (Peters et al. 2010), but such is not the case when dioleoyl PA is used as substrate (Nakamura et al. 2005). NPC5, the closest homolog of NPC4, has ~40-fold lower NPC activity than NPC4 (Gaude et al. 2008). Similar to NPC4, NPC5 cleaves PE as well as PC although the specific activity with either substrate is quite low. NPC3 is highly homologous to NPC4 and NPC5, and yet it exhibits lysoPA phosphatase activity (~500 pmol Pi formed/min/mg) (Reddy et al. 2010). NPC3 dephosphorylates PA and sphingosine 1-phosphate at relatively lower activity, but it cannot dephosphorylate glycerol 3-phosphate, lysoPC, lysoPE, or DAG-pyrophosphate. NPC3 has no apparent specificity for any particular acyl group except that 18:0-lysoPA is slightly less preferred than the others. To date, no in vitro enzyme activity has been demonstrated for NPC1, NPC2, or NPC6.

3.2 Intracellular Localization

The NPCs 1–6 are considered either membrane-associated or cytosolic proteins, as none of their amino acid sequences contain a transmembrane domain (Nakamura et al. 2005). NPC4-specific antibodies revealed NPC4 localization at the plasma

membrane (Nakamura et al. 2005), and fluorescence of an NPC4-GFP fusion protein also revealed localization at the plasma membrane of leaf epidermal cells (Gaude et al. 2008). Unlike NPC4, NPC5-GFP fluorescence is evident at multiple intracellular sites, particularly surrounding chloroplasts, suggesting that NPC5 resides in the cytosol adjacent to chloroplasts. Subcellular fractionation revealed that NPC5-GFP is recovered in the endoplasmic reticulum-enriched fraction as well as the soluble fraction. NPC5 may thus play a role in phospholipid trafficking between the endoplasmic reticulum and the outer envelope of chloroplasts. In summary, NPC4 and NPC5 have distinct intracellular localization patterns that do not overlap. The localization of the remaining NPC isoforms remains unknown, however, although NPC6 has a putative transit peptide for chloroplasts or mitochondria (Pokotylo et al. 2013).

3.3 Tissue Specificity

Apart from tissue-specificity data from microarrays, two independent studies used quantitative reverse transcription (qRT)-PCR analyses (Peters et al. 2010; Wimalasekera et al. 2010). *NPC1* and *NPC6* are ubiquitously expressed, whereas the other isoforms have more specific tissue expression. *NPC2* is highly expressed in fertile organs such as flowers and siliques but not in roots, whereas *NPC3* is highly expressed in roots. *NPC4* is expressed in mature leaves and non-photosynthetic organs such as flowers and roots, whereas *NPC5* expression is specific to flowers. GUS reporter gene expression analyses revealed *NPC3* and *NPC4* tissue-specific staining in the leaf margins, root meristem, and immature anthers, although qRT-PCR data showed distinct expression patterns (Wimalasekera et al. 2010). The detailed tissue-specific expression of the other isoforms is unknown.

4 NPC4 and NPC5 Involvement in Phosphate Starvation-Induced Membrane Lipid Remodeling

Phosphate is an essential nutrient for plant growth. Phosphate bioavailability is often limited in many soils because phosphate may form insoluble salts that cannot be assimilated by plant roots (Raghothama 1999). In response to phosphate starvation, a significant portion of membrane phospholipids, particularly PC, is replaced by the nonphosphate-containing galactolipid digalactosyldiacylglycerol (DGDG) (Essigmann et al. 1998; Nakamura 2013). This membrane lipid remodeling is indicated by the induction of galactolipid biosynthetic activity and gene expression of type B monogalactosyldiacylglycerol (MGDG) synthases (*MGD2* and *MGD3*)

and DGDG synthase 2 (*DGD2*) (Awai et al. 2001; Kelly and Dormann 2002; Kelly et al. 2003; Kobayashi et al. 2004, 2006).

A transient increase in PC occurs in plant cell culture upon phosphate starvation (Jouhet et al. 2003). Because PC and DAG have similar acyl profiles, PC was suggested as a precursor to DAG that can be galactosylated to produce DGDG (Jouhet et al. 2003). Upon phosphate starvation in Arabidopsis seedlings, the activity that produces DAG from PC is upregulated both in shoots and roots (Nakamura et al. 2005). At least two pathways were suggested for this PC-to-DAG conversion, namely a one-step reaction catalyzed by PLC or a two-step reaction in which PLD hydrolyzes PC to yield PA, which is further dephosphorylated by PA phosphatase to produce DAG. Assaying PLD using 1-butanol did not reveal a significant increase in PLD activity, suggesting that PC-to-DAG conversion is mainly catalyzed by PLC (Nakamura et al. 2005). PC-PLC activity, which hydrolyzes PC and other primary membrane phospholipids, has only been found in bacteria (see Sect. 1). Based on homology, six putative PLC genes (*NPC1–6*) have been identified in the Arabidopsis genome (Nakamura et al. 2005).

Northern blotting has revealed that *NPC4* is upregulated upon phosphate starvation. A recombinant NPC4 showed significant hydrolytic activity for PC and PE but not PA or PI(4,5)P_2 (Nakamura et al. 2005). Using an NPC4-specific antibody, subcellular fractionation showed that NPC4 as well as total NPC activity are enriched mainly at the plasma membrane (Nakamura et al. 2005). *NPC4* knockout mutants have lower total NPC activity induced upon phosphate starvation, suggesting that *NPC4* encodes the major PC-hydrolyzing PLC activity induced by phosphate starvation (Nakamura et al. 2005). However, there is little effect on DGDG levels and other lipids in T-DNA-tagged *NPC4* mutants (Nakamura et al. 2005). An independent study using microarray analysis found that *NPC5* is another phosphate starvation-inducible NPC gene, with a soluble recombinant NPC5 showing 40-fold lower PC-PLC activity than plasma membrane-bound NPC4 (Gaude et al. 2008). Interestingly, two knockout mutant alleles of NPC5, *npc5-1* and *npc5-2*, significantly reduced DGDG accumulation in response to phosphate starvation, although total PC-PLC activity was not reduced in these mutants (Gaude et al. 2008).

In conclusion, NPC4 and NPC5 are both induced by phosphate starvation. NPC4 has substantial NPC activity and localizes to the plasma membrane, although its contribution to DGDG accumulation remains elusive. NPC5 is a soluble protein with minor activity, although its disruption significantly affects DGDG accumulation upon phosphate starvation (Fig. 2).

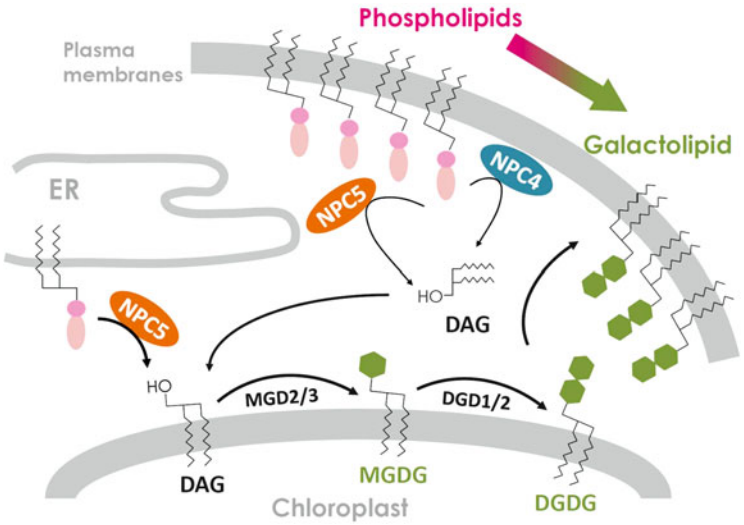

Fig. 2 Differential roles for the two NPC isoforms, NPC4 and NPC5, in membrane lipid remodeling upon phosphate starvation. NPC4 localizes to the plasma membrane, where it hydrolyzes local phospholipids to yield diacylglycerol (DAG). NPC5 is soluble and hence hydrolyzes phospholipids on the cytoplasmic side of several organelles, including the endoplasmic reticulum (ER). The DAG produced is galactosylated at the chloroplast envelope to yield digalactosyldiacylglycerol (DGDG), which is subsequently transferred back to the plasma membrane and other organelles. *NPC* nonspecific phospholipase C, *MGDG* monogalactosyldiacylglycerol, *MGD* MGDG synthase, *DGD* DGDG synthase

5 NPC4 Involvement in Salt Stress

High-salt conditions stimulate metabolism of choline-containing compounds such as phosphocholine, PC, and glycerophosphocholine (Summers and Weretilnyk 1993). Indeed, expression of many of the genes encoding choline metabolic enzymes is upregulated in high-NaCl conditions (Tasseva et al. 2004), implying rapid phosphocholine production from PC via NPC. Expression analysis of the *NPC* family in response to different salt concentrations, including 100 mM NaCl, revealed marked induction of *NPC4* in roots, with a transient peak 3 h after treatment (Kocourkova et al. 2011). The other isoforms did not show significant induction, suggesting that *NPC4* is the main isoform responding to high salt. Arabidopsis seedlings treated with 100 mM NaCl showed a fourfold increase in total PC-hydrolyzing PLC activity compared with the untreated control (Kocourkova et al. 2011). Although involvement of PLD-mediated DAG production was not examined, these data suggest that NPC activity contributes to DAG production in high-salt conditions. In addition, DAG production was elevated fourfold at 90 min after 100 mM NaCl treatment. *pNPC4*::GUS expression only changed in roots in response to high salt, with expression not only in untreated root tips but also in root epidermal and vascular tissues and throughout lateral root

primordia of NaCl-treated plants (Kocourkova et al. 2011). The fresh weight and root length in the *npc4-2* mutant were reduced by 25 % and 55 %, respectively, in 100 mM NaCl compared with wild type, and the seed germination rate after 24 h in 150 mM NaCl was only 27 % in *npc4-2* whereas wild type attained 82 % (Kocourkova et al. 2011). Compared with wild type, seedling viability in 200 mM NaCl was also reduced in the *npc4-1* mutant whereas *NPC4* overexpressors showed enhanced seedling viability with longer primary roots than wild type, suggesting that NPC4 has a dose-dependent effect on plant tolerance to high salt (Peters et al. 2010). The *npc4* mutants have reduced or no expression of salt overly sensitive (SOS) genes (*SOS1–3*) and ABA-related genes (*ABI1*, *ABI2*, *PP2CA*, and *SOT12*), but the expression of ion transporter genes and phospholipid signaling-related genes is unaffected (Kocourkova et al. 2011). The contribution of NPC4 to choline metabolism under high-NaCl conditions is unknown because of the lack of lipid analysis data in salt-stressed *npc4* mutants.

6 NPC4 Involvement in ABA Signaling

Phospholipids play an important role in ABA signaling. An *NPC4* T-DNA knockout mutant, *npc4-1*, showed reduced sensitivity to ABA; *npc4-1* germinated in the presence of ABA but had longer primary roots and more lateral branches than wild type (Peters et al. 2010). In the presence of 25 mM ABA, *npc4-1* flowering was not inhibited, and plants exhibited two- to threefold higher dry weight, reduced seed dormancy, enhanced seed germination, and compromised stomatal movement compared with wild type. These phenotypic features of *npc4* mutants were not evident in mutants of other NPCs, suggesting a specific role for NPC4 in ABA signaling (Peters et al. 2010). Indeed, ABA levels in freshly picked *npc4-1* seeds were 20 % higher than wild type. Basal expression of ABA-responsive genes in *npc4-1* was lower for *RD29B* and *ABI2* but higher for *RCN1*. Upon ABA treatment, expression of ABA-responsive genes (*ABI1*, *ABI2*, *RAB18*, *OST1*, *RD29B*, and *ERA1*) was reduced in *npc4-1* compared with wild type, suggesting that this *npc4* mutant has reduced ABA sensitivity. The *npc4-1* showed lower DAG level compared with wild type. Since DAG level is reduced upon ABA treatment, it suggests reduced DAG production owing to the *NPC4* knockout causes reduced sensitivity to ABA signaling. Indeed, overexpression of *NPC4* (*NPC4-OE*) greatly enhanced sensitivity to ABA, resulting in a lower rate of seed germination and suggesting that NPC4 promotes ABA action and seed dormancy (Peters et al. 2010).

Salinity and drought stresses involve ABA signaling. Under high-NaCl conditions, *npc4-1* was found to be hypersensitive to salt stress with significantly reduced seedling viability, 80 % reduced root length, and 70 % reduced biomass, whereas NPC4-OE seedlings were more viable than wild type with 55 % longer root length and twofold increased biomass (Peters et al. 2010). Transpiration rate and stomatal conductance under watered conditions were lower in *npc4-1* but higher in NPC4-OE. In addition, *npc4-1* exhibited a lower photosynthetic rate, water efficiency, and

leaf turgor, whereas NPC4-OE exhibited higher water use efficiency and more turgid leaves. Exogenous supplementation with di8:0-DAG or di8:0-PA restored root length of *npc4-1* plants in the presence of ABA. Addition of a DAG kinase inhibitor together with di8:0-DAG abolished this effect, suggesting that DAG produced by NPC4 is converted to bioactive PA. The DAG level was lower in *npc4-1* rosettes and roots, but DAG was at a similar level in roots and at higher level in rosettes of NPC4-OE plants compared with wild type. The PA level upon ABA treatment was dependent on NPC4, as *npc4-1* plants had an increased PA level whereas NPC4-OE plants had a decreased PA level compared with wild type (Peters et al. 2010).

7 NPC4 and NPC3 Involvement in Brassinolide Signaling

As described earlier, histochemical GUS staining showed overlapping tissue specificity of NPC3 and NPC4 (Wimalasekera et al. 2010). Brassinolide treatment of *pNPC3*::GUS and *pNPC4*::GUS plants induced GUS staining in the primary root tips, cotyledon margins, and developing and mature leaves. Compared with wild type, *npc3-1* and *npc4-1* mutants treated with 0.05 μM 24-epi-brassinolide showed increased lateral root density, suggesting their reduced sensitivity to this brassinolide (Wimalasekera et al. 2010). Indeed, expression of a brassinolide-regulated gene, *LRX2*, was partially compromised in these mutants (Wimalasekera et al. 2010).

8 NPC4 Involvement in Auxin Signaling

NPC expression in response to auxin treatment has been studied using RT-PCR and promoter::GUS staining. Among the six isoforms tested, only *NPC4* showed marked induction after 1 h of treatment with indole acetic acid (IAA), 1-NAA, or 2,4-D (Wimalasekera et al. 2010). In response to auxin treatment, *pNPC4*::GUS was expressed throughout the root, the hypocotyl root-shoot junction, shoot meristematic region, and emerging young leaves (Wimalasekera et al. 2010). *pNPC3*::GUS had the same expression pattern, although no transcript increase was observed using RT-PCR. Roots were slightly shorter in *npc3-1* and *npc4-1* mutants compared with wild type. *npc3-1* and *npc4-1* mutants treated with 1-NAA showed no significant phenotypic changes, although expression of the auxin-regulated genes *IAA19* and *IAA20* in *npc3-1* was lower than in wild type (Wimalasekera et al. 2010).

9 Future Perspectives

Since the isolation of six Arabidopsis NPCs in 2005 (Nakamura et al. 2005), gene knockout studies of these isoforms has revealed the critical involvement of NPCs in diverse physiological processes such as phosphate starvation response, salt stress

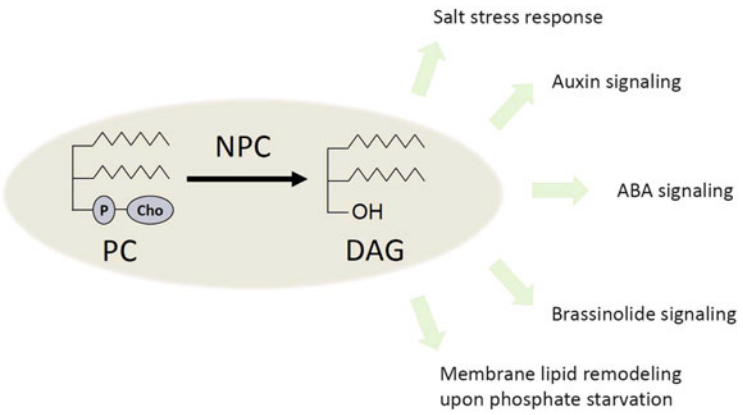

Fig. 3 Suggested physiological functions of NPCs in Arabidopsis. *DAG* diacylglycerol (DAG), *NPC* nonspecific phospholipase C, *PC* phosphatidylcholine

tolerance, and hormonal signaling (Fig. 3). Recent advances in understanding NPC functions may help shed light on several issues.

First, fundamental studies of the uncharacterized isoforms, NPC1, NPC2, and NPC6, are of great importance. These isoforms are highly homologous and thus likely encode functional NPCs. However, their intracellular/tissue distribution patterns may differ as NPC6 has a putative transit peptide for plastid or mitochondrial targeting, and NPC1/2 are predicted to localize at the endoplasmic reticulum (Pokotylo et al. 2013). *NPC1* and *NPC6* are expressed more ubiquitously compared with *NPC2*, but the former two isoforms have different tissue specificities. The fact that single T-DNA mutants of all these isoforms were previously isolated suggests that there are no known lethal isoforms in the NPC family (Peters et al. 2010). Therefore, testing various stress conditions to identify conditional phenotypes or creating double or multiple mutants with different combinations of isoforms would help to dissect their putative differential functions.

Both NPC4 and NPC5 are involved in phosphate starvation. Whereas the role of NPC5 in membrane lipid remodeling has been clearly demonstrated based on compromised DGDG accumulation (Gaude et al. 2008), the role of NPC4 is uncertain because lipid metabolism/composition is not affected in the *npc4* mutant (Nakamura et al. 2005). Because NPC4 is responsible for the majority of upregulated PC-PLC activity under phosphate starvation, NPC4 may have a distinct role other than membrane lipid remodeling. The released P-choline may serve as an internal phosphate reserve because an enzyme was found that dephosphorylates P-choline to produce free inorganic phosphate (May et al. 2012). An alternative possibility is that P-choline produced by NPC4 serves a distinct function. In PI-PLC, a reaction product (inositol 1,4,5-trisphosphate) serves as an important signaling molecule that triggers Ca^{2+} influx (Boss and Im 2012). Likewise, a phosphor-base produced by NPC (e.g., phosphocholine in the case of PC) may have a distinct biological function. It will be important to investigate whether DAG

or a phosphor base plays a major role in response to environmental stimuli that activates NPC function. Producing and characterizing an *npc4 npc5* double knockout mutant under phosphate starvation, together with the metabolic fate of phosphocholine produced by NPC4, are important future experiments.

The involvement of NPCs in hormonal signaling needs to be studied more extensively. The roles of NPC4 and NPC3 in signaling mediated by ABA, brassinolides, and auxin have been described, but involvement of the remaining isoforms and other hormones is not known. Similarly, the roles of NPC in stress conditions other than high salinity need to be determined. Based on the known transcriptional data, targeting specific stresses for each isoform and examining respective mutants in these conditions would further our understanding of NPC isoform functions in hormonal or stress signaling.

In addition to their signaling role, the involvement of NPCs in glycerolipid metabolism is an important issue. PC can be a substrate for plastidic galactolipid biosynthesis (PC hypothesis) (Roughan 1970; Heinz 1977; Douce and Joyard 1980; Roughan and Slack 1982) based on an in situ pulse-chase tracer experiment in which radiolabeled PC was incorporated primarily into MGDG. Because DAG is the substrate for MGDG synthesis, the initial step of PC-to-DAG conversion can be catalyzed by one or multiple NPC isoforms. Measuring the galactolipid content in each knockout mutant is an approach to resolve this issue. As in studies with other phospholipases for which functions have been more fully established, e.g., PLD, phospholipase A1/2, or PI-PLC, further investigation is needed to reveal the importance of NPCs in various aspects of plant physiology.

Acknowledgments The author thanks Yu-chi Liu for assistance with the manuscript formatting. The author is supported by a core research grant from the Institute of Plant and Microbial Biology, Academia Sinica, Taiwan, and the Japan Science and Technology Agency, PRESTO, Japan.

References

Awai K, Marechal E, Block MA, Brun D, Masuda T, Shimada H, Takamiya K, Ohta H, Joyard J (2001) Two types of MGDG synthase genes, found widely in both 16:3 and 18:3 plants, differentially mediate galactolipid syntheses in photosynthetic and nonphotosynthetic tissues in Arabidopsis thaliana. Proc Natl Acad Sci U S A 98:10960–10965

Boss WF, Im YJ (2012) Phosphoinositide signaling. Annu Rev Plant Biol 63:409–429

Chrastil J, Parrish FW (1987) Phospholipases C and D in rice grains. J Agric Food Chem 35:624–627

Douce R, Joyard J (1980) Lipids: structure and function. In: Stumpf PK (ed) The biochemistry of plant, vol 4. Academic, New York, NY, pp 321–362

Essigmann B, Guler S, Narang RA, Linke D, Benning C (1998) Phosphate availability affects the thylakoid lipid composition and the expression of SQD1, a gene required for sulfolipid biosynthesis in Arabidopsis thaliana. Proc Natl Acad Sci U S A 95:1950–1955

Gaude N, Nakamura Y, Scheible WR, Ohta H, Dormann P (2008) Phospholipase C5 (NPC5) is involved in galactolipid accumulation during phosphate limitation in leaves of Arabidopsis. Plant J 56:28–39

Heinz E (1977) Enzymatic reactions in galactolipid biosynthesis. In: Licthenthaler HK, Tevini M (eds) Lipids and lipid polymers. Springer, Berlin, pp 102–120
Jouhet J, Marechal E, Bligny R, Joyard J, Block MA (2003) Transient increase of phosphatidylcholine in plant cells in response to phosphate deprivation. FEBS Lett 544:63–68
Kates M (1955) Hydrolysis of lecithin by plant plastid enzymes. Can J Biochem Physiol 33:575–589
Kelly AA, Dormann P (2002) DGD2, an arabidopsis gene encoding a UDP-galactose-dependent digalactosyldiacylglycerol synthase is expressed during growth under phosphate-limiting conditions. J Biol Chem 277:1166–1173
Kelly AA, Froehlich JE, Dormann P (2003) Disruption of the two digalactosyldiacylglycerol synthase genes DGD1 and DGD2 in Arabidopsis reveals the existence of an additional enzyme of galactolipid synthesis. Plant Cell 15:2694–2706
Kobayashi K, Awai K, Takamiya K, Ohta H (2004) Arabidopsis type B monogalactosyldiacylglycerol synthase genes are expressed during pollen tube growth and induced by phosphate starvation. Plant Physiol 134:640–648
Kobayashi K, Masuda T, Takamiya K, Ohta H (2006) Membrane lipid alteration during phosphate starvation is regulated by phosphate signaling and auxin/cytokinin cross-talk. Plant J 47:238–248
Kocourkova D, Krckova Z, Pejchar P, Veselkova S, Valentova O, Wimalasekera R, Scherer GF, Martinec J (2011) The phosphatidylcholine-hydrolysing phospholipase C NPC4 plays a role in response of Arabidopsis roots to salt stress. J Exp Bot 62:3753–3763
Macfarlane MG, Knight BCJG (1941) The biochemistry of bacterial toxins I. The lecithinase activity of *Cl. welchii* toxins. Biochem J 35:884–902
May A, Spinka M, Kock M (2012) Arabidopsis thaliana PECP1: enzymatic characterization and structural organization of the first plant phosphoethanolamine/phosphocholine phosphatase. Biochim Biophys Acta 1824:319–325
Nakamura Y (2013) Phosphate starvation and membrane lipid remodeling in seed plants. Prog Lipid Res 52:43–50
Nakamura Y, Ohta H (2007) The diacylglycerol forming pathways differ among floral organs of Petunia hybrida. FEBS Lett 581:5475–5479
Nakamura Y, Awai K, Masuda T, Yoshioka Y, Takamiya K, Ohta H (2005) A novel phosphatidylcholine-hydrolyzing phospholipase C induced by phosphate starvation in Arabidopsis. J Biol Chem 280:7469–7476
Peters C, Li M, Narasimhan R, Roth M, Welti R, Wang X (2010) Nonspecific phospholipase C NPC4 promotes responses to abscisic acid and tolerance to hyperosmotic stress in Arabidopsis. Plant Cell 22:2642–2659
Pokotylo I, Pejchar P, Potocky M, Kocourkova D, Krckova Z, Ruelland E, Kravets V, Martinec J (2013) The plant non-specific phospholipase C gene family. Novel competitors in lipid signalling. Prog Lipid Res 52:62–79
Raghothama KG (1999) Phosphate acquisition. Annu Rev Plant Physiol Plant Mol Biol 50:665–693
Reddy VS, Rao DK, Rajasekharan R (2010) Functional characterization of lysophosphatidic acid phosphatase from Arabidopsis thaliana. Biochim Biophys Acta 1801:455–461
Rouet-Mayer MA, Valentova O, Simond-Cote E, Daussant J, Thevenot C (1995) Critical analysis of phospholipid hydrolyzing activities in ripening tomato fruits. Study by spectrofluorimetry and high-performance liquid chromatography. Lipids 30:739–746
Roughan PG (1970) Turnover of the glycerolipids of pumpkin leaves. The importance of phosphatidylcholine. Biochem J 117:1–8
Roughan PG, Slack CR (1982) Cellular organization of glycerolipid metabolism. Annu Rev Plant Physiol 33:97–132
Scherer GF, Paul RU, Holk A, Martinec J (2002) Down-regulation by elicitors of phosphatidylcholine-hydrolyzing phospholipase C and up-regulation of phospholipase A in plant cells. Biochem Biophys Res Commun 293:766–770

Strauss H, Leibovitz-Ben Gershon Z, Heller M (1976) Enzymatic hydrolysis of 1-monoacyl-SN-glycerol-3-phosphorylcholine (1-lysolecithin) by phospholipases from peanut seeds. Lipids 11:442–448

Summers PS, Weretilnyk EA (1993) Choline synthesis in spinach in relation to salt stress. Plant Physiol 103:1269–1276

Tasseva G, Richard L, Zachowski A (2004) Regulation of phosphatidylcholine biosynthesis under salt stress involves choline kinases in Arabidopsis thaliana. FEBS Lett 566:115–120

Titball RW (1993) Bacterial phospholipases C. Microbiol Rev 57:347–366

Wimalasekera R, Pejchar P, Holk A, Martinec J, Scherer GF (2010) Plant phosphatidylcholine-hydrolyzing phospholipases C NPC3 and NPC4 with roles in root development and brassinolide signaling in Arabidopsis thaliana. Mol Plant 3:610–625

Yang SF, Freer S, Benson AA (1967) Transphosphatidylation by phospholipase D. J Biol Chem 242:477–484

Phosphatidic Acid as Lipid Messenger and Growth Regulators in Plants

Xuemin Wang, Yuan Su, Yu Liu, Sang-Chul Kim, and Brian Fanella

Abstract Phosphatidic acid (PA) has emerged as a class of important growth mediators involved in various cellular and physiological processes. The cellular level of PA is highly dynamic, increasing transiently in response to hormones, abiotic cues, and biotic challenges. Several families of enzymes can contribute to the production of PA, and phospholipase D and phospholipase C/diacylglycerol kinases play a major role in the stimulus-induced generation of signaling PA. The identification and analyses of PA-interacting proteins provide insights into the cellular and physiological processes in which PA is involved and the mechanism of PA action. The PA–protein interactions help tether proteins to membranes and/or modulate enzymatic activities. In addition, PA has been implicated in modulating proteolytic cleavage and inter-organelle translocation of target proteins. Manipulations of the reactions involved in PA production and removal have provided evidence for the involvement of PA in plant growth, development, and stress responses.

Keywords Phosphatidic acid • Phospholipase D • Phospholipase C • Diacylglycerol kinase • Lipid signaling • Stress response

1 Introduction

Membrane lipids provide essential boundaries that separate the cell from the outside environment and form intracellular compartments. In addition to the well-known structural functions, membrane lipids have many other crucial regulatory functions (Wallis and Browse 2002; Wang 2004). Lipids are rich sources for the

X. Wang (✉) • Y. Su • Y. Liu • S.-C. Kim • B. Fanella
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

X. Wang (ed.), *Phospholipases in Plant Signaling*, Signaling and Communication in Plants 20, DOI 10.1007/978-3-642-42011-5_4,

production of hormones and secondary messengers, while polyphosphoinositides and oxylipins are extensively studied for their effects in diverse cellular and physiological processes (Munnik and Nielsen 2011; Erb et al. 2012). In recent years, phosphatidic acid (PA) has been identified as an important cellular mediator in regulating cell growth, development, and stress responses in plants, animals, and fungi (Wang et al. 2006; Li et al. 2009; Hong et al. 2010; Testerink and Munnik 2011; Shin and Loewen 2011; Kolesnikov et al. 2012; Jang et al. 2012; Peng and Frohman 2012).

PA is a minor class of membrane phospholipids with the simplest head group. PA occupies a central step in glycerolipid metabolism, and its regulatory function has only recently been appreciated. PA in plants is produced under a wide variety of stress conditions. Advances in the knowledge of phospholipases have facilitated the understanding of PA production and function. Analyses of PA-interacting proteins and their physiochemical properties have yielded mechanistic insights into how PA functions as a class of cellular mediators. Manipulations of PA production and catabolism have identified the involvement of PA in specific cellular and physiological processes.

2 PA Production and Measurements in Plants

The cellular level of PA in plant tissues is highly dynamic, increasing rapidly under various stress and growth conditions. Different approaches have been used to quantify PA levels in plants, but accurate measurements of stimulus-induced PA in plants remain challenging due to the rapid activation of lipolytic enzymes, especially phospholipase D (PLD), during tissue handling.

2.1 Cellular Dynamics of PA Levels

PA consists of two fatty acid chains esterified to a phosphoryl glycerol (Fig. 1). The cellular level of PA is estimated to range from 50 to 150 μM, constituting approximately 1 % of total phospholipids in *Arabidopsis* leaves (Welti et al. 2002; Zhang et al. 2004). However, the cellular PA can increase rapidly under various conditions, such as hormone treatments, wounding, freezing, drought, and pathogen elicitation (Wang et al. 2006). The magnitude of PA increase varies greatly, ranging from a few percentage points to several folds, depending upon the types of stress and tissues. For example, the amount of PA increased by approximately 50 % in *Arabidopsis* leaves treated with abscisic acid (ABA) (Zhang et al. 2004; Guo et al. 2012b), while the PA level was elevated by more than fivefold in *Arabidopsis* leaves during freezing (Welti et al. 2002). When *Arabidopsis* plants were sprayed with ABA, the increase in PA peaked at 10 min, but by 20 min the level of PA had returned to the pretreatment level (Zhang et al. 2004; Guo et al. 2012b). In castor

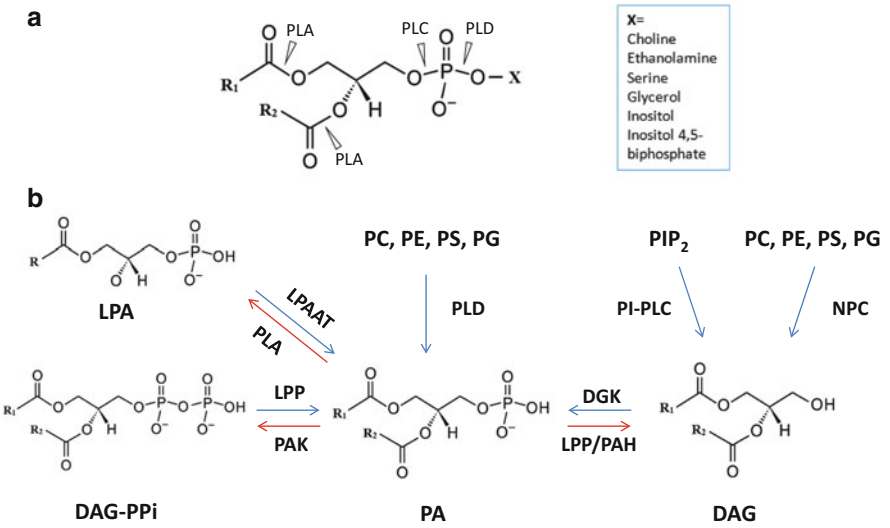

Fig. 1 PA structure, production, and metabolism. (**a**) Molecular structure of phospholipids and the hydrolysis sites of phospholipases. (**b**) Metabolic reactions and enzymes that produce and metabolize PA in the cell. The *blue arrows* indicate PA-producing pathways whereas the *red arrows* indicate PA-removing pathways. Enzymes are abbreviated as follows: *PLA* phospholipase A, *LPAAT* LPA acyltransferase, *PLD* phospholipase D, *PI-PLC* phosphoinositide-phospholipase C, *NPC* nonspecific PLC, *DGK* DAG kinase, *LPP* lipid phosphate phosphatase, *PAK* PA kinase, *PAH* PA phosphohydrolase. Metabolites are abbreviated as follows: *DAG* diacylglycerol, *DAG-PPi* DAG pyrophosphate, *LPA* lyso-PA, *PC* phosphatidylcholine, *PE* phosphatidylethanolamine, *PS* phosphatidylserine, *PG* phosphatidylglycerol, *PIP2* phosphatidylinositol 4,5-bisphosphate

bean leaves, wounding-induced PA production plateaued 10 min after wounding and then decreased (Ryu and Wang 1998). The transient changes indicate that PA is actively metabolized after its production.

PA is a class of phospholipids with different molecular species because the two acyl chains can differ in the number of carbons and double bonds. The major species in *Arabidopsis* tissues are 34:2 (mostly 18:2/16:0), 34:3 (mostly 18:3/16:0), 36:4 (mostly 18:2/18:2), and 36:5 (mostly 18:3/18:2), and the relative abundance varies depending on the tissue type (Welti et al. 2002; Devaiah et al. 2006). The different species may have different functions, because different proteins interact with PA species differently (Zhang et al. 2004; Guo et al. 2011). Different PA species also display various abilities to stimulate superoxide production (Sang et al. 2001). Likewise, the various relative abundance among tissues indicates possible tissue-specific function of different PA species that remains to be determined.

2.2 Measurements of PA

Different approaches have been used to quantify PA levels in plant tissues. A conventional method involves solvent extraction of total lipids, followed by thin layer chromatography (TLC) separation and quantification of PA based on the level of phosphate and/or fatty acids (Ryu and Wang 1996; Zien et al. 2001). To assess new PA produced in response to a stimulus, membrane lipids may be prelabeled with radioisotopes (e.g., ^{32}Pi or ^{3}H-fatty acids) or a fluorescent lipid, such as NBD-PC, followed by quantification of radioactive or fluorescent PA produced after treatments (Arisz and Munnik 2013; Zhang et al. 2004; Fig. 2b). In recent years, mass spectrometry has been used increasingly to detect and measure PA. In particular, lipid profiling using electrospray ionization tandem mass spectrometry (ESI-MS/MS) has proven to be sensitive, quantitative, and efficient (Welti et al. 2002). One major advantage of the mass spectrometry-based method is that it allows the quantification of PA molecular species (Fig. 2a). To detect PA changes in living cells, the use of intracellular PA reporters or sensors is being explored. In this method, a PA-binding protein motif is fused to a fluorescent protein. The fluorescently labeled, PA-binding proteins are used to image PA dynamics in living cells (Vermeer and Munnik 2013). One motif in the PA-binding region of Raf-1 was sufficient to target green fluorescent protein to membranes (Rizzo et al. 2000).

The rapid activation of lipolytic enzymes, specifically PLD, during tissue handling has made accurate measurements of cellular PA a challenge. Precaution must be taken to minimize the PLD activation during tissue handling. One effective approach is to submerge plant tissues quickly in heated isopropanol (75 °C) to inactivate nonspecific lipolytic activities (Welti et al. 2002).

3 Cellular Production and Removal of Signaling PA

Cellular PA may be produced by multiple enzymes: (1) PLD, (2) phospholipase C (PLC) coupled with diacylglycerol (DAG) kinase (DGK), (3) lysoPA acyl transferase (LPAAT), and (4) lipid phosphate phosphatases (LPP) that dephosphorylate DAG pyrophosphates to PA (Fig. 1b). PLD and PLC/DGK are the two major routes that produce signaling PA. Multiple enzymes are also involved in the PA removal. Each class of these enzymes is comprised of multiple forms. The activation and regulation of these enzymes play important roles in the location and timing of signaling PA production.

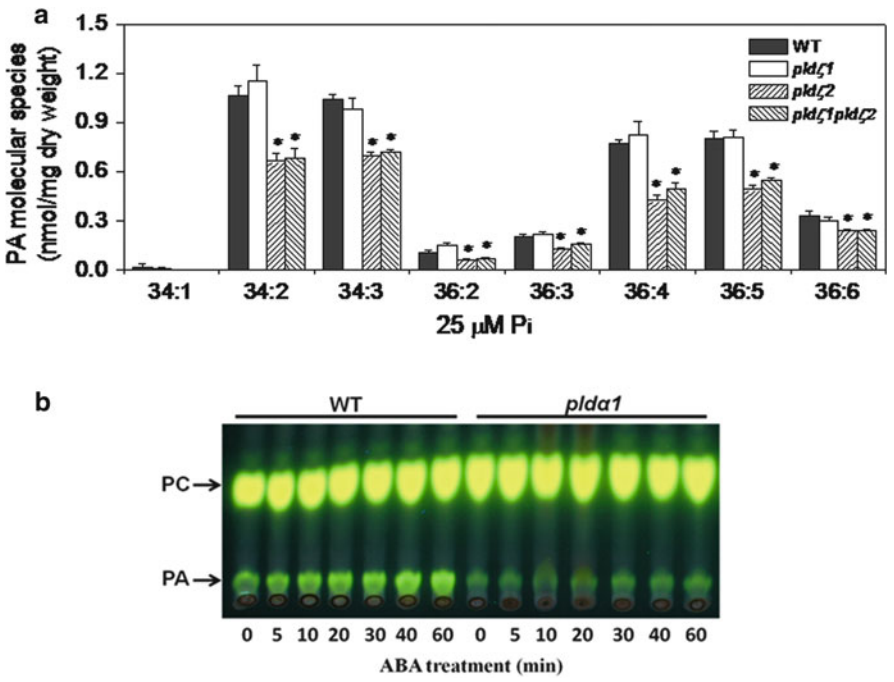

Fig. 2 Molecular species of PA measured by different methods. (**a**) PA molecular species were analyzed by ESI-MS/MS in roots of wild type, *pldζ1*, *pldζ2*, and *pldζ1 pldζ2* under Pi deprivation condition. Values are means ± se (n=5). The *stars* indicate that mutant and wild-type values are significantly different ($P < 0.05$; modified from Li et al. 2006). (**b**) Representative image of fluorescent-based assay of PLD activity using NBD-PC-labeled protoplasts of wild type and *pldα1* treated with 50 μM ABA over different times. PA was identified by TLC (modified from Guo et al. 2012a, b)

3.1 The PLD Multi-gene Family

The activation of PLD produces PA directly through hydrolysis of common membrane lipids, such as PC and PE. PLD is a family of multiple enzymes that is subject to complex control, as described in chapter "PLD: Phospholipase Ds in Plant Signaling". The different subcellular locales, expression patterns, and substrate selectivities of PLDs play critical roles in regulating the spatial and temporal production of PA and its molecular species.

3.2 Two Distinctively Different PLC Families

The activity of PLC can contribute to PA production by generating DAG that is phosphorylated by DGK to PA. Plants have two distinct families of PLCs,

phosphoinositide-hydrolyzing PLC (PI-PLC, described in chapter "PI-PLC: Phosphoinositide-Phospholipase C in Plant Signaling") and *n*onspecific *PLC* (NPC, in chapter "NPC: Nonspecific Phospholipase Cs in Plant Functions") that hydrolyze common membrane phospholipids, such as PC. Both families of PLCs have been reported to produce DAG as a substrate for DGK phosphorylation to PA, but the exact contribution remains to be determined.

Worth noting is that in animal cells, DAG itself is a potent messenger, activating certain members of the protein kinase C (PKC) family and other proteins, including G-proteins and DGKs (Colón-González and Kazanietz 2006; Pu et al. 2009). DAG modulates protein functions primarily via binding to the C1 domain, along with PE in a phospholipid and zinc-dependent fashion. The C1 domain is cysteine-rich, about 50 amino-acid residues long, and this lipid–C1 interaction is critical for governing protein association to membranes and often leads to enzyme activation (Pu et al. 2009). In plants however, no canonical PKCs have been documented. Plants lack highly conserved C1 domains, but C1-like, divergent C1 (DC1) domains exist in some proteins. It remains to be examined whether DC1s or other domains interact with DAG in plants.

3.3 The DGK Family

Multiple DGKs have been grouped into three subgroups DGKI, II, and III in plants (Arisz et al. 2009; Shulga et al. 2011; Gómez-Merino et al. 2004; Liu et al. 2013). In addition to PI-PLC and NPC, DGK may use DAG resulting from activation of other enzymes. For example, 18:3/16:3-PA produced during low temperature stress in *Arabidopsis* is likely to be derived from galactolipids via 18:3/16:3-DAG and DGK, as the 18:3/16:3 species is abundant in galactolipids and nearly undetectable in phospholipids (Welti et al. 2002).

3.4 Enzymes Involved in PA Removal

As signaling messengers, PA is expected to be removed after its function, and the transient increase of PA in plants supports this notion. The removal of PA can be accomplished by several enzymes: (1) lipid phosphate phosphatase (LPP) and PA hydrolase (PAH) that dephosphorylates PA to produce DAG; (2) PA kinase that phosphorylates PA to form DAG pyrophosphate; (3) PA-selective A type phospholipases that deacylate PA to produce lysoPA and free fatty acids (FFAs) (Fig. 1b). In addition to attenuating PA function, these enzyme activities can also generate new lipid messengers such as DAG, FFAs, DAG pyrophosphate, and lysoPA (Wang et al. 2006).

4 Mechanisms of PA Action as Cellular Mediators

Recent results have begun to shed light on how PA acts as a messenger in the cells. One main mode of PA action is its direct interaction with proteins, and its unique physicochemical properties distinguish PA from other membrane lipids in its ability to interact with proteins. The PA–protein interaction may modulate the catalytic activity of target proteins, tether proteins to the membrane, or promote the formation and/or stability of protein complexes. In addition, PA, as a hexagonal type II lipid, can also alter membrane structure, which can then affect membrane–protein interactions and membrane budding and fusion.

4.1 PA Interactions with Proteins

PA has been shown to bind a number of proteins (Table 1). Different approaches have been used to identify and characterize PA–protein interactions, including nitrocellulose filter binding (commonly referred to as lipid strips or fat-blotting) (Zhang et al. 2004), liposomal binding (Guo et al. 2011), and PA immobilized to beads or membranes followed by mass spectrometry (Manifava et al. 2001; Testerink et al. 2004). In addition to the qualitative approaches, the PA interaction has been quantitatively characterized using surface plasmon resonance (SPR), isothermal titration calorimetry (ITC), and other approaches (Mishra et al. 2006; Guo et al. 2011; Wang and Benning 2012; Kim et al. 2013). Many PA-interacting proteins exhibit binding specificity to PA but not to other membrane phospholipid classes such as PC, PE, phosphatidylglycerol (PG), phosphatidylinositol (PI), or phosphatidylserine (PS). Different PA molecular species have been shown to differ in their affinity or ability to bind different proteins (Guo et al. 2011).

The protein motifs that bind PA are not highly conserved. Some phox homology (PX) domains also bind PA (Karathanassis et al. 2002), but consensus sequences for binding specifically to PA have yet to be defined. In general, basic, positively charged amino acids, such as arginine and lysine, are involved in the binding to PA (Fig. 3a). For instance, the protein kinase Raf-1 that binds to PA requires a 35 amino acid motif (Rizzo et al. 2000). A similar PA binding motif is found in the protein phosphatase 2C abscisic acid insensitive 1 (ABI1) and protein kinase constitutive triple response 1 (CTR1) in plants (Zhang et al. 2004; Wang et al. 2006; Testerink et al. 2007). The motif contains polybasic amino acid residues followed by a stretch of hydrophobic amino acids, suggesting that a specific structural fold, rather than a simple electrostatic interaction, is required for a PA-effector protein interaction. An “electrostatic/hydrophobic switch” has been suggested as a mechanism for PA interaction with some proteins, such as ABI1 and Raf1. In this model, the PA–protein interaction is initiated by electrostatic attraction followed by hydrophobic partitioning of the hydrophobic residues of the PA-binding region to membranes (Wang et al. 2006).

Table 1 PA binding proteins in plants

PA-binding proteins	PA effect on proteins	Protein functions	Reference
14-3-3 protein	Inhibition	Protein interaction, activation of membrane H^+-ATPase	Camoni et al. (2012)
ABI1	Translocation	Protein phosphatase 2 family, ABA signaling	Zhang et al. (2004)
ACBP1	Binding	Acyl-CoA-binding protein	Du et al. (2013)
AGD7	Activation	Arf GTPase activation protein	Min et al. (2007)
AtCHC1/ AtCHC2	Binding	Clathrin heavy chain, coated vesicle formation	McLoughlin et al. (2013)
AtPDK1	Activation	Phospholipid-binding 3-phosphoinositide-dependent protein kinase, pathogen response	Anthony et al. (2004)
GAPC1/GAPC2	Cleavage	Cytosolic glycerolaldehyde dehydrogenase in glycolysis	Kim et al. (2013), McLoughlin et al. (2013)
CdeT11-24	Binding	The late embryogenesis abundant-like protein	Petersen et al. (2012)
CP	Inhibition	Heterodimeric capping protein, F-actin binding and inhibition	Huang et al. (2006)
CTR1	Inhibition	Raf-like protein kinase	Testerink et al. (2007)
MAP65-1	Activation	Microtubule-associated protein	Zhang et al. (2012)
MGD1	Activation	MGDG synthase	Dubots et al. (2010)
MPK6	Activation	Mitogen-activated protein kinase phosphorylates SOS1 in salt response	Yu et al. (2010)
PEPC	Inhibition	Phosphoenolpyruvate carboxylase	Testerink et al. (2004)
PIPKIγ	Activation	PI4P-5-kinase produces PI(4,5)P2	Roach et al. (2012)
PP2AA1	Binding	PP2A scaffolding subunit A1 regulates PP2A-mediated PIN1 dephosphorylation	Gao et al. (2013)
RbohD/F	Activation	NADPH oxidases	Zhang et al. (2009)
SnRK2.10/ SnRK2.4	Translocation	The sucrose nonfermenting-1-related protein kinases	McLoughlin et al. (2012)
SPHK1/SPHK2	Activation	Phytosphingosine kinase, ABA signaling	Guo et al. (2011)
TaPEAMT1/ TaPEAMT2	Inhibition	Methylation of phosphoethanolamine, PC production	Jost et al. (2009)
TGD2	Binding	PA transportation to chloroplast	Awai et al. (2006)
TGD4	Binding	Lipid trafficking	Wang et al. (2012)
ZmCPK11	Activation, stimulate transcription	Calcium-dependent protein kinase, plant wounding response	Klimecka et al. (2011)

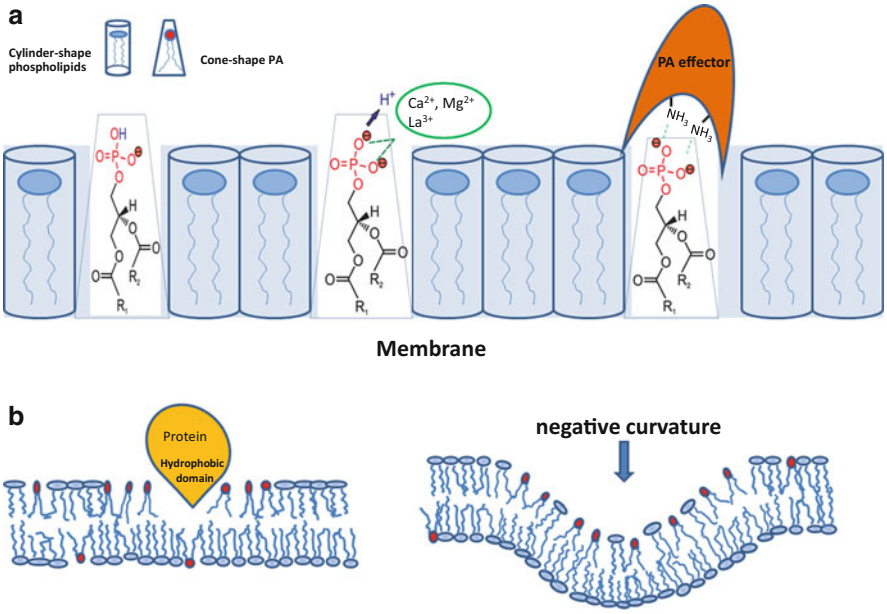

Fig. 3 Proposed model for PA interaction with proteins and structural effect on membranes. (**a**) Dual negative charges of PA due to loss of one or two protons. The negative one and two charges are influenced by cellular pH and the presence of cations. The electrostatic and hydrogen bonding switch facilitates PA interaction with basic amino acid residues on PA-effector proteins. In comparison to PC, the cone-shaped PA has a smaller head group and is more stable in the negative curvature. (**b**) PA-mediated alterations of membrane structures. The local distortion of membrane structure promotes protein association with membranes (*left*) and lead to negative membrane curvature (*right*), potentially involved in membrane budding and fusion in vesicular trafficking. The *red head* molecules represent PA, whereas the *blue head* molecules are other phospholipids

Recent studies of PA structure and behavior have led to the proposition of an "electrostatic/hydrogen bond switch mechanism" for PA interaction with effector proteins (Kooijman et al. 2007; Fig. 3a). In this model, a PA-binding protein first binds to a membrane region with negative charges via electrostatic interactions. Then, the protein swings to the bilayer until it encounters a protonated PA which is −1 charged. A hydrogen bond will be formed between the side chain of the basic amino acids of the protein and the phosphomonoester head group of PA, which dissociates its remaining proton, switching the charge of PA from −1 to −2. This interaction leads to stronger electrostatic attraction between PA and proteins (Kooijman et al. 2007; Shin and Loewen 2011; Fig. 3a). Besides hydrogen bonding with proteins, the deprotonation status of phosphomonoester head group of PA can be induced by bivalent cations, such as Ca^{2+} or Mg^{2+} (Fig. 3a) (Kooijman et al. 2007). The electrostatic/hydrogen bond switch mechanism may explain the specificity of effector proteins for PA over other anionic membrane phospholipids, such as PS, with a maximum charge of −1. Overall, the electrostatic/hydrogen bond and availability of the hydrophobic area around the PA-binding region in a protein

may determine the specificity of PA-protein binding (Wang et al. 2006; Shin and Loewen 2011).

The ability of PA to change its negative charge numbers has also been proposed as a base for PA to act as a pH sensor in yeast cells. The charge number on PA's head group is different from any other phospholipid and can change under physiological pH (Shin and Loewen 2011). Such changes may render PA itself "active" or "inactive" under different environmental conditions. PA contains a phosphomonoester head group and thus has a second pK_a within the physiological range (6.9–7.9; Kooijman et al. 2005). PA can be more or less deprotonated when the intracellular pH (pH_i) goes up or down, respectively. For example, Opi1p, a transcriptional suppressor for phospholipid biosynthesis, displays a higher affinity with deprotonated PA compared with protonated PA, and the binding is pH dependent (Young et al. 2010). The lower pH_i, caused by glucose starvation, alleviates PA-Opi1p binding. The released Opi1p enters the nucleus where it suppresses the expression of genes for phospholipid biosynthesis. Hence, PA acts as a pH_i sensor that connects glucose metabolism to membrane lipid biogenesis (Loewen 2012; Young et al. 2010).

4.2 PA Tethering Proteins to the Membrane

One major effect of the PA–protein interaction is the tethering of proteins to the membrane, thus modulating the protein's intracellular location and protein–protein association. Signal transduction, vesicular trafficking, and many other critical cellular functions require targeting proteins to specific locations. The membrane tethering by PA helps to direct proteins to a membrane and/or to a specific membrane region. The cellular levels of PA also support the hypothesis that membrane PA binds effector proteins. The basal level of PA in plant cells (50–150 μM) (Zhang et al. 2004) is considerably above a phospholipid's critical micelle concentration in the sub-nanomolar range. Thus, the stimulus-induced increase in PA should not affect the monomeric PA concentration because the concentration of PA monomer in solution is constant when the level of PA is above its critical micelle concentration. Therefore, the membrane is likely the primary site for PA signaling and interaction with target proteins (Wang et al. 2006).

In the PA-ABI1 interaction in ABA signaling, the PA binding tethers ABI1 to the plasma membrane and decreases its translocation to the nucleus (Zhang et al. 2004). Under salt stress, PA binds a cytosolic glyceraldehyde dehydrogenase and recruits it to the membrane (McLoughlin et al. 2013). In response to auxin, PA stimulates the membrane accumulation of protein phosphatase 2A (PP2A) and enhances the PP2A activity at the membrane (Gao et al. 2013). In *Saccharomyces cerevisiae*, increased PA tethers the transcriptional repressor Opi1p to the endoplasmic reticulum, thus keeping it from functioning in the nucleus (Loewen et al. 2004). The PA binding to Raf1 and sphingosine kinases regulates their specific functions associated with protein interactions and membranes (Rizzo et al. 2000;

Delon et al. 2004). PA binds to a cytoplasmic region of the integral membrane protein NADPH oxidase to promote ROS production in plants (Zhang et al. 2009). Thus, besides regulating the intracellular translocation of soluble proteins to the membranes and protein assembly on the membrane, the PA tethering of proteins can also modulate the membrane association of cytosolic regions of integral membrane proteins.

4.3 PA Modulation of Enzymatic Activities

In addition to membrane tethering, PA–protein interactions modulate the catalytic activity of the protein. PA may inhibit or stimulate the protein catalytic activity, depending on the target protein. PA promotes the phytosphingosine kinase activity by enhancing its binding to its substrate (Guo et al. 2011). PA also stimulates NADPH oxidase activity, whereas it inhibits the activity of ABI1 phosphatase and protein phosphatase 1 (PP1) (Jones et al. 2005). PP1 is involved in promoting blue light-induced stomatal opening. In the ABA signaling cascade, ABI1 is a negative effector, but NADPH oxidase and phytosphingosine kinase are positive effectors (Zhang et al. 2004, 2009; Guo et al. 2011). Thus, the differential modulation of enzyme activities by PA could be an important cellular mechanism for coordinated regulation of a specific cellular process that involves a number of proteins. PA-effector protein interaction has also been proposed to act as a coordinator in promoting protein phosphorylation in specific cellular responses (Wang et al. 2006).

4.4 PA Alteration of Membrane Structures

One of PA's effects on protein interaction and cellular processes may result from its structural effect on cellular membranes. PA, with its small polar head group and two relatively bulky acyl tails, is a hexagonal type II, cone-shape anionic lipid (Fig. 3a) (van den Brink-van der Laan et al. 2004; Kooijman et al. 2003). The cone shape of PA can prevent tight packing with head groups of neighboring phospholipids, resulting in a loosened structure around PA and exposure of the hydrophobic zone to the effector proteins (Fig. 3b, *left*). Most PAs in *Arabidopsis* contain two unsaturated acyl chains (Wang et al. 2006), which expand the hydrophobic region in the planar bilayer compared with phospholipids containing two saturated chains. Thus, the aggregation of cone-shaped PA in the bilayer confers a unique platform that attracts proteins enriched with basic amino acid motifs and hydrophobic domains (Fig. 3b, *left*) (Roth 2008). The hydrophobic region of proteins can insert into the bilayer and bind to PA tightly. In addition, the accumulation of the cone-shaped PA at a certain region in the lipid bilayer induces negative curvature, which is usually found at the neck of a vesicle during the process of fusing to an acceptor

or budding from a donor membrane (Fig. 3b, *right*) (Roth 2008). Thus, through its structural effect, PA may affect membrane–protein interactions and membrane budding and fusion (Fig. 3b).

5 PA Effects on Cellular and Metabolic Processes

The analyses of PA interaction with proteins have provided valuable insights into the specific cellular processes (Table 1). PA binds to various proteins including those involved in cytoskeletal organization, vesicular trafficking, regulatory cascades such as transcriptional factors/regulators, protein kinases, and protein phosphatases, metabolic catalysis, and transport. Here we will highlight the role of PA in cytoskeleton organization, vesicular trafficking, lipid transport, and metabolism.

5.1 Cytoskeletal Rearrangements

Manipulating PA levels in the cell has led to alterations of cytoskeletal networks in plants. An increase in PA levels is generally associated with an increase in the density of actin filament arrays, possibly via actin filament polymerization, while a decrease in PA levels is associated with disassembly of actin filaments (Li et al. 2012). PA binds to the heterodimeric capping protein (CP) and inhibits the end-capping activity of CP (Huang et al. 2006). CP inhibits filament–filament annealing and filament elongation, reducing actin cytoskeletal dynamics (Huang et al. 2006; Pleskot et al. 2012). Thus, the effect of PA inhibition of CP is promotion of actin reorganization (Li et al. 2012). CP has been proposed as a PA biosensor that transduces cues from cell membranes into changes in actin cytoskeleton dynamics (Li et al. 2012). In addition, PA enhances the binding of the microtubule-associated protein, MAP65-1, to microtubules (Zhang et al. 2012). The PA-MAP65-1 binding promotes microtubule polymerization and bundling. Thus, PA is involved in both actin and microtubule cytoskeletal dynamics in plants. Proteomic studies identified cytoskeletal proteins annexin and tubulin as PA-binding protein candidates, further supporting the possible regulatory function of PA associated with cytoskeletons (Testerink et al. 2004; Kim et al. 2013).

5.2 Vesicular Trafficking

PA in plants has also been implicated in vesicular trafficking. PA interacts with AGD7, an ADP ribosylation factor (ARF) GTPase-activating protein (GAP) homolog in *Arabidopsis*, and promotes the hydrolysis of GTP bound to ARFs to GDP (Min et al. 2007, 2013). ARF GAPs regulate ARFs by converting the active

GTP-bound forms of these proteins into their inactive GDP-bound forms. ARF is known to play important roles in vesicular trafficking in animal and plant cells. In animal cells ARF binds to and activates PLD to produce PA at specific regions of a given membrane (Liu et al. 2010). The increase in PA accelerates negative curvature of the membrane (Roth 2008). PA recruits and activates proteins important for vesicle formation to the appropriate site in the membrane. PA and its binding partners facilitate vesicle formation in the vesicle budding and fusion processes that are critical for endocytosis and exocytosis. In plants, whether ARF interacts with and activates PLD is not yet determined. PLD and PA have been suggested to regulate auxin polar transportation through the cycling of the auxin efflux carrier PIN-FORMED2 (PIN2) (Li and Xue 2007).

5.3 Lipid Transport and Membrane Biogenesis

Fatty acids in plants are synthesized exclusively in the plastid and then exported to the ER for the synthesis of glycerolipids. Some of the glycerolipids are transported back to the plastid where they are used for the synthesis of galactolipids and sulfolipids, the abundant and essential components of photosynthetic membranes (Wang and Benning 2012). Recent studies indicate that lipids from the ER are transported into the plastids in the form of PA (Wang et al. 2012). The PA mediation of the interorganelle transport of lipids raises an intriguing question of whether PA regulates lipid production and photosynthetic membrane biogenesis. PA is a central intermediate in glycerolipid biosynthesis and a key cellular mediator. PA and PG increase the activity of chloroplast monogalactosyldiacylglycerol synthase (MGD1) that synthesizes MGDG (Dubots et al. 2010). In yeast, PA has been documented to mediate the transcriptional regulation of phospholipid synthesis. PA binds to the transcriptional repressor Opi1p, serving as part of a lipid sensor complex in the ER (Young et al. 2010). The enriched PA in the ER keeps Opi1p out of the nucleus, leading to the increase in the transcription of genes encoding phospholipid-metabolizing enzymes. When the PA level at the ER membrane decreases, Opi1p translocates into the nucleus, where it represses a transcriptional activator complex and, consequently, represses the expression of genes for phospholipid metabolism (Loewen et al. 2004; Young et al. 2010). The data suggest that PA plays a key role in membrane lipid synthesis and biogenesis.

5.4 Modulating Cellular Metabolism

In addition to lipid metabolism, PA has been implicated in regulating central carbohydrate metabolism. PA interacts with C_4 phosphoenolpyruvate carboxylase (PEPC) and cytosolic glyceraldehyde dehydrogenases (GAPC) (Monreal et al. 2010; Kim et al. 2013; McLoughlin et al. 2013). PA inhibits the activity of

C_4 PEPC, which catalyzes carboxylation of phosphoenolpyruvate to oxaloacetate, a reaction for the initial fixation of CO_2 in photosynthesis in C_4 plants. GAPC catalyzes a glycolytic, NAD-dependent conversion of glyceraldhyde-3-Pi (G3P) to 1,3-bisphosphoglycerate. Glycolysis breaks down glucose and provides a central link to energy production and cellular metabolism, such as the synthesis of fatty acids and amino acids. PA does not affect GAPC activity but promotes GAPC cleavage (Kim et al. 2013). GAPC is found in the cytosol and nucleus, and its movement to the nucleus is promoted by oxidative stress. In response to salt stress, PA interacts with GAPC and recruits it to the membrane (McLoughlin et al. 2013). In mammalian cells, GAPDH, besides being a metabolic enzyme, has many regulatory functions including gene transcription, DNA replication, nuclear tRNA export, and DNA repair (Sirover 1999; Nicholls et al. 2012). Thus, PA interaction with metabolic enzymes may function as a key modulator coordinating cellular metabolism. In addition, the lipid–protein interactions may play a role in the conversion of some of the "classic" metabolic enzymes into regulatory proteins.

6 PA Involvements in Hormonal and Physiological Processes

Genetic manipulations of genes encoding enzymes for PA production and removal have been particularly informative in understanding the physiological functions of PA. The function of PLD, PLC, and NPC has been described elsewhere in the volume. Here we describe briefly the role of PA in plant response to selected hormones, drought, salinity, nitrogen availability, phosphate deficiency, oxidative stress, and microbial interaction, as well as seed germination, pollen tube growth, root growth, and root hair patterning (Fig. 4).

6.1 PA in ABA Signaling, Transpirational Water Loss, and Seed Germination

Plant hormones play important roles in growth, development, and stress responses. ABA is a stress hormone well documented for its effect on promoting stomatal closure, and PA can mimic the effect of ABA on stomatal movement (Ritchie and Gilroy 1998; Zhang et al. 2004; Mishra et al. 2006). PA interacts with and inhibits the protein phosphatase 2C ABI1, a negative effector in ABA response (Zhang et al. 2004). PA binds to NADPH oxidase to promote the production of the reactive oxygen species such as H_2O_2 (Zhang et al. 2009). PA also promotes nitric oxide (NO) production. NO and H_2O_2 are positive mediators in ABA responses (Yan et al. 2007; Cho et al. 2009). PA binds to SPHKs and stimulates their activity to produce phytosphingosine-phosphate and stimulate stomata closure (Guo

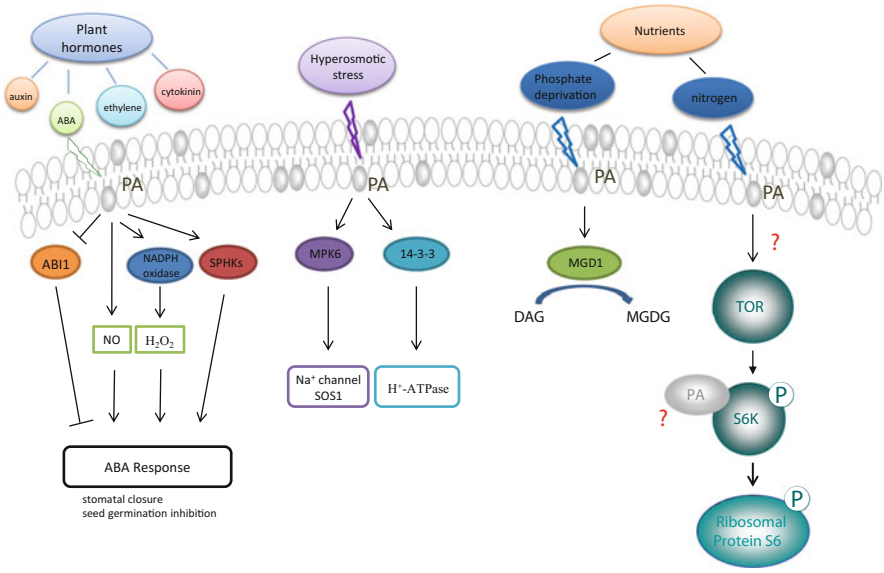

Fig. 4 The roles of PA in the various physiological processes. PA functions as a mediator in response to different plant hormones. Particularly in ABA response, PA interacts with ABI1, NADPH oxidase, and SPHKs to regulate ABA effectors. Under the hyperosmotic stress, PA binds to and stimulates MPK6 and 14-3-3 proteins to regulate Na^+ channel SOS1 and plasma membrane H^+-ATPase, respectively. When phosphate is deficient, PA production is induced as the precursor for DAG synthesis. Also, PA binds to and promotes MGD1 to enhance MGDG production. In the case of nitrogen supply, PA stimulates the nitrogen uptake; however, the underlying mechanism is not clear. The TOR-S6K pathway could be a candidate as the pathway in animals has the similar functions

et al. 2011, 2012b; Guo and Wang 2012). In addition, PA inhibits blue light-induced stomatal opening via inhibition of protein phosphatase 1 (Takemiya and Shimazaki 2010). Thus, PA closes stomata by promoting the closure of open stomata and also inhibiting the opening of closed stomata.

Terrestrial plants lose most of their water via transpiration through open stomata. The effect of PA on decreasing stomatal aperture plays an important role in decreasing water loss. Suppression of enzymes PLDα1, PLDδ, or both and thus PA production have been shown to increase transpirational water loss using whole plants or detached leaves, whereas overexpression of PLDα1 decreased transpirational water loss (Sang et al. 2001; Guo et al. 2012a, b; Hong et al. 2008). A recent study using guard cell-specific expression of PLDα1 demonstrated decreased water loss and increased canola growth and yield under drought (Lu et al. 2013).

Another well-documented effect of ABA is its suppression of seed germination. PA can mimic the effects of ABA, including inhibition of α-amylase production in aleurone cells and suppression of seed germination. Knockout mutation of PA-producing PLDα1 and PLDδ renders seeds less sensitive to ABA inhibition of seed germination (Uraji et al. 2012). However, mutation of PA-removing

enzymes lipid phosphate phosphatases (LPP2), which catalyze the conversion of PA to DAG, renders seeds hypersensitive to ABA (Katagiri et al. 2005). The *ABA-insensitive 4* (*ABI4*), which encodes an AP2-type transcription factor, is epistatic to LPP2, suggesting that LPP2 and PA removal act as negative regulators upstream of ABI4 in ABA signaling during germination. On the other hand, PA and also PC have been found to interact with ACBP1, one of the six acyl-CoA-binding proteins (ACBPs) (Du et al. 2013). In addition, ACBP1 interacts directly with PLDα1. *Arabidopsis* ACBP1-over-expressors showed increased sensitivity to ABA during germination and seedling development, whereas the *acbp1* mutant showed decreased ABA sensitivity during these processes. Similarly, *Arabidopsis npc4* displayed reduced sensitivity to ABA and this was fully restored by exogenous PA application, implying the involvement of PLC/DGK pathway in ABA signaling (Peters et al. 2010). These results indicate that the production and removal of PA play important roles in regulating seed germination and seedling development.

6.2 PA in Ethylene and Auxin Signaling

Ethylene promotes senescence and an early study showed that suppression of PLDα1 expression delayed ethylene- and also ABA-promoted senescence in detached leaves, suggesting that PLDα1 and its product PA mediate leaf's response to ABA and ethylene (Fan et al. 1997). PA has been shown to bind to and repress constitutive triple response 1 (CTR1), a protein kinase that negatively regulates plant responses to ethylene (Testerink et al. 2007). It has been proposed that stress-induced PA formation may switch on downstream ethylene responses via interaction with CTR1 (Testerink et al. 2008).

PA and its producing enzyme PLDζ2 have been reported to modulate the cycling of the auxin efflux carrier PIN-FORMED2 (PIN2) and auxin polar movement (Li and Xue 2007). A recent study showed that PA binds to the scaffolding A1 subunit of PP2A, recruits it to the membrane, enhances PP2A-mediated PIN1 dephosphorylation, and regulates the polar localization of PIN1 protein in *Arabidopsis* (Gao et al. 2013).

6.3 Plant Response to High Salinity

Increases in PA have been reported under salt stresses in different plants using different approaches. Genetic ablation of several PA-producing enzymes, including PLDα1, PLDα3, PLDδ, or PLDε, compromises *Arabidopsis* seedling growth under high salinity (Bargmann et al. 2009; Zhang et al. 2012), while plants overexpressing PLDα3 or PLDε show salt tolerance (Hong et al. 2008, 2010). High salinity results in hyperosmotic stress and ionic toxicity. PA has been reported to modulate the activity of ion channels/transporters on the membranes that remove excess ions

such as Na^+. PA binds to and stimulates the MAP kinase MPK6, which regulates the Na^+ channel SOS1 (Yu et al. 2010). Recently, it is reported that PA binds to the sucrose non-fermenting-1-related protein kinase 2 (SnRK2), SnRK2.4 and SnRK2.10 (McLoughlin et al. 2012). Binding of PA to 14-3-3 proteins hampers their ability to activate the plant plasma membrane H^+-ATPase (Camoni et al. 2012). The lysine-rich motif of the intrinsically disordered stress protein CDeT11-24 from *Craterostigma plantagineum* is responsible for PA binding and protection of enzymes from damaging effects caused by desiccation (Peterson et al. 2012).

6.4 Plant Response to Nutrient Availability

Phosphate (Pi) is an essential macronutrient for plants, and membrane phospholipids contain approximately one quarter of total phosphate in the cell. Under phosphate deprivation, a portion of membrane phospholipids is hydrolyzed whereas the relative content of galactolipids and sulfolipids is increased. PA has both metabolic and signaling roles in plant response to Pi deficiency. The activation of PLDs, particularly PLDζ2, under Pi deprivation hydrolyzes PC to PA, which can be further dephosphorylated to provide DAG for MGDG synthesis (Li et al. 2006). Meanwhile, PA binds to and stimulates MGD1 activity for the synthesis of MGDG and subsequently DGDG. On the other hand, PA inhibits phosphoethanolamine N-methyltransferases that provide phosphocholine for PC production (Jost et al. 2009). These effects of PA can contribute to the decrease in PC and increase in DGDG under Pi limitation.

PA is involved in plant response to nitrogen availability (Hong et al. 2009). The mechanism by which PA enhances plants' response to nitrogen is unclear but may share some similarities with mammalian mTOR, a serine/threonine kinase that integrates a wide range of signals such as nutrient, growth factors, and mitogen stimuli (Foster 2007). PA competes with rapamycin to bind the FKBP12-rapamycin binding (FRB) domain of mTOR (Fang et al. 2001). The PA binding stabilizes mTOR complex formation and also activates the mTOR kinase activity (Foster 2009). Plants have the TOR kinase but the components in the TOR signaling complex are not well defined. In addition, the inhibition of plant TOR requires much higher concentrations of rapamycin (Xiong and Sheen 2012). It would be of interest to determine if PA modulates TOR function in regulating plant response to nutrient availability.

6.5 Wounding and Freezing Responses

PA levels were found to be upregulated rapidly after plant wounding (Ryu and Wang 1996). A recent study in maize identifies that PA binds and regulates a

wound-inducible, calcium-dependent protein kinase (CPDK), ZmCPK11 (Klimecka et al. 2011). PA increases ZmCPK11 activity and also upregulates the transcript level of ZmCPK11.

During cold acclimation, both PLD and PLC/DGK are involved in the PA production (Ruelland et al. 2002; Welti et al. 2002), but the function of PA accumulation in the process is unknown. The PA increase in cold acclimation was modest when comparing to freezing under which large increases in PA occurred (Welti et al. 2002; Li et al. 2008). PLDα1 contributed most PC hydrolysis to PA during freezing, and the increased PA to PC ratio has been proposed to result in structural change and the potential disruption of the plasma membrane (Welti et al. 2002).

6.6 Plant–Microbial Interactions

When exposed to microbial pathogens, rapid PA production occurs in plants. The source of PA may come from the reaction of PLD and/or PLC/DGK. In rice, PLDα1-like PLDs distribute differently on the cell membrane in resistant and sensitive interactions with bacteria pathogen *Xanthomonas oryzae* (Young et al. 1996). PLC/DGK derived PA accumulates in tomato during *C. fulvum* invasion (de Jong et al. 2004). Suppression of *LePLDβ1* resulted in a strong decrease in xylanase-induced PLD activity and a disproportionate oxidative burst (Bargmann et al. 2009). Infection of *PLDβ1*-deficient plants by *Pseudomonas syringae* pv. DC3000 resulted in less bacterial growth than in WT plants, and the effect was more profound in virulent *Pst* DC3000 than avirulent *Pst* DC3000 (*avrRpt2*) infection. 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. PLDβ1 is responsible for the majority of increased PA in response to necrotrophic *B. cinerea* and virulent *Pst* DC3000 infection but contributes less to the avirulent *Pst* DC3000 (*avrRpt2*)-induced PA production (Zhao et al. 2013).

6.7 Pollen Tube and Root Hair Growth

PA also regulates cell expansion in root hairs and pollen tubes (Anthony et al. 2004; Hong et al. 2009; Pleskot et al. 2013). PA has been shown to interact with phosphoinositide-dependent protein kinase 1 (PDK1), which activates AGC2-1 kinase to promote root hair growth in *Arabidopsis* (Anthony et al. 2004). It has been reported that PA promotes pollen tube elongation and germination partially by modulating microtubule integrity in tobacco (Potocky et al. 2003). *Arabidopsis*

pollen tube also requires PA for the maintenance of Ca^{2+} gradient and vesicle secretion, both essential for pollen tube growth and reorientation (Monteiro et al. 2005). Taken together, PA, as noted, plays a crucial role in promoting cytoskeletal rearrangements and vesicle trafficking, thus affecting cell growth and changing cellular architecture.

7 Perspectives

PA is a new class of cellular mediators involved in a wide range of metabolic, cellular, and physiological processes in plants. Genetic manipulations of the genes encoding PA-producing and -removing enzymes have been highly informative, providing direct evidence for cellular and physiological functions of PA. However, multiple PLDs, PLCs, and DGKs, as well as other enzymes, may contribute to the production of signaling PA, which makes it challenging to define the role of PA in the specific processes of growth, development, and stress responses. Inhibitors specific to mammalian PLD1 and PLD2 have been identified (O'Reilly et al. 2013), but their efficacy on plant PLDs is yet to be determined. Direct testing of the functions of PA in the cell is challenging because PA is water insoluble and can be metabolized rapidly. Importantly, the location and timing of PA production are critical to the PA function. Analyses of PA-interacting proteins have been informative to PA's mode of actions. A systematic characterization of the PA-interactome will provide valuable insights into the processes that PA is involved in and the mechanisms by which PA functions in the cell. Genes in PA production have been manipulated to explore the application of PA alteration for crop improvement. Further advances in the study of PA as a growth mediator will enhance current understanding of growth and stress signaling, opening more avenues for crop improvement.

Acknowledgments The work resulting from Wang laboratory was supported by grants from the National Science Foundation Grant IOS-0818740, the US Department of Agriculture Grant 2007-35318-18393, and the work on PA-GAPC interaction was supported by the U.S. Department of Energy (DOE), Office of Science, Office of Basic Energy Sciences (BES), Materials Sciences and Engineering Division under Award # DE-SC0001295 to XW. We thank Brian Fanella for critical reading of the manuscript.

References

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

Arisz SA, Munnik T (2013) Distinguishing phosphatidic acid pools from de novo synthesis, PLD, and DGK. Methods Mol Biol 1009:55–62

Arisz SA, Testerink C, Munnik T (2009) Plant PA signaling via diacylglycerol kinase. Biochim Biophys Acta 1791:869–875

Awai K, Xu C, Tamot B, Benning C (2006) A phosphatidic acid-binding protein of the chloroplast inner envelope membrane involved in lipid trafficking. Proc Natl Acad Sci U S A 103:10817–10822

Bargmann BO, Laxalt AM, ter Riet B, van Schooten B, Merquiol E, Testerink C, Haring MA, Bartels D, Munnik T (2009) Multiple PLDs required for high salinity and water deficit tolerance in plants. Plant Cell Physiol 50:78–89

Camoni L, Di Lucente C, Pallucca R, Visconti S, Aducci P (2012) Binding of phosphatidic acid to 14-3-3 proteins hampers their ability to activate the plant plasma membrane H^+-ATPase. IUBMB Life 64:710–716

Cho D, Shin DJ, Jeon BW, Kwak JM (2009) ROS-mediated ABA signaling. J Plant Biol 52:102–113

Colón-González F, Kazanietz GM (2006) C1 domains exposed: from diacylglycerol binding to protein–protein interactions. Biochim Biophys Acta 1761:827–837

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

Devaiah SP, Roth MR, Baughman E, Li M, Tamura P, Jeannotte R, Welti R, Wang X (2006) Quantitative profiling of polar glycerolipid species from organs of wild-type *Arabidopsis* and a phospholipase Dalpha1 knockout mutant. Phytochemistry 67:1907–1924

Du ZY, Chen MX, Chen QF, Xiao S, Chye ML (2013) *Arabidopsis* acyl-CoA-binding protein ACBP1 participates in the regulation of seed germination and seedling development. Plant J 74:294–309

Dubots E, Audry M, Yamaryo Y, Bastien O, Ohta H, Breton C, Maréchal E, Block MA (2010) Activation of the chloroplast monogalactosyldiacylglycerol synthase MGD1 by phosphatidic acid and phosphatidylglycerol. J Biol Chem 285:6003–6011

Erb M, Meldau S, Howe GA (2012) Role of phytohormones in insect-specific plant reactions. Trends Plant Sci 17:250–259

Fan L, Zheng S, Wang X (1997) Antisense suppression of phospholipase D alpha retards abscisic acid- and ethylene-promoted senescence of postharvest *Arabidopsis* leaves. Plant Cell 9:2183–2196

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

Foster DA (2007) Regulation of mTOR by phosphatidic acid? Cancer Res 67(1):1–4

Foster DA (2009) Phosphatidic acid signaling to mTOR: signals for the survival of human cancer cells. Biochim Biophys Acta 1791:949–955

Gao HB, Chu YJ, Xue HW (2013) Phosphatidic acid (PA) binds PP2AA1 to regulate PP2A activity and PIN1 polar localization. Mol Plant 6:1692–1702

Gómez-Merino FC, Brearley CA, Ornatowska M, Abdel-Haliem ME, Zanor MI, Mueller-Roeber B (2004) AtDGK2, a novel diacylglycerol kinase from Arabidopsis thaliana, phosphorylates 1-stearoyl-2-arachidonoyl-sn-glycerol and 1,2-dioleoyl-sn-glycerol and exhibits cold-inducible gene expression. J Biol Chem 279:8230–8241

Guo L, Wang X (2012) Crosstalk between phospholipase D and sphingosine kinase in plant stress signaling. Front Plant Sci 3:51

Guo L, Mishra G, Taylor K, Wang X (2011) Phosphatidic acid binds and stimulates *Arabidopsis* sphingosine kinases. J Biol Chem 286:13336–13345

Guo L, Devaiah SP, Narasimhan R, Pan X, Zhang Y, Zhang W, Wang X (2012a) Cytosolic glyceraldehyde-3-phosphate dehydrogenases interact with phospholipase Dδ to transduce hydrogen peroxide signals in the *Arabidopsis* response to stress. Plant Cell 24:2200–2212

Guo L, Mishra G, Markham JE, Li M, Tawfall A, Welti R, Wang X (2012b) Connections between sphingosine kinase and phospholipase D in the abscisic acid signaling pathway in *Arabidopsis*. J Biol Chem 287:8286–8296

Hong Y, Pan X, Welti R, Wang X (2008) The effect of phospholipase Dα3 on *Arabidopsis* response to hyperosmotic stress and glucose. Plant Signal Behav 3:1099–1100

Hong Y, Devaiah SP, Bahn SC, Thamasandra BN, Li M, Welti R, Wang X (2009) Phospholipase Dε and phosphatidic acid enhance *Arabidopsis* nitrogen signaling and growth. Plant J 58:376–387

Hong Y, Zhang W, Wang X (2010) Phospholipase D and phosphatidic acid signalling in plant response to drought and salinity. Plant Cell Environ 33:627–635

Huang S, Gao L, Blanchoin L, Staiger CJ (2006) Heterodimeric capping protein from *Arabidopsis* is regulated by phosphatidic acid. Mol Biol Cell 17:1946–1958

Jang JH, Lee CS, Hwang D, Ryu SH (2012) Understanding of the roles of phospholipase D and phosphatidic acid through their binding partners. Prog Lipid Res 51:71–81

Jones JA, Rawles R, Hannun YA (2005) Identification of a novel phosphatidic acid binding domain in protein phosphatase-1. Biochemistry 44:13235–13245

Jost R, Berkowitz O, Shaw J, Masle J (2009) Biochemical characterization of two wheat phosphoethanolamine N-methyltransferase isoforms with different sensitivities to inhibition by phosphatidic acid. J Biol Chem 284:31962–31971

Karathanassis D, Stahelin RV, Bravo J, Perisic O, Pacold CM, Cho W, Williams RL (2002) Binding of the PX domain of p47(phox) to phosphatidylinositol 3,4-bisphosphate and phosphatidic acid is masked by an intramolecular interaction. EMBO J 21(19):5057–5068

Katagiri T, Ishiyama K, Kato T, Tabata S, Kobayashi M, Shinozaki K (2005) An important role of phosphatidic acid in ABA signaling during germination in *Arabidopsis thaliana*. Plant J 43 (1):107–117

Kim SC, Guo L, Wang X (2013) Phosphatidic acid binds to cytosolic glyceraldehyde-3-phosphate dehydrogenase and promotes its cleavage in *Arabidopsis*. J Biol Chem 288:11834–11844

Klimecka M, Szczegielniak J, Godecka L, Lewandowska-Gnatowska E, Dobrowolska G, Muszyńska G (2011) Regulation of wound-responsive calcium-dependent protein kinase from maize (ZmCPK11) by phosphatidic acid. Acta Biochim Pol 58:589–595

Kolesnikov YS, Nokhrina KP, Kretynin SV, Volotovski ID, Martinec J, Romanov GA, Kravets VS (2012) Molecular structure of phospholipase D and regulatory mechanisms of its activity in plant and animal cells. Biochemistry (Mosc) 77:1–14

Kooijman EE, Chupin V, de Kruijff B, Burger KN (2003) Modulation of membrane curvature by phosphatidic acid and lysophosphatidic acid. Traffic 4:162–174

Kooijman EE, Carter KM, Van Laar EG, Chupin V, Burger KN, de Kruijff B (2005) What makes the bioactive lipids phosphatidic acid and lysophosphatidic acid so special? Biochemistry 44:17007–17015

Kooijman EE, Tieleman DP, Testerink C, Munnik T, Rijkers DT, Burger KN, de Kruijff B (2007) An electrostatic/hydrogen bond switch as the basis for the specific interaction of phosphatidic acid with proteins. J Biol Chem 282:11356–11364

Li G, Xue HW (2007) *Arabidopsis* PLDζ2 regulates vesicle trafficking and is required for auxin response. Plant Cell 19:281–295

Li M, Qin C, Welti R, Wang X (2006) Double knockouts of phospholipases Dζ1 and Dζ2 in *Arabidopsis* affect root elongation during phosphate-limited growth but do not affect root hair patterning. Plant Physiol 140:761–770

Li W, Wang R, Li M, Li L, Wang C, Welti R, Wang X (2008) Differential degradation of extraplastidic and plastidic lipids during freezing and post-freezing recovery in *Arabidopsis thaliana*. J Biol Chem 283:461–468

Li M, Hong Y, Wang X (2009) Phospholipase D- and phosphatidic acid-mediated signaling in plants. Biochim Biophys Acta 1791:927–935

Li J, Henty-Ridilla JL, Huang S, Wang X, Blanchoin L, Staiger CJ (2012) Capping protein modulates the dynamic behavior of actin filaments in response to phosphatidic acid in *Arabidopsis*. Plant Cell 24:3742–3754

Liu HY, Lee N, Tsai TY, Ho SY (2010) Zebrafish fat-free, a novel Arf effector, regulates phospholipase D to mediate lipid and glucose metabolism. Biochim Biophys Acta 1801:1330–1340

Liu Y, Su Y, Wang X (2013) Phosphatidic acid-mediated signaling. Adv Exp Med Biol 991:159–176

Loewen CJ (2012) Lipids as conductors in the orchestra of life. F1000 Biol Rep 4:4

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

Lu S, Bahn SC, Qu G, Qin H, Hong Y, Xu Q, Zhou Y, Hong Y, Wang X (2013) Increased expression of phospholipase Dα1 in guard cells decreases water loss with improved seed production under drought in *Brassica napus*. Plant Biotechnol J 11:380–389

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

McLoughlin F, Galvan-Ampudia CS, Julkowska MM, Caarls L, van der Does D, Laurière C, Munnik T, Haring MA, Testerink C (2012) The Snf1-related protein kinases SnRK2.4 and SnRK2.10 are involved in maintenance of root system architecture during salt stress. Plant J 72:436–449

McLoughlin F, Arisz SA, Dekker HL, Kramer G, de Koster CG, Haring MA, Munnik T, Testerink C (2013) Identification of novel candidate phosphatidic acid-binding proteins involved in the salt-stress response of *Arabidopsis thaliana* roots. Biochem J 450:573–581

Min MK, Kim SJ, Miao Y, Shin J, Jiang L, Hwang I (2007) Overexpression of *Arabidopsis* AGD7 causes relocation of Golgi-localized proteins to the endoplasmic reticulum and inhibits protein trafficking in plant cells. Plant Physiol 143:1601–1614

Min MK, Jang M, Lee M, Lee J, Song K, Lee Y, Choi KY, Robinson DG, Hwang I (2013) Recruitment of Arf1-GDP to Golgi by Glo3p-type ArfGAPs is crucial for golgi maintenance and plant growth. Plant Physiol 161:676–691

Mishra G, Zhang W, Deng F, Zhao J, Wang X (2006) A bifurcating pathway directs abscisic acid effects on stomatal closure and opening in *Arabidopsis*. Science 312:264–266

Monreal JA, McLoughlin F, Echevarría C, García-Mauriño S, Testerink C (2010) Phosphoenolpyruvate carboxylase from C4 leaves is selectively targeted for inhibition by anionic phospholipids. Plant Physiol 152:634–638

Monteiro D, Liu Q, Lisboa S, Scherer GEF, Quader H, Malho R (2005) Phosphoinositides and phosphatidic acid regulate pollen tube growth and reorientation through modulation of $[Ca^{2+}]_c$ and membrane secretion. J Exp Bot 56:1665–1674

Munnik T, Nielsen E (2011) Green light for polyphosphoinositide signals in plants. Curr Opin Plant Biol 14:489–497

Nicholls C, Li H, Liu JP (2012) GAPDH: a common enzyme with uncommon functions. Clin Exp Pharmacol Physiol 39:674–679

O'Reilly MC, Scott SA, Brown KA, Oguin TH 3rd, Thomas PG, Daniels JS, Morrison R, Brown HA, Lindsley CW (2013) Development of dual PLD1/2 and PLD2 selective inhibitors from a common 1,3,8-Triazaspiro[4.5]decane Core: discovery of Ml298 and Ml299 that decrease invasive migration in U87-MG glioblastoma cells. J Med Chem 56:2695–2699

Peng X, Frohman MA (2012) Mammalian phospholipase D physiological and pathological roles. Acta Physiol (Oxf) 204:219–226

Peters C, Li M, Narasimhan R, Roth M, Welti R, Wang X (2010) Nonspecific phospholipase C NPC4 promotes response to abscisic acid and tolerance to hyperosmotic stress in Arabidopsis. Plant Cell 22:2642–2659

Petersen J, Eriksson SK, Harryson P, Pierog S, Colby T, Bartels D, Röhrig H (2012) The lysine-rich motif of intrinsically disordered stress protein CDeT11-24 from Craterostigma plantagineum is responsible for phosphatidic acid binding and protection of enzymes from damaging effects caused by desiccation. J Exp Bot 63:4919–4929

Pleskot R, Pejchar P, Žárský V, Staiger CJ, Potocký M (2012) Structural insights into the inhibition of actin-capping protein by interactions with phosphatidic acid and phosphatidylinositol (4,5)-bisphosphate. PLoS Comput Biol 8(11):e1002765

Pleskot R, Li J, Zárský V, Potocký M, Staiger CJ (2013) Regulation of cytoskeletal dynamics by phospholipase D and phosphatidic acid. Trends Plant Sci 18(9):496–504

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

Pu Y, Garfield HS, Kedei N, Blumberg MP (2009) Characterization of the differential roles of the twin C1a and C1b domains of protein kinase C. J Biol Chem 284:1302–1312

Ritchie S, Gilroy S (1998) Abscisic acid signal transduction in the barley aleurone is mediated by phospholipase D activity. Proc Natl Acad Sci U S A 95:2697–2702

Rizzo MA, Shome K, Watkins SC, Romero G (2000) The recruitment of Raf-1 to membranes is mediated by direct interaction with phosphatidic acid and is independent of association with Ras. J Biol Chem 275:2391–2398

Roach AN, Wang Z, Wu P, Zhang F, Chan RB, Yonekubo Y, Di Paolo G, Gorfe AA, Du G (2012) Phosphatidic acid regulation of PIPKI is critical for actin cytoskeletal reorganization. J Lipid Res 53:2598–2609

Roth MG (2008) Molecular mechanisms of PLD function in membrane traffic. Traffic 9:1233–1239

Ruelland E, Cantrel C, Gawer M, Kader JC, Zachowski A (2002) Activation of phospholipases C and D is an early response to a cold exposure in *Arabidopsis* suspension cells. Plant Physiol 130:999–1007

Ryu SB, Wang X (1996) Activation of phospholipase D and the possible mechanism of activation in wound-induced lipid hydrolysis in castor bean leaves. Biochim Biophys Acta 1303:243–250

Ryu SB, Wang X (1998) Increase in free linolenic and linoleic acids associated with phospholipase D-mediated hydrolysis of phospholipids in wounded castor bean leaves. Biochim Biophys Acta 1393:193–202

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

Shin JJ, Loewen CJ (2011) Putting the pH into phosphatidic acid signaling. BMC Biol 9:85

Shulga YV, Topham MK, Epand RM (2011) Regulation and functions of diacylglycerol kinases. Chem Rev 111:6186–6208

Sirover MA (1999) New insights into an old protein: the functional diversity of mammalian glyceraldehyde-3-phosphate dehydrogenase. Biochim Biophys Acta 1432:159–184

Takemiya A, Shimazaki K (2010) Phosphatidic acid inhibits blue light-induced stomatal opening via inhibition of protein phosphatase 1 [corrected]. Plant Physiol 153:1555–1562

Testerink C, Munnik T (2011) Molecular, cellular, and physiological responses to phosphatidic acid formation in plants. J Exp Bot 62:2349–2361

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, Larsen PB, van der Does D, van Himbergen JA, Munnik T (2007) Phosphatidic acid binds to and inhibits the activity of *Arabidopsis* CTR1. J Exp Bot 58:3905–3914

Testerink C, Larsen PB, McLoughlin F, van der Does D, van Himbergen JA, Munnik T (2008) PA, a stress-induced short cut to switch-on ethylene signalling by switching-off CTR1? Plant Signal Behav 3:681–683

Uraji M, Katagiri T, Okuma E, Ye W, Hossain MA, Masuda C, Miura A, Nakamura Y, Mori IC, Shinozaki K, Murata Y (2012) Cooperative function of PLDδ and PLDα1 in abscisic acid-induced stomatal closure in *Arabidopsis*. Plant Physiol 159:450–460

van den Brink-van der Laan E, Killian JA, de Kruijff B (2004) Nonbilayer lipids affect peripheral and integral membrane proteins via changes in the lateral pressure profile. Biochim Biophys Acta 1666:275–288

Vermeer JE, Munnik T (2013) Using genetically encoded fluorescent reporters to image lipid signalling in living plants. Methods Mol Biol 1009:283–289

Wallis JG, Browse J (2002) Mutants of *Arabidopsis* reveal many roles for membrane lipids. Prog Lipid Res 41:254–278

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

Wang Z, Benning C (2012) Chloroplast lipid synthesis and lipid trafficking through ER-plastid membrane contact sites. Biochem Soc Trans 40:457–463

Wang X, Devaiah SP, Zhang W, Welti R (2006) Signaling functions of phosphatidic acid. Prog Lipid Res 45:250–278

Wang Z, Xu C, Benning C (2012) TGD4 involved in endoplasmic reticulum-to-chloroplast lipid trafficking is a phosphatidic acid binding protein. Plant J 70:614–623

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

Xiong Y, Sheen J (2012) Rapamycin and glucose-target of rapamycin (TOR) protein signaling in plants. J Biol Chem 287:2836–2842

Yan J, Tsuichihara N, Etoh T, Iwai S (2007) Reactive oxygen species and nitric oxide are involved in ABA inhibition of stomatal opening. Plant Cell Environ 30:1320–1325

Young SA, Wang X, Leach JE (1996) Changes in the plasma membrane distribution of rice phospholipase D during resistant interactions with Xanthomonas oryzae pv oryzae. Plant Cell 8:1079–1090

Young BP, Shin JJH, Orij R, Chao JT, Li SC, Guan XL, Khong A, Jan E, Wenk MR, Prinz WA, Smits GJ, Loewen CJ (2010) Phosphatidic acid is a pH biosensor that links membrane biogenesis to metabolism. Science 329:1085–1088

Yu L, Nie J, Cao C, Jin Y, Yan M, Wang F, Liu J, Xiao Y, Liang Y, Zhang W (2010) Phosphatidic acid mediates salt stress response by regulation of MPK6 in *Arabidopsis thaliana*. New Phytol 188:762–773

Zhang W, Qin C, Zhao J, Wang X (2004) Phospholipase Dα1-derived phosphatidic acid interacts with ABI1 phosphatase 2C and regulates abscisic acid signaling. Proc Natl Acad Sci U S A 101:9508–9513

Zhang Y, Zhu H, Zhang Q, Li M, Yan M, Wang R, Wang L, Welti R, Zhang W, Wang X (2009) Phospholipase Dα1 and phosphatidic acid regulate NADPH oxidase activity and production of reactive oxygen species in ABA-mediated stomatal closure in *Arabidopsis*. Plant Cell 21:2357–2377

Zhang Q, Lin F, Mao T, Nie J, Yan M, Yuan M, Zhang W (2012) Phosphatidic acid regulates microtubule organization by interacting with MAP65-1 in response to salt stress in *Arabidopsis*. Plant Cell 24:4555–4576

Zhao J, Devaiah SP, Wang C, Welti R, Wang X (2013) Phospholipase Dβ1 modulates defense responses to bacterial and fungal pathogens in *Arabidopsis*. New Phytol 199:228–240

Zien CA, Wang C, Wang X, Welti R (2001) In-vivo substrates and the contribution of the common phospholipase D, PLDα, to wound-induced metabolism of lipids in *Arabidopsis*. Biochim Biophys Acta 1530:236–248

pPLA: Patatin-Related Phospholipase As with Multiple Biological Functions

Maoyin Li and Xuemin Wang

Abstract The release of fatty acids from membranous and storage lipids has been implicated in a variety of cellular functions, including carbon partitioning, cell elongation, defense response, seedling establishment, and plant growth. Patatin-related proteins are the major enzymes that catalyze the release of fatty acids and are present in various organisms, including plants and mammals. The *Arabidopsis* genome has 13 patatin-like genes encoding proteins including three groups of patatin-related phospholipases, pPLAI, pPLAII (α,β,γ,δ,ε), and pPLAIII (α,β,γ,δ). The pPLAI, pPLAIIs, and pPLAIIIs possess phospholipase and galactolipase activities, while pPLAIIIs have an additional acyl-CoA thioesterase activity. The fourth group of patatin-related proteins possesses triacylglycerol lipase activity and includes SDP1, SDP1-L, and ATGL-L. Phenotypic analyses of *Arabidopsis* deficient in and overexpressing specific patatin-related genes have revealed their important roles in stress responses, plant development, and lipid homeostasis. This chapter aims to summarize current knowledge of patatin-related proteins and document their emerging importance in plant growth and lipid metabolism.

Keywords Lipid metabolism • Patatin-related protein • Phospholipase A • Plant growth • Stress response

1 Introduction

Lipid acyl hydrolases are diverse enzymes that hydrolyze the ester or amide bonds of fatty acids (FA) in lipids. These include triacylglycerol lipases, phospholipases, galactolipases, ceraminidases, cholesterol ester hydrolases, and retinol ester

M. Li (✉) • X. Wang
Donald Danforth Plant Science Center, St. Louis, MO 63132, USA

Department of Biology, University of Missouri-St. Louis, St. Louis, MO 63121, USA
e-mail: mali@danforthcenter.org

X. Wang (ed.), *Phospholipases in Plant Signaling*, Signaling and Communication in Plants 20, DOI 10.1007/978-3-642-42011-5_5, © Springer-Verlag Berlin Heidelberg 2014

hydrolases. The function of these enzymes is diverse, including the generation of lipid messengers, remodeling of biological membranes, lipid degradation, and regulation of energy homeostasis. Patatin-related phospholipase A (pPLA) is a major family of lipid acyl hydrolases and plays important roles in lipid metabolism, signal transduction, cell growth, and plant response to environmental stresses. The genetic manipulation of various pPLAs has shed light on their metabolic, cellular, and stress responsive functions.

During the last decade, much knowledge has been gained regarding the biochemical properties and biological functions of plant patatin-related enzymes, and several reviews have highlighted their importance in signal transduction (Ryu 2004; Matos and Pham-Thi 2009; Scherer et al. 2010, 2012), plant immunity (Grienenberger et al. 2010; described in chapter "Phospholipase A in Plant Immunity"), and seedling growth (Quettier and Eastmond 2009). This chapter aims to summarize recent advances in the understanding of the biochemical properties and the physiological significance of patatin-related enzymes in lipid turnover, cellulose production, drought response, and plant growth.

2 pPLA Classification and Subcellular Localization

The acronym pPLA was recently introduced to designate patatin-related proteins in *Arabidopsis* (Scherer et al. 2010). The eponym of this protein family, potato patatin, possesses an evolutionarily conserved esterase motif GxSxG (Andrews et al. 1988), but variations occur in some members with demonstrated enzymatic activity (Li et al. 2011). Patatin in potato tubers is localized in vacuoles, but pPLAs are associated with different subcellular fractions.

2.1 Subclasses of Patatin-Related Proteins

Thirteen patatin-related genes have been identified in the *Arabidopsis* genome database (Fig. 1; Eastmond 2006; Scherer et al. 2010; Kelly et al. 2011). Based on their gene structure, deduced protein sequences, and enzymatic properties, they are divided into pPLAI, pPLAII (α,β,γ,δ,ε), and pPLAIII (α,β,γ,δ) (Scherer et al. 2010). The fourth subclass includes SDP1, SDP1-L, and adipose triglyceride lipase-like (ATGL-L; Eastmond 2006). pPLAI is the only member that contains a C-terminal leucine-rice repeat domain and an N-terminal ankyrin-like domain that are involved in protein–protein interactions. Based on protein sequence analysis, pPLAI is more closely related to the human patatin-like phospholipase domain containing proteins (PNPLA6–9, calcium-independent iPLAs) (Fig. 1). The *pPLAIIs* have five to six introns while *pPLAIIIs* have only one intron (Scherer et al. 2010). *pPLAIIIs* share less overall similarity to patatin (Holk et al. 2002; La Camera et al. 2005; Yang et al. 2007; Scherer et al. 2010). They possess a noncanonical esterase motif GxGxG instead of GxSxG in other family members

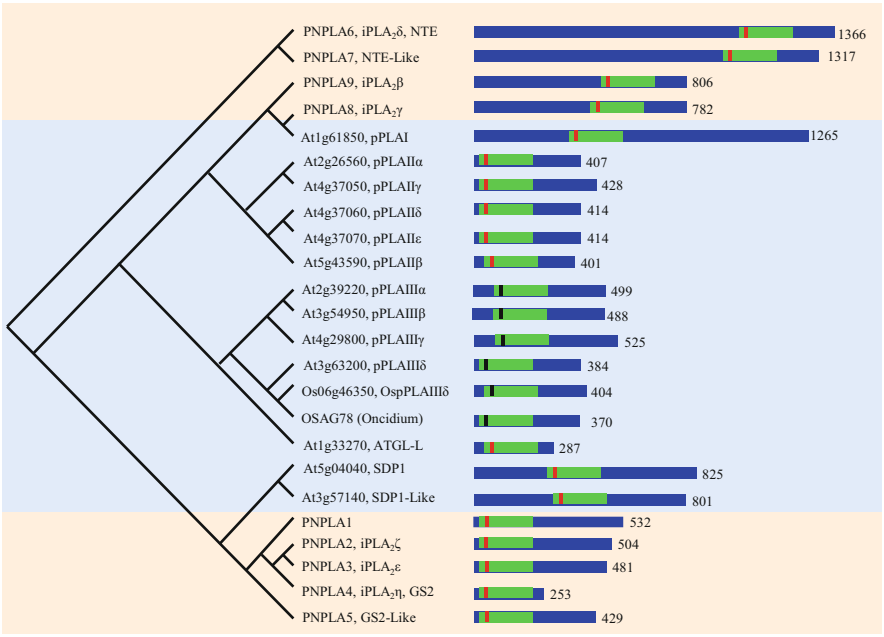

Fig. 1 Phylogenetic relationship and structural comparison of plant and human patatin proteins. The plant patatin proteins, pPLAs, are highlighted under *blue* background, including 13 proteins in *Arabidopsis*, one in rice, and one in oncidium flower. PNPLA1-9 refer to nine human patatin-related proteins and are highlighted under *pink* background. The patatin domain in the full-length proteins is shown as *green box*. The *red vertical line* indicates the canonical esterase motif GxSxG. The *dark vertical line* indicates the noncanonical esterase motif GxGxG which is specific for pPLAIIIs. The number on the right denotes the predicted protein length in amino acids. Multiple sequence alignment was done with MUSCLE, and corresponding tree was generated in MEGA5.05

(Scherer et al. 2010; Li et al. 2011). The fourth subclass members have triacylglycerol lipase activity that other family members do not have (Eastmond 2006; Kelly et al. 2011). This group is more closely related to human PNPLA1–5 based on protein sequences analysis (Fig. 1).

Besides *Arabidopsis*, function of patatin-related genes has been described in other organisms, such as cowpea (Matos et al. 2001), tobacco (Dhondt et al. 2002), rubber tree (Jekel et al. 2003), cucumber (Rudolph et al. 2011), oncidium (Lin et al. 2011), rice (Singh et al. 2012), and mammals (Kienesberger et al. 2009). Sixteen *pPLA*s have been identified from rice genome (Singh et al. 2012). They are classified into three groups as one *OspPLAI*, nine *OspPLAII*, and six *OspPLAIII*. Nine patatin-related proteins, designated PNPLA1–9, have been identified in the human genome which possess acyl hydrolase activities with diverse substrates, including triacylglycerols, phospholipids, and retinol esters (Wilson et al. 2006; Kienesberger et al. 2009; Moon et al. 2012). Since some PNPLAs were originally discovered to have Ca^{2+}-independent phospholipase A_2 activity, they are referred to

as iPLA$_2$ as opposed to the cytosolic, Ca^{2+}-dependent cPLA$_2$ and secreted, Ca^{2+}-dependent sPLA$_2$ (Six and Dennis 2000; Schaloske and Dennis 2006).

2.2 Subcellular Localization

Using transiently produced and green fluorescence protein (GFP) fused pPLAs in tobacco leaf cells, pPLAI-GFP was found in the cytosol and also to be associated with chloroplasts, while pPLAIIα-GFP, pPLAIIγ-GFP, and pPLAIIε-GFP were potentially associated with the plasma membrane or endoplasmic reticulum (Holk et al. 2002). The plasma membrane location of pPLAIIα-GFP has been further proved in *Arabidopsis* leaf cells (La Camera et al. 2005). SDP1-GFP fusion protein associates with the oil body surface in *Arabidopsis* seedlings (Eastmond 2006).

The majority of pPLAIIIβ-GFP protein is associated with the plasma membrane as observed in epidermal cells of *Arabidopsis* leaves and roots (Li et al. 2011). When total proteins from *Arabidopsis* leaf were subcellularly fractionated, approximately 80 % of pPLAIIIβ-GFP was localized on the plasma membrane with the remainder on intracellular membranes (Li et al. 2011). OSAG78 in *Oncidium* is a patatin-related protein and is evolutionally close to pPLAIIIδ (Lin et al. 2011). OSAG78-GFP associates with the plasma membrane in onion epidermal cells and *Arabidopsis* root epidermal cells; it also associates with the chloroplast membrane in *Arabidopsis* leaf guard cells (Lin et al. 2011). Rice OspPLAIIIδ-GFP protein associates with the plasma membrane in onion epidermal cells (Singh et al. 2012).

3 Enzymatic Properties of Patatin-Related Proteins

When they are assayed in vitro, patatin-related proteins general possess acyl hydrolase activity toward a broad range of substrates, such as phospholipids and galactolipids, but they display differences in lipid substrate selectivity. Moreover, the lipid substrates in vivo for these enzymes are difficult to determine and mostly unknown.

3.1 Enzyme Activities of pPLAI, pPLAIIs, and pPLAIIIs

A pPLA in cowpea, VuPAT1, is specifically active with galactolipids and sulfolipids but not with phospholipids (Matos et al. 2001). pPLAI and pPLAIIs use phospholipids and galactolipids as substrates (Holk et al. 2002; La Camera et al. 2005; Yang et al. 2007, 2012; Rietz et al. 2010). pPLAI exhibits much higher galactolipase activity versus phospholipase activity and preferentially hydrolyzes oxidized galactolipids over nonoxidized ones (Yang et al. 2007). pPLAIIγ, δ, and ε

display higher galactolipase activity than phospholipase (Rietz et al. 2010). pPLAIIδ and pPLAIIε can be phosphorylated by Ca^{2+}-dependent protein kinases in vitro and the phosphorylated proteins have enhanced activity toward phosphatidylcholine (PC) but not phosphatidylglycerol (PG; Rietz et al. 2010), implying a phosphorylation-regulated substrate selectivity in vivo.

pPLAIIIs have the noncanonical esterase motif GxGxG, and their enzyme activity was not demonstrated until the recent characterization of *Arabidopsis* pPLAIIIβ (Li et al. 2011). pPLAIIIs hydrolyze phospholipids and galactolipids. Recombinant pPLAIIIβ purified from *E. coli* and *Arabidopsis* plants hydrolyzes phospholipids and galactolipids. In addition, it hydrolyzes acyl-CoAs (Li et al. 2011). These activities are also shown with pPLAIIIδ (Li et al. 2013). pPLAIIIβ releases more fatty acids from the *sn*-2 than *sn*-1 position in PC hydrolysis (Li et al. 2011). Knockout of *pPLAIIIβ* in *Arabidopsis* results in a 20 % reduction of the total free fatty acids in leaves (Li et al. 2011). The oncidium pPLA, OSAG78, is closely related to pPLAIIIδ and has acyl-hydrolyzing activity as shown by an artificial substrate *p*-nitrophenyl palmitate (pNPP) (Lin et al. 2011). The level of free 18:2 and 18:3 fatty acids in 35S::OSAG78-GFP overexpressed plants is twofold higher than that of wild-type *Arabidopsis* (Lin et al. 2011).

The substrate uses of pPLAI, pPLAII, and pPLAIII are distinguishable in vitro. pPLAI hydrolyzes monogalactosyldiacylglycerol (MGDG) at levels fourfold higher than PG (Yang et al. 2007), whereas pPLAIIIβ hydrolyzes PG fourfold greater than MGDG (Li et al. 2011). pPLAIIγ,δ, and ε have similar activities on both substrates (Rietz et al. 2010). The specific activity of pPLAIIα is much higher than that of pPLAIIIβ (Yang et al. 2007; Li et al. 2011). These differences suggest that individual pPLAs may selectively hydrolyze different classes of membrane lipids. It should be noted that substrate presentation affects greatly the lipid uses of lipolytic enzymes in vitro and that the substrate selectivity of these enzymes *in planta* remains to be determined. None of the pPLAI, IIs, and IIIs examined so far possesses triacylglycerol lipase activity.

3.2 Enzyme Activities of Group 4 Patatin-Related Proteins

The group 4 patatin-related proteins possess triacylglycerol (TAG) lipase activities. In a mutant screening for sucrose-dependent germination, *sugar-dependent 1* (*sdp1*) was identified as an *Arabidopsis* mutant with retarded postgerminative growth (Eastmond 2006). SDP1 has a patatin domain and exhibits a strong specificity toward TAG and a weak activity toward diacylglycerols (DAG), but no activity toward monoacylglycerols (MAG), phospholipids, galactolipids, or cholesterol esters (Eastmond 2006). Based on homology with SDP1, *sugar-dependent 1-like* (*SDP1-L*) was cloned from *Arabidopsis* (Eastmond 2006; Kelly et al. 2011). SDP1-L displays higher activity toward TAG than DAG but no activity on MAG (Kelly et al. 2011). The TAG preference of SDP1 and SDP1-L resembles that of human *a*dipose *t*riacyl*g*lycerol *l*ipase (ATGL, PNPLA2). Lipase activity of SDP1 and

Table 1 Distinct biochemical properties of patatin-related proteins

pPLAI (AtPLAI)	Phospholipase; galactolipase (Yang et al. 2007)
pPLAIIα (AtPLAIIA)	Phospholipase; galactolipase (La Camera et al. 2005; Matos et al. 2008; Yang et al. 2012)
pPLAIIε (AtPLAIVA)	Phospholipase; calcium dependent and inhibited by BEL (Holk et al. 2002)
pPLAIIγ (AtPLAIVC); pPLAIIδ (AtPLAIVB); pPLAIIε (AtPLAIVA)	More galactolipase activity over phospholipase (Rietz et al. 2010)
pPLAIIβ (AtPLAV)	
VuPAT1 (Cowpea)	Galactolipase; sulfolipase; but not phospholipase; evolutionally closed to pPLAII (Matos et al. 2001)
pPLAIIIβ (AtPLAIIIA)	Phospholipase; galactolipase; acyl-CoAs thioesterase (Li et al. 2011)
pPLAIIIδ (pPLAIIIB)	Phospholipase; acyl-CoAs thioesterase (Li et al. 2013)
pPLAIIIα (AtPLAIIB); pPLAIIIγ (AtPLAIVD)	
OSAG78 (Oncidium)	Lipase activity as assayed by pNPP; evolutionally closed to pPLAIIIs (Lin et al. 2011)
OspPLAIIIδ (DEP3, Rice)	
SDP1	Triacylglycerol lipase; inhibited by BEL (Eastmond 2006)
SDP1-like	Triacylglycerol lipase (Kelly et al. 2011)
ATGL-like	
Patatin (Potato)	Phospholipase; galactolipase; transacetylase (Andrews et al. 1988)
NtPAT (Tobacco)	Phospholipase (Dhondt et al. 2002)
CaPLA1 (Pepper)	
CsPAT (Cucumber)	Phospholipase (Rudolph et al. 2011)
PNPLA1	
PNPLA2 (iPLA$_2$ζ; PLA$_2$GVIE)	Acylglycerol transacylase; triacylglycerol lipase; transacetylase; inhibited by BEL (Jenkins et al. 2004)
PNPLA3 (iPLA$_2$ε; PLA$_2$GVID)	Acylglycerol transacylase; triacylglycerol lipase; transacetylase; inhibited by BEL (Jenkins et al. 2004)
PNPLA4 (iPLA$_2$η; GS2; PLA$_2$GVIF)	Acylglycerol transacylase; triacylglycerol lipase; transacetylase; inhibited by BEL (Jenkins et al. 2004)
PNPLA5 (GS2-like)	
PNPLA6 (iPLA$_2$δ; NTE; PLA$_2$GVIC)	Neuropathy target esterase; phospholipase; lysophospholipase (van Tienhoven et al. 2002)
PNPLA7 (NTE-like)	Lysophospholipase; but not phospholipase and triacylglycerol lipase (Kienesberger et al. 2008)
PNPLA8 (iPLA$_2$γ; PLA$_2$GVIB)	Lysophospholipase; phospholipase; inhibited by BEL (Yan et al. 2005; Moon et al. 2012)
PNPLA9 (iPLA$_2$β; PLA$_2$GVIA; PLA$_2$G6)	Phospholipase; acyl-CoA thioesterase; inhibited by BEL (Jenkins et al. 2006)

BEL (E)-6-(bromomethylene)-3-(1-naphthalenyl)-2H-tetrahydropyran-2-one, *NTE* Neuropathy target esterase, *GS2* gene sequence 2, *pNPP* *p*-nitrophenyl palmitate

PNPLA2 can be inhibited by the mechanistic inhibitor *b*romo*e*nol *l*actone (BEL, or HELSS), implying a conserved catalytic mechanism among them (Table 1). Based on sequence homology to ATGL, an ATGL-like protein was deduced in *Arabidopsis* (Eastmond 2006), but its enzyme activity remains unknown.

3.3 Enzyme Activities of Human Patatin-Related Protein PNPLAs

Since mammalian PNPLAs are extensively studied and are evolutionarily close to plant pPLAs, some recent progresses on PNPLA are highlighted here to shed some light onto the enzymatic properties and function of plant patatin-related proteins (Fig. 1; Table 1). Like pPLAs, PNPLA contain a patatin domain and a conserved lipase motif GxSxG (Kienesberger et al. 2009). Human patatin-related proteins, PNPLA1-9, have diverse lipolytic activities and important biological functions (Cedars et al. 2009; Kienesberger et al. 2009; Cohen et al. 2011). PNPLA2, 3, and 4 possess activities of TAG lipase and acylglycerol transacylase and are sensitive to BEL inhibition (Jenkins et al. 2004). PNPLA2 is the principal enzyme for fat cell lipolysis, and its polymorphisms are associated with increased risk for type 2 diabetes (Schoenborn et al. 2006). PNPLA6, 7, 8, and 9 display phospholipase and/or lysophospholipase activity (van Tienhoven et al. 2002; Yan et al. 2005; Kienesberger et al. 2008; Moon et al. 2012). PNPLA9 also has acyl-CoA thioesterase activity (Jenkins et al. 2006). Male mice deficient in PNPLA9 produce spermatozoa with impaired motility and have greatly reduced fertility (Bao et al. 2004). Enzyme activity remains unknown for PNPLA1.

The GxSxG motif in PNPLA3 is critical in its substrate selection. PNPLA3 has a strong TAG lipase activity but a weak activity toward acyl-CoA-dependent *l*yso*p*hosphatidic *a*cid *a*cyl*t*ransferase (LPAAT; Huang et al. 2011; Kumari et al. 2012). PNPLA3 (I148M), a variant of PNPLA3, possess strong LPAAT activity but no detectable TAG lipase activity (Kumari et al. 2012). Modeling analysis on protein structures suggests that the I148M substitution between catalytic dyad S47 and D166 could interfere with the configuration of the catalytic center (He et al. 2010). PNPLA3 (I148M) has a strong genetic association with *n*on*a*lcoholic *f*atty *l*iver *d*isease NAFLD, which results in an aberrant TAG accumulation in the liver, and affects one-third of human population (Romeo et al. 2008; Cohen et al. 2011; Sookoian and Pirola 2012).

4 Physiological Functions of Patatin-Related Proteins

pPLAs are involved in a wide range of physiological processes. The effect of pPLAs on plant–pathogen interactions are described in chapters "Phospholipase A in Plant Immunity" and "Lipases in Signaling Plant Defense Responses". Here we focus on the role of patatin-related proteins in plant metabolism, growth, and plant response to abiotic stress.

4.1 pPLAIIs in Drought Stress and Root Development

pPLAIIα plays a role in pathogen infection and drought stress (Yang et al. 2012). Knockout of *pPLAIIα* increases water loss and drought sensitivity in *Arabidopsis* plants (Yang et al. 2012). pPLAIIα hydrolyzes oxylipin-containing galactolipids, such as 12-*o*xo-*p*hyto*d*ienoic *a*cid (OPDA)- and dnOPDA-containing galactolipids (Yang et al. 2012). OPDA is a precursor for synthesis of jasmonic acid (JA). Therefore, it is proposed that the ablation of *pPLAIIα* could result in less JA production during drought stress. Interestingly however, detached leaves of *pPLAIIα* knockout mutants produced more JA than wild-type ones (Yang et al. 2012). This observation suggests that pPLAIIα plays a role in the removal of oxylipins from oxidized lipid to maintain membrane integrity but does not influence the JA production during drought stress.

Functions of *pPLAIIγ,δ,ε* have been investigated in *Arabidopsis* root in responses to hormone treatment and nutrient starvation. *pPLAIIγ* is highly expressed in the floral gynoecia and is upregulated in roots by abscisic acid (ABA) and phosphorus-deficient conditions (Rietz et al. 2010). *pPLAIIγ*-deficient mutant exhibits a normal ABA response but an impaired growth of primary roots under phosphorus-deficient conditions and a 50 % increase in hypocotyl length (Rietz et al. 2010). The transcript level of *pPLAIIδ* is low in roots but increased by auxin treatment. *pPLAIIδ*-deficient *Arabidopsis* seedlings were normal in primary root growth but with an increased lateral root density (Rietz et al. 2010). *pPLAIIε* is expressed strongly in roots, and *pPLAIIε*-deficient mutants display a decreased number of lateral roots (Rietz et al. 2010). These results suggest that *pPLAIIs* play important roles in root development.

4.2 *pPLAIIIs in Stress Responses, Lipid Metabolism, and Plant Growth*

4.2.1 pPLAIIIs on Stress Response

pPLAIIIs are also implicated in pathogen defense and abiotic stresses. Overexpression of *OSAG78* in *Arabidopsis* leads to more than a tenfold induction of pathogenic inducible genes *PDF1.2* and pathogenesis-related 4 (PR4) (Lin et al. 2011). Rice *OspPLAIIIα* and *OspPLAIIIδ* (DEP3) are induced two- and fourfold, respectively, to salt stress, and 4- and 30-fold, respectively, to drought stress, implying their potential functions in osmotic stresses (Singh et al. 2012). *pPLAIIIδ* deficiency in *Arabidopsis* impedes early auxin-induced gene expression and increases lateral root numbers under auxin treatment (Labusch et al. 2013).

4.2.2 pPLAIIIs on Lodging Capacity and Grain Yield

An early mutant screening for enhanced lodge tolerance identified a dominant *Arabidopsis* mutant, *STURDY*. *STURDY* has a stiff inflorescence stem, thick leaves, short siliques, large seeds, and round flower (Huang et al. 2001). Map-based cloning shows that the multiple tandem 35S mosaic promoters are inserted into the promoter region of *pPLAIIIδ* and results in an increased expression of *pPLAIIIδ* (Huang et al. 2001). A recessive mutant, *dense and erect panicle 3* (*dep3*), was isolated in a screening for changes in rice panicle architectures (Qiao et al. 2011). The gene structure and deduced amino acid sequence of DEP3 are closely related to those of pPLAIIIδ (Qiao et al. 2011; Figs. 1 and 2). The panicle of the *dep3* remains erect from flowering to full maturation, and the *dep3* mutant displays shorter panicle length, rounder grain, more grain number per panicle, and higher grain yield per plant (Qiao et al. 2011). The *dep3* mutant has more small vascular bundles and thicker culm than wild-type plants, which may explain the greater stem mechanical strength of *dep3* (Qiao et al. 2011).

4.2.3 pPLAIIIs on Flower Time

OSAG78 was isolated from a suppression subtractive hybridization between fully opened and partially senescent oncidium flower (Lin et al. 2011). Analysis of deduced protein sequence indicates that OSAG78 is closely related to pPLAIIIδ with a noncanonical esterase motif GxGxG (Figs. 1 and 2). Transgenic *Arabidopsis* plants overexpressing *OSAG78*, 35S::OSAG78, had smaller leaves, rounder flowers, and a late flowering phenotype which are rescued by an application of gibberellin A_3 (GA_3). The expression levels of gibberellin metabolism genes are decreased in 35S::OSAG78, including genes of *GA2ox1*, *GA2ox2*, *GA3ox1*, and *GA20ox1*. 35S::OSAG78 plant has a decreased level of active gibberellin GA_4 and

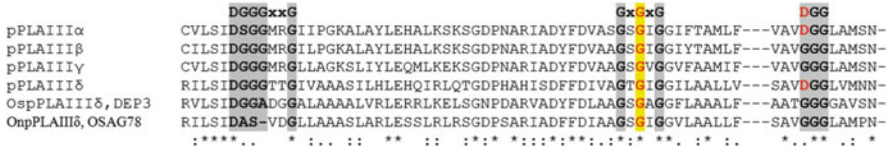

Fig. 2 Sequence alignment of conserved motifs in pPLAIIIs. The conserved motifs are highlighted for four *Arabidopsis* protein pPLAIII (α,β,γ,δ), one rice protein DEP3 (OspPLAIIIδ), and one oncidium protein OSAG78 (OnpPLAIIIδ), including the phosphate or anion binding element DGGGxxG, the noncanonical esterase box GxGxG, and the catalytic dyad-containing motif DGG (in pPLAIIIα,δ). The *gray* highlighting indicates the region of conserved motif. The *bold* highlighting indicates the amino acids in the conserved motif

decreased gene expression levels in gibberellin-responsive gene *SUPPRESSOR OF CO OVEREXPRESSION 1* (*SOC1*) and *GALABAR1* (*GL1*) (Lin et al. 2011). These results suggest OSAG78 and its lipid products suppress gibberellin synthetic gene expression, which can result in a lower level of active gibberellin, a decreased expression level of gibberellin-responsive genes, and subsequently a late flower phenotype in 35S::OSAG78 plants (Lin et al. 2011).

4.2.4 pPLAIIIs on Lipid and Cellulose Production

pPLAIIIs have been shown to regulate membrane lipid metabolism and plant growth. Knockout of *pPLAIIIβ* in *Arabidopsis* results in longer leaves, petioles, hypocotyls, primary roots, and root hairs than in WT plants, while overexpression of it leads to opposite effects (Li et al. 2011). The epidermal cells in leaf and hypocotyl are significantly longer in *pPLAIIIβ* knockout mutants than in overexpressors (Li et al. 2011). Knockout of *pPLAIIIβ* results in lower levels of leaf free fatty acids and a higher stem cellulose content (Li et al. 2011). On the other hand, overexpression of *pPLAIIIβ* leads to a significantly lower cellulose content and weaker stem mechanical strength than that of WT plants (Li et al. 2011). Multiple lines of evidence indicate that *pPLAIIIs* suppress plant growth. Overexpression of *pPLAIIIδ*, *pPLAIIIβ*, and *OSAG78* (*OnpPLAIIIδ*) leads to a shorter *Arabidopsis* plant stature (Huang et al. 2001; Li et al. 2011; Lin et al. 2011), while a recessive rice mutant *dep3*, deficiency of *OspPLAIIIδ*, displays a taller plant stature (Qiao et al. 2011). These results suggest that pPLAIII-mediated lipid metabolism is involved in cellulose production and plant growth.

Overexpression of *pPLAIIIδ* in *Arabidopsis* resulted in higher oil content with increased long chain fatty acids (20C and 22C) (Li et al. 2013). It is hypothesized that the acyl-hydrolase activity of pPLAIIIδ enhances the PC turnover and facilitates the fatty acyl elongation (Li et al. 2013). The TAG synthesis enzymes, diacylglycerol acyltransferases (DGAT), have selectivity on long chain fatty acyl CoAs (Weselake et al. 2006; Siloto et al. 2008). Increased availability of long chain fatty acids could facilitate DGAT to synthesize TAG.

4.3 Functions of Group 4 Patatin-Related Proteins in Seed Storage Lipid Mobilization

SDP1 and SDP1-L play major roles in TAG hydrolysis during *Arabidopsis* seed germination (Eastmond 2006; Kelly et al. 2011). During the first 5 days of postgerminative growth, the TAG level drops 95 % in wild-type seedlings while only 20 % in *SDP1*-deficient seedlings (Eastmond 2006). The postgerminative growth is retarded but not arrested in *SDP1*-deficent mutants, implying that SDP1-like protein might account for the resting TAG hydrolysis which could be sufficient for seedling establishment (Eastmond 2006). Indeed, double mutants with deficiency in both SDP1 and its homologous SDP1-L display a more severe phenotype with respect to postgerminative growth arrestment and storage lipid breakdown (Quettier and Eastmond 2009; Kelly et al. 2011).

Patatin-related TAG lipases also play important roles in seed oil metabolism and accumulation. Three cDNAs in *Brassica napus* have been identified to be closely related to *Arabidopsis* SDP1 (Kelly et al. 2013). There is approximately a 10 % decline in oil content during the final stage of rapeseed maturation, suggesting a potential oil degradation at this stage (Kelly et al. 2013). However, RNAi suppression of BnSDP1 alleviates this oil decrease; BnSDP1-RNAi seeds harvested both in green house and in field have an 8 % increase in seed oil content (Kelly et al. 2013). There is no observable retardation on germination or plant growth for transgenic seeds which are stored for as long as 2 years (Kelly et al. 2013).

Different patatin-related proteins may function at different steps of storage lipid mobilization. A patatin-related protein in cucumber, CsPAT, possesses PLA activity but not TAG lipase activity; however, it facilitates TAG breakdown in cucumber cotyledons (Rudolph et al. 2011). The PLA activity is co-localized with isolated lipid bodies (Rudolph et al. 2011), suggesting that CsPAT decomposes the oil body membrane that would allow a better access to TAG by TAG lipases. ATGL-L was identified due to its homology to human ATGL (PNPLA2; Eastmond 2006). ATGL-L is more closely related to pPLAIIIs, but its esterase motif is canonical GxSxG which is different from pPLAIII's GxGxG (Fig. 1). Neither enzyme activity nor biological function has been identified for ATGL-L.

5 Conclusions and Perspectives

Many exciting advances have been made recently on the biochemical properties and biological functions of patatain-related proteins (Fig. 3). pPLAIIs selectively hydrolyze oxylipin-containing galactolipids which suggests a role in basal production of JA and maintenance of cellular membrane integrity. Some of the functions of pPLAIIs have been revealed in drought stress (Matos et al. 2008; Yang et al. 2012), pathogen defense (La Camera et al. 2009), and root development (Rietz et al. 2004, 2010; Scherer et al. 2012). The plant unique subclass pPLAIIIs

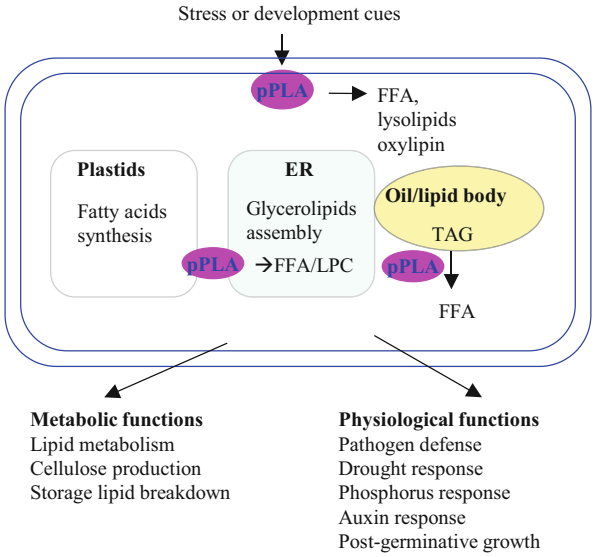

Fig. 3 Metabolic and physiological functions of pPLAs. In response to the stress or development signals, pPLA can hydrolyze membrane lipids to generate lipid signaling molecules. pPLA may be involved in lipid synthesis by facilitating fatty acids flux from plastids to ER, in which the newly fatty acids synthesized in plastids can be acylated to LPC to form PC, and the fatty acids can be released from PC and form LPC in ER by pPLA. pPLA can also mobilize fatty acids from storage lipids in oil bodies during postgerminative growth

with noncanonical esterase motif have been proven to have phospholipase, galactolipase, and acyl-CoA esterase activities (Li et al. 2011, 2013). The involvement of pPLAIIIs on plant growth and development has been revealed by forward and reverse genetic studies in multiple plant species, including *Arabidopsis* (Huang et al. 2001; Li et al. 2011), rice (Qiao et al. 2011), and oncidium (Lin et al. 2011). Some plant patatin proteins have also been shown to have unique TAG lipase activity and are involved in seed oil mobilization and postgerminative growth (Eastmond 2006; Kelly et al. 2011, 2013).

It is still unknown whether the noncanonical esterase motif in pPLAIIIs impacts their specific activity, particularly their substrate selection in vivo. The noncanonical esterase motif may have a unique configuration critical for substrate accessibility. The substitution I148M in PNPLA3 can interfere with the configuration of its esterase motif (He et al. 2010). PNPLA3 has strong TAG lipase activity and weak LPAAT activity, while PNPLA3 (I148M) possesses strong LPAAT activity but lacks TAG lipase activity (Huang et al. 2011; Kumari et al. 2012). It is necessary to understand the specific configurations in different pPLAs in an attempt to study their biochemical and biological functions.

A highly intriguing finding from recent investigation is that overexpression of pPLAIIIs decreases cellulose content. How do the enzymes that catalyze fatty acid release impact cellulose production. It would be of great interest to determine if

pPLA-derived products, such as fatty acids and lysolipids, impede the cellulose deposition machinery on the plasma membrane. Such investigations may reveal important metabolic and/or regulatory link between lipid and cellulose metabolism. In addition, some of the pPLA members can selectively hydrolyze oxylipin-containing substrates. Considering the induction by different stress conditions, it would also be interesting to study the metabolic mechanisms between the biochemical properties and biological functions. Novel technologies such as the high throughput lipidomics, proteomics, and reverse genetics will be powerful tools in these studies.

In addition, the components that interact with pPLAs have been scarcely identified. The chemical BEL can be useful to characterize pPLA-mediated pathways. BEL is a potent, irreversible mechanism-based PLA_2 inhibitor that possesses greater than 1,000-fold selectivity for Ca^{2+}-independent $iPLA_2$ (PNPLA) compared with Ca^{2+}-dependent ones (Jenkins et al. 2004, 2006). BEL inhibits PLA_2 activity in a very low dosage as reported in PNPLA2,3,4,8,9, pPLAIIε, and SDP1 (Table 1). BEL inhibits plant root elongation as assayed in agar plates (Holk et al. 2002). It will be advantageous to use chemical genetic methods to screen plant mutants that have altered growth in response to BEL.

Therefore, it will be necessary in the future to characterize the relationship between protein structure, biochemical properties, and biological functions of pPLAs. It will also be interesting to identify the up- and downstream components in pPLA-mediated pathways. The next few years will likely witness significant advances in the elucidation of pPLA-mediated metabolic, cellular, and physiological responses during plant growth and development and environmental stresses.

Acknowledgments The work is supported by grants from Natural Science Foundation (MCB-0922879 and IOS-0818740 to X.W.) and work on lipid production was supported by the U.S. Department of Energy (DOE), Office of Science, Office of Basic Energy Sciences (BES), Materials Sciences and Engineering Division under Award # DE-SC0001295 to X.W.

References

Andrews DL, Beames B, Summers MD, Park WD (1988) Characterization of the lipid acyl hydrolase activity of the major potato (*Solanum tuberosum*) tuber protein, patatin, by cloning and abundant expression in a baculovirus vector. Biochem J 252:199–206

Bao S, Miller DJ, Ma Z, Wohltmann M, Eng G, Ramanadham S et al (2004) Male mice that do not express group VIA phospholipase A2 produce spermatozoa with impaired motility and have greatly reduced fertility. J Biol Chem 279:38194–38200

Cedars A, Jenkins CM, Mancuso DJ, Gross RW (2009) Calcium-independent phospholipases in the heart: mediators of cellular signaling, bioenergetics, and ischemia-induced electrophysiologic dysfunction. J Cardiovasc Pharmacol 53:277–289

Cohen JC, Horton JD, Hobbs HH (2011) Human fatty liver disease: old questions and new insights. Science 332:1519–1523

Dhondt S, Gouzerh G, Müller A, Legrand M, Heitz T (2002) Spatio-temporal expression of patatin-like lipid acyl hydrolases and accumulation of jasmonates in elicitor-treated tobacco leaves are not affected by endogenous levels of salicylic acid. Plant J 32:749–762

Eastmond PJ (2006) SUGAR-DEPENDENT1 encodes a patatin domain triacylglycerol lipase that initiates storage oil breakdown in germinating Arabidopsis seeds. Plant Cell 18:665–675

Grienenberger E, Geoffroy P, Mutterer J, Legrand M, Heitz T (2010) The interplay of lipid acyl hydrolases in inducible plant defense. Plant Signal Behav 5:1181–1186

He S, McPhaul C, Li JZ, Garuti R, Kinch L, Grishin NV et al (2010) A sequence variation (I148M) in PNPLA3 associated with nonalcoholic fatty liver disease disrupts triglyceride hydrolysis. J Biol Chem 285:6706–6715

Holk A, Rietz S, Zahn M, Quader H, Scherer GF (2002) Molecular identification of cytosolic, patatin-related phospholipases A from Arabidopsis with potential functions in plant signal transduction. Plant Physiol 130:90–101

Huang S, Cerny RE, Bhat DS, Brown SM (2001) Cloning of an Arabidopsis patatin-like gene, STURDY, by activation T-DNA tagging. Plant Physiol 125:573–584

Huang Y, Cohen JC, Hobbs HH (2011) Expression and characterization of a PNPLA3 protein isoform (I148M) associated with nonalcoholic fatty liver disease. J Biol Chem 286:37085–37093

Jekel PA, Hofsteenge J, Beintema JJ (2003) The patatin-like protein from the latex of Hevea brasiliensis (Hev b 7) is not a vacuolar protein. Phytochemistry 63:517–522

Jenkins CM, Mancuso DJ, Yan W, Sims HF, Gibson B, Gross RW (2004) Identification, cloning, expression, and purification of three novel human calcium-independent phospholipase A2 family members possessing triacylglycerol lipase and acylglycerol transacylase activities. J Biol Chem 279:48968–48975

Jenkins CM, Yan W, Mancuso DJ, Gross RW (2006) Highly selective hydrolysis of fatty acyl-CoAs by calcium-independent phospholipase A2β. Enzyme autoacylation and acyl-CoA-mediated reversal of calmodulin inhibition of phospholipase A2 activity. J Biol Chem 281:15615–15624

Kelly AA, Quettier AL, Shaw E, Eastmond PJ (2011) Seed storage oil mobilization is important but not essential for germination or seedling establishment in Arabidopsis. Plant Physiol 157:866–875

Kelly AA, Shaw E, Powers SJ, Kurup S, Eastmond PJ (2013) Suppression of the SUGAR-DEPENDENT1 triacylglycerol lipase family during seed development enhances oil yield in oilseed rape (Brassica napus L.). Plant Biotechnol J 11:355–361

Kienesberger PC, Lass A, Preiss-Landl K, Wolinski H, Kohlwein SD, Zimmermann R, Zechner R (2008) Identification of an insulin-regulated lysophospholipase with homology to neuropathy target esterase. J Biol Chem 283:5908–5917

Kienesberger PC, Oberer M, Lass A, Zechner R (2009) Mammalian patatin domain containing proteins: a family with diverse lipolytic activities involved in multiple biological functions. J Lipid Res 50:S63–S68

Kumari M, Schoiswohl G, Chitraju C, Paar M, Cornaciu I, Rangrez AY et al (2012) Adiponutrin functions as a nutritionally regulated lysophosphatidic acid acyltransferase. Cell Metab 15:691–702

La Camera S, Geoffroy P, Samaha H, Ndiaye A, Rahim G, Legrand M et al (2005) A pathogen-inducible patatin-like lipid acyl hydrolase facilitates fungal and bacterial host colonization in Arabidopsis. Plant J 44:810–825

La Camera S, Balagué C, Göbel C, Geoffroy P, Legrand M, Feussner I et al (2009) The Arabidopsis patatin-like protein 2 (PLP2) plays an essential role in cell death execution and differentially affects biosynthesis of oxylipins and resistance to pathogens. Mol Plant Microbe Interact 22:469–481

Labusch C, Shishova M, Effendi Y, Li M, Wang X, Scherer GF (2013) Patterns and timing in expression of early auxin-induced genes imply involvement of phospholipases A (pPLAs) in the regulation of auxin responses. Mol Plant 6(5):1473–1486, PMID: 23519456

Li M, Bahn SC, Guo L, Musgrave W, Berg H, Welti R et al (2011) Patatin-related phospholipase pPLAIIIβ-induced changes in lipid metabolism alter cellulose content and cell elongation in Arabidopsis. Plant Cell 23:1107–1123

Li M, Bahn SC, Fan C, Li J, Phan T, Ortiz M et al (2013) Patatin-related phospholipase pPLAIIIδ increases seed oil content with long-chain fatty acids in Arabidopsis. Plant Physiol 162:39–51

Lin CC, Chu CF, Liu PH, Lin HH, Liang SC, Hsu WE et al (2011) Expression of an Oncidium gene encoding a patatin-like protein delays flowering in Arabidopsis by reducing gibberellin synthesis. Plant Cell Physiol 52:421–435

Matos AR, Pham-Thi AT (2009) Lipid deacylating enzymes in plants: old activities, new genes. Plant Physiol Biochem 47:491–503

Matos AR, d'Arcy-Lameta A, França M, Pêtres S, Edelman L, Kader J et al (2001) A novel patatin-like gene stimulated by drought stress encodes a galactolipid acyl hydrolase. FEBS Lett 491:188–192

Matos AR, Gigon A, Laffray D, Pêtres S, Zuily-Fodil Y, Pham-Thi AT (2008) Effects of progressive drought stress on the expression of patatin-like lipid acyl hydrolase genes in Arabidopsis leaves. Physiol Plant 134:110–120

Moon SH, Jenkins CM, Liu X, Guan S, Mancuso DJ, Gross RW (2012) Activation of mitochondrial calcium-independent phospholipase A2γ (iPLA2γ) by divalent cations mediating arachidonate release and production of downstream eicosanoids. J Biol Chem 287:14880–14895

Qiao Y, Piao R, Shi J, Lee SI, Jiang W, Kim BK et al (2011) Fine mapping and candidate gene analysis of dense and erect panicle 3, DEP3, which confers high grain yield in rice (Oryza sativa L.). Theor Appl Genet 122:1439–1449

Quettier AL, Eastmond PJ (2009) Storage oil hydrolysis during early seedling growth. Plant Physiol Biochem 47:485–490

Rietz S, Holk A, Scherer GF (2004) Expression of the patatin-related phospholipase A gene AtPLA IIA in Arabidopsis thaliana is up-regulated by salicylic acid, wounding, ethylene, and iron and phosphate deficiency. Planta 219:743–753

Rietz S, Dermendjiev G, Oppermann E, Tafesse FG, Effendi Y, Holk A et al (2010) Roles of Arabidopsis patatin-related phospholipases a in root development are related to auxin responses and phosphate deficiency. Mol Plant 3:524–538

Romeo S, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA et al (2008) Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. Nat Genet 40:1461–1465

Rudolph M, Schlereth A, Körner M, Feussner K, Berndt E, Melzer M et al (2011) The lipoxygenase-dependent oxygenation of lipid body membranes is promoted by a patatin-type phospholipase in cucumber cotyledons. J Exp Bot 62:749–760

Ryu SB (2004) Phospholipid-derived signaling mediated by phospholipase A in plants. Trends Plant Sci 9:229–235

Schaloske RH, Dennis EA (2006) The phospholipase A_2 superfamily and its group numbering system. Biochim Biophys Acta 1761:1246–1259

Scherer GF, Ryu SB, Wang X, Matos AR, Heitz T (2010) Patatin-related phospholipase A: nomenclature, subfamilies and functions in plants. Trends Plant Sci 15:693–700

Scherer GF, Labusch C, Effendi Y (2012) Phospholipases and the network of auxin signal transduction with ABP1 and TIR1 as two receptors: a comprehensive and provocative model. Front Plant Sci 3:56

Schoenborn V, Heid IM, Vollmert C, Lingenhel A, Adams TD, Hopkins PN et al (2006) The ATGL gene is associated with free fatty acids, triglycerides, and type 2 diabetes. Diabetes 55:1270–1275

Siloto RM, Madhavji M, Wiehler WB, Burton TL, Boora PS, Laroche A et al (2008) An N-terminal fragment of mouse DGAT1 binds different acyl-CoAs with varying affinity. Biochem Biophys Res Commun 373:350–354

Singh A, Baranwal V, Shankar A, Kanwar P, Ranjan R, Yadav S et al (2012) Rice phospholipase A superfamily: organization, phylogenetic and expression analysis during abiotic stresses and development. PLoS One 7:e30947

Six DA, Dennis EA (2000) The expanding superfamily of phospholipase A(2) enzymes: classification and characterization. Biochim Biophys Acta 1488:1–19

Sookoian S, Pirola CJ (2012) PNPLA3, the triacylglycerol synthesis/hydrolysis/storage dilemma, and nonalcoholic fatty liver disease. World J Gastroenterol 18:6018–6026

van Tienhoven M, Atkins J, Li Y, Glynn P (2002) Human neuropathy target esterase catalyzes hydrolysis of membrane lipids. J Biol Chem 277:20942–20948

Weselake RJ, Madhavji M, Szarka SJ, Patterson NA, Wiehler WB, Nykiforuk CL et al (2006) Acyl-CoA-binding and self-associating properties of a recombinant 13.3 kDa N-terminal fragment of diacylglycerol acyltransferase-1 from oilseed rape. BMC Biochem 7:24

Wilson PA, Gardner SD, Lambie NM, Commans SA, Crowther DJ (2006) Characterization of the human patatin-like phospholipase family. J Lipid Res 47:1940–1949

Yan W, Jenkins CM, Han X, Mancuso DJ, Sims HF, Yang K et al (2005) The highly selective production of 2-arachidonoyl lysophosphatidylcholine catalyzed by purified calcium-independent phospholipase A2γ: identification of a novel enzymatic mediator for the generation of a key branch point intermediate in eicosanoid signaling. J Biol Chem 280:26669–26679

Yang W, Devaiah SP, Pan X, Isaac G, Welti R, Wang X (2007) AtPLAI is an acyl hydrolase involved in basal jasmonic acid production and Arabidopsis resistance to Botrytis cinerea. J Biol Chem 282:18116–18128

Yang W, Zheng Y, Bahn SC, Pan X, Li M, Vu H et al (2012) The patatin-containing phospholipase A pPLAIIα modulates oxylipin formation and water loss in Arabidopsis thaliana. Mol Plant 5:452–460

sPLA$_2$ and PLA$_1$: Secretory Phospholipase A$_2$ and Phospholipase A$_1$ in Plants

Hae Jin Kim and Stephen Beungtae Ryu

Abstract Plant phospholipase As (PLAs) are classified into two major types, PLA$_1$ and PLA$_2$, according to the hydrolysis sites of their membrane lipids. The lipid products released by PLAs have been suggested to act as bioactive molecules that mediate cellular signaling pathways functioning in plant growth and development, as well as responses to abiotic and biotic stimuli. The past few years have witnessed a wealth of new information regarding the function of these phospholipases in various biological processes. In this chapter, we discuss recent insights into lipid-based signaling mediated by PLAs and their lipid products, with particular emphasis on their emerging role as lipid mediators.

Keywords PLA$_2$ • PLA$_1$ • Signaling • Cellular roles Lipid mediators

1 Introduction

Phospholipid-derived products generated by phospholipase A (PLA), such as free fatty acids (FFAs) and lysophospholipids, play critical roles in plants, as these products are the precursors of second messengers in signal transduction pathways. In addition, these signaling molecules function in important physiological processes such as cell elongation, gravitropism, anther dehiscence, biosynthesis of jasmonic acid (JA), and defense signaling (Chen et al. 2011; Ryu 2004; Wang et al. 2012). To understand the highly regulated production of these lipid mediators in response to diverse extracellular stimuli, it is important to study the functions and regulatory mechanisms of the diverse PLA enzymes. Recent developments in

H.J. Kim
Department of Biochemistry, University of Nebraska-Lincoln, Lincoln, NE 68588, USA

S.B. Ryu (✉)
Environmental Biotechnology Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon 305-806, South Korea
e-mail: sbryu@kribb.re.kr

X. Wang (ed.), *Phospholipases in Plant Signaling*, Signaling and Communication in Plants 20, DOI 10.1007/978-3-642-42011-5_6,

genetic and biochemical analysis have facilitated our understanding of the cellular functions of plant PLAs. The PLA superfamily has three subtypes, i.e., phospholipase A_1 (PLA_1), phospholipase A_2 (PLA_2), and patatin-like PLA (pPLA). PLA_1 and PLA_2 catalyze the hydrolysis of membrane glycerophospholipids at their sn-1 or sn-2 positions, respectively, while pPLA shows activity at both positions. In this chapter, we will describe the current classifications of PLA_1s and PLA_2s that have been identified in plants, especially in *Arabidopsis*, and recent advances in our understanding of how these PLAs are involved in various cellular signaling pathways.

2 Phospholipase A_2

Based on the functional, structural, and catalytic properties of lipolytic enzymes, the PLA_2 superfamily can generally be divided into five principal families in animals, categorized as secretory PLA_2s ($sPLA_2$), cytosolic PLA_2s ($cPLA_2$), Ca^{2+}-independent PLA_2s ($iPLA_2$s), platelet-activating factor acetyl hydrolases (PAF-AHs), and lysosomal PLA_2s (Schaloske and Dennis 2006). Only low molecular weight secretory PLA_2s have been reported in plants, although patatin-like PLAs have been identified, which are similar to the $iPLA_2$s or $cPLA_2$ but exhibit both PLA_1- and PLA_2-like activity (Lee et al. 2005; Ryu 2004).

2.1 $sPLA_2$ Grouping, Expression, and Localization

Four and three secretory PLA_2 paralogs were identified in the *Arabidopsis* and rice genomes, respectively. Based on their primary structures, plant $sPLA_2$s are equivalent to animal group XI $sPLA_2$s, which are further divided into groups XIA and XIB. The former group includes $AtsPLA_2$-α, $OsPLA_2$-α, and $OsPLA_2$-β, while the latter group includes $AtsPLA_2$-β, $AtsPLA_2$-γ, $AtsPLA_2$-δ, and $OsPLA_2$-γ (Lee et al. 2005; Schaloske and Dennis 2006; Singh et al. 2012).

$AtsPLA_2$s exhibit different spatial and temporal expression patterns. *$AtsPLA_2$-α* and *AtsPLA2-β* are expressed in all sporophytic tissues. Unlike *$AtsPLA_2$-α*, *$AtsPLA_2$-β* is strongly expressed in actively growing young tissues and pollen. *$AtsPLA_2$-γ* and *$AtsPLA_2$-δ* are exclusively expressed in pollen. While *$AtsPLA_2$-β* is expressed during all stages of pollen development, *$AtsPLA_2$-γ* is expressed at low levels during the early stage of pollen formation and is strongly expressed at the mature stage, and *$AtsPLA_2$-δ* is expressed at the mature pollen stage (Bahn et al. 2003; Kim et al. 2011b; Lee et al. 2003).

Studies of the subcellular localizations of plant $sPLA_2$ have been performed using fused fluorescence proteins. These studies provide important insights into the functions of these proteins in plants. Early experiments involving transient expression analysis of green fluorescence protein (GFP) in onion epidermal cells showed

that AtsPLA$_2$-β and AtsPLA$_2$-γ are secreted into the cell wall/extracellular space (Bahn et al. 2003; Lee et al. 2003), but this localization pattern is regarded as a result of the masking of the ER retention signal (KTEL) by that of the C terminal GFP fusion protein of AtsPLA$_2$-β. More recently, Seo et al. (2008) reexamined the subcellular localization of AtsPLA$_2$-β with N terminal GFP fusion protein of AtsPLA$_2$-β and observed that it indeed localizes to the ER in *Vicia faba* guard cells, and the result was supported in AtsPLA$_2$-β:YFP transgenic plants (Lee et al. 2010). Transient expression of *AtsPLA$_2$-γ:YFP* and *AtsPLA$_2$-δ:YFP* in tobacco leaf epidermal cells demonstrated that AtsPLA$_2$-γ localizes to the ER and Golgi, while AtsPLA$_2$-δ localizes to the ER and is found in the pollen of transgenic plants (Kim et al. 2011b). Subcellular localization of AtsPLA$_2$-α revealed a rather complicated localization pattern unlike that of its paralogs. An analysis of *AtsPLA$_2$-α:DsRed2* transgenic *Arabidopsis* plants suggests that AtsPLA$_2$-α localizes to the Golgi in root tissues (Lee et al. 2010). In another study, AtsPLA$_2$-α exhibited different localizations in a time-dependent manner when this gene was introduced into *Arabidopsis* seedlings and tobacco leaves via *Agrobacterium*-mediated transient transformation (Froidure et al. 2010). The AtsPLA$_2$-α:YFP signal was detected in cytoplasmic vesicles around the nucleus 36 h after inoculation and was detected at the extracellular spaces outside of the cells at 48 h after inoculation. AtsPLA$_2$-α was partially localized to the cell nucleus when it was co-expressed with AtMYB30.

A more recent study shows that subcellular localization of AtsPLA$_2$-α is dependent on the developmental stage of the leaf tissue. Fluorescence signals are present primarily at the Golgi apparatus in premature young leaves in transgenic AtsPLA$_2$-α:RFP plants, while these signals are detected primarily in the apoplasts in mature leaves (Jung et al. 2012). Also, translocation of AtsPLA$_2$-α to the apoplast is stimulated by bacterial infection in premature young leaves. In contrast, the signal of GFP:OssPLA$_2$-α merges with that of an endoplasmic marker in onion epidermal cells, which indicates that the transiently expressed OssPLA$_2$-α localizes to the ER (Singh et al. 2012). RFP fusion protein, but not GFP and YFP, is relatively stable in the apoplasts. Thus, it is desirable to use RFP fusion protein rather than GFP or YFP fusion proteins for apoplastic localization studies.

2.2 *sPLA$_2$ Functions*

Plant PLA$_2$s have been implicated in important physiological processes such as development, senescence, biotic and abiotic stress responses, and the induction of secondary metabolite accumulation (Lee et al. 2005; Mansfeld 2009; Ryu 2004; Wang 2001). Lysophosphatidylcholine (LPC) and linolenic acid, which are the products of PLA$_2$, induce a decline in pH and accelerate the elongation of corn coleoptiles (Yi et al. 1996). Also, the PLA$_2$ inhibitors aristolochic acid and manoalide inhibit the auxin-induced pH decrease and coleoptile elongation. These results suggest that PLA$_2$ is activated by auxin, and its products induce

acidification of the apoplast by activating the H^+ pump through the signal transduction pathway of protein kinase, which in turn promotes corn coleoptile elongation (Yi et al. 1996). This hypothesis was supported by Lee et al. (2003) using transgenic plants. These authors reported that *AtsPLA$_2$-β* transcripts are induced by auxin treatment. In addition, RNA interference-mediated silencing (RNAi) of *AtsPLA$_2$-β* expression retards cell elongation, while overexpression of *AtsPLA$_2$-β* promotes cell elongation. Moreover, *AtsPLA$_2$-β* overexpressors exhibit faster stomatal opening than wild type, and AtsPLA$_2$-β-RNAi plants exhibit delayed light-induced stomatal opening, which can be rescued by exogenous application of LPC or lysophosphatidylethanolamine (LPE). Also, exogenous applications of LPC or LPE enhance stomatal opening in wild-type plants (Seo et al. 2008). In contrast, AtsPLA$_2$-β, AtsPLA$_2$-γ, and AtsPLA$_2$-δ are involved in pollen development and pollen germination (Kim et al. 2011b). *AtsPLA$_2$-β* may play a more vital role in pollen development, while *AtsPLA$_2$-γ* and *-δ* may function in pollen germination and pollen tube growth. Also, pollen germination is inhibited by the application of sPLA$_2$ inhibitors and is recovered by exogenous application of LPE but not of LPC or lysophosphatidic acid (LPA). These results indicate that LPE in particular is a key signal molecule in pollen germination and tube growth (Kim et al. 2011b).

Both AtsPLA$_2$-α-RNAi *Arabidopsis* plants and *Arabidopsis* seedlings treated with the sPLA$_2$ inhibitor ONO-RS-082 exhibit significantly disrupted plasma membrane (PM) localization of PINs in the root tissues, causing internal PIN compartments to form (Lee et al. 2010). Application of exogenous LPE restores the PM localization of PINs in an *AtsPLA$_2$-α* mutant and in ONO-RS-082-treated seedling. These results indicate that AtsPLA$_2$-α modulates PIN-FORMED protein trafficking to the PM in *Arabidopsis* roots, thereby revealing that sPLA$_2$ also plays an important role in intracellular membrane trafficking in plants (Lee et al. 2010). AtsPLA$_2$-α was also suggested to be as a negative regulator of the defense response through its interaction with AtMYB30, a transcription factor that functions in the hypersensitive response in short-day conditions (Froidure et al. 2010). Intriguingly, the regulation does not depend on the enzymatic activity of AtsPLA$_2$-α but on the just physical binding of AtsPLA$_2$-α to A+MYB30.

Aristolochic acid reduces root elongation and causes radial swelling of the root tip caused by microtubule disorganization (Gardiner et al. 2008). This indicates that PLA$_2$ is involved in microtubule organization and anisotropic growth. Another study employing aristolochic acid suggests that PLA$_2$ plays a critical role in programmed cell death induced by misexpression of fatty acid elongation, likely involving the exchange of very long chain fatty acids (VLCFAs) between phospholipids and the acyl-CoA pool (Reina-Pinto et al. 2009). PLA$_2$ is involved in salt stress-induced LPA production in the unicellular green alga *Chlamydomonas* (Meijer et al. 2001). LPA accumulates in *Chlamydomonas* under conditions of salt and nonionic hyperosmotic stress. The fact that LPA is generated by PLA$_2$ was recently confirmed using differential ^{32}P-radiolabeling experiments (Arisz and Munnik 2011).

3 Phospholipase A1

Plant PLA$_1$s are classified based on the presence of N terminal stretches, sequence similarities in the catalytic region, and substrate specificity. These PLA$_1$s include group I, II, and III PLA$_1$, phosphatidic acid-specific PLA$_1$ (PA-PLA$_1$), and lecithin: cholesterol acyltransferase-like PLA$_1$ (LCAT-PLA$_1$) in *Arabidopsis* and group I and II PLA$_1$ and PA-PLA$_1$ in rice (Chen et al. 2011; Singh et al. 2012).

3.1 Class I PLA$_1$s

Class I PLA$_1$s include seven PLA$_1$ genes in *Arabidopsis* and eight in rice. Class I PLA$_1$s are defined by the presence of a putative N terminal chloroplast-targeting signal (Chen et al. 2011; Seo et al. 2009). All class I PLA$_1$s have a GXSXG motif in the lipase class 3 domain and catalytic triad (Ser, Asp and His residues). All GFP-class I PLA$_1$ fusion proteins localize to the chloroplast (Ellinger et al. 2010; Grienenberger et al. 2010; Hyun et al. 2008; Ishiguro et al. 2001; Seo et al. 2009). However, a second opinion about the subcellular localization of AtPLA$_1$-Iα1 has recently emerged (Ellinger et al. 2010). In this study, AtPLA$_1$-Iα1 was co-localized with cytoplasmic lipid bodies instead of localizing to chloroplasts.

Defective in Anther Dehiscence1 (DAD1, *AtPLA$_1$-Iβ1*) was the first reported PLA$_1$ enzyme in *Arabidopsis*. This enzyme preferentially hydrolyzes phosphatidylcholine (PC) at the sn-1 position and regulates anther dehiscence in flowers by releasing linolenic acid for the initial step of JA biosynthesis (Ishiguro et al. 2001). DONGLE (DGL, *AtPLA$_1$-I*α1) and DAD1 are necessary and sufficient for JA production (Hyun et al. 2008). DGL plays a specific role in maintaining basal JA content under normal conditions. During wounding, DGL was shown to be required for the rapid JA burst in the early phase, and DAD1 was shown to play a role in the late phase of JA production. However, the initial step in the biosynthesis of wound- and pathogen-induced JA production remains controversial. Ellinger et al. (2010) suggest that *AtPLA$_1$-Iγ1* is a novel target gene for the manipulation of jasmonate biosynthesis and that, in addition to DAD1 and AtPLA$_1$-Iγ1, as yet unidentified enzymes with sn-1 and sn-2 hydrolase activity are involved in stress-induced JA formation, indicating that there is functional redundancy within the lipase family.

3.2 Class II PLA$_1$s

Class II PLA$_1$s comprise four PLA$_1$ genes in *Arabidopsis* and three in rice (Chen et al. 2011; Singh et al. 2012). Based on their sequence homology, PLA$_1$s from other species are also included in class II, including *Dclipase* from *Dianthus caryophyllus* (carnation) (Hong et al. 2000), *LeLID1* from *Lycopersicon esculentum*

(tomato) (Matsui et al. 2004) and *CaPLA$_1$* from *Capsicum annuum* (hot pepper) (Seo et al. 2007). Class II PLA$_1$s lack N terminal signal peptides and are predicted to localize to the cytosol. Cytosolic localization of AtPLA$_1$-IIδ and DAD1-like Seedling Establishment-related Lipase (AtDSEL, AtPLA$_1$-IIγ) were confirmed through GFP fusion protein studies (Kim et al. 2011a; Lo et al. 2004). *AtPLA$_1$-IIδ* expression is induced by treatment with sublethal levels of UV-B. Plants with suppressed *AtPLA$_1$-IIδ* expression via antisense technology exhibit increased tolerance to sublethal levels of UV-B stress and are unable to upregulate the expression of *pathogenesis-related protein 1* (*PR-1*) in response to UV-B treatment. These results indicate that AtPLA$_1$-IIδ is capable of deesterifying membrane phospholipids and is induced in response to UV-B irradiation (Lo et al. 2004). Recombinant AtDSEL expressed in *Escherichia coli* (*E. coli*) shows a preference for 1,3-diacylglycerol and 1-monoacylglycerol over PC, which suggests that AtDSEL is a sn-1-specific lipase.

AtDSEL overexpressors are defective in post-germinative seedling growth on medium lacking an exogenous carbon source; this phenotype is rescued by the addition of sucrose to the growth medium. By contrast, *atdsel-1* and *atdsel-2* exhibit a mildly fast-growing phenotype in the absence of an exogenous carbon source. *AtDSEL*-overexpressors retained numerous peroxisomes and oil bodies in their 5-day-old cotyledons, while these organelles are exhausted in wild-type or mutant cotyledons. These results suggest that AtDSEL is involved in the negative regulation of seedling establishment by inhibiting the breakdown of storage oils (Kim et al. 2011a). *Dclipase* transcript levels increase just as carnation flowers begin to senesce, and the expression of this gene is also induced by ethylene treatment. Southern blot analysis confirmed that these flowers contain a single copy of *Dclipase*. *Dclipase* is predicted to be involved in mediating the onset of senescence (Hong et al. 2000). Recombinant LeLID1 protein exhibits high activity against triacylglycerols (TAGs) with long acyl chains but little activity against PC or monogalactosyldiacylglycerol. Transcript levels of *LeLID1* increase rapidly in seeds during germination, reaching a maximum level just before cotyledon opening, followed by a rapid decrease. Low levels of *LeLID1* expression are detected in flowers and fruits, while none can be detected in roots. LeLID1 is thought to function as a lipase during lipid mobilization to liberate fatty acids from TAG's stored in oil bodies (Matsui et al. 2004).

3.3 Class III PLA$_1$

There is only one class III PLA$_1$ in the *Arabidopsis* genome and none in the rice genome (Chen et al. 2011; Singh et al. 2012). Recombinant DAD1-like acylhydrolase (AtDLAH, AtPLA$_1$-III) displays a stronger preference for 1-lysophosphatidylcholine, 1-monoacylglycerol, and phosphatidic acid than for PC, which indicates that AtDLAH is an sn-1-specific acylhydrolase. AtDLAH exclusively localizes to the mitochondria. Seeds of *Arabidopsis AtDLAH*

overexpressors are more tolerant to accelerated-aging treatment than wild type, and thus these seeds have higher germination rate than wild-type seeds. By contrast, *atdlah* knockout mutant seeds are susceptible to accelerated-aging conditions. These results suggest that AtDLAH plays an important role in *Arabidopsis* seed viability (Seo et al. 2011).

4 PA-PLA$_1$

SGR2 in *Arabidopsis* is classified as a PA-PLA$_1$ based on the similarity of its domain structures to that of a mammalian PA-PLA$_1$. One gene of PA-PLA$_1$ was found to be present in the *Arabidopsis* and rice genome. The fusion protein of AtPA-PLA$_1$ and GFP localizes to the membranes of vacuoles and small organelles. However, the localization of OsPA-PLA$_1$ was predicted to be nuclear using in silico tools. AtPA-PLA$_1$ mutants exhibit abnormal gravitropism in inflorescence stems and hypocotyls and irregularly shaped seeds. Mutant analysis suggests that AtPA-PLA$_1$ is involved in a vacuolar membrane system that affects the early step of shoot gravitropism. Microarray data and quantitative RT-PCR in rice show that *OsPA-PLA$_1$* transcripts are upregulated in response to salt and drought stress (Kato et al. 2002; Morita et al. 2002; Singh et al. 2012). In animals, LPA is a phospholipid mediator with multiple biological roles, functions via interactions with G protein-coupled seven-transmembrane receptors (GPCRs), and is implicated in various human diseases. While two pathways for LPA production have been identified in animal cells, little is known about the function of LPA at the molecular level. It was demonstrated that LPA produced by hair follicle-specific PA-PLA$_1$ is an important signaling molecule for hair follicle development, which functions by modulating epidermal growth factor receptor signaling (Aoki et al. 2008; Chen et al. 2011; Inoue et al. 2011). However, PLA$_1$ activity was not detected with recombinant AtPA-PLA$_1$ proteins expressed in *E. coli*. Moreover, GPCRs are not found in plants. Elucidating the mechanisms of PA-PLA$_1$ function in plants will be challenging.

5 LCAT-PLA1

During a search for plant genes encoding enzymes involved in sterol esterification by free acids, At3g03310 was identified as lecithin:cholesterol acyltransferase (LCAT) (Noiriel et al. 2004). This gene has sequence homology with the recently discovered gene encoding phosphatidylcholine:diacylglycerol acyltransferase from *Saccharomyces cerevisiae* (*ScPDAT*). PLA$_1$ activity was first revealed by LCAT or PDAT assays, and the gene encoding PLA$_1$ was designated *AtLCAT-PLA$_1$* (Chen et al. 2011; Noiriel et al. 2004). Yeast expressing *AtLCAT-PLA$_1$* accumulates TAG

(Noiriel et al. 2004). Thus, AtLCAT-PLA$_1$ may play a role in acyl-editing during TAG formation (Bates et al. 2009; Lu et al. 2009) but not in signaling.

6 Conclusions

Recent discoveries have significantly advanced our understanding of the biochemical and genetic requirements of distinct phospholipid signaling in plants. However, several unanswered questions still remain. For example, which isoforms of the PLA families are activated in response to specific external stimuli? What are the downstream targets of the lipid signals that are generated by PLA? These targets may be lysophospholipid-specific receptors, protein kinases, mitogen-activated protein kinase, or other signaling enzymes. And what are the upstream regulators of PLA activation? These regulators may take the form of receptors, activators, inhibitors, or hormones. The next coming years will likely produce significant advances towards the elucidation of PLA-mediated membrane phospholipid signaling in plants at both the biochemical and genetic levels. Moreover, the construction of knockout mutants and activation tagging lines, as well as the analysis of rapid protein activation without involving gene expression, will help clarify the phospholipid-derived signaling cascades.

References

Aoki J, Inoue A, Okudaira S (2008) Two pathways for lysophosphatidic acid production. Biochim Biophys Acta 1781:513–518

Arisz SA, Munnik T (2011) The salt stress-induced LPA response in Chlamydomonas is produced via PLA2 hydrolysis of DGK-generated phosphatidic acid. J Lipid Res 52:2012–2020

Bahn SC, Lee HY, Kim HJ, Ryu SB, Shin JS (2003) Characterization of Arabidopsis secretory phospholipase A2-γ cDNA and its enzymatic properties. FEBS Lett 553:113–118

Bates PD, Durrett TP, Ohlrogge JB, Pollard M (2009) Analysis of acyl fluxes through multiple pathways of triacylglycerol synthesis in developing soybean embryos. Plant Physiol 150:55–72

Chen G, Snyder CL, Greer MS, Weselake RJ (2011) Biology and biochemistry of plant phospholipases. Crit Rev Plant Sci 30:239–258

Ellinger D, Stingl N, Kubigsteltig II, Bals T, Juenger M, Pollmann S, Berger S, Schuenemann D, Mueller MJ (2010) DONGLE and DEFECTIVE IN ANTHER DEHISCENCE1 lipases are not essential for wound-and pathogen-induced jasmonate biosynthesis: redundant lipases contribute to jasmonate formation. Plant Physiol 153:114–127

Froidure S, Canonne J, Daniel X, Jauneau A, Brière C, Roby D, Rivas S (2010) AtsPLA2-α nuclear relocalization by the Arabidopsis transcription factor AtMYB30 leads to repression of the plant defense response. Proc Natl Acad Sci U S A 107:15281–15286

Gardiner J, Andreeva Z, Barton D, Ritchie A, Overall R, Marc J (2008) The phospholipase A2 inhibitor, aristolochic acid, disrupts cortical microtubule arrays and root growth in Arabidopsis. Plant Biol 10:725–731

Grienenberger E, Geoffroy P, Mutterer J, Legrand M, Heitz T (2010) The interplay of lipid acyl hydrolases in inducible plant defense. Plant Signal Behav 5:1181–1186

Hong Y, Wang T-W, Hudak KA, Schade F, Froese CD, Thompson JE (2000) An ethylene-induced cDNA encoding a lipase expressed at the onset of senescence. Proc Natl Acad Sci U S A 97:8717–8722

Hyun Y, Choi S, Hwang H-J, Yu J, Nam S-J, Ko J, Park J-Y, Seo YS, Kim EY, Ryu SB (2008) Cooperation and functional diversification of two closely related galactolipase genes for jasmonate biosynthesis. Dev Cell 14:183–192

Inoue A, Arima N, Ishiguro J, Prestwich GD, Arai H, Aoki J (2011) LPA-producing enzyme PA-PLA1a regulates hair follicle development by modulating EGFR signalling. EMBO J 30:4248–4260

Ishiguro S, Kawai-Oda A, Ueda J, Nishida I, Okada K (2001) The DEFECTIVE IN ANTHER DEHISCENCE1 gene encodes a novel phospholipase A1 catalyzing the initial step of jasmonic acid biosynthesis, which synchronizes pollen maturation, anther dehiscence, and flower opening in Arabidopsis. Plant Cell 13:2191–2209

Jung J, Kumar K, Lee HY, Park Y-I, Cho H-T, Ryu SB (2012) Translocation of phospholipase A2α to apoplasts is modulated by developmental stages and bacterial infection in Arabidopsis. Front Plant Sci 3:126

Kato T, Morita MT, Fukaki H, Yamauchi Y, Uehara M, Niihama M, Tasaka M (2002) SGR2, a phospholipase-like protein, and ZIG/SGR4, a SNARE, are involved in the shoot gravitropism of Arabidopsis. Plant Cell 14:33–46

Kim EY, Seo YS, Kim WT (2011a) AtDSEL, an *Arabidopsis* cytosolic DAD1-like acylhydrolase, is involved in negative regulation of storage oil mobilization during seedling establishment. J Plant Physiol 168:1705–1709

Kim HJ, Ok SH, Bahn SC, Jang J, Oh SA, Park SK, Twell D, Ryu SB, Shin JS (2011b) Endoplasmic reticulum–and golgi-localized phospholipase A2 plays critical roles in Arabidopsis pollen development and germination. Plant Cell 23:94–110

Lee HY, Bahn SC, Kang YM, Lee KH, Kim HJ, Noh EK, Palta JP, Shin JS, Ryu SB (2003) Secretory low molecular weight phospholipase A2 plays important roles in cell elongation and shoot gravitropism in Arabidopsis. Plant Cell 15:1990–2002

Lee HY, Bahn SC, Shin JS, Hwang I, Back K, Doelling JH, Ryu SB (2005) Multiple forms of secretory phospholipase A2 in plants. Prog Lipid Res 44:52–67

Lee O, Kim S, Kim H, Hong J, Ryu S, Lee S, Ganguly A, Cho H (2010) Phospholipase A2 is required for PIN-FORMED protein trafficking to the plasma membrane in the Arabidopsis root. Plant Cell 22:1812–1825

Lo M, Taylor C, Wang L, Nowack L, Wang T-W, Thompson J (2004) Characterization of an ultraviolet B-induced lipase in Arabidopsis. Plant Physiol 135:947–958

Lu C, Xin Z, Ren Z, Miquel M (2009) An enzyme regulating triacylglycerol composition is encoded by the ROD1 gene of Arabidopsis. Proc Natl Acad Sci U S A 106:18837–18842

Mansfeld J (2009) Plant phospholipases A2: perspectives on biotechnological applications. Biotechnol Lett 31:1373–1380

Matsui K, Fukutomi S, Ishii M, Kajiwara T (2004) A tomato lipase *homologous to DAD1*(*LeLID1*) is induced in post-germinative growing stage and encodes a triacylglycerol lipase. FEBS Lett 569:195–200

Meijer HJ, Arisz SA, Van Himbergen JA, Musgrave A, Munnik T (2001) Hyperosmotic stress rapidly generates lyso-phosphatidic acid in Chlamydomonas. Plant J 25:541–548

Morita MT, Kato T, Nagafusa K, Saito C, Ueda T, Nakano A, Tasaka M (2002) Involvement of the vacuoles of the endodermis in the early process of shoot gravitropism in Arabidopsis. Plant Cell 14:47–56

Noiriel A, Benveniste P, Banas A, Stymne S, Bouvier-Nave P (2004) Expression in yeast of a novel phospholipase A1 cDNA from *Arabidopsis thaliana*. Eur J Biochem 271:3752–3764

Reina-Pinto JJ, Voisin D, Kurdyukov S, Faust A, Haslam RP, Michaelson LV, Efremova N, Franke B, Schreiber L, Napier JA (2009) Misexpression of FATTY ACID ELONGATION1 in the Arabidopsis epidermis induces cell death and suggests a critical role for phospholipase A2 in this process. Plant Cell 21:1252–1272

Ryu SB (2004) Phospholipid-derived signaling mediated by phospholipase A in plants. Trends Plant Sci 9:229–235

Schaloske R, Dennis E (2006) The phospholipase A2 superfamily and its group numbering system. Biochim Biophys Acta 1761:1246–1259

Seo YS, Kim EY, Mang HG, Kim WT (2007) Heterologous expression, and biochemical and cellular characterization of CaPLA1 encoding a hot pepper phospholipase A1 homolog. Plant J 53:895–908

Seo J, Lee HY, Choi H, Choi Y, Lee Y, Kim YW, Ryu SB, Lee Y (2008) Phospholipase A2-b mediates light-induced stomatal opening in Arabidopsis. J Exp Bot 59:3587–3594

Seo YS, Kim EY, Kim JH, Kim WT (2009) Enzymatic characterization of class I DAD1-like acylhydrolase members targeted to chloroplast in *Arabidopsis*. FEBS Lett 583:2301–2307

Seo YS, Kim EY, Kim WT (2011) The Arabidopsis sn-1-specific mitochondrial acylhydrolase AtDLAH is positively correlated with seed viability. J Exp Bot 62:5683–5698

Singh A, Baranwal V, Shankar A, Kanwar P, Ranjan R, Yadav S, Pandey A, Kapoor S, Pandey GK (2012) Rice phospholipase A superfamily: organization, phylogenetic and expression analysis during abiotic stresses and development. PLoS One 7:e30947

Wang X (2001) Plant phospholipases. Annu Rev Plant Physiol Plant Mol Biol 52:211–231

Wang G, Ryu S, Wang X (2012) Plant phospholipases: an overview. Methods Mol Biol 861:123–137

Yi HJ, Park D, Lee Y (1996) In vivo evidence for the involvement of phospholipase A and protein kinase in the signal transduction pathway for auxin-induced corn coleoptile elongation. Physiol Plant 96:359–368

Part II
Phospholipase Signalling in Response to Environmental Stresses

Phospholipase Ds in Plant Response to Hyperosmotic Stresses

Qun Zhang, Yana Qu, Wen Jing, Li Li, and Wenhua Zhang

Abstract Many adverse environmental conditions, including drought, high salinity, and freezing, induce hyperosmotic stresses to plant cells. Extensive efforts have been made to elucidate sensory and signal transduction mechanisms that perceive hyperosmotic stress and control cellular homeostasis in plants. In these cellular processes, phospholipase D (PLD) and its lipid product phosphatidic acid (PA) act as essential signal transducers. Recent studies have identified and characterized an increasing number of targets for both PLD and PA, but especially PA. These targets include protein kinases and phosphatases, NADPH oxidase, G proteins, and microtubule- and actin-related proteins. Through interaction with these targets, PLD/PA regulate a variety of cellular activities, including sodium transport, oxidative burst, cytoskeletal organization (and reorganization), and ABA signaling. This chapter will be focused on the advances in knowledge of molecular and cellular mechanisms and physiological functions of PLD/PA in plant response to hyperosmotic stresses.

Keywords Phospholipase D • Hyperosmotic stress • PA targets

1 Introduction

Hyperosmotic stresses such as high salinity, drought, and freezing are major determinants of crop yield throughout the world. These adverse conditions induce osmotic stresses in plant cells by decreasing water availability, leading to a loss of cell turgor. To survive in a changing environment, plants must initiate intracellular and physiological signaling networks to respond rapidly and efficiently to such stresses (Zhu 2002). These responses are manifested as changes in the activation of

Q. Zhang (✉) • Y. Qu • W. Jing • L. Li • W. Zhang (✉)
State Key Laboratory of Crop Genetics and Germplasm Enhancement, College of Life Science, Nanjing Agricultural University, Nanjing 210095, China
e-mail: zhangqun@njau.edu.cn; whzhang@njau.edu.cn

X. Wang (ed.), *Phospholipases in Plant Signaling*, Signaling and Communication in Plants 20, DOI 10.1007/978-3-642-42011-5_7,

ion channels, reorganization of membrane trafficking, changes in gene expression, and increased biosynthesis of osmoprotectants such as sucrose, betaines, and proline in the cytosol as a protective mechanism (Thiery et al. 2004).

During the past 2 decades, many key components of the signaling networks have been identified, including the stress hormone, abscisic acid (ABA), and its receptors, G proteins, protein kinases (calcium-dependent protein kinase and sucrose non-fermenting-1-related protein kinase 2, SnRK2), protein phophatases (ABI1 and ABI2), lipid-signaling molecules such as phosphatidic acid (PA) and sphingosine-1-phosphate, reactive oxygen species (ROS), and nitric acid (Ng et al. 2001; Santner and Estelle 2009, and references therein; Cutler et al. 2010, and references therein). Some of the interconnections among key components have been defined. Several reviews outlining lipid signaling in plants have been presented (Li et al. 2009; Munnik and Vermeer 2010; Zhang et al. 2010; Hong et al. 2010). This chapter highlights the components, mechanisms, and functions of phospholipase D (PLD) signaling in plant response to hyperosmotic stresses.

2 Multiple PLD Isoforms are Involved in Hyperosmotic Stress Response

2.1 Hyperosmotic Stress Induces PLD Activation

PLD is activated by hyperosmotic stresses or ABA in *Vicia faba*, tomato, tobacco, alfalfa, *Arabidopsis*, and resurrection plant *Craterostigma plantagineum* (Jacob et al. 1999; Munnik et al. 2000; Katagiri et al. 2001; Dhonukshe et al. 2003; Zhang et al. 2004, 2012; Bargmann et al. 2009). The *Arabidopsis* genome contains 12 *PLD* genes that are grouped into six types: three αs, two βs, three γs, one δ, one ε, and two ζs, based on gene architecture, sequence similarity, domain structure, biochemical properties, and the order of cDNA cloning (Qin and Wang 2002; Zhang et al. 2010). Different PLDs participate in different stress responses. The transcript of *PLDδ* increases under dehydration, NaCl, and ABA, but not cold treatments, whereas the transcript level of *PLDα* is not affected under these conditions (Katagiri et al. 2001). PLDα protein level remains unchanged but the activity increases transiently in *Arabidopsis* cells treated with NaCl (Zhang et al. 2012). Knockdown or knockout of *PLDα1* gene renders plants sensitive to drought and salinity stresses (Sang et al. 2001; Yu et al. 2009), and knockout of *PLDδ* leads to an increased sensitivity to freezing (Li et al. 2004). These results support the notion that PLDα1 and PLDδ are important components in plant response to hyperosmotic stresses.

There are 17 PLD genes in rice (*Oryza sativa*) and 18 in soybean (*Glycine max*) (Li et al. 2007; Zhao et al. 2012). Similar to *Arabidopsis*, the mRNA level of *OsPLDα1* was unchanged after NaCl treatments (Li et al. 2007). Under high salinity stress, the mRNA level of *OsPLDα2* and *OsPLDα3* increased but that of

OsPLDα4 and *OsPLDα5* decreased. In soybean, however, the mRNA level of *GmPLDαs* (*α1*, *α2*, and *α3*) increased during the NaCl treatment (Zhao et al. 2012).

2.2 Some PLDs Mutually Interact in Response to Stresses

PLDα1 and PLDδ interact in response to salt and drought stresses (Zhang et al. 2004, 2009; Bargmann et al. 2009; Guo et al. 2012a, b; Uraji et al. 2012). For example, although *pldδ* seedlings show the same survival as wild type under salt stress (Zhang et al. 2012), double mutant *pldα1pldδ* displays hypersensitivity of root growth compared to the single mutant (Bargmann et al. 2009). These results imply that PLDα and PLDδ interact directly or indirectly in response to salt stress. With regard to reduced, ABA-induced stomatal closing, the double mutants also show an additive phenotype as compared with single mutants, suggesting that PLDα1 and PLDδ cooperate in ABA signaling in guard cells.

The mechanisms of cooperation between PLDα1 and PLDδ in response to ABA were reported recently (Guo et al. 2012b). It is well known that ROS are key regulators of ABA-regulated signal pathways in guard cells (Pei et al. 2000; Zhang et al. 2001). Genetic ablation of *PLDα1* or *PLDδ* impedes stomatal response to ABA (Zhang et al. 2004; Guo et al. 2012b). Knockout of *PLDα1*, but not *PLDδ*, impairs ABA-induced ROS generation. ROS can induce stomatal closing in both mutants, thus placing PLDα1 upstream of ROS production, while PLDδ acts downstream of ROS in the signal transduction of ABA-induced stomatal closure (Guo et al. 2012b). When PLDα1 is activated by ABA, the produced PA binds to and activates NADPH oxidase to produce ROS. ROS transduces the signal by promoting the interaction of PLDδ and cytosolicglyceraldehyde-3-phosphate dehydrogenase (GAPC) that catalyzes the conversion of glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate in the glycolytic pathway. Such interactions may provide a direct connection between lipid signaling, energy metabolism, and growth control in the plant response to hyperosmotic stress (Guo et al. 2012b).

Interestingly, PLDα and PLDδ may act oppositely in response to freezing (Li et al. 2004, 2008). Knockout of *PLDα1* results in increased freezing tolerance, while PLD*δ* knockouts are more sensitive (Welti et al. 2002; Li et al. 2004; Rajashekar et al. 2006), suggesting a negative role for PLDα1 and a positive role for PLDδ in Arabidopsis freezing tolerance. Although an exact mechanism for such an opposite effect of the two PLDs has yet to be uncovered, PLDα1- and PLDδ-induced lipid compositional changes could be responsible (Li et al. 2008). Lipid profiling analysis has shown that freezing treatment induces a sustained PA increase, and 50 % of the PA formed during freezing is derived primarily from PC hydrolysis by PLDα1. The lower ratio of PA to PC after freezing reduces the propensity for formation of non-lamellar phase, hexagonal II phase, and thus enhances *PLDα1*-null plant tolerance to freezing. In contrast, in *PLDδ*-null seedlings, there is little reduction in PA levels compared with wild type. It seems that

PLD positively regulates freezing tolerance, perhaps by mitigating postfreezing damage and cell death (Li et al. 2004, 2008).

2.3 Manipulation of PLD to Improve Stress Tolerance

As stated above, knockdown of *PLDα1* increases water loss and renders plants more drought sensitive (Sang et al. 2001). On the other hand, overexpression of PLDα1 in tobacco promotes stomatal closure and decreases water loss at early phases of water deficits. With prolonged drought stress, however, the high PLDα1 activity in the overexpressed plants leads to more severe damage due to increased lipid hydrolysis and membrane degradation (Hong et al. 2008). However, when PLDα1 is expressed under the control of a guard cell-specific promoter *AtKatIpro*, the $AtKat1_{pro}$*::PLDα1*-expressed canola (*Brassica napus*) plants display decreased water loss, improved biomass accumulation, and higher seed yield under drought and high salinity (Lu et al. 2013). Thus, like the ROS, PLDα1 seems to act as a double-edged sword: When signaling molecules are at low levels, the PLD may activate downstream adaptive responses, but a sustained lipid hydrolysis by PLDα1 may lead to membrane damage or other degradative cellular responses (Welti et al. 2002).

Another PLD member, PLDα3, shows a different pattern of response to salinity and water deficit (Hong et al. 2008). Quantitative real-time PCR data show that the expression level of PLDα3 in most tissues is about 1,000-fold lower than that of PLDα1 in the absence of any stress. The *PLDα3*-KO plants display increased sensitivities to salt stress and water deficiency, while *PLDα3*-OE plants show decreased sensitivities. Moreover, *PLDα3*-KO plants flower later than wild-type plants under slightly dry conditions, whereas PLDα3-OE plants flower earlier (Hong et al. 2008).

3 The Mechanisms of Stress Signaling by PLDs

3.1 Protein Phosphorylation

3.1.1 MAP Kinase Cascades

Mitogen-activated protein kinase (MAPK) cascades play an essential role in plant signaling of various abiotic and biotic stresses. The Arabidopsis genome contains about 20 MAPKs, 10 MAPKKs, and more than 80 MAPKKKs (Tena et al. 2011). It has been reported that MAPKs, such as MAPK3, MAPK4, and MAPK6, are activated by salt and cold stresses (Yuasa et al. 2001). Recent studies show that PLDα1 and PA play an important role in regulating MAPK6 activity in response to

NaCl stress (Yu et al. 2010). PA binds to MAPK6. MAPK6 phosphorylates the plasma membrane Na^+/H^+ antiporter (SOS1), which mediates the extrusion of Na^+ from plant cells (Shi et al. 2000). These results establish a link among salt stress, PLD, MAPK6 activity, and the SOS pathway (Morris 2010).

CTR1 (CONSTITUTIVE TRIPLE RESPONSE1) is a potential PA target in response to the plant hormone ethylene (Testerink et al. 2007). CTR1 is most similar in sequence to the Raf protein kinase family in animal cells and believed to function like Raf, as a MAP kinase kinase kinase (MAPKKK). However, the existence of such a MAPK cascade in ethylene signaling is controversial, and no authenticated substrate MAPKK of CTR1 has been identified. Instead, the recently identified target EIN2 is a NRAMP-like, integral ER membrane protein, which shows CTR1-dependent phosphorylation in the absence of ethylene. By binding to ethylene, the receptor ETR1 at the ER is inactivated, and CTR1 also becomes inactive. This triggers dephosphorylation of EIN2, resulting in EIN2 C terminus cleavage, and subsequent nuclear translocation where it activates the transcriptional factor EIN3 (Ju et al. 2012; Qiao et al. 2012).

Genetic evidence shows that CTR1 acts as a negative regulator in hyperosmotic stress response, as *ctr1-1* mutants display increased salt and osmotic tolerance during germination (Achard et al. 2006; Wang et al. 2007). Using ^{32}P labeling or ESI/MS/MS analysis methods, it was shown that salt stress transiently stimulates PA increase (Testerink et al. 2008; Yu et al. 2010), and PLDα1 is a contributor of the salt-induced PA generation (Yu et al. 2010). In vitro experiments show that PA inhibits CTR1's kinase activity and disrupts the interaction between CTR1 and ETR1 (Testerink et al. 2007). Therefore, it is possible that PLDα regulates salt signaling through PA inhibition of CTR1 in vivo, although the exact mechanism has yet to be determined.

3.1.2 SnRKs and SPHKs

The sucrose non-fermenting-1-related protein kinase 2 family (SnRK2) is a unique family of protein kinases regulating cellular response to osmotic stress (McLoughlin et al. 2012). The SnRK2 family is grouped into three classes according to phylogeny. *Arabidopsis* class 3 members, SnRK2.2, -2.3, and -2.6 (OST1) are activated by ABA. In the absence of ABA, protein phosphatase 2C (PP2C) binds to SnRK2 (SnRK2.6) kinase domain and inhibits its kinase activity. When ABA increases under stress conditions, it binds to soluble receptor (PYL or RCAR) which interacts with PP2C (ABI1, ABI2, HAB1) and inhibits PP2C activity. The SnRK2 released from PP2C is activated to transmit the ABA signal via phosphorylation of downstream targets (Soon et al. 2012).

Arabidopsis class 1 members, SnRK2s, SnRK2.4, and SnRK2.10, are activated within 1 min of salt treatment. Upon salt exposure, SnRK2.4 is targeted to the membrane structures (McLoughlin et al. 2012). Interestingly, both SnRK2.4 and SnRK2.10 bind to PA in vitro, suggesting PA may act to recruit SnRK2s to membranes during stress response (McLoughlin et al. 2012). However, more

physiological and cellular evidence is needed to unravel the detailed interaction between PA and SnRK2.

Recent studies have shown that PA interacts with sphingosine kinase (SPHK) in *Arabidopsis* (Guo et al. 2011, 2012a). SPHK phosphorylates long-chain bases to generate long-chain base-1-phosphates such as phytosphingosine-1-phosphate (phyto-S1P). There are four SPHK members similar to human SPHK in *Arabidopsis*, but only two have sphingosine phosphorylating activity (Worrall et al. 2008). SPHK1 and SPHK2 can phosphorylate phytosphingosine into phyto-S1P. Phyto-S1P has been identified as a lipid messenger mediating plant response to ABA (Coursol et al. 2005). Evidence shows that PA binds to and promotes SPHK's activity by increasing the specificity constant by decreasing K_m^B in vitro (Guo et al. 2011). Further cellular and physiological studies reveal that phyto-S1P induces stomatal closure in *sphk1-1* and *sphk2-1*, but not in *pldα1*, while PA promotes stomatal closure in *sphk1-1, sphk2-1*, and *pldα1,* suggesting that SPHK and phyto-S1P are upstream of PLDα1 and PA in ABA-induced stomatal movement (Guo et al. 2012a).

3.2 Protein Dephosphorylation

Ablation of *PLDα1* increases water loss and decreases ABA-induced stomatal closure in *Arabidopsis* (Zhang et al. 2004). PA derived from PLDα1 binds to ABI1, a negative regulator of ABA signaling (Raghavendra et al. 2010), and inhibits its phosphatase activity. The PA–ABI1 interaction results in the tethering of ABI1 to the plasma membrane, inhibiting its negative effects within the nucleus, thereby inducing stomatal closure (Zhang et al. 2004). On the other hand, PLDα1 itself interacts with heterotrimeric G protein α-subunit (Gα/GPA1) through its DRY motif. Biochemical data indicate that PLDα1 activates the intrinsic guanosine triphosphatase activity that converts active Gα-GTP to inactive Gα-GDP (Zhao and Wang 2004). In turn, Gα-GDP binds to PLDα1 and decreases its activity. When GPA1 is bound by GTP (Gα-GTP), it is dissociated from PLDα1, and the latter is activated (Zhao and Wang 2004). The PA resulting from PLDα1 activity promotes inhibition of stomatal opening. By promotion of stomatal closing and inhibition of stomatal opening, PLDα1/PA regulate the bifurcating signaling pathway during ABA-regulated stomatal movement, thereby reducing water loss (Mishra et al 2006).

3.3 ROS

ROS are regarded as an important class of secondary messengers in response to stresses (Delledonne et al. 2001). Antisense suppression of *PLDα* lowers the level of superoxide production in *Arabidopsis*, while addition of PA enhances superoxide

burst in leaves (Sang et al. 2001). A recent study indicates that PA regulates the activity of NADPH oxidase RbohD (respiratory burst oxidase homolog D) in ABA-induced stomatal closure (Zhang et al. 2009). There are ten *Rboh* genes in the *Arabidopsis* genome, with *RbohD* and *RbohF* mainly expressed in guard cells (Torres and Dangl 2005). The *plda1* show similar phenotypes of insensitivy to ABA-induced stomatal closure with *rbohD/F* double mutants (Kwak et al. 2003; Zhang et al. 2004). Genetic and cellular evidence shows that ABA promotes PLDα1 activity, producing PA, which binds to the N-terminal region of RbohD in the cytosol to promote the NADPH oxidase activity and ROS production in guard cells (Zhang et al. 2009).

PA–ABI1 interaction affects ROS or NO-induced stomatal closure, but not ROS or NO production, suggesting that ROS and NO may act upstream of the PA–ABI1 interaction in ABA signaling (Zhang et al. 2009). Other components such as small G protein Rac, Ca^{2+}, CDPK, and MAPK may work together with PLD/PA to regulate NADPH oxidase activity and ROS production in stomatal movement response to hyperosmotic stresses (Ogasawara et al. 2008; Zhang et al. 2009).

Besides NADPH oxidases, apoplast amine oxidases, including copper-containing amine oxidase (CuAO) and polyamine oxidase (PAO) are also important sources of ROS production (Mittler 2002). ABA treatment stimulated apoplast CuAO activity to increase production and Ca^{2+} levels in *Vicia faba* guard cells (An et al. 2008) and nitric oxide (NO) production in roots (Wimalasekera et al. 2011). Whether CuAO (PAO) is regulated by PLD/PA is an interesting question that has arisen.

3.4 Cytoskeleton

Microtubule dynamics and organization regulate cell growth, division, and development, in response to biotic and abiotic stresses (Dixit and Cyr 2004; Ehrhardt and Shaw 2006). For example, deletion of Na^+/H^+ antiporter protein (*sos1* mutant) results in microtubule depolymerization and salt sensitivity (Shoji et al. 2006). In contrast, stabilization of microtubules by paclitaxel results in increased seedling death under salt stress (Wang et al. 2007). These results suggest that precise control of microtubule organization is essential for cells to survive under hyperosmotic stress (Wang et al. 2011).

A microtubule-associated 90-kD polypeptide isolated from tobacco cells displayed sequence similarity to PLDδ in *Arabidopsis* (Marc et al. 1996). PLDδ was later shown to be associated with microtubules and plasma membranes (Gardiner et al. 2001). *Arabidopsis* PLDδ was shown to be associated with the plasma membrane as visualized with yellow fluorescence protein (eYFP) (Guo et al. 2011). The tobacco 90-kD PLD can also associate with the preprophase band and spindle. Gardiner et al. (2001) suggested that this protein dissociates from the plasma membrane at the onset of mitosis and reattaches at the end of cell division. Later, Dhonukshe et al. (2003) found that PLD activators such as

mastoparan, xylanase, NaCl, and hypo-osmotic stress can induce the release of microtubules from plasma membrane, resulting in their reorganization. However, direct evidence was lacking for the direct involvement of PLD in the regulation of microtubule organization.

A more recent study has shown that PA derived from PLDα1 regulates microtubule organization by interacting with a microtubule-associated protein 65-1 (MAP65-1) (Zhang et al. 2012). The cortical microtubules in *pldα1* mutant are sensitive to NaCl treatment, being depolymerized into dot-like structures in plants exposed to NaCl. In addition, application of PA restores the sensitive phenotype of *pldα1* mutant (Zhang et al. 2012). PA binds to a microtubule-associated protein, MAP65-1, which can then bind to and bundle microtubules stabilizing them (Chang-Jie and Sonobe 1993; Smertenko et al. 2004; Mao et al. 2005; Lucas et al. 2011). PA promotes this polymerization and bundle activity of MAP65-1 both in vitro and in vivo, and mutations of the PA-binding amino acids disrupted the binding of PA and MAP65-1, and the organization of microtubules in response to salt stress (Zhang et al. 2012). PLDα1 itself does not bind to MAP65-1 or microtubules, and PA does not bind directly to microtubules either. Furthermore, unlike PLDα1, PLDδ is not involved directly in microtubule organization under salt stress response. Knockout of PLDδ does not change microtubule patterns or salt tolerance when compared with wild type (Zhang et al. 2012). Therefore, PA from PLDα1 hydrolysis specifically regulates microtubule organization in response to salt stress. It is unclear whether other PLDs, for example, PLDα3 which is involved in salt and osmotic stress (Hong et al. 2008), regulate microtubule organization during salt or other stress responses.

In contrast to the regulation of microtubule organization by PA–MAP65-1 interaction during salt stress response, MAP65-1-bundling activity is negatively regulated by MAPK cascades during cell division and development. The phosphorylation of MAP65-1 by MAPKs reduces its microtubule-bundling activity, thereby enhancing destabilization of microtubules and promoting mitosis (Sasabe et al. 2006; Smertenko et al. 2006). The deficiency of a MAPKKK (ANP2 or ANP3) induces a lesser phosphorylation status of MAP65-1, resulting in heavy bundling of microtubules and abnormal root growth (Beck et al. 2010). These results indicate that the dynamic organization of microtubules is controlled by complex mechanisms of the cellular response to developmental and environmental signals.

It is well known that plant cells remodel their actin cytoskeleton in response to biotic and abiotic stresses. PA has been recently reported to bind a capping protein (CP) and dissociate it from the ends of actin, thus enhancing actin filament–filament annealing (Huang et al. 2006; Li et al. 2012). However, a direct link between PA–actin filaments and any stress signal is yet to be established.

3.5 Proline

Proline has been thought of as a compatible osmolyte which preserves protein activity, maintains pH, and prevents oxidative damage (Delauney and Verma 1993; Amtmann 2009). The significance of proline accumulation as an osmolyte has recently been debated because the proline metabolism intermediate pyrroline-5-carboxylate (P5C) is highly toxic to the cell, directly or indirectly triggering apoptosis (Thiery et al. 2004). PLD and PLC have been found to be related to the regulation of proline accumulation. The effect of PLD and PLC on proline accumulation is dependent on both the degree of stress and the plant species (Szabados and Savouré 2009). Using 1-butanol to inhibit PA generation, Thiery et al. (2004) found that PLD functions as a negative regulator of proline accumulation in *Arabidopsis* plants under non-stressed conditions. Treatments of plants with 200 mM NaCl or 400 mM mannitol induced an increase in proline, of which PLD was probably not involved (Thiery et al. 2004), whereas PLC triggered P5CS transcription and proline accumulation during salt stress (Parre et al. 2007).

In model halophyte, *Thellungiella halophila/salsuginea*, plants accumulate high levels of proline even in the absence of stress. Pharmacological evidence shows that PLDs positively control the proline accumulation under severe stress (400 mM NaCl or 400 mM mannitol) but have no effect on its accumulation in non-stressed conditions. Inhibition of PLC by the inhibitor U73122 leads to more proline accumulation under unstressed or moderate salt stress (200 mM NaCl) conditions, suggesting that PLC is a negative regulator (Ghars et al. 2012).

4 Conclusions and Perspectives

Plant PLDs are a family of multifarious enzymes with different biochemical, regulatory, and structural properties. Different PLDs show unique functions in response to hyperosmotic stresses and other stresses (e.g., PLDε for nutrition deficiency) and development signals (e.g., PLDζ for root hair development, which are described in other chapters in this book). With the extensive investigation in recent years, increasing numbers of components in PLD/PA-regulated signaling pathways are characterized. As outlined in Fig. 1, PLDs transmit osmotic stress signals by regulating of the activity of protein kinases and phosphatases, NADPH oxidase, as well as cytoskeleton organization. Genetic evidence indicates that the activation of SPHKs by ABA produces phyto-S1P which in turn activates PLDα1 to produce PA (Guo et al. 2012a). Early findings indicate that phyto-S1P activate G protein (GPA1) (Coursol et al. 2003), while activated GPA1 probably release and stimulate PLDα1 (Fig. 1). However, whether this loop exists in a cell in response to a specific stimulus still needs to be investigated.

Aside from phospholipase activity, mammalian PLD (PLD2) has recently been reported to bear an additional and novel catalytic function. The PLD2 acts as a

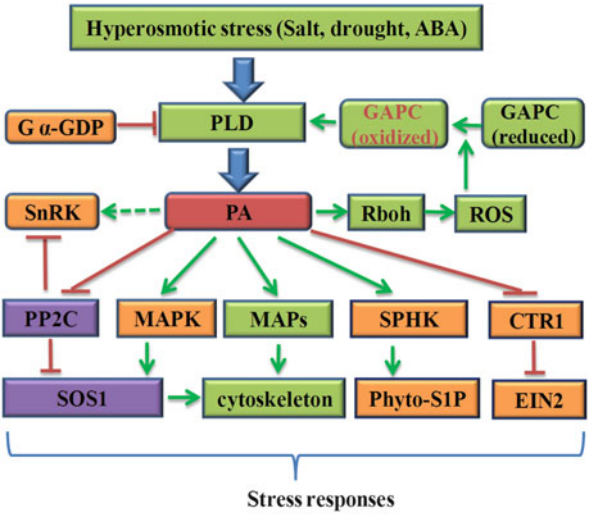

Fig. 1 Proposed model depicting the functions of PLD/PA in response to hyperosmotic stresses. When plant cells sense hyperosmotic or other stresses, PLDs are regulated (activated) by upstream factors. For example, PLDα1 is activated with the stimulation of G protein by the conversation of Gα-GDP to Gα-GTP and release of PLDα1 from Gα-GDP binding. Different stresses may stimulate the appropriate PLDs. For example, ABA-promoted PA binds to and regulates ABI1 and NADPH oxidase in plasma membrane and interacts with SPHK in tonoplast, respectively. In response to NaCl stress, PA may bind to and activate MAPK6 to regulate SOS1antiporter and interact with MAP65-1 to stabilize microtubule, improving salt tolerance. Note that this model is not comprehensive and only includes some of the signaling components implicated in the hyperosmotic stress and ABA responses. *Arrows with solid lines* indicate established links and *arrows with dashed lines* denote putative links

guanine nucleotide exchange factor (GEF) for the small GTPase Rac2 by turnover of the inactive GDP-bound GTPase to the active GTP-bound GTPase (Mahankali et al. 2012). In *Arabidopsis*, the sequences of PLDζ1 and ζ2 are most similar to those of mammalian PLDs, raising intriguing questions of whether plant PLDζs also have GEF activity.

Therefore, in future work, it is necessary to further clarify PLD properties, including biochemical characteristics, localization, and cellular functions. Compared with identified PLD/PA targets, we know much less about the upstream effectors of PLDs such as receptors and the components connecting receptors and PLDs. More detailed and precise networks among PLDs and other signal molecules should be established using combined genetic, molecular, cellular, and physiological methods.

Acknowledgments The work is supported by grants from National Natural Science Foundation of China (31171461 and 91117003), grants from the Ministry of Science and Technology in China (2012CB114200) to W.Z., and grants from National Natural Science Foundation of China (31100194) and State Key Laboratory of Plant Physiology and Biochemistry (SKLPPBKF11002) to Q.Z.

References

Achard P, Cheng H, De Grauwe L, Decat J, Schoutteten H, Moritz T, Van Der Straeten D, Peng J, Harberd NP (2006) Integration of plant responses to environmentally activated phytohormonal signals. Science 311:91–94

Amtmann A (2009) Learning from evolution: Thellungiella generates new knowledge on essential and critical components of abiotic stress tolerance in plants. Mol Plant 2:3–12

An Z, Jing W, Liu Y, Zhang W (2008) Hydrogen peroxide generated by copper amine oxidase is involved in abscisic acid-induced stomatal closure in Vicia faba. J Exp Bot 59:815–825

Bargmann BO, Laxalt AM, ter Riet B, van Schooten B, Merquiol E, Testerink C, Haring MA, Bartels D, Munnik T (2009) Multiple PLDs required for high salinity and water deficit tolerance in plants. Plant Cell Physiol 50:78–89

Beck M, Komis G, Muller J, Menzel D, Sama J (2010) Arabidopsis homologs of nucleus- and phragmoplast-localized kinase 2 and 3 and mitogen-activated protein kinase 4 are essential for microtubule organization. Plant Cell 22:755–771

Chang-Jie J, Sonobe S (1993) Identification and preliminary characterization of a 65 kDa higher-plant microtubule-associated protein. J Cell Sci 105:891–901

Coursol S, Fan LM, Stuff HL, Spiegel S, Gilroy A, Assmann SM (2003) Sphingolipid signaling in Arabidopsis guard cells involves heterotrimeric G proteins. Nature 423:651–654

Coursol S, Le Stunff H, Lynch DV, Gilroy S, Assmann SM, Spiegel S (2005) Arabidopsis sphingosine kinase and the effects of phytosphingosine-1-phosphate on stomatal aperture. Plant Physiol 137:724–737

Cutler SR, Rodriguez PL, Finkelstein RR, Abram SR (2010) Abscisic acid: emergence of a core signaling network. Annu Rev Plant Biol 61:651–679

Delauney AJ, Verma DPS (1993) Proline biosynthesis and osmoregulation in plants. Plant J 4:215–223

Delledonne M, Zeier J, Marocco A, Lamb C (2001) Signal interactions between nitric oxide and reactive oxygen intermediates in the plant hypersensitive disease resistance response. Proc Natl Acad Sci U S A 98:13454–13459

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

Dixit R, Cyr R (2004) The cortical microtubule array: from dynamics to organization. Plant Cell 16:2546–2552

Ehrhardt DW, Shaw SL (2006) Microtubule dynamics and organization in the plant cortical array. Annu Rev Plant Biol 57:859–875

Gardiner JC, Harper JD, Weerakoon ND, Collings DA, Ritchie S, Gilroy S, Cyr RJ, Marc J (2001) A 90-kD phospholipase D from tobacco binds to microtubules and the plasma membrane. Plant Cell 13:2143–2158

Ghars MA, Richard L, Lefebvre-De Vos D, Leprince AS, Parre E, Bordenave M, Abdelly C, Savoure A (2012) Phospholipases C and D modulate proline accumulation in *Thellungiella halophila/salsuginea* differently according to the severity of salt or hyperosmotic stress. Plant Cell Physiol 53:183–192

Guo L, Mishra G, Taylor K, Wang X (2011) Phosphatidic acid binds and stimulates Arabidopsis sphingosine kinases. J Biol Chem 286:13336–13345

Guo L, Mishra G, Markham JE, Li M, Tawfall A, Welti R, Wang X (2012a) Connections between sphingosine kinase and phospholipase D in the abscisic acid signaling pathway in Arabidopsis. J Biol Chem 287:8286–8296

Guo L, Devaiah SP, Narasimhan R, Pan P, Zhang Y, Zhang Z, Wang X (2012b) Cytosolic glyceraldehyde-3-phosphate dehydrogenases interact with phospholipase Dδ to transduce hydrogen peroxide signals in Arabidopsis response to stress. Plant Cell 24:2200–2212

Hong Y, Pan X, Welti R, Wang X (2008) Phospholipase Dalpha3 is involved in the hyperosmotic response in Arabidopsis. Plant Cell 20:803–816

Hong Y, Zhang W, Wang X (2010) Phospholipase D and phosphatidic acid signaling in plant response to drought and salinity. Plant Cell Environ 33:627–635

Huang S, Gao L, Blanchoin L, Staiger CJ (2006) Heterodimeric capping protein from Arabidopsis is regulated by phosphatidic acid. Mol Biol Cell 17:1946–1958

Jacob T, Ritchie S, Assmann SM, Gilroy S (1999) Abscisic acid signal transduction in guard cells is mediated by phospholipase D activity. Proc Natl Acad Sci U S A 96:12192–12197

Ju C, Yoon GM, Shemansky JM, Lin DY, Ying ZI, Chang J, Garrett WM, Kessenbrock M, Groth G, Tucker ML, Cooper B, Kieber JJ, Chang C (2012) CTR1 phosphorylates the central regulator EIN2 to control ethylene hormone signaling from the ER membrane to the nucleus in Arabidopsis. Proc Natl Acad Sci U S A 109:19486–19491

Katagiri T, Takahashi S, Shinozaki K (2001) Involvement of a novel Arabidopsis phospholipase D, AtPLDδ, in dehydration-inducible accumulation of phosphatidic acid in stress signalling. Plant J 26:595–605

Kwak JM, Mori IC, Pei ZM, Leonhardt N, Torres MA, Dangl JL, Bloom RE, Bodde S, Jones JD, Ji S (2003) NADPH oxidase AtrbohD and AtrbohF genes function in ROS-dependent ABA signaling in Arabidopsis. EMBO J 22:2623–2633

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

Li G, Lin F, Xue HW (2007) Genome-wide analysis of the phospholipase D family in *Oryza sativa* and functional characterization of PLDβ1 in seed germination. Cell Res 17:881–894

Li W, Wang R, Li M, Li L, Wang C, Welti R, Wang X (2008) Differential degradation of extraplastidic and plastidic lipids during freezing and post-freezing recovery in *Arabidopsis thaliana*. J Biol Chem 283:461–468

Li M, Hong Y, Wang X (2009) Phospholipase D- and phosphatidic acid-mediated signaling in plants. Biochim Biophys Acta 1791:927–935

Li J, Henty-Ridilla JL, Huang S, Wang X, Blanchoin L, Staiger CJ (2012) Capping protein modulates the dynamic behavior of actin filaments in response to phosphatidic acid in Arabidopsis. Plant Cell 24:3742–3754

Lu S, Bahn SC, Qu G, Qin H, Hong Y, Xu Q, Zhou Y, Wang X (2013) Increased expression of phospholipase Dα1 in guard cells decreases water loss with improved seed production under drought in Brassica napus. Plant Biotechnol J 11:380–389

Lucas JR, Courtney S, Hassfurder M, Dhingra S, Bryant A, Shaw SL (2011) Microtubule-associated proteins MAP65-1 and MAP65-2 positively regulate axial cell growth in etiolated Arabidopsis hypocotyls. Plant Cell 23:1889–1903

Mahankali M, Henkels KM, Alter G, Gomez-Cambronero JG (2012) Identification of the catalytic site of phospholipase D2 (PLD2) newly described guanine nucleotide exchange factor activity. J Biol Chem 287:41417–41431

Mao T, Jin L, Li H, Liu B, Yuan M (2005) Two microtubule-associated proteins of the Arabidopsis MAP65 family function differently on microtubules. Plant Physiol 138:654–662

Marc J, Sharkey DE, Durso NA, Zhang M, Cyr RJ (1996) Isolation of a 90-kD microtubule-associated protein from tobacco membranes. Plant Cell 8:2127–2138

McLoughlin F, Galvan-Ampudia CS, Julkowska MM, Caarls L, van der Does D, Laurière C, Munnik T, Haring MA, Testerink C (2012) The Snf1-related protein kinases SnRK2.4 and SnRK2.10 are involved in maintenance of root system architecture during salt stress. Plant J 72:436–449

Mishra G, Zhang W, Deng F, Zhao J, Wang X (2006) A bifurcating pathway directs abscisic acid effects on stomatal closure and opening in Arabidopsis. Science 312:264–266

Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci 7:405–410

Morris PC (2010) Integrating lipid signalling, mitogen-activated protein kinase cascades and salt tolerance. New Phytol 188:640–643

Munnik T, Vermeer JEM (2010) Osmotic stress-induced phosphoinnositide and inositol phosphate signaling in plants. Plant Cell Environ 33:655–669

Munnik T, Meijer HJ, TerRiet B, Hirt H, Frank W, Bartels D, Musgrave A (2000) Hyperosmotic stress stimulates phospholipase D activity and elevates the levels of phosphatidic acid and diacylglycerolpyrophosphate. Plant J 22:147–154

Ng CKY, Carr K, McAinsh MR, Powell B, Hetherington AM (2001) Drought-induced guard cell signal transduction involves sphigosine-1-phosphate. Nature 410:596–599

Ogasawara Y, Kaya H, Hiraoka G, Yumoto F, Kimur S, Kadota Y, Hishinuma H, Senzaki E, Yamagoe S, Nagata K, Nara M, Suzuki K, Tanokura M, Kuchitsu K (2008) Synergistic activation of the Arabidopsis NADPH oxidase AtrbohD by Ca^{2+} and phosphorylation. J Biol Chem 283:8885–8892

Parre E, Ghars MA, Leprince A-S, Thiery L, Lefebvre D, Bordenave M, Richard L, Mazars C, Abdell C, Savouré A (2007) Calcium signaling via phospholipase C is essential for proline accumulation upon ionic but not nonionic hyperosmotic stresses in arabidopsis. Plant Physiol 144:503–512

Pei Z-M, Murata Y, Benning G, Thomine S, Klusener B, Allen GJ, Grill E, Schroeder JI (2000) Calcium channels activated by hydrogen peroxide mediate abscisic acid signalling in guard cells. Nature 406:731–734

Qiao H, Shen Z, Huang SS, Schmitz RJ, Urich MA, Briggs SP, Ecker JR (2012) Processing and subcellular trafficking of ER-tethered EIN2 control response to ethylene gas. Science 338:390–393

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

Raghavendra AS, Gonugunta VK, Christmann A, Grill E (2010) ABA perception and signalling. Trends Plant Sci 15:395–401

Rajashekar CB, Zhou HE, Zhang Y, Li W, Wang X (2006) Suppression of phospholipase Dα1 induces freezing tolerance in Arabidopsis: response of cold-responsive genes and osmolyte accumulation. J Plant Physiol 163:916–926

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

Santner A, Estelle M (2009) Recent advances and emerging trends in plant hormone signalling. Nature 459:1071–1078

Sasabe M, Soyano T, Takahashi Y, Sonobe S, Igarashi H, Itoh TJ, Hidaka M, Machida Y (2006) Phosphorylation of NtMAP65-1 by a MAP kinase down-regulates its activity of microtubule bundling and stimulates progression of cytokinesis of tobacco cells. Genes Dev 20:1004–1014

Shi H, Ishitani M, Kim C, Zhu JK (2000) The Arabidopsis thaliana salt tolerance gene SOS1 encodes a putative Na^+/H^+ antiporter. Proc Natl Acad Sci U S A 97:6896–6901

Shoji T, Suzuki K, Abe T, Kaneko Y, Shi H, Zhu JK, Rus A, Hasegawa PM, Hashimoto T (2006) Salt stress affects cortical microtubule organization and helical growth in Arabidopsis. Plant Cell Physiol 47:1158–1168

Smertenko AP, Chang HY, Wagner V, Kaloriti D, Fenyk S, Sonobe S, Lloyd C, Hauser MT, Hussey PJ (2004) The Arabidopsis microtubule-associated protein AtMAP65-1: molecular analysis of its microtubule bundling activity. Plant Cell 16:2035–2047

Smertenko AP, Chang HY, Sonobe S, Fenyk SI, Weingartner M, Bogre L, Hussey PJ (2006) Control of the AtMAP65-1 interaction with microtubules through the cell cycle. J Cell Sci 119:3227–3237

Soon FF, Ng LM, Zhou XE, West GM, Kovach A, Tan MH, Suino-Powell KM, He Y, Xu Y, Chalmers MJ, Brunzelle JS, Zhang H, Yang H, Jiang H, Li J, Yong EL, Cutler S, Zhu JK, Griffin PR, Melcher K, Xu HE (2012) Molecular mimicry regulates ABA signaling by SnRK2 kinases and PP2C phosphatases. Science 335:85–88

Szabados L, Savouré A (2009) Proline: a multifunctional amino acid. Trends Plant Sci 15:89–97

Tena G, Boudsocq M, Sheen J (2011) Protein kinase signaling networks in plant innate immunity. Curr Opin Plant Biol 14:519–529

Testerink C, Larsen PB, van der Does D, van Himbergen JA, Munnik T (2007) Phosphatidic acid binds to and inhibits the activity of Arabidopsis CTR1. J Exp Bot 58:3905–3914

Testerink C, Larsen PB, McLoughlin F, van der Does D, van Himbergen JA, Munnik T (2008) PA, a stress-induced short cut to switch-on ethylene signalling by switching-off CTR1? Plant Signal Behav 3:681–683

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

Torres MA, Dangl JL (2005) Functions of the respiratory burst oxidase in biotic interactions, abiotic stress and development. Curr Opin Plant Biol 18:397–403

Uraji M, Katagiri T, Okuma E, Ye W, Hossain MA, Masuda C, Miura A, Nakamura Y, Mori IC, Shinozaki K, Murata Y (2012) Cooperative function of PLDδ and PLDα1 in abscisic acid-induced stomatal closure in Arabidopsis. Plant Physiol 159:450–460

Wang C, Li J, Yuan M (2007) Salt tolerance requires cortical microtubule reorganization in Arabidopsis. Plant Cell Physiol 48:1534–1547

Wang S, Kurepa J, Hashimoto T, Smalle JA (2011) Salt stress-induced disassembly of Arabidopsis cortical microtubule arrays involves 26S proteasome-dependent degradation of SPIRAL1. Plant Cell 23:3412–3427

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 Dα in freezing-induced lipid changes in Arabidopsis. J Biol Chem 277:31994–32002

Wimalasekera R, Villar C, Begum T, Scherer GF (2011) COPPER AMINE OXIDASE1 (CuAO1) of Arabidopsis thaliana contributes to abscisic acid- and polyamine-induced nitric oxide biosynthesis and abscisic acid signal transduction. Mol Plant 4:663–678

Worrall D, Liang YK, Alvarez S, Holroyd GH, Spiegel S, Panagopulos M, Gray JE, Hetherington AM (2008) Involvement of sphingosine kinase in plant cell signaling. Plant J 56:64–72

Yu L, Nie J, Cao C, Jin Y, Yan M, Wang F, Liu J, Xiao Y, Liang Y, Zhang W (2010) Phosphatidic acid mediates salt stress response by regulation of MPK6 in *Arabidopsis thaliana*. New Phytol 188:762–773

Yuasa T, Ichimura K, Mizoguchi T, Shinozaki K (2001) Oxidative stress activates ATMPK6, an Arabidopsis homologue of MAP kinase. Plant Cell Physiol 42:1012–1016

Zhang X, Zhang L, Dong F, Gao J, Galbraith DW, Song C-P (2001) Hydrogen peroxide is involved in abscisic acid-induced stomatal closure in vicia faba. Plant Physiol 126:1438–1448

Zhang W, Qin C, Zhao J, Wang X (2004) Phospholipase Dα1-derived phosphatidic acid interacts with ABI1 phosphatase 2C and regulates abscisic acid signaling. Proc Natl Acad Sci U S A 101:9508–9513

Zhang Y, Zhu H, Zhan Q, Li M, Yan M, Wang R, Wang L, Welti R, Zhang W, Wang X (2009) Phospholipase Dα1 and phosphatidic acid regulate NADPH oxidase activity and production of reactive oxygen species in ABA-mediated stomatal closure in Arabidopsis. Plant Cell 21:2357–2377

Zhang W, Wan X, Hong Y, Li W, Wang X (2010) Plant phospholipase. In: Munnik DT (ed) Lipid signaling in plants, plant cell monographs16. Springer, Berlin, pp 39–62

Zhang Q, Lin F, Mao T, Nie J, Yan M, Yuan M, Zhang W (2012) Phosphatidic acid regulates microtubule organization by interacting with MAP65-1 in response to salt stress in Arabidopsis. Plant Cell 24:4555–4576

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

Zhao J, Zhou D, Zhang Q, Zhang W (2012) Genomic analysis of phospholipase D family and characterization of GmPLDalphas in soybean (Glycine max). J Plant Res 125:569–578

Zhu JK (2002) Salt and drought stress signal transduction in plants. Annu Rev Plant Biol 53:247–273

Phospholipases in Nitric Oxide-Mediated Plant Signaling

Gabriela Gonorazky, Ayelen M. Distéfano, Carlos García-Mata, Lorenzo Lamattina, and Ana M. Laxalt

Abstract Nitric Oxide (NO) is an important redox-based regulator of cell physiology involved in many signaling processes in plants. The precise molecular mechanism of how NO interacts with or activates different targets is still poorly understood. The polar lipid phosphatidic acid (PA) is another molecule involved in plant signaling. NO and PA have been independently regarded as general, multifunctional stress-signaling molecules in plants. Results obtained in our laboratory revealed that NO induces PA formation during plant-defense responses, stomatal closure, and adventitious root formation. Conversely, during extracellular ATP perception, PA modulates NO production. PA is generated via phospholipase D and phospholipase C in concerted action with diacylglycerol kinase. In this chapter, we discuss how NO might act on PA-generating enzymes as well as their common downstream effectors like Ca^{2+}, reactive oxygen species, kinases, and phosphatases.

Keywords Adventitious root formation • Auxin • Extracellular ATP • Nitric oxide • Phosphatidic acid • Phospholipases • Plant-defense • Stomatal closure

1 Introduction

Nitric Oxide (NO) is a short-lived, bioactive gas, able to cross biological membranes (Stamler et al. 1992). NO is a well-established second messenger in animals (Davis et al. 2001) and has been shown to be involved in different physiological and developmental processes in plants (Lamattina et al. 2003). NO belongs to a family of reactive nitrogen species (RNS) such as peroxynitrite ($ONOO^-$), nitrogen dioxide (NO_2), dinitrogen trioxide (N_2O_3) and *S*-nitrosoglutathione (GSNO). RNS

G. Gonorazky • A.M. Distéfano • C. García-Mata • L. Lamattina • A.M. Laxalt (✉)
Facultad de Ciencias Exactas y Naturales, Instituto de Investigaciones Biológicas, Universidad Nacional de Mar del Plata, CC 1245, 7600 Mar del Plata, Argentina
e-mail: amlaxalt@mdp.edu.ar

X. Wang (ed.), *Phospholipases in Plant Signaling*, Signaling and Communication in Plants 20, DOI 10.1007/978-3-642-42011-5_8,

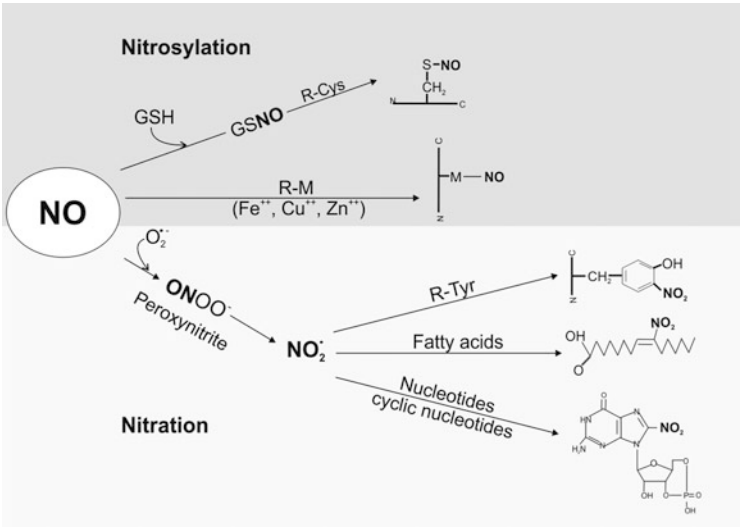

Fig. 1 Chemical Biology of NO. NO reacts with molecules in two basic ways: via nitrosylation (*upside box*) or nitration (*downside box*). Nitrosylation is the reversible addition of NO radical to (a) thiol groups of Cys residues (R-Cys) of proteins, known as S-nitrosylation or (b) to transition metals (R-M), like iron sulfur clusters, heme, and zinc-finger proteins. In S-nitrosylation NO first reacts with S-nitrosoglutathione (GSH) to form GSNO, which then reacts with R-Cys. Nitration is the incorporation of a nitro ($-NO_2$) group by a double bond. This is mediated by NO-derived species, peroxynitrite (ONOO−), formed by NO in the presence of superoxide anions ($O2^{\bullet -}$), and subsequently $NO_2^{\bullet}$. $NO_2^{\bullet}$ could react with: (1) the aromatic ring of tyrosine residues (R-Tyr), (2) different unsaturated fatty acids, and (3) cyclic nucleotides or the nucleotides in the DNA or RNA such as guanine

mediates signaling processes at low and localized concentrations and promotes nitro-oxidative damage when they are over produced. The involvement of NO and derived RNS in a wide variety of physiological processes is achieved via a rich redox and additive chemistry (oxidation, nitration, or nitrosylation) toward its targets (proteins, lipids, and nucleic acids), (Fig. 1) (Stamler 1994). Plant NO research has focused on post-translational modifications (PTM) in proteins, mainly S-nitrosylation and nitration. S-nitrosylation is the covalent binding of NO to a free thiol group of a cysteine residue (Cys) (Astier and Lindermayr 2012). The nitration of tyrosine residues (Tyr) is induced by $^{\bullet}$NO-derived species (homolytic addition of $^{\bullet}NO_2$). Proteins containing transition metals, thiols, or tyrosine residues strategically located at either allosteric or active sites are potential NO targets and essential components of NO signaling.

NO concentrates in membranes where it more readily reacts with oxygen generating oxidizing, nitrosylating, and nitrating species (Moller et al. 2005). Therefore, membrane proteins, lipoproteins, and lipids are theoretically more exposed to NO. Membrane NO targets have been described in animals (Stamler 1994). So far, only two membrane proteins were identified in plants: NADPH oxidase and ion channels (Table 1). There are no reports of S-nitrosylation or nitration of plant phospholipases. In animals, the only phospholipase reported to

Table 1 Examples of proteins involved in plant signaling that are regulated by S-nitrosylation

Protein	Nitrosylation stimuli	Plant species	References
-Ascorbate peroxidase	GSNO/NO gas	*A. thaliana*/ Potato	Bai et al. (2011), Fares et al. (2011)
	Salt Stress	Citrus/ *G. max*	Tanou et al. (2009)
-Auxin receptor (TIR1)	GSNO	*A. thaliana*	Terrile et al. (2012)
-Catalase	GSNO/ Pathogen	*A. thaliana* Pea/Potato	Maldonado-Alconada et al. (2011), Kato et al. (2013), Ortega-Galisteo et al. (2012)
-GAPDH	**GSNO/ DEANO salt**	***A. thaliana*/ Potato/ *G. max*/ *N. tabacum***	Tanou et al. (2009), Wawer et al. (2010)
-HSP90	GSNO	*A. thaliana*	Lindermayr et al. (2005)
-MYB2	GSNO	*A. thaliana*	Serpa et al. (2007)
-NADPH oxidase (RBOH D)	**GSNO/ Pathogens**	***A. thaliana***	Yun et al. (2011)
-NPR1 (Nonexpressor of pathogenesis-related gene 1)	GSNO	*A. thaliana*	Tada et al. (2008)
-PEPC (Phosphoenol pyruvate Carbaxylase)	**Pathogens**	***A. thaliana***	Maldonado-Alconada et al. (2011)
-SABP3 (SA-binding protein 3)	GSNO/ Pathogen	*A. thaliana*	Wang et al. (2009)
-Thioredoxin	GSNO	Potato	Kato et al. (2013)
-TGA1 (Transcription factor)	GSNO	*A. thaliana*	Lindermayr et al. (2010)
-Voltage-Gated Ion Channels	GSNO	Citrus	Tanou et al. (2009)

Summary of those plant proteins involved in signaling processes that were reported to be a target of *S*-nitrosylation either in vitro or in vivo. Bold denotes proteins that bind PA as well

be S-nitrosylated is phospholipase A2α (cPLA2α; Xu et al. 2008). Besides reacting with membrane proteins, NO can react with lipid peroxyl radicals, effectively inhibiting lipid peroxidation chain reactions, displaying an oxidant-protective and anti-inflammatory role in animals (Rubbo et al. 2000). Fatty acid (FA) nitration is induced by $^{\bullet}$NO-derived species yielding NO_2-FAs (Fig. 1). Nitration of unsaturated FAs was shown to be a new signaling mechanism in pathological processes in animals (Freeman et al. 2008). So far, there are no plant reports on NO_2-FAs. RNS can also react with nucleic acids, generating DNA lesions on the bases and/or sugar moieties (Kamiya 2003). Base nitration has been shown in cGMP in animals, and nitrated-cGMP has previously been reported as a signal in animals and more recently in plants (Joudoi et al. 2013).

1.1 NO Production and Localization in Plants

Several nonenzymatic or enzymatic sources of NO have been described in plants (Besson-Bard et al. 2008). They are either L-Arginine or nitrite-dependent. L-Arginine-dependent sources involve NO synthase (NOS)-like enzyme. NOS-like activity has been detected in several plant tissues and it plays a role in NO synthesis during hormone treatments and (a)biotic stress (Neill et al. 2003). Recently, Foresi et al. (2010) described a NOS enzyme from *Ostreococcus tauri* (OtNOS), a green algae that branches near the base in the phylogenetic tree of photosynthetic organisms (Foresi et al. 2010). OtNOS has most of the characteristics ascribed to animal NOS. However, a NOS gene has not been identified in higher plants yet. A decade ago an Arabidopsis gene was reported as the first plant NOS, when mutant plants displayed reduced NO production. However, later on, this NOS-like enzyme was proven to be a GTPase and renamed as AtNOA1 (Nitric Oxide-Associated1) (Moreau et al. 2008). Other processes of NO production use hydroxylamines and polyamines as substrates (Yamasaki and Cohen 2006). In both cases aerobic NO formation is stimulated after external addition of hydroxylamines or polyamines; however, the enzymatic/chemical reactions have not been described yet. Exogenous polyamines rapidly induce NO production, either by triggering NO production via an unknown enzyme or as substrate for NO production by themselves (Frohlich and Durner 2011).

The other NO source is nitrite. Nitrite can accumulate at significant levels under anaerobic conditions as well as when photosynthetic activity is inhibited. The nitrite-dependent NO production can be either enzymatic, catalyzed by the enzymes nitrate reductase (NR) and nitrite-NO reductase (Ni-NOR), or nonenzymatic at low pH (Bethke et al. 2004). The cytosolic enzyme NR reduces nitrite to NO in a NADP (H)-dependent reaction, when nitrite concentrations are high and O_2 concentrations are low (Frohlich and Durner 2011). *Arabidopsis* has two NR genes. NR-deficient double mutants, *nia1*/*nia2*, show low NO levels and are impaired in abscisic acid (ABA)-induced stomatal closure (Desikan et al. 2002), root hair elongation (Lombardo et al. 2006), and lateral root formation (Kolbert et al. 2007). A plasma membrane-bound Ni-NOR, distinct from the plasma membrane NR, has been shown to convert nitrite to NO in tobacco (Stohr and Stremlau 2006). Mitochondrial electron transport-dependent reductase is also capable of reducing nitrite to NO (Planchet et al. 2005). However, these latter routes seem to be localized in the roots of higher plants, where the oxygen tension is low (Gupta et al. 2005).

NO has been detected in the cytosol, nucleus, peroxisomes, mitochondria, and chloroplasts (Neill et al. 2003). The specificity of NO signaling can be explained by this temporal and spatial NO distribution, superimposed to the spatial distribution of its effectors (Besson-Bard et al. 2008). Such spatial versatility can possibly be ascribed to the variety of NO sources.

1.2 Post-translational Modification of Plant Proteins by NO

S-nitrosylation is considered the most important of the NO-dependent PTMs due to its higher reactivity and occurrence under physiological conditions (Astier and Lindermayr 2012). In this sense, S-nitrosylation/denitrosylation has been proposed to be as important as phosphorylation/dephosphorylation (Martinez-Ruiz and Lamas 2004). Several proteomic analysis of S-nitrosylated proteins have been performed in plants (Astier and Lindermayr 2012). There are reports of proteins which are nitrosylated in vitro using nitrosylating agents like GSNO or Cys-NO and proteins which are endogenously nitrosylated in response to different stimuli like plant pathogen infection and salt stress. Some of the S-nitrosylation target proteins, particularly those involved in signaling processes, are listed in Table 1. The factors that make this PTM a specific process are still under debate (Marino and Gladyshev 2010). It is speculated that the colocalization of NO sources and targets within subcellular compartments is important. In this regard, given (Hess et al. 2005) that NO partitions better in membranes than in cytosol (Moller et al. 2005), the role of phospholipids and phospholipases might be of great importance. In addition, there is a technical constraint in the identification of PTM of membrane proteins, since the current methodologies used for NO targets (2D gel electrophoresis and mass spectroscopy) are biased toward the detection of highly abundant proteins (Sun and Murphy 2010). Therefore, it is not common to see low abundant proteins such as ion channels or phospholipases in the lists of S-nitrosylated proteins.

Plant NADPH-dependent oxidases RboHD and RboHF play an active role in the production of reactive oxygen species (ROS) associated with plant defense responses (Torres et al. 2002). Recently it was demonstrated that the ability of *Arabidopsis* RbohD to form ROS is negatively regulated by the S-nitrosylation (Yun et al. 2011). Interestingly, PA binds to both RbohD and RbohF, increasing their activity and thus promoting stomatal closure (Zhang et al. 2009). Another example is phosphoenol pyruvate carboxylase (PEPC). This enzyme, which has increased affinity to PA under hypo-osmotic stress (Testerink et al. 2004), was also reported to be S-nitrosylated in Arabidopsis, C3, and CAM plants (Abat et al. 2008). Finally, glyceraldehyde 3 phosphate dehydrogenase (GAPDH), one of the first proteins to be identified as an S-nitrosylation target (Molina y Vedia et al. 1992), is detected in the PA-binding fraction of root extracts of salt-stressed plants (Yun et al. 2011).

Another form of PTM is metal nitrosylation. In this case, an NO moiety binds to redox active metal centers of proteins, mainly those in heme proteins (Ford 2010). Some of the target proteins of metal nitrosylation in plants are hemoglobins, aconitase, and NO-dependent guanylate cyclase (Astier and Lindermayr 2012). Finally NO can indirectly modify tyrosine residues. In this case NO reacts with superoxide to form peroxynitrite (ONOO-) which reacts with the tyrosine of the target proteins. Some examples of tyrosine nitration are Ferredoxin-NADP oxido-reductase (FNR), O-acetylserine(thiol)lyase (OASTL), and Peroxide reductase II (PrxII) (Astier and Lindermayr 2012).

1.3 Phosphatidic Acid Signaling

PA has emerged as a second messenger in plants as described in Chap. 4 (Testerink and Munnik 2011). It is formed within minutes in response to drought stress, ABA treatments, salt stress, as well as during pathogenic and mutualistic interactions. Increases in PA are transient since PA can be converted to diacylglycerol pyrophosphate by PA kinase or to diacylglycerol (DAG) by lipid phosphate phosphatases.

In stress-induced signal transduction, PA responses have been mainly attributed to two pathways: (a) via phospholipase D (PLD) hydrolysis of structural phospholipids or (b) via phospholipase C (PLC) that hydrolyzes polyphosphoinositides (PPIs) to DAG which is subsequently phosphorylated to PA by diacylglycerol kinase (DGK). In addition, the hydrolysis of PPIs by PLCs results in water-soluble inositol polyphosphates (InsPPs), Ins(1,4)P_2 and Ins(1, 4, 5)P_3, that diffuse into the cytosol. In animals, $InsP_3$ release Ca^{2+} from intracellular stores by activating a ligand-gated calcium channel at the endoplasmic reticulum. To date, no clear homologue of the $InsP_3$-activated Ca^{2+} channel was identified in plant genomic sequences. Instead, $InsP_6$ was shown to stimulate the release of Ca^{2+} from intracellular stores in guard cells, strongly pointing to a signaling function in plants (Lemtiri-Chlieh et al. 2003). Further highlighting the case for $InsP_6$ and other InsPPs involvement during plant signaling is the recent discovery of $InsP_6$ in the crystal structure of the auxin receptor, TIR1, and of $InsP_5$ in the jasmonate receptor, COI1 (Munnik and Nielsen 2011). TIR1 and COI1 are F-box proteins and function as part of SCF ubiquitin–ligase complexes. The role of InsPPs in the TIR1 and COI1 protein functions remains unknown. Recently it was shown that two Cys of TIR1 are S-nitrosylated, and targets of NO function in auxin signaling (Terrile et al. 2012). In addition, phosphatidylinositol 4-phosphate (PtdIns4P) and phsphatidylinositol 4,5-biphosphate PtdIns(4,5)P_2, originally characterized as PLC substrates, have roles as signaling molecules, since many proteins involved in signaling and membrane trafficking have domains that bind to these lipids (see Chap. 2).

Arabidopsis has seven putative PLC genes and 2 pseudogenes (Mueller-Roeber and Pical 2002). PLCs contain a Ca^{2+}-dependent phospholipid-binding domain (C2) and are activated by Ca^{2+}. Until now, many aspects regarding the regulation of plant PLCs were unknown. Very few regulatory mechanisms have been described. In Arabidopsis, AtPLC2 gets phosphorylated upon elicitation (Nuhse et al. 2007) and, in pea, one of the PLCs interacts with Gα protein (Misra et al. 2007). Characterization of each PLC isoform, their mechanisms of regulation, and their putative role in a specific physiological response, remains to be elucidated.

A family of 12 PLD isoforms with specific biochemical, regulatory, and structural properties has been described in *Arabidopsis* (Elias et al. 2002; Qin and Wang 2002). PLD isoforms are classified into two classes. PX/PH-PLDs class (PLDζ has N-terminal phox homology (PX) and pleckstrin homology (PH) lipid-binding

domains, see Chap. 1), whereas the C2-PLDs, unique to plants, have a C2 domain as described above. The latter is further classified into PLDα, β, γ, δ, ε, and ζ classes. Therefore, lipids and Ca^{2+} regulate PLDs (described in Chap. 1). The isoform PLDδ is activated by oleic acid (Wang and Wang 2001) and μM Ca^{2+} concentrations. PtdIns(4,5)P_2 activates the isoforms PLDβ1, PLDγ1, PLDγ2, and PLDδ (Li et al. 2009). Genetically modified plants have been successfully used to address the roles of individual members of the PLD family during a number of stress responses.

1.4 NO Signaling: Connecting to PA

NO has been shown to be involved in the $InsP_3$ and cADPR-dependent increase of cytosolic Ca^{2+} (Garcia-Mata et al. 2003; Lanteri et al. 2006). Ca^{2+} is a regulator of PLD and PLC, as well as many other enzymes such as NADPH oxidase. NADPH oxidase is involved in ROS production. ROS production occurs concomitantly with NO generation during several physiological processes (Delledonne et al. 2001). PA has been shown to trigger an oxidative burst suggesting a similar activation of NADPH oxidase (Sang et al. 2001; de Jong et al. 2004; Park et al. 2004). This suggested a putative cross talk between NO, Ca^{2+}, PA, and ROS. NO and PA share other downstream effectors like an inward-rectifying K^+ channel, ABI1 protein phosphatase 2C, mitogen-activated protein kinase (MAPK), and Ca^{2+}-dependent protein kinase (CDPK). There are a number of processes in which NO- and PA-generating enzymes have been shown to participate (Distéfano et al. 2010). Much evidence supports the link between NO and phospholipid signaling (Distéfano et al. 2010).

2 Plant Defense

NO is involved in the plant defense response of a growing list of plant–pathogen interactions. Accumulation of NO has been observed in plant systems inoculated with *Pseudomonas syringae* (Delledonne et al. 1998), the biotroph fungus *Blumeria graminis* (Prats et al. 2005), and the necrotroph *Botrytis cinerea* (van Baarlen et al. 2007). Rapid NO production has been reported as well in response to treatment with several pathogen-associated molecular pattern (PAMP) molecules. These include the bacterial PAMPs lipopolysaccharides and flagellin (Gust et al. 2007), elicitin INF1 and cryptogein from *Phytophthora* (Foissner et al. 2000; Yamamoto et al. 2004), as well as the fungal PAMPs xylanase and chitosan (Laxalt et al. 2007; Manjunatha et al. 2009; Raho et al. 2011). Downstream of NO, diverse plant defense responses are activated including ROS production, expression of defense-related genes, and cell death (Gaupels et al. 2011). Increased production of NO and ROS is necessary to trigger HR (Delledonne et al. 2001).

Distinct evidence indicates that NO also negatively regulates the induction of plant defense. This regulation would occur through the S-nitrosylation of crucial components of the antioxidant defense system. For instance, S-nitrosylation of NADPH oxidase inhibits its activity, thus reducing ROS production and therefore plant defense responses during plant–pathogen interaction (Yun et al. 2011). Nonexpressor of PR1 (NPR1) is a central salicylic acid-responsive coactivator of the transcription factor TGA1 that regulates *PR1* expression. NPR1 is present in the cytosol as an oligomer and dissociates into monomers that move into the nucleus and activate *PR1* expression. During induction of SA-induced disease resistance, S-nytrosilation of NPR1 induces its oligomerization, thus inhibiting the transcription of *PR1* (Tada et al. 2008). The activities of SA-binding protein 3 (SABP3), GAPDH, prometacaspase 9, *S*-adenosyl-methionine synthetase1 (SAMS1), and constitutive triple response 1 (CTR1) are also regulated by S-nitrosylation during HR induction (Astier and Lindermayr 2012).

The accumulation of PA is one of the earliest host responses as evidenced upon treatment with several PAMPs. The elicitors xylanase, chitosan, *N*-acetyloligosaccharides, and flagellin induce PA production in tomato, alfalfa, and rice cells (Testerink and Munnik 2011). Moreover, the race-specific pathogen effector, Avr4 from the fungus *Cladosporium fulvum* induces PLC/DGK activity in *Cf4*-expressing tobacco cells (de Jong et al. 2004). Similar results have been obtained in *RPM1*/*RPS2*-expressing *Arabidopsis* cells treated with the specific effectors AvrRpm1 and AvrRpt2 from *P. syringae* (Andersson et al. 2006). Nod factors have also been reported to induce PA accumulation in alfalfa cells (den Hartog et al. 2003). Flagellin treatment triggers the phosphorylation of AtPLC2 (Nuhse et al. 2003, 2007) which may indicate its involvement in PA production. Surprisingly, AtPLC2 knockout mutants cannot survive (Munnik, personal communication). Downstream of PA, ROS production, expression of defense-related genes, and cell death are triggered (Testerink and Munnik 2011). Some of the target proteins of PA have been previously described for their role in plant defense. These include phosphoinositide-dependent kinase 1 (PDK1), mitogen-activated protein kinase 6 (MAPK6), CTR1, NADPH oxidase, and GAPDH (reviewed by Testerink and Munnik 2011).

Currently, the elicitors for which an interaction between PA and NO has been established are xylanase and chitosan (Laxalt et al. 2007; Lanteri et al. 2011; Raho et al. 2011). Both PAMPs activate rapid PA production in tomato cells via two enzymatic pathways, PLD and PLC/DGK. Xylanase-induced PLC/DGK-derived PA is NO dependent, while the triggering of PLD activity appears to be independent of NO. In addition, pharmacological approaches indicate that protein kinase activity upstream of NO production as well as protein S-nitrosylation events downstream of NO are required for xylanase-induced PA production. On the other hand, chitosan activates both PLC/DGK and PLD via NO. This suggests that, depending on the elicitor, there is specificity on the requirement of NO for the activation of the phospholipase pathway. Inhibition of NO accumulation or inhibition of either the PLC or the DGK enzyme diminished xylanase-induced ROS production, gene expression, and cell death (Laxalt et al. 2007). Similarly, PLC inhibitors blocked

chitosan-induced ROS production (Raho et al. 2011). This suggests that NO-dependent, PLC/DGK-generated PA is involved in the induction of plant defense triggered by xylanase and chitosan treatment. Recent experiments have shown that AVR4 induces NO production and that this response is required for PLC/DGK activation (Laxalt et al. unpublished). Vossen et al. (2010) by performing silencing assays demonstrated that *SlPLC6* is required for general resistance during incompatible interaction between *Cf4*-tomato plants and *C. fulvum* expressing *Avr4*, while *SlPLC4* is specifically involved in the induction of HR triggered upon AVR4 perception (Vossen et al. 2010).

The oxidative burst in plant–pathogen interactions mediated by specific elicitors occurs in two phases. The first phase shows a rapid, transient, and low ROS production, whereas the second phase shows a prolonged and massive ROS production (Lamb and Dixon 1997). The activation of PLC and PLD was shown for the first phase of ROS generation in rice cells induced by *N*-acetylchitooligosaccharide elicitor, whereas for the second phase only the activation of PLD was evident (Yamaguchi et al. 2005). Recognition of *Pseudomonas syringae* AvrRpm1 or AvrRpt2 in *Arabidopsis* induced a biphasic accumulation of PA. The first wave was attributed to the PLC/DGK pathway and the second to PLD, and both act upstream of ROS formation (Andersson et al. 2006). We suggest that PLC/DGK-generated PA is involved in the induction of ROS production during the first peak of the oxidative burst in xylanase and chitosan treated cells and that it is NO dependent.

The proposed signaling is subject to several regulatory mechanisms, as illustrated in the model of the xylanase-induced signaling pathway (Fig. 2). Distinct feedback loops are proposed in this signaling pathway. An increase in Ca^{2+} concentrations might stimulate PLC or C2-PLDs, thereby establishing possible signal amplification via a positive feedback; however, this mechanism has not yet been proven. A second positive feedback could be established between ROS and NO, based on the observation that H_2O_2 triggers NO production in different biological systems (Lum et al. 2002; Bright et al. 2006; de Pinto et al. 2006). H_2O_2 also induces PA formation (Yamaguchi et al. 2004). In addition, NO negatively modulates the induction of defense responses by inhibiting NADPH oxidase activity and NPR1 monomerization through S-nitrosylation events (Tada et al. 2008; Yun et al. 2011). Although this data suggests a feedback between ROS, NO, and PA, genetic approaches are required to unequivocally demonstrate the order of events following elicitor perception.

3 Stomatal Closure

Stomatal pores, located in the epidermis of plants, are form by a pair of guard cells. The opening and closure of stomata is regulated by guard cell turgor, in rapid response to several signals such as light, temperature, and humidity, and to the hormones ABA, auxin, and ethylene. During drought stress conditions, ABA

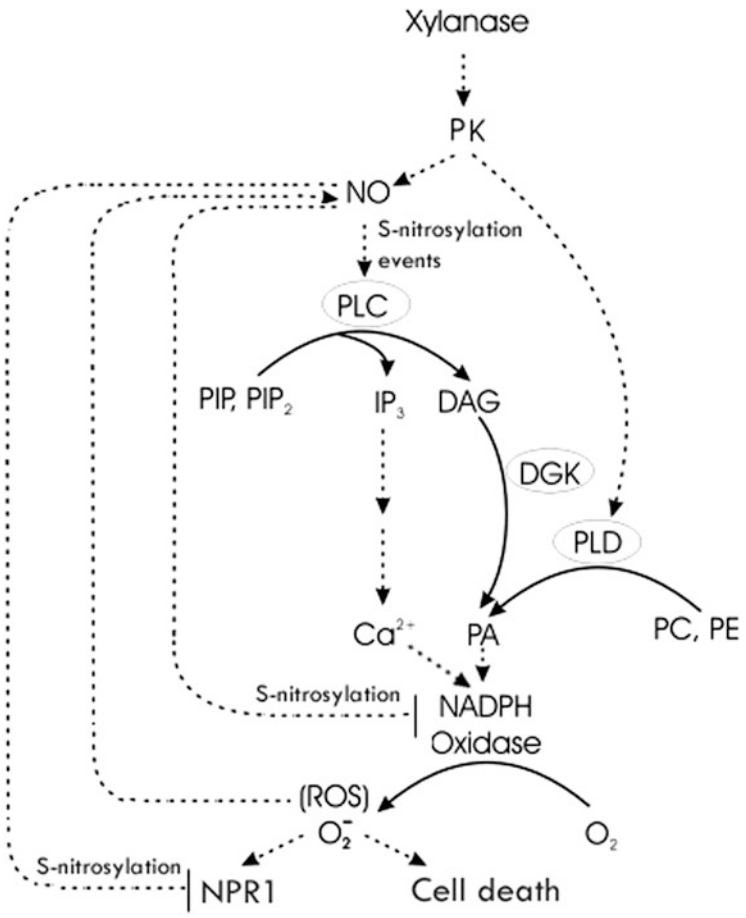

Fig. 2 Model of NO and PA signaling downstream of xylanase perception in cultured tomato cells. Xylanase induces NO and PA production in a protein kinase (PK) activation-dependent manner. NO directly or indirectly triggers PLC/DGK activity through S-nitrosylation events. PLC catalyzes the formation of DAG, which is then converted by DGK to PA. Xylanase also induces PLD to produce PA in an apparent NO-independent manner. PA in turn activates NADPH oxidase, generating superoxide and related ROS. The resulting ROS induces cell death and *PR1* expression through translocation of NPR1 monomers to the nucleus. PLC signaling also results in $InsP_3$ formation. $InsP_3$-derived products induce the release of Ca^{2+} from internal stores and Ca^{2+} further stimulates NADPH oxidase leading to plant defense responses. ROS production forms a positive feedback via stimulation of NO production. NO negatively modulates defense responses by S-nitrosylation of NADPH oxidase and NPR1. *Solid arrows* indicate metabolic conversion; *dashed arrows* indicate activation (directly or indirectly)

regulates water loss by two different process, induction of stomatal closure, and inhibition of stomatal opening (Mishra et al. 2006). Stomatal closure involves several processes resulting in inactivation of inward-rectifying K^+ (K^+_{in}) channels,

activation of outward-rectifying K^+ (K^+_{out}) channels, and activation of slow- and fast-anion channels (MacRobbie 2006).

NO is produced during ABA-induced stomatal closure, methyl jasmonate, UV-B light, and bicarbonate (Distéfano et al. 2010). Cross talk of NO and ABA has been thoroughly studied. In ABA-treated guard cells, endogenous NO has been shown to induce stomatal closure. NO, in turn, participates in a subset of ABA-evoked responses by inactivating K^+_{in} channels via a cGMP/cADPR-dependent increase of cytosolic Ca^{2+} concentration (Garcia-Mata et al. 2003). Sokolovski et al. (2005) showed that several NO-dependent signals could be modulated through protein phosphorylation upstream of intracellular Ca^{2+} release. We have shown that NO induces PA production in guard cells, (Distéfano et al. 2008), and when either PLC or PLD activity was inhibited, NO failed to induce stomatal closure. This evidence suggests that PLD and PLC are participating in the NO-signaling pathway and agrees with similar evidence presented for ABA-induced stomatal closure (Jacob et al. 1999).

Recently, research has focused on the study of PLD isoenzymes involved in phospholipid signaling during stomatal closure. Zhang et al. (2004) showed that PLDα1 is downstream of ABA-induced stomatal closure. Afterwards, the same group showed that upon ABA treatment, PLDα1-derived PA triggers NADPH oxidase activation, with the consequent H_2O_2 production, which in turn induces NO increase (Zhang et al. 2009). PA binds to recombinant *Arabidopsis* NADPH oxidase RbohD and RbohF (Zhang et al. 2009). Our results show that *PLDα1* knockout mutants do close their stomata in response to NO treatments (Distefano et al. 2012), placing PLDα1 upstream of ABA-induced NO production, as reported earlier (Zhang et al. 2009). In contrast, *PLDδ* knockout mutants do not close their stomata in response to ABA, H_2O_2, and NO, placing PLDδ downstream of ABA-induced NO production (Distefano et al. 2012). Two independent groups, Uraji et al. (2012) and Guo et al. (2012), have obtained similar results (Guo et al. 2012; Uraji et al. 2012). Guo and colleagues established that PLDδ is downstream of H_2O_2 in mediating ABA-induced stomatal closure, further supporting our results. H_2O_2 promoted GAPDH–PLDδ interaction and PLDδ activity. Interestingly, GAPDH is regulated by S-nitrosylation (Lindermayr et al. 2005), thus NO could modulate GAPDH–PLDδ interaction. All together, these results suggest that PLDδ might have a role on regulation of stomatal closure downstream of ABA-induced ROS and NO production via PLDα1 activation. Direct evidences will be needed to confirm this hypothesis. However, Uraji et al. (2012) suggested that PLDα1 and PLDδ have a cooperative function in ABA-induced stomatal closure, since single mutants close the stomata upon ABA treatment, but the double mutant does not.

Several components can be proposed as putative downstream effectors of NO–PA production. It has been reported that PA inhibits the function of the PP2C phosphatase ABI1, which is a negative regulator of ABA responses (Zhang et al. 2004). Since *abi1* mutant is able to produce NO in response to ABA, but is impaired in ABA induction of stomatal closure, NO has been proposed to act upstream of ABI1 (Desikan et al. 2002, 2004). Thus, it is speculated that NO

inactivates ABI1 via PA production. In addition, MAPKs, which are involved in ABA-promoted stomatal closure, have been implicated as targets of PA (Testerink and Munnik 2011) and stimulated by NO (Neill et al. 2008). Thus, MAPKs could be targets regulated by NO-induced PLDδ-mediated stomatal closure. K^+_{in} channel could be proposed as another target, because the channel activity is regulated by both PA and NO (Jacob et al. 1999; Garcia-Mata et al. 2003). PLDδ-dependent regulation of stomatal closure can also be linked to cytoskeletal changes, since it has been proposed that PLDδ has a role in microtubule reorganization (Gardiner et al. 2003; Andreeva et al. 2009; Ho et al. 2009). PLD activation induces microtubules to be release from the plasma membrane and incremental F-actin polymerization (Dhonukshe et al. 2003; Gardiner et al. 2003; Smertenko et al. 2004; Motes et al. 2005). Interestingly, F-actin polymerization and changes in vacuolar volume occur in NO-induced stomatal closure (Huang et al. 2009). Considering this evidence, it would be interesting to study the role of PLDδ in actin polymerization and microtubule organization during NO-induced stomatal closure.

Different proteins have been proposed to be the ABA receptor; however, only one is now recognized as a bona fide receptor. The receptor is formed by soluble proteins named PYR/PYL/RCAR (Ma et al. 2009; Park et al. 2009), which interacts with a protein phosphatase–kinase complex, functioning as a double negative regulatory system (Vlad et al. 2009; Umezawa et al. 2013). The phosphatases ABI1, ABI2, and HAB1 belong to clade A type 2C protein phosphatase (PP2C) and the kinases are SNF1-related protein kinase 2 type III (SnRK2-2; 2.3 and the 2.6 OST1) (Kulik et al. 2011). ABA promotes the interaction of PYR/PYL/RCAR and PP2C, resulting in PP2C inhibition and SnRK2 activation**.** Downstream, SnRK2 phosphorylate numerous target proteins involved in ABA responses, including transcription factors that bind to ABA responsive elements, anion and potassium channels, and the NADPH oxidase RbohF (Weiner et al. 2010). Yet, there is no conclusive evidence that NO and PA production occurs through PYL/PYR/RCAR receptor. Interestingly, PA interacts with the SnRK2s type I SnRK2.4 and 2.10 (Testerink et al. 2004) and, as we have mentioned above, PA interacts and regulates ABI1 (Zhang et al. 2004). In addition, NO activates type I SnRK2 in tobacco (Lamotte et al. 2006).

It has been also reported that both NO (Garcia-Mata and Lamattina 2007; Zhang et al. 2007) and PA (Mishra et al. 2006) are involved in the inhibition of light-induced stomatal opening. After we reported the link between NO and PA during stomatal closure, Takemiya and Shimazaki (2010) showed that NO also induces PA during inhibition of light-induced stomatal opening. The authors showed that PLD activity is required for NO induced PA; however, PLDα1 is not involved (Takemiya and Shimazaki 2010). Thus it would be interesting to determine if PLDδ participates in this process. The proposed roles of NO and PA during stomatal movements are summarized in Fig. 3.

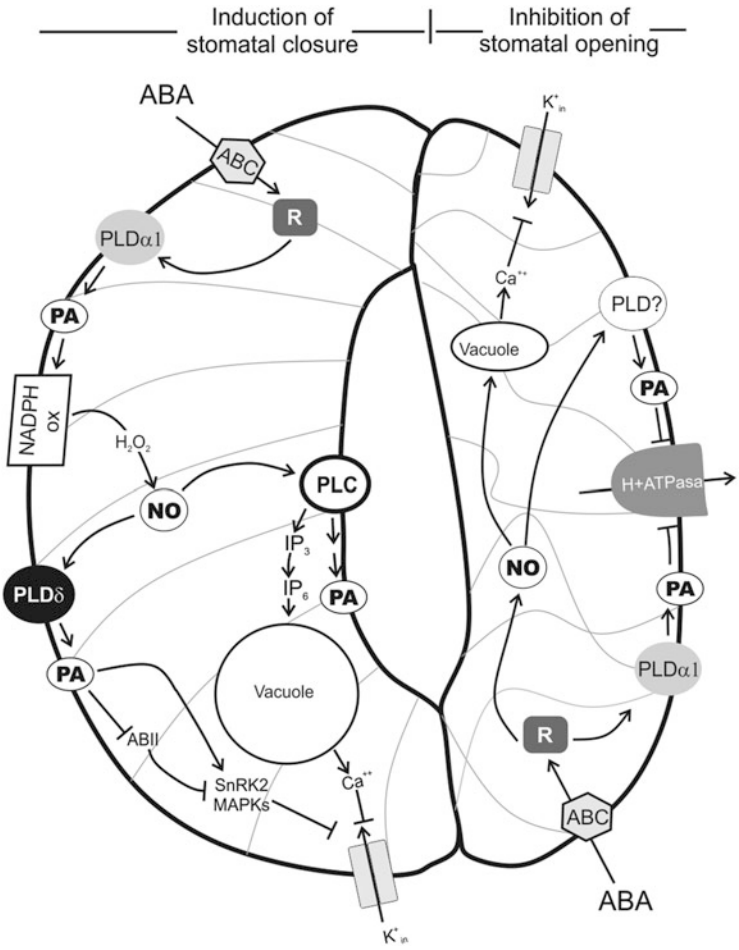

Fig. 3 Simplified model of phospholipid signaling in NO regulation of stomatal movement. In guard cells, NO production is induced upon ABA detection. ABA could be internalized by an ABC transporter (ABC) and then interacts with a soluble receptor (R). NO participates in the regulation of stomatal movements by two different ways, by inducing closure (*left guard cell*) or inhibiting opening (*right guard cell*). NO regulates phospholipases in both processes. In a closed stoma, K^+ uptake and solute accumulation are inhibited. Because of that the osmotic pressure diminishes as water leaves and the cell shrinks. The closed stoma has a big number of small vacuoles and a random distribution of actin filaments. In an opened stoma an increase in the turgor pressure occurs, due to the H^+ATPasa activation, which in turns provokes the uptake of ions, mainly K^+, and the accumulation of solutes. In consequence water enters through guard cells, the cell swell, and the stomata opens. Other characteristic of the open stoma is the low number of large vacuoles and the radial distribution of actin filaments. For details of how NO and phospholipases regulates these processes see the text

4 Auxin and Adventitious Root Formation

The plant hormone auxin modulates diverse aspects of root growth and development. NO is a second messenger in the auxin signal transduction leading to lateral root formation (Correa-Aragunde et al. 2004), root gravitropism (Hu et al. 2005), root hair formation (Lombardo et al. 2006), and adventitious root (AR) formation (Pagnussat et al. 2003).

Auxin induces a transient increase in the level of NO in the tip of cucumber (*Cucumis sativus*) hypocotyls, where ARs are formed. It was proposed that auxin-induced NO triggers cGMP production (Pagnussat et al. 2003). NO also activates a MAPK cascade in a cGMP-independent pathway (Pagnussat et al. 2004). A third signaling pathway that is required is via the increase in cytosolic Ca^{2+} concentration and CDPK activity (Lanteri et al. 2006). Pharmacological data indicate that inhibitors of $InsP_3$-regulated Ca^{2+} channels suppress auxin- and NO-induced AR formation in cucumber explants suggesting the participation of PLC activity (Lanteri et al. 2006). Recent evidence indicates that auxin-induced NO production triggers a rapid and transient accumulation of PA in cucumber hypocotyls and PA stimulated AR formation (Lanteri et al. 2008). Before an increase in PA, auxin induces a NO-dependent accumulation of PtdInsP and $PtdInsP_2$ in cucumber explants (Lanteri et al. 2008). Both PtdInsP and $PtdInsP_2$ act as secondary messengers in a wide scope of processes critical for cell survival, environmental adaptation, and growth (Meijer and Munnik 2003). An increase of phosphatidylinositol 3-phosphate (PtdIns3P) was found upon auxin treatment in isolated membranes of *Arabidopsis* roots, with a role in signaling during the root gravitropic response (Joo et al. 2005). We still do not know which isomers accumulate in response to auxin and NO treatments in cucumber (Lanteri et al. 2008). As mentioned earlier, PtdInsP and $PtdInsP_2$ induce activation of several PLDs in vitro. PLD is activated in vivo during auxin and NO treatments generating PA in cucumber hypocotyls; however, the activation of the PLC/DGK pathway could not be demonstrated (Lanteri et al. 2008). PLD could be directly activated by NO via nitrosylation or nitration and/or indirectly activated via elevations in Ca^{2+} and/or PtdInsP and $PtdInsP_2$. PA could possibly activate CDPK- and MAPK-signaling pathways, as was previously shown (Farmer and Choi 1999; Lee et al. 2001).

Genetic evidence in *Arabidopsis* suggested an involvement of the PLDξ class in root architecture and auxin responses. PLDξ1 is likely to participate in both initiation and maintenance of root hair morphogenesis (Ohashi et al. 2003). PLDξ2 has a role in auxin-induced primary root elongation, hypocotyl elongation, and gravitropism (Li and Xue 2007). Inhibition of PLD with 1-butanol in wild-type *Arabidopsis* seedlings showed stronger repression of gravitropism and lateral root formation than is observed in *pldξ2* mutant, indicating that other PLD members may be involved in auxin responses (Li and Xue 2007). It will be interesting to analyze the level of PA upon auxin treatment in *pldξ2* mutant. A subsequent paper by Mancuso et al. (2007) provides evidence that specifically PLDξ2, and no other PLD, is specialized in the vesicular regulation of polar auxin transport in the distal

portion of the transition zone in root apices. PLDξ2 may regulate auxin transport through modulation of the activity of RCN1 and PID1 (Li and Xue 2007). RCN1, a protein phosphatase 2A regulatory subunit involved in auxin transport (Rashotte et al. 2001), is a potential target protein of PA (Testerink et al. 2004). PID1, a serine/threonine protein kinase that acts as a regulator of polar auxin transport (Bennett et al. 1995) is activated by PDK1 (Zegzouti et al. 2006) which is directly stimulated by PA (Anthony et al. 2004). Since auxin induces PA formation via NO, one can speculate that downstream of this NO-induced PA, auxin transport is regulated via the phosphorylation of auxin transport proteins PIN.

5 Extracellular ATP

In animal cells, ATP is well established as an extracellular signal in a number of cellular responses (Fredholm et al. 1994; Burnstock and Williams 2000). Extracellular ATP (eATP) is perceived through the purine P2 receptors P2X (ion channels) and P2Y (G protein-coupled receptors) (Ralevic and Burnstock 1998). Both P2X and P2Y receptors affect directly or indirectly intracellular Ca^{2+} signaling resulting in a variety of downstream cellular responses. P2Y receptors act via G protein coupling to activate PLC, leading to the formation of DAG in the membrane and $InsP_3$ in the cytosol. $InsP_3$ triggers Ca^{2+} release from intracellular stores, stimulating a variety of signaling pathways including phospholipase A_2, Ca^{2+}-dependent K^+ channels, and NOS. Downstream, DAG, phosphatidylcholine-specific PLC, PLD, the MAPK pathway, and Ca^{2+} influx via voltage-operated Ca^{2+} channels are all being activated. eATP have been detected in plants and shown to participate in different processes. Even though the presence of plant P2-like receptors has been suggested (Song et al. 2006), the molecular components involved in the eATP signaling pathway are poorly known. During wounding, the cell membrane is broken and millimolar ATP concentrations could be released into the extracellular space (Jeter et al. 2004). In addition, other stress conditions like mechanical stimuli and osmotic stress induce ATP release (Jeter et al. 2004; Weerasinghe et al. 2009). More recently, eATP levels were closely correlated with regions of active growth and cell expansion (Kim et al. 2006). Remarkably, increases of PA production have been reported in the abovementioned processes (Munnik and Meijer 2001; Bargmann et al. 2009a, b; Han and Yuan 2009; Zhang et al. 2009). Considering that phospholipases are downstream components of eATP perception in animals (Ralevic and Burnstock 1998) and that in plants phospholipases and lipid-derived molecules regulate diverse responses, we hypothesized a similar scenario in eATP-induced plant responses. The addition of exogenous ATP activated the PLC/DGK and PLD pathways, generating PA (Sueldo et al. 2010). In animals, one of the downstream effectors of eATP-induced PLC activation is NO (Ralevic and Burnstock 1998). In plants, NO production upon eATP treatments was reported in tomato cells suspensions, as well as in *Salvia miltiorrhiza* hairy roots and in *Arabidopsis* pollen tubes (Foresi et al. 2007; Wu and Wu 2008; Reichler

et al. 2009). In tomato cell suspensions, ATP-induced NO production requires PLC/DGK activation (Sueldo et al. 2010). NO production required Ca^{2+} influx from the extracellular in ATP-treated hairy roots (Wu and Wu 2008). In tomato cells, ATP-induced NO accumulation but not PA, requires the activation of plasma membrane Ca^{2+}-permeable channels (Sueldo et al. 2010). Ca^{2+} and PA have been shown to activate NADPH oxidase in *Arabidopsis* (Ogasawara et al. 2008; Takeda et al. 2008; Zhang et al. 2009). Thus, during eATP perception the secondary messengers PA, Ca^{2+}, ROS, and NO are interconnected.

6 Conclusions and Perspectives

In recent years, important efforts have been made to address the role of PA-generating enzymes in different physiological processes. During this time, the targets of PA in plants are being unraveled as well. The study of upstream regulation of the PA-generating enzymes is a less explored area. We postulated NO as an upstream regulator of PA signaling in plants and have demonstrated such a role in four different physiological processes. How NO activates phospholipid-related enzymes is still unknown, although a number of direct or indirect mechanisms by which NO activates PLC/DGK or PLD can be envisaged. NO could act (a) directly on PLC, DGK, or PLD enzymes by S-nitrosylation of cysteines or nitration of tyrosines; (b) indirectly on phospholipases and DGKs by regulating Ca^{2+} homeostasis, or (c) indirectly via protein kinases. Even though NO is regarded as a diffusible signal molecule with a promiscuous sphere of influence, NO signaling is characterized by a set of restricted and highly specific effects. NO activates the PLC/DGK and PLD pathway in plant defense; NO activates PLD in auxin treatments; and NO activates both PLC and PLD during stomatal closure. The fact that PA is localized in membranes, seemingly confines NO–PA signaling to membrane systems. NO activation of different phospholipase and/or kinase isoforms will depend on which isoforms are present at/near the location where NO is generated. Future work should focus on elucidating the specific phospholipase isoforms activated by NO and the mechanisms of activation.

Acknowledgments This work was financially supported by Universidad Nacional de Mar del Plata, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), and Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT).

References

Abat JK, Mattoo AK, Deswal R (2008) S-nitrosylated proteins of a medicinal CAM plant Kalanchoe pinnata- ribulose-1,5-bisphosphate carboxylase/oxygenase activity targeted for inhibition. FEBS J 275:2862–2872

Andersson MX, Kourtchenko O, Dangl JL, Mackey D, Ellerstrom M (2006) Phospholipase-dependent signalling during the AvrRpm1- and AvrRpt2-induced disease resistance responses in Arabidopsis thaliana. Plant J 47:947–959

Andreeva Z, Ho AYY, Barthet MM, Potocký M, Bezvoda R, Žárský V, Marc J (2009) Phospholipase D family interactions with the cytoskeleton: isoform δ promotes plasma membrane anchoring of cortical microtubules. Funct Plant Biol 36:600–612

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

Astier J, Lindermayr C (2012) Nitric oxide-dependent posttranslational modification in plants: an update. Int J Mol Sci 13:15193–15208

Bai X, Yang L, Tian M, Chen J, Shi J, Yang Y, Hu X (2011) Nitric oxide enhances desiccation tolerance of recalcitrant Antiaris toxicaria seeds via protein S-nitrosylation and carbonylation. PLoS One 6:e20714

Bargmann BO, Laxalt AM, Riet BT, Testerink C, Merquiol E, Mosblech A, Leon-Reyes A, Pieterse CM, Haring MA, Heilmann I, Bartels D, Munnik T (2009a) Reassessing the role of phospholipase D in the Arabidopsis wounding response. Plant Cell Environ 32:837–850

Bargmann BOR, Laxalt AM, Bt R, van Schooten B, Merquiol E, Testerink C, Haring MA, Bartels D, Munnik T (2009b) Multiple PLDs required for high salinity and water deficit tolerance in plants. Plant Cell Physiol 50:78–89

Bennett SRM, Alvarez J, Bossinger G, Smyth DR (1995) Morphogenesis in pinoid mutants of *Arabidopsis thaliana*. Plant J 8:505

Besson-Bard A, Pugin A, Wendehenne D (2008) New insights into nitric oxide signaling in plants. Annu Rev Plant Biol 59:21–39

Bethke PC, Badger MR, Jones RL (2004) Apoplastic synthesis of nitric oxide by plant tissues. Plant Cell 16:332–341

Bright J, Desikan R, Hancock JT, Weir IS, Neill SJ (2006) ABA-induced NO generation and stomatal closure in Arabidopsis are dependent on H_2O_2 synthesis. Plant J 45:113–122

Burnstock G, Williams M (2000) P2 purinergic receptors: modulation of cell function and therapeutic potential. J Pharmacol Exp Ther 295:862–869

Correa-Aragunde N, Graziano M, Lamattina L (2004) Nitric oxide plays a central role in determining lateral root development in tomato. Planta 218:900–905

Davis KL, Martin E, Turko IV, Murad F (2001) Novel effects of nitric oxide. Annu Rev Pharmacol Toxicol 41:203–236

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

de Pinto MC, Paradiso A, Leonetti P, De Gara L (2006) Hydrogen peroxide, nitric oxide and cytosolic ascorbate peroxidase at the crossroad between defence and cell death. Plant J 48:784–795

Delledonne M, Xia Y, Dixon RA, Lamb C (1998) Nitric oxide functions as a signal in plant disease resistance. Nature 394:585–588

Delledonne M, Zeier J, Marocco A, Lamb C (2001) Signal interactions between nitric oxide and reactive oxygen intermediates in the plant hypersensitive disease resistance response. Proc Natl Acad Sci U S A 98:13454–13459

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

Desikan R, Graffiths R, Hancock J, Neill S (2002) A new role for an old enzyme: nitrate Reductase- mediated nitric oxide generation is required for abscisic acid-induced stomatal closure in *Arabidopsis thaliana*. Proc Natl Acad Sci U S A 99:16314–16318

Desikan R, Cheung M-K, Bright J, Henson D, Hancock JT, Neill SJ (2004) ABA, hydrogen peroxide and nitric oxide signalling in stomatal guard cells. J Exp Bot 55:205–212

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

Distéfano AM, Garcia-Mata C, Lamattina L, Laxalt AM (2008) Nitric oxide-induced phosphatidic acid accumulation: a role for phospholipases C and D in stomatal closure. Plant Cell Environ 31:187–194

Distéfano A, Lanteri M, ten Have A, García-Mata C, Lamattina L, Laxalt A (2010) Nitric oxide and phosphatidic acid signaling in plants. In: Munnik T (ed) Lipid signaling in plants, plant cell monographs, vol 16. Springer, Berlin, pp 223–242

Distefano AM, Scuffi D, Garcia-Mata C, Lamattina L, Laxalt AM (2012) Phospholipase D delta is involved in nitric oxide-induced stomatal closure. Planta 236:1899–1907

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

Fares A, Rossignol M, Peltier JB (2011) Proteomics investigation of endogenous S-nitrosylation in Arabidopsis. Biochem Biophys Res Commun 416:331–336

Farmer PK, Choi JH (1999) Calcium and phospholipid activation of a recombinant calcium-dependent protein kinase (DcCPK1) from carrot (*Daucus carota* L.). Biochim Biophys Acta 1434:6–17

Foissner I, Wendehenne D, Langebartels C, Durner J (2000) In vivo imaging of an elicitor-induced nitric oxide burst in tobacco. Plant J 23:817–824

Ford PC (2010) Reactions of NO and nitrite with heme models and proteins. Inorg Chem 49:6226–6239

Foresi NP, Laxalt AM, Tonon CV, Casalongue CA, Lamattina L (2007) Extracellular ATP induces nitric oxide production in tomato cell suspensions. Plant Physiol 145:589–592

Foresi N, Correa-Aragunde N, Parisi G, Calo G, Salerno G, Lamattina L (2010) Characterization of a nitric oxide synthase from the plant kingdom: NO generation from the green alga Ostreococcus tauri is light irradiance and growth phase dependent. Plant Cell 22:3816–3830

Fredholm BB, Lindstrom K, Wallman-Johansson A (1994) Propentofylline and other adenosine transport inhibitors increase the efflux of adenosine following electrical or metabolic stimulation of rat hippocampal slices. J Neurochem 62:563–573

Freeman BA, Baker PR, Schopfer FJ, Woodcock SR, Napolitano A, D'Ischia M (2008) Nitro-fatty acid formation and signaling. J Biol Chem 283:15515–15519

Frohlich A, Durner J (2011) The hunt for plant nitric oxide synthase (NOS): is one really needed? Plant Sci 181:401–404

Garcia-Mata C, Lamattina L (2007) Abscisic acid (ABA) inhibits light-induced stomatal opening through calcium- and nitric oxide-mediated signaling pathways. Nitric Oxide 17:143–151

Garcia-Mata C, Gay R, Sokolovski S, Hills A, Lamattina L, Blatt MR (2003) Nitric oxide regulates K^+ and Cl^- channels in guard cells through a subset of abscisic acid-evoked signaling pathways. Proc Natl Acad Sci U S A 100:11116–11121

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

Gaupels F, Kuruthukulangarakoola GT, Durner J (2011) Upstream and downstream signals of nitric oxide in pathogen defence. Curr Opin Plant Biol 14:707–714

Guo L, Devaiah SP, Narasimhan R, Pan X, Zhang Y, Zhang W, Wang X (2012) Cytosolic glyceraldehyde-3-phosphate dehydrogenases interact with phospholipase Dδ to transduce hydrogen peroxide signals in the Arabidopsis response to stress. Plant Cell 24:2200–2212

Gupta KJ, Stoimenova M, Kaiser WM (2005) In higher plants, only root mitochondria, but not leaf mitochondria reduce nitrite to NO, in vitro and in situ. J Exp Bot 56:2601–2609

Gust AA, Biswas R, Lenz HD, Rauhut T, Ranf S, Kemmerling B, Gotz F, Glawischnig E, Lee J, Felix G, Nurnberger T (2007) Bacteria-derived peptidoglycans constitute pathogen-associated molecular patterns triggering innate immunity in Arabidopsis. J Biol Chem 282:32338–32348

Han PP, Yuan YJ (2009) Lipidomic analysis reveals activation of phospholipid signaling in mechanotransduction of Taxus cuspidata cells in response to shear stress. FASEB J 23:623–630

Hess DT, Matsumoto A, Kim SO, Marshall HE, Stamler JS (2005) Protein S-nitrosylation: purview and parameters. Nat Rev Mol Cell Biol 6:150–166

Ho AYY, Day DA, Brown MH, Marc J (2009) Arabidopsis phospholipase Ddelta as an initiator of cytoskeleton-mediated signalling to fundamental cellular processes. Funct Plant Biol 36:190–198

Hu X, Neill SJ, Tang Z, Cai W (2005) Nitric oxide mediates gravitropic bending in soybean roots. Plant Physiol 137:663–670

Huang AX, She XP, Cao B, Zhang B, Mu J, Zhang SJ (2009) Nitric oxide, actin reorganization and vacuoles change are involved in PEG 6000-induced stomatal closure in *Vicia faba*. Physiol Plant 136:45–56

Jacob T, Ritchie S, Assmann SM, Gilroy S (1999) Abscisic acid signal transduction in guard cells is mediated by phospholipase D activity. Proc Natl Acad Sci U S A 96:12192–12197

Jeter CR, Tang W, Henaff E, Butterfield T, Roux SJ (2004) Evidence of a novel cell signaling role for extracellular adenosine triphosphates and diphosphates in Arabidopsis. Plant Cell 16:2652–2664

Joo JH, Yoo HJ, Hwang I, Lee JS, Nam KH, Bae YS (2005) Auxin-induced reactive oxygen species production requires the activation of phosphatidylinositol 3-kinase. FEBS Lett 579:1243–1248

Joudoi T, Shichiri Y, Kamizono N, Akaike T, Sawa T, Yoshitake J, Yamada N, Iwai S (2013) Nitrated cyclic GMP modulates guard cell signaling in Arabidopsis. Plant Cell 25:558–571

Kamiya H (2003) Mutagenic potentials of damaged nucleic acids produced by reactive oxygen/nitrogen species: approaches using synthetic oligonucleotides and nucleotides: survey and summary. Nucleic Acid Res 31:517–531

Kato H, Takemoto D, Kawakita K (2013) Proteomic analysis of S-nitrosylated proteins in potato plant. Physiol Plant 148(3):371–386

Kim SY, Sivaguru M, Stacey G (2006) Extracellular ATP in plants. Visualization, localization, and analysis of physiological significance in growth and signaling. Plant Physiol 142:984–992

Kolbert Z, Bartha B, Erdei L (2007) Exogenous auxin-induced NO synthesis is nitrate reductase-associated in Arabidopsis thaliana root primordia. J Plant Physiol 165:967–975

Kulik A, Wawer I, Krzywinska E, Bucholc M, Dobrowolska G (2011) SnRK2 protein kinases–key regulators of plant response to abiotic stresses. OMICS 15:859–872

Lamattina L, Garcia-Mata C, Graziano M, Pagnussat G (2003) Nitric oxide: the versatility of an extensive signal molecule. Annu Rev Plant Biol 54:109–136

Lamb C, Dixon R (1997) The oxidative burst in plant disease resistance. Annu Rev Plant Physiol Plant Mol Biol 48:251–275

Lamotte O, Courtois C, Dobrowolska G, Besson A, Pugin A, Wendehenne D (2006) Mechanisms of nitric-oxide-induced increase of free cytosolic Ca^{2+} concentration in Nicotiana plumbaginifolia cells. Free Radic Biol Med 40:1369–1376

Lanteri ML, Pagnussat GC, Lamattina L (2006) Calcium and calcium-dependent protein kinases are involved in nitric oxide- and auxin-induced adventitious root formation in cucumber. J Exp Bot 57:1341–1351

Lanteri ML, Laxalt AM, Lamattina L (2008) Nitric oxide triggers phosphatidic acid accumulation via phospholipase D during auxin-induced adventitious root formation in cucumber. Plant Physiol 147:188–198

Lanteri ML, Lamattina L, Laxalt AM (2011) Mechanisms of xylanase-induced nitric oxide and phosphatidic acid production in tomato cells. Planta 234(4):845–855. doi:10.1007/s00425-011-1446-4

Laxalt AM, Raho N, Have AT, Lamattina L (2007) Nitric oxide is critical for inducing phosphatidic acid accumulation in xylanase-elicited tomato cells. J Biol Chem 282:21160–21168

Lee S, Hirt H, Lee Y (2001) Phosphatidic acid activates a wound-activated MAPK in Glycine max. Plant J 26:479–486

Lemtiri-Chlieh F, MacRobbie EAC, Webb AAR, Manison NF, Brownlee C, Skepper JN, Chen J, Prestwich GD, Brearley CA (2003) Inositol hexakisphosphate mobilizes an endomembrane store of calcium in guard cells. Proc Natl Acad Sci U S A 100:10091

Li G, Xue HW (2007) Arabidopsis PLD{zeta}2 regulates vesicle trafficking and is required for auxin response. Plant Cell 19:281

Li M, Hong Y, Wang X (2009) Phospholipase D- and phosphatidic acid-mediated signaling in plants. Biochim Biophys Acta 1791:927–935

Lindermayr C, Saalbach G, Durner J (2005) Proteomic identification of S-Nitrosylated proteins in Arabidopsis. Plant Physiol 137:921–930

Lindermayr C, Sell S, Muller B, Leister D, Durner J (2010) Redox regulation of the NPR1-TGA1 system of Arabidopsis thaliana by nitric oxide. Plant Cell 22:2894–2907

Lombardo M, Graziano M, Polacco J, Lamattina L (2006) Nitric oxide functions as a positive regulator of root hair development. Plant Signal Behav 1:28–33

Lum HK, Butt YKC, Lo SCL (2002) Hydrogen peroxide induces a rapid production of nitric oxide in mung bean (*Phaseolus aureus*). Nitric Oxide 6:205–213

Ma Y, Szostkiewicz I, Korte A, Dl M, Yang Y, Christmann A, Grill E (2009) Regulators of PP2C phosphatase activity function as abscisic acid sensors. Science 324:1064–1068

MacRobbie E (2006) Control of volume and turgor in stomatal guard cells. J Membr Biol 210:131

Maldonado-Alconada A, Echevarría-Zomeño S, Lindermayr C, Redondo-López I, Durner J, Jorrín-Novo J (2011) Proteomic analysis of Arabidopsis protein S-nitrosylation in response to inoculation with Pseudomonas syringae. Physiol Plant 33:1493–1514

Mancuso S, Marras AM, Mugnai S, Schlicht M, Zársky V, Li G, Song L, Xue H, Baluska B (2007) Phospholipase Dζ2 drives vesicular secretion of auxin for its polar cell-cell transport in the transition zone of the root apex. Plant Signal Behav 2:240–244

Manjunatha G, Niranjan-Raj S, Prashanth GN, Deepak S, Amruthesh KN, Shetty HS (2009) Nitric oxide is involved in chitosan-induced systemic resistance in pearl millet against downy mildew disease. Pest Manag Sci 65:737–743

Marino SM, Gladyshev VN (2010) Structural analysis of cysteine S-nitrosylation: a modified acid-based motif and the emerging role of trans-nitrosylation. J Mol Biol 395:844–859

Martinez-Ruiz A, Lamas S (2004) S-nitrosylation: a potential new paradigm in signal transduction. Cardiovasc Res 62:43–52

Meijer HJ, Munnik T (2003) Phospholipid-based signaling in plants. Annu Rev Plant Biol 54:265–306.

Mishra G, Zhang W, Deng F, Zhao J, Wang X (2006) A bifurcating pathway directs abscisic acid effects on stomatal closure and opening in Arabidopsis. Science 312:264–266

Misra S, Wu Y, Venkataraman G, Sopory SK, Tuteja N (2007) Heterotrimeric G-protein complex and G-protein-coupled receptor from a legume (Pisum sativum): role in salinity and heat stress and cross-talk with phospholipase C. Plant J 51:656–669

Moller M, Botti H, Batthyany C, Rubbo H, Radi R, Denicola A (2005) Direct measurement of nitric oxide and oxygen partitioning into liposomes and low density lipoprotein. J Biol Chem 280:8850

Molina y Vedia L, McDonald B, Reep B, Brüne B, Di Silvio M, Billiar TR, Lapetina EG (1992) Nitric oxide-induced S-nitrosylation of glyceraldehyde-3-phosphate dehydrogenase inhibits enzymatic activity and increases endogenous ADP-ribosylation. J Biol Chem 267:24929–24932

Moreau M, Lee GI, Wang Y, Crane BR, Klessig DF (2008) AtNOS/AtNOA1 is a functional Arabidopsis thaliana cGTPase and not a nitric-oxide synthase. J Biol Chem 283:32957–32967

Motes CM, Pechter P, Yoo CM, Wang YS, Chapman KD, Blancaflor EB (2005) Differential effects of two phospholipase D inhibitors, 1-butanol and N-acylethanolamine, on in vivo cytoskeletal organization and Arabidopsis seedling growth. Protoplasma 226:109–123

Mueller-Roeber B, Pical C (2002) Inositol phospholipid metabolism in Arabidopsis. Characterized and putative isoforms of inositol phospholipid kinase and phosphoinositide-specific phospholipase C. Plant Physiol 130:22–46

Munnik T, Meijer HJ (2001) Osmotic stress activates distinct lipid and MAPK signalling pathways in plants. FEBS Lett 498:172–178

Munnik T, Nielsen E (2011) Green light for polyphosphoinositide signals in plants. Curr Opin Plant Biol 14:489–497

Neill SJ, Desikan R, Hancock JT (2003) Nitric oxide signalling in plants. New Phytol 159:11–35

Neill S, Barros R, Bright J, Desikan R, Hancock J, Harrison J, Morris P, Ribeiro D, Wilson I (2008) Nitric oxide, stomatal closure, and abiotic stress. J Exp Bot 59:165–176

Nuhse TS, Boller T, Peck SC (2003) A plasma membrane syntaxin is phosphorylated in response to the bacterial elicitor flagellin. J Biol Chem 278:45248–45254

Nuhse TS, Bottrill AR, Jones AM, Peck SC (2007) Quantitative phosphoproteomic analysis of plasma membrane proteins reveals regulatory mechanisms of plant innate immune responses. Plant J 51:931–940

Ogasawara Y, Kaya H, Hiraoka G, Yumoto F, Kimura S, Kadota Y, Hishinuma H, Senzaki E, Yamagoe S, Nagata K, Nara M, Suzuki K, Tanokura M, Kuchitsu K (2008) Synergistic activation of the Arabidopsis NADPH oxidase AtrbohD by Ca^{2+} and phosphorylation. J Biol Chem 283:8885–8892

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

Ortega-Galisteo AP, Rodriguez-Serrano M, Pazmino DM, Gupta DK, Sandalio LM, Romero-Puertas MC (2012) S-Nitrosylated proteins in pea (Pisum sativum L.) leaf peroxisomes: changes under abiotic stress. J Exp Bot 63:2089–2103

Pagnussat GC, Lanteri ML, Lamattina L (2003) Nitric oxide and cyclic GMP are messengers in the indole acetic acid-induced adventitious rooting process. Plant Physiol 132:1241–1248

Pagnussat GC, Lanteri ML, Lombardo MC, Lamattina L (2004) Nitric oxide mediates the indole acetic acid induction activation of a mitogen-activated protein kinase cascade involved in adventitious root development. Plant Physiol 135:279–286

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

Park S-Y, Fung P, Nishimura N, Jensen DR, Fujii H, Zhao Y, Lumba S, Santiago J, Rodrigues A, T-fF C, Alfred SE, Bonetta D, Finkelstein R, Provart NJ, Desveaux D, Rodriguez PL, McCourt P, Zhu J-K, Schroeder JI, Volkman BF, Cutler SR (2009) Abscisic acid inhibits type 2c protein phosphatases via the PYR/PYL Family of START Proteins. Science 324:1068–1071

Planchet E, Jagadis Gupta K, Sonoda M, Kaiser WM (2005) Nitric oxide emission from tobacco leaves and cell suspensions: rate limiting factors and evidence for the involvement of mitochondrial electron transport. Plant J 41:732–743

Prats E, Mur LA, Sanderson R, Carver TL (2005) Nitric oxide contributes both to papilla-based resistance and the hypersensitive response in barley attacked by *Blumeria graminis* f. sp. hordei. Mol Plant Pathol 6:65–78

Qin C, Wang X (2002) The Arabidopsis phospholipase D family. Characterization of a calcium-independent and phosphatidylcholine-selective PLD zeta 1 with distinct regulatory domains. Plant Physiol 128:1057–1068

Raho N, Ramirez L, Lanteri ML, Gonorazky G, Lamattina L, ten Have A, Laxalt AM (2011) Phosphatidic acid production in chitosan-elicited tomato cells, via both phospholipase D and phospholipase C/diacylglycerol kinase, requires nitric oxide. J Plant Physiol 168:534–539

Ralevic V, Burnstock G (1998) Receptors for purines and pyrimidines. Pharmacol Rev 50:413–492

Rashotte AM, DeLong A, Muday GK (2001) Genetic and chemical reductions in protein phosphatase activity alter auxin transport, gravity response, and lateral root growth. Plant Cell 13:1683–1697

Reichler SA, Torres J, Rivera AL, Cintolesi VA, Clark G, Roux SJ (2009) Intersection of two signalling pathways: extracellular nucleotides regulate pollen germination and pollen tube growth via nitric oxide. J Exp Bot 60:2129–2138

Rubbo H, Radi R, Anselmi D, Kirk M, Barnes S, Butler J, Eiserich JP, Freeman BA (2000) Nitric oxide reaction with lipid peroxyl radicals spares alpha -tocopherol during lipid peroxidation. Greater oxidant protection from the pair nitric oxide/alpha-tocopherol than alpha -tocopherol/ascorbate. J Biol Chem 275:10812

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

Serpa V, Vernal J, Lamattina L, Grotewold E, Cassia R, Terenzi H (2007) Inhibition of AtMYB2 DNA-binding by nitric oxide involves cysteine S-nitrosylation. Biochem Biophys Res Commun 361:1048–1053

Smertenko AP, Chang H-Y, Wagner V, Kaloriti D, Fenyk S, Sonobe S, Lloyd C, Hauser M-T, Hussey PJ (2004) The Arabidopsis microtubule-associated protein AtMAP65-1: molecular analysis of its microtubule bundling activity. Plant Cell 16:2035–2047

Sokolovski S, Hills A, Gay R, Garcia-Mata C, Lamattina L, Blatt MR (2005) Protein phosphorylation is a prerequisite for intracellular Ca^{2+} release and ion channel control by nitric oxide and abscisic acid in guard cells. Plant J 43:520–529

Song CJ, Steinebrunner I, Wang X, Stout SC, Roux SJ (2006) Extracellular ATP induces the accumulation of superoxide via NADPH oxidases in Arabidopsis. Plant Physiol 140:1222–1232

Stamler JS (1994) Redox signaling: nitrosylation and related target interactions of nitric oxide. Cell Dev Biol 78:931–936

Stamler JS, Singel DJ, Loscalzo J (1992) Biochemistry of nitric oxide and its redox-activated forms. Science 258:1898–1902

Stohr C, Stremlau S (2006) Formation and possible roles of nitric oxide in plant roots. J Exp Bot 57:463–470. doi:10.1093/jxb/erj058

Sueldo D, Foresi N, Casalongué C, Lamattina L, Laxalt AM (2010) Phosphatidic acid formation is required for extracellular ATP-mediated nitric oxide production in suspension-cultured tomato cells. New Phytol 185:909–916

Sun J, Murphy E (2010) Protein S-nitrosylation and cardioprotection. Circ Res 106(2):285–296. doi:10.1161/CIRCRESAHA.109.209452, Review

Tada Y, Spoel SH, Pajerowska-Mukhtar K, Mou Z, Song J, Wang C, Zuo J, Dong X (2008) Plant immunity requires conformational changes of NPR1 via S-nitrosylation and thioredoxins. Science 321:952–956

Takeda S, Gapper C, Kaya H, Bell E, Kuchitsu K, Dolan L (2008) local positive feedback regulation determines cell shape in root hair cells. Science 319:1241–1244

Takemiya A, Shimazaki K (2010) Phosphatidic acid inhibits blue light-induced stomatal opening via inhibition of protein phosphatase 1 [corrected]. Plant Physiol 153:1555–1562

Tanou G, Job C, Rajjou L, Arc E, Belghazi M, Diamantidis G, Molassiotis A, Job D (2009) Proteomics reveals the overlapping roles of hydrogen peroxide and nitric oxide in the acclimation of citrus plants to salinity. Plant J 60:795–804

Terrile MC, Paris R, Calderon-Villalobos LI, Iglesias MJ, Lamattina L, Estelle M, Casalongue CA (2012) Nitric oxide influences auxin signaling through S-nitrosylation of the Arabidopsis TRANSPORT INHIBITOR RESPONSE 1 auxin receptor. Plant J 70:492–500

Testerink C, Munnik T (2011) Molecular, cellular, and physiological responses to phosphatidic acid formation in plants. J Exp Bot 62:2349–2361

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

Torres MA, Dangl JL, Jones JD (2002) Arabidopsis gp91phox homologues AtrbohD and AtrbohF are required for accumulation of reactive oxygen intermediates in the plant defense response. Proc Natl Acad Sci USA 99:517–522

Umezawa T, Sugiyama N, Takahashi F, Anderson JC, Ishihama Y, Peck SC, Shinozaki K (2013) Genetics and phosphoproteomics reveal a protein phosphorylation network in the abscisic acid signaling pathway in Arabidopsis thaliana. Sci Signal 6:rs8

Uraji M, Katagiri T, Okuma E, Ye W, Hossain MA, Masuda C, Miura A, Nakamura Y, Mori IC, Shinozaki K, Murata Y (2012) Cooperative function of PLDdelta and PLDalpha1 in abscisic acid-induced stomatal closure in Arabidopsis. Plant Physiol 159:450–460

Van Baarlen P, Woltering EJ, Staats M, van Kan JAL (2007) Histochemical and genetic analysis of host and non-host interactions of Arabidopsis with three Botrytis species: an important role for cell death control. Mol Plant Pathol 8:41–54

Vlad F, Rubio S, Rodrigues A, Sirichandra C, Belin C, Robert N, Leung J, Rodriguez PL, Lauriere C, Merlot S (2009) Protein phosphatases 2C regulate the activation of the Snf1-related kinase OST1 by abscisic acid in Arabidopsis. Plant Cell 21:3170–3184

Vossen JH, Abd-El-Haliem A, Fradin EF, van den Berg GC, Ekengren SK, Meijer HJ, Seifi A, Bai Y, Ten Have A, Munnik T, Thomma BP, Joosten MH (2010) Identification of tomato phosphatidylinositol-specific phospholipase-C (PI-PLC) family members and the role of PLC4 and PLC6 in HR and disease resistance. Plant J 62:224–239

Wang C, Wang X (2001) A novel phospholipase D of Arabidopsis that is activated by oleic acid and associated with the plasma membrane. Plant Physiol 127:1102–1112

Wang YQ, Feechan A, Yun BW, Shafiei R, Hofmann A, Taylor P, Xue P, Yang FQ, Xie ZS, Pallas JA, Chu CC, Loake GJ (2009) S-nitrosylation of AtSABP3 antagonizes the expression of plant immunity. J Biol Chem 284:2131–2137

Wawer I, Bucholc M, Astier J, Anielska-Mazur A, Dahan J, Kulik A, Wysłouch-Cieszynska A, Zareba-Kozioł M, Krzywinska E (2010) Regulation of Nicotiana tabacum osmotic stress-activated protein kinase and its cellular partner GAPDH by nitric oxide in response to salinity. Biochem J 429:73–83

Weerasinghe RR, Swanson SJ, Okada SF, Garrett MB, Kim SY, Stacey G, Boucher RC, Gilroy S, Jones AM (2009) Touch induces ATP release in Arabidopsis roots that is modulated by the heterotrimeric G-protein complex. FEBS Lett 583:2521–2526

Weiner JJ, Peterson FC, Volkman BF, Cutler SR (2010) Structural and functional insights into core ABA signaling. Curr Opin Plant Biol 13:495–502

Wu SJ, Wu JY (2008) Extracellular ATP-induced NO production and its dependence on membrane Ca^{2+} flux in Salvia miltiorrhiza hairy roots. J Exp Bot 59:4007–4016

Xu L, Han C, Lim K, Wu T (2008) Activation of cytosolic phospholipase A2alpha through nitric oxide-induced S-nitrosylation. Involvement of inducible nitric-oxide synthase and cyclooxygenase-2. J Biol Chem 283:3077–3087

Yamaguchi T, Tanabe S, Minami E, Shibuya N (2004) Activation of phospholipase D induced by hydrogen peroxide in suspension-cultured rice cells. Plant Cell Physiol 45:1261–1270

Yamaguchi T, Minami E, Ueki J, Shibuya N (2005) Elicitor-induced activation of phospholipases plays an important role for the induction of defense responses in suspension-cultured rice cells. Plant Cell Physiol 46:579–587

Yamamoto A, Katou S, Yoshioka H, Doke N, Kawakita K (2004) Involvement of nitric oxide generation in hypersensitive cell death induced by elicitin in tobacco cell suspension culture. J Gen Plant Pathol 70:85–92

Yamasaki H, Cohen MF (2006) NO signal at the crossroads: polyamine-induced nitric oxide synthesis in plants? Trends Plant Sci 11:522–524

Yun BW, Feechan A, Yin M, Saidi NB, Le Bihan T, Yu M, Moore JW, Kang JG, Kwon E, Spoel SH, Pallas JA, Loake GJ (2011) S-nitrosylation of NADPH oxidase regulates cell death in plant immunity. Nature 478:264–268

Zegzouti H, Anthony RG, Jahchan N, Bogre L, Christensen SK (2006) Phosphorylation and activation of PINOID by the phospholipid signaling kinase 3-phosphoinositide-dependent protein kinase 1 (PDK1) in Arabidopsis. Proc Natl Acad Sci U S A 103:6404–6409

Zhang W, Qin C, Zhao J, Wang X (2004) Phospholipase D alpha 1-derived phosphatidic acid interacts with ABI1 phosphatase 2C and regulates abscisic acid signaling. Proc Natl Acad Sci U S A 101:9508–9513

Zhang X, Takemiya A, Kinoshita T, Shimazaki K-I (2007) Nitric oxide inhibits blue light-specific stomatal opening via abscisic acid signaling pathways in vicia guard cells. Plant Cell Physiol 48:715

Zhang Y, Zhu H, Zhang Q, Li M, Yan M, Wang R, Wang L, Welti R, Zhang W, Wang X (2009) Phospholipase Dα1 and phosphatidic acid regulate NADPH oxidase activity and production of reactive oxygen species in ABA-mediated stomatal closure in Arabidopsis. Plant Cell 21:2357–2377

Phospholipases in Plant Response to Nitrogen and Phosphorus Availability

Yueyun Hong and Shaoping Lu

Abstract Nitrogen and phosphorus are vital elements with key roles in cellular structure and metabolism and are essential nutrients required for plant growth and development. Membrane phospholipids function as units of cellular structure and also as perception sites that initiate signaling transduction in cells. Recent studies of metabolism combined with genetic, molecular, and cellular analyses have determined that phospholipase-mediated signaling and metabolism play important roles in nitrogen signaling and internal phosphate recycling. The involvement of phospholipases in nitrogen or phosphate starvation occurs likely through different mechanisms. Nitrogen response is through phospholipase Dε- and phosphatidic acid-mediated signaling, which promotes root growth and nitrogen acquisition to enhance N use efficiency and plant growth. Phospholipase Dζ2 and nonspecific phospholipase Cs are involved in plant response to phosphate deprivation through lipid remodeling to enhance internal Pi use efficiency.

Keywords Phospholipases • Nitrogen signaling • Phosphorus recycle • Plant nutrient use efficiency

1 Introduction

Nitrogen (N) and phosphorus are essential, major elements for plant growth and development. Plants frequently suffer from nutrient deficiency, especially in nitrogen (N) and phosphate (Pi), due to their low availability and heterogeneous distribution in soil. To survive in adverse environments, plants have evolved multiple mechanisms including altering their morphological patterns, molecular regulation, metabolism, and physiology to enhance nutrient capture and use

Y. Hong (✉) • S. Lu
National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan 430070, China
e-mail: hongyy@mail.hzau.edu.cn

X. Wang (ed.), *Phospholipases in Plant Signaling*, Signaling and Communication in Plants 20, DOI 10.1007/978-3-642-42011-5_9,

efficiency. Lipid signaling and lipid remodeling play important roles in such responses. Membrane phospholipids function in cell structure and also as perception sites that initiate signaling transduction in cells. Phospholipids constitute approximately 30 % of total internal Pi in plant tissues. In addition to Pi, several classes of phospholipids also contain nitrogen, including phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylserine (PS). Moreover, membranes are enriched in signaling molecules involved in various signaling cascades. Increasing evidence shows that phosphatidic acid (PA) is an important messenger in different biological processes, such as plant growth, development, and responses to stress and nutrient limitation (Wang 2004; Testerink and Munnik 2005; Wang et al. 2006; Li et al. 2006a, b; Hong et al. 2008, 2009; Guo et al. 2012). Recent genetic, molecular, and metabolic analyses have revealed that phospholipase-mediated lipid messengers and metabolism play important roles in nitrogen signaling and internal Pi recycling. Here we highlight recent advances in nutrient sensing and metabolism mediated by phospholipase Dε (PLDε), PLDζ, and nonspecific phospholipase C (NPC).

2 PLDε Is Involved in N Signaling and Enhances N Use Efficiency

Plant growth and development are tightly regulated by N availability, and N limitation is a major factor adversely impacting agricultural productivity. Recent studies have shed light on N uptake, transport, and assimilation (Crawford 1995; Zhang et al. 1999; Walch-Liu et al. 2006; Hirel et al. 2007). However, there is still little known about the signaling events involving plant response to N availability. The activation of PLD is an early, critical step in many signaling cascades regarding nutrient sensing and stress responses (Wang et al. 2006; Li et al. 2006a, b; Hong et al. 2008, 2009; Guo et al. 2012). PLD-derived PA has been implicated in the regulation of protein phosphorylation, transcription, and translation processes that enhance cell proliferation and growth (Potocky et al. 2003; Fang et al. 2001). In animal cells, PLD1-derived PA activates mammalian target of rapamycin (mTOR) signaling to promote protein synthesis in response to nutrient availability (Fang et al. 2001, 2003).

Plant PLD family is heterogeneous and involved in various stress responses including drought, salt, freezing, and senescence and also is implicated to regulate growth and development. PLDε is quite distinct from the other 11 PLDs in terms of its domain structure, subcellular localization, and regulation (Hong et al. 2009, 2010). Recently, PLDε has been found to promote whole organismal growth and enhance biomass and seed yield in *Arabidopsis* (Hong et al. 2009). This growth promotion by PLDε is mediated by PLDε-derived PA. Lateral root elongation in response to external N availability is regarded as an indicator of N signaling (Zhang et al. 2007; Desnos 2008). Enhanced lateral root elongation by PLDε-derived PA

suggests that PLDε and its PA are involved in N signaling to promote plant growth and N use efficiency.

2.1 Activation and Regulation of PLDε

The expression pattern and subcellular localization of PLDε are distinctively different from other PLDs. Under nitrogen-sufficient conditions, the relative expression level of *PLDε* in tissues is much lower than that of *PLDα1*, and its tissue distribution is different from other *PLDs* as shown by RT-PCR as well as data from Genevestigator (http://www.genevestigator.ethz.ch). The Arabidopsis *PLDε* mRNA level is highest in roots, but low in leaves. The expression of *PLDε* was induced by N limitation but not by other nutrient deficiencies such as sulfur (S) or Pi. PLDε is exclusively localized at the plasma membrane as revealed by subcellular fractionation and also visualization of YFP fused protein under confocal microscopy (Hong et al. 2009).

Of 12 PLDs in Arabidopsis, 10 PLDs contain a C2 domain, which serves as a structural fold for Ca^{2+}-dependent phospholipid binding. Four to five acidic residues in the C2 domain are important for Ca^{2+} binding (Qin et al. 1997). Unlike PLDβs, γs, and δ, which all contain Ca^{2+}-binding residues, PLDε does not. Of 10 C2-PLDs, the sequence of PLDε is most closely related to that of the PX/PH containing PLDζs (Qin et al. 1997; Qin and Wang 2002). Sequence analysis also showed that PLDε is a unique protein that shares no more than 55 % similarity to other PLDs (Qin and Wang 2002). The level of PLDε activity depends on reaction conditions, but is most active under the PLDα1 condition that uses 50 mM Ca^{2+}, SDS, and single-class lipid vesicles. Under micromolar concentrations of Ca^{2+}, PLDε was also activated by either oleic acid or PE plus phosphatidylinositol 3,4-bisphosphate (PIP_2). In vitro assay showed that PLDε hydrolyzed membrane phospholipids PC, PE, and phosphatidyl-glycerol (PG) to produce PA (Hong et al. 2009). The results suggest that PLDε activation is more permissive compared to other PLDs, responding to a broad range of reaction conditions. The membrane association of PLDε may facilitate its rapid access to membrane substrates for activation without the relocation to the membrane.

2.2 PLDε Is Involved in Root Architecture in Response to N Availability

In response to low N, plant root architecture is altered, including increases in lateral root length and number, in order to enhance nutrient acquisition. It was shown that the alteration of PLDε resulted in a significant difference in root growth in response to N availability. When seedlings were grown in severe (0.6 mM N, NO_3^-:

NH_4^+ = 2:1) or mild (6 mM N) N-limited conditions (N levels that are 100- and 10-fold lower than those of 1 × MS media), the lateral root elongation was markedly higher in *PLDε*-OE, but was lower in *PLDε*-KO, as compared with WT seedlings. The PLDε effect is specific to N, but not to other nutrients deficiencies, such as Pi, potassium (K), or S (Hong et al. 2009). When seedlings were supplied with only nitrate, the root length was also greater in OE and shorter in KO than that of WT. The result suggests that PLDε enhanced lateral root elongation and number in response to N deficiency. PLDε also promotes primary root and root hair growth under severe N stress (0.1 mM N) (Hong et al. 2009).

These results suggest that the basal level of *PLDε* expression is important for maintaining normal primary and lateral root growth, especially under N-limited conditions. Overexpression of *PLDε* enhances lateral root number and root hair length. In addition, the enhanced root growth by PLDε was also found in conditions of hyperosmotic stress including high salinity and water deficiency. Drought usually induces N deficiency due to the interplay between water availability and nutrient transport (Heckathorn et al. 1997; Foyer et al. 1998). The positive effect of PLDε on hyperosmotic stress may result from the enhanced root growth by *PLDε* in N response.

2.3 PLDε Enhances N Uptake and Assimilation

The alteration of PLDε resulted in changes in nitrogen acquisition in response to N availability. The gene expression of dual-affinity nitrate transporter *NRT1.1* was suppressed in all genotypes and was significantly lower in *PLDε*-KO mutant plants than in WT under N-limited condition (0.6 mM). The gene encoding high-affinity, low-capacity nitrate transporter *NRT2.1* was induced in all genotypes and was twofold higher in *PLDε*-OE than in WT plants under N starvation (0.6 mM N) (Huang et al. 1996; Cerezo et al. 2001; Hong et al. 2009). OE plants also exhibited 1.5-fold higher nitrate uptake than WT after transferring nitrate-starved seedlings to nitrate-sufficient medium for 6–7 h (Hong et al. 2009). The enhanced nitrate transporter expression and uptake in *PLDε*-OE plants suggest that *PLDε* promotes nitrogen acquisition. Further enzymatic analyses indicate that PLDε plays a positive role in N assimilation under N-limited conditions. Nitrate assimilation consists of several reactions, including the reductions of NO_3^- to NO_2^- by nitrate reductase (NR) and then to NH_4^+ by nitrite reductase (NiR). NH_4^+ is then incorporated into organic molecules by glutamine synthetase (GS)/glutamine synthase (Crawford 1995; Takahashi et al. 2001; Walch-Liu et al. 2006). Analysis on *PLDε*-altered plants showed that NiR and GS activities in PLD-OE were significantly higher than in WT plants under severe and mild N starvation. However, the activity of glutamate dehydrogenase (GDH), which is responsible for oxidative deamination of glutamate, was lower in OE than in WT. The difference in NiR, GS, and GDH was greatest between OE and WT seedlings under severe N deficiency (0.6 mM). Thus,

PLDε enhances both N acquisition and N assimilation and meanwhile decreases glutamate catabolism.

2.4 PLDε Enhances Plant Growth Under N-Enriched Condition

It was shown that *PLDε*-OE plants grew faster and larger than WT plants in N-enriched soil or agar plates under sufficient light conditions (Hong et al. 2009). The fresh and dry weights of rosettes of *PLDε*-OE were 192 % and 212 % of those of WT, respectively, after 5 weeks grown in well-fertilized conditions, and seed yield of *PLDε*-OE was 25 % higher than that of WT. Further investigation revealed that the enhanced growth resulted from an increase in both leaf size and leaf number. The increased cell size and cell number contributed to the larger plant size in *PLDε*-OE plants. When *PLDε*-OE, -KO, and WT plants were planted side by side under low fertilizer and low light intensity conditions, *PLDε*-KO plants were much smaller in size and were outcompeted by WT and OE plants. The growth enhanced by *PLDε* was demonstrated by biomass accumulation in *PLDε*-altered plant under different N supply conditions. The biomass of *PLDε*-KO was 20 % less than that of WT under various levels of N (0.6, 2, 6, or 60 mM), whereas *PLDε*-OE plants accumulated substantially more biomass as N concentrations increased. The dry weight of OE plants was approximately 20, 30, and 40 % higher than that of WT plants at N levels of 0.6, 2, and 6 mM (Hong et al. 2009). The described results suggest that *PLDε* enhances N acquisition, which leads to improved plant growth in response to N availability. This suggests that *PLDε* is required for plant growth under severe N-limited conditions, whereas a high level of PLDε promotes plant growth under N-enriched conditions.

Plant hormones auxin and abscisic acid (ABA) are often associated with growth and morphological alterations under adverse conditions. Further investigation revealed that PLDε may cross talk with hormones to regulate cell and plant growth in response to N availability. When plants were grown in N-enriched soil, the level of auxin (IAA) was higher in OE plants than in WT plants; however, the levels of the stress hormone ABA were not different under the same conditions (Hong et al. unpublished data). However, ABA content in OE plants was significantly lower than in WT when seedlings were grown in agar plates under N limitation, whereas PLDε-KO plants accumulated more ABA under high N (60 mM) condition (Hong et al. unpublished data). This suggests that PLDε also affects the levels of IAA and ABA to regulate plant growth and root architecture in response to N availability (Fig. 1).

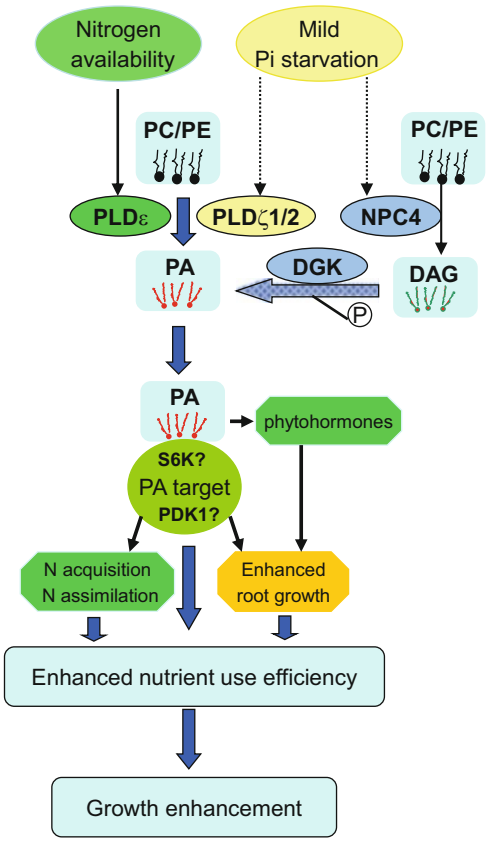

Fig. 1 PLDε, PLDζ and NPC derived PA is involved in nutrient signaling and growth regulation. In response to nitrogen availability, PLDε is activated to hydrolyze phospholipids PC, PE to produce PA. PA subsequently activates its potential targets such as S6K, PDK1 to promote nitrogen uptake, transport and assimilation to enhance nitrogen use efficiency. The enhanced root growth by PA could also improve nutrient acquisition to promote plant growth. In addition, under mild Pi starvation, PLDζ1/2 and NPC4 are also involved in PA signaling to promote root growth, thus enhance Pi acquisition rather than involvement of lipid remodeling. PA may also have crosstalk with phytohormone signaling to regulate growth

2.5 *PLDε-Derived PA Is Important for N Response and Growth*

Pharmacological treatments indicate that PLDε-derived PA is important for plant growth regulation. PLD can transfer the phosphatidyl group to a primary alcohol, forming phosphatidylalcohol (PEOH) at the expense of PA when a primary alcohol was present. To distinguish the effect of PLDε itself or its product PA, 1-butanol was used to suppress PLD-mediated PA formation. As a result, 1-butanol inhibited the number and length of lateral roots in all genotypes. The application of 1-butanol attenuated the difference in the number and length of lateral roots and biomass among OE, WT, and KO plants under N-limited condition (Fig. 1).

Lipid profiling revealed that PA concentrations in OE and KO plants are different from WT. When plants were grown in N-enriched soil, PA level in leaves of *PLDε*-KO mutant was approximately 50 % lower, whereas that of OE was 15 % higher than in WT. PA level in roots of *PLDε*-KO was only 67 % of that in WT, whereas that of OE was slightly higher than that of WT under mild N-limited

conditions. The levels of major membrane lipids in roots, including PC, PG, monogalactosyl-diacylglycerol (MGDG), and digalactosyl-diacylglycerol (DGDG), were not significantly different among KO, OE, and WT. However, the PE level in roots was substantially higher in KO, but lower in OE than in WT. The inverse changes in PA and PE in roots suggest that most PA was derived from PE hydrolysis by PLDε. Molecular species analysis revealed the concomitant changes in the most abundant species 34:3-, 34:2-PE, and PA, suggesting that most PA is derived from PLDε-catalyzed PE hydrolysis (Hong et al. 2009). Taken together, the results from alcohol treatment and lipid analysis indicate that PLDε contributes to PA production and that PLDε-produced PA is involved in growth promotion in response to N availability (Fig. 1).

In addition, under severe N-deficient conditions (0.6 mMN), PG level was lower in leaves of *PLDε*-OE than that of WT, with a concomitant increase in the level of MGDG and DGDG (Hong et al. unpublished data). The decrease of PG in OE seedlings was 13 %, whereas the increases in MGDG and DGDG were about 5 %. The amount of PG lost was almost fully compensated for by the gain of galactolipids. The effect of *PLDε* alterations on PG level also occurred in plants grown in N-enriched soil (Hong et al. unpublished data). These results could mean that PLDε is also involved in the turnover of plastidic lipids involved in photosynthesis in response to nutrient availability. Since MGDG and DGDG are important components in photosynthetic membranes, a higher level of MGDG and DGDG may benefit plant growth under severe nitrogen deficiency. Plants are autotrophic, and their growth relies on photosynthesis and carbon fixation. Enhanced growth in response to N availability requires enhanced photosynthesis because C/N ratio is important for many biosynthetic processes. However, further investigations are needed to determine how the plasma membrane-associated PLDε is involved in the change in plastidic lipids.

2.6 *Downstream Processes Regulated by PLDε in N Response*

The activation of PLD is an early response to extracellular signal stimuli. How do PLDε and its produced PA mediate N sensing and plant growth regulation? Previous studies have revealed that PA interacts with phosphoinositide-dependent protein kinase 1 (PDK1) to activate AGC2-1 kinase and thus promotes root hair growth (Anthony et al. 2004). *PLDε*-OE displayed a similar effect on root hair growth, suggesting that PLDε-derived PA may be also involved in the PDK1 activation pathway, and PDK1 may function as a PLDε-derived PA downstream target in N response (Fig. 1). It was shown that PDK1 also phosphorylates ribosomal S6 kinase (S6K) in Arabidopsis (Mahfouz et al. 2006). In mammalian cells, PA binds to TOR and S6K, a master regulator, to promote cell growth and nutrient utilization (Fang et al. 2001, 2003; Wullschleger et al. 2006; Lehman et al. 2007).

PLDε-derived PA was shown to promote cell growth and enhance osmotic tolerance in Arabidopsis. The alterations of PLDε affected the phosphorylation of S6K, and the level of phosphorylated S6K in PLDε-KO plants was lower than that of WT plants when plants were grown under severe N-limited conditions, whereas the level of phosphorylated S6K in OE plants was higher than in WT and KO under higher N levels (Hong et al. unpublished data). These results imply that PLDε plays a positive role in growth response to N availability through S6K (Fig. 1). The growth effect of PLDε raises intriguing questions as to whether PLD- and PA-mediated signaling plays a role in connecting the sensing of external nutrient cues at membranes to translational regulation and growth alterations.

3 Phospholipases in Lipid Remodeling in Pi Starvation

Pi is an important cellular component of membrane lipids, nucleic acids, and energy supply sources. Pi is also a key component in various biological processes such as protein phosphorylation and substrate activation for metabolism and signaling. Due to its immobilization in soil by binding to other compounds, phosphorus in soil is often unavailable for direct plant use in natural conditions (Raghothama 1999; Poirier and Bucher 2002). To cope with Pi limitation, plants have developed a series of mechanisms to enhance Pi use efficiency (Raghothama 1999; Theodorou and Plaxton 1993; Vance et al. 2003). Membrane lipid remodeling is an important strategy of plant adaptation to Pi deprivation through relocalization and recycling of internal Pi within different cells or cellular compartments (Li et al. 2006a, b; Cruz-Ramirez et al. 2006; Gaude et al. 2008). In response to Pi starvation, Pi is released from membrane phospholipids for critical cell activities, and degraded phospholipids are replaced by non-phosphorus containing lipids, particularly DGDG and sulfoquinovosyldiacylglycerol (SQDG), to maintain membrane integrity (Benning 1998; Hartel et al. 2000; Andersson et al. 2005; Russo et al. 2007; Tjellstrom et al. 2008). Recent studies have shed light on molecular regulation mediated by phospholipases in internal Pi use efficiency. Two phospholipase-mediated pathways have been determined to be involved in phosphate recycling through membrane remodeling in plants. One is through the activation of PLD to hydrolyze phospholipids to produce PA, followed by the activity of PA phosphohydrolase (PAH) to generate DAG for galactolipid production (Cruz-Ramirez et al. 2006; Li et al. 2006a; Nakamura et al. 2009). The other is via a nonspecific phospholipase C (NPC) to hydrolyze phospholipids to generate diacylglycerol (DAG) that is then used to form DGDG (Nakamura et al. 2005; Gaude et al. 2008).

3.1 Alteration in Root Architecture and Lipid Remodeling Under Pi Starvation

In response to Pi starvation, plants change their physiology and morphology to enhance Pi acquisition and use efficiency. These changes include the inhibition of primary root elongation, increases in lateral root length and number, and arrestment of shoot growth, resulting in the increase of root/shoot ratio to enhance Pi acquisition (Desnos 2008). The alteration in root growth results from reduced cell elongation and retarded meristem growth (Williamson et al. 2001; Reymond et al. 2006; Lai et al. 2007). The growth inhibition is a rapid response mediated by root tip sensing of the low Pi supply (Linkohr et al. 2002; Svistoonoff et al. 2007). The defect on the primary root growth cannot be recovered when plants suffer from long-term Pi starvation due to root meristem exhaustion (Sanchez-Calderon et al. 2005).

In response to Pi deficiency, lipid remodeling consisting of a decrease in phospholipids and an increase in non-phosphorus containing lipids occurs in all plant tissues, such as roots and leaves (Li et al. 2006a; Cruz-Ramirez et al. 2006; Gaude et al. 2008). Quantitative analysis showed that Arabidopsis galactolipid content in roots increased from 0.6 to 15 % for DGDG, and from 0.5 to 6.6 mol% for sulfolipid SQDG, whereas phospholipid content in roots decreased from 33.8 to 21.8 mol% for PC and from 51.9 to 21.9 % for PE when seedlings were transferred to Pi-starved condition (1 μM Pi) (Cruz-Ramirez et al. 2006). Li and his colleagues also found that the DGGD increased tenfold in roots, whereas phospholipids, PC, PE, and PG were decreased by 51 %, 65 %, and 49 % when Arabidopsis seedlings were grown in low Pi for 7 days (Li et al. 2006a, b). Similar alterations in lipid composition have been found in various plant species (Andersson et al. 2005; Russo et al. 2007; Yamaryo et al. 2008). These lines of evidence suggest that lipid remodeling is a ubiquitous response by plants to enhance internal Pi use efficiency, under Pi-limited condition.

The decrease in phospholipids was replaced by galactolipids such that total membrane lipids remained unchanged in shoot tissues between Pi-deprived and Pi-replete condition. However, the magnitude of decreased phospholipids in roots was more than that of increased DGDG. As a result degraded phospholipids could not be compensated for completely by accumulated DGDG in roots (Li et al. 2006a, b). The finding suggests that the effect of Pi starvation on lipid turnover in roots is more impactful than in shoots. Lipid profiling revealed that the decrease in particular fatty acyl species in phospholipids was identical to that of the increased galactolipid species under Pi starvation, indicating that DGDG is synthesized from recycled component of phospholipids (Li et al. 2006a, b; Nakamura et al. 2009). The acyl chain species 34:2, 34:3, 36:4, 36:5, and 36:6 that are mainly derived from eukaryotic pathway contribute the most to lipid remodeling under Pi starvation (Li et al. 2006a, b). The evidence suggests that most DGDG derived from extraplastidic lipid and DAG produced by phospholipases may serve as substrates for MGDG and DGDG synthesis (Fig. 2).

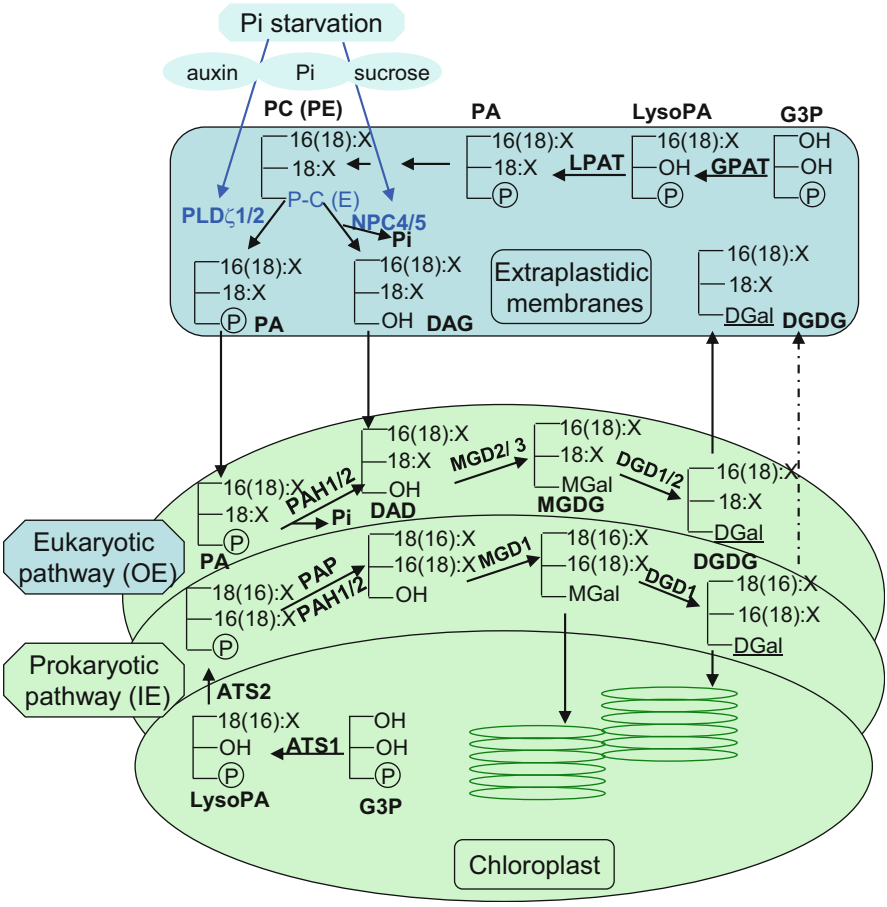

Fig. 2 PLDζ and NPC are involved in lipid remodeling under Pi starvation. In response to Pi starvation, PLDζs are activated to hydrolyze phospholipids PC and PE to PA, which is subsequently dephosphorylated to DAG by PAH1/2. NPCs hydrolyze phospholipids to generate DAG. The PA and DAG derived from extraplasitdic phospholipids are transported to chloroplast for galactolipid synthesis in outer envelope membrane of chloroplast by galactolipid synthases MGD2/3 and DGD1/2 (eukaryotic derived pathway) under Pi starved condition, which is distinct from prokaryotic pathway localized in inner envelope membrane of chloroplast by MGD1 and DGD1. The synthesized DGDG could be exported to extraplastidic membranes such as plasma membrane, ER, tonoplast, and mitochondrial membrane to compensate degraded phospholipids for maintaining membrane integrity. Pi released from phospholipids provides Pi source for critical cell activities during Pi starvation

3.2 *Phospholipase Dζ1 and 2 Are Involved in Lipid Remodeling Under Pi Starvation*

One pathway involved in lipid remodeling during Pi starvation is controlled by PLDζs. Both PLDζ1 and PLDζ2 contain phox homology and pleckstrin homology domains similar to mammalian PLDs and are distinct from other C2-PLDs in plants (Qin and Wang 2002). Biochemical assays showed that PLDζ1 activity is independent of Ca^{2+} and selectively uses PC as a substrate to generate PA (Qin and Wang 2002). *PLDζ2* is markedly induced by Pi limitation, as shown by different plant species including Arabidopsis (Cruz-Ramirez et al. 2006; Li et al. 2006a, b), oat (Nakamura et al. 2005), bean (Russo et al. 2007), and rice (Sun et al. unpublished data). Of 12 Arabidopsis *PLDs*, the expression level of *PLDζ2* is rather small under Pi-sufficient condition, but is markedly induced in all tissues examined including cotyledon, shoot apical meristem, primary root, lateral root, and differentiation zone in response to Pi deprivation (Cruz-Ramirez et al. 2006; Li et al. 2006a, b). *PLDζ2* is specifically induced by Pi limitation and had minor induction by nitrogen, but not with other nutrient components such as C, S, K, and Fe (Cruz-Ramirez et al. 2006). It was shown that *pldζ1/pldζ2* double mutants exhibited shorter primary root and longer lateral root under Pi-limiting conditions (Li et al. 2006a, b). Loss of *PLDζ2* conferred the primary root meristem exhaustion in longer term of Pi starvation (Cruz-Ramirez et al. 2006). Lipid profiling showed that DGDG in *pldζ2* mutant roots accumulated only 60 % of that in WT under Pi limitation, while corresponding PC and PE levels in *pldζ2* mutant were less reduced than that of WT. The amount of decreased DGDG is almost equal to that of increased PC and PE regulated by PLDζ2 activity (Cruz-Ramirez et al. 2006). Other lipids such as PG, phosphatidylserine (PS), phosphatidylinositol (PI), PA, MGDG, and SQDG are not significantly different between *pldζ2* mutant and WT under both Pi-sufficient and Pi limitation conditions (Cruz-Ramirez et al. 2006; Li et al. 2006a, b). The reciprocal alterations between PC, PE, and DGDG suggest phospholipids hydrolyzed by PLDζ1/2 are compensated by newly synthesized DGDG to maintain membrane integrity for plant adaptation to Pi-starved condition.

Molecular species profiling revealed that compared to WT, *pldz2* mutant had decreased DGDG species in 34:2, 34:3, 36:4, 36:5, and 36:6 with a corresponding increase in these molecular species of PC and PE (Cruz-Ramirez et al. 2006). The finding indicates that 34:2, 34:3, 36:4, 36:5, and 36:6 acyl chain species are the substrates for lipid remodeling during Pi limitation. The involvement of PLDζ2 in Pi starvation was demonstrated by another study of PLDζ1/2 using mutant analysis and lipid profiling (Li et al. 2006a, b). The results indicate that PLDζ selectively hydrolyzed phospholipids PC to produce PA. PA was rapidly converted by a phosphatase into DAG which can act as a substrate for DGDG synthesis in response to Pi deficiency (Nakamura et al. 2009). The root meristem exhaustion and primary root growth retardation in *pldζ2* and *pldζ1/2* mutants (Cruz-Ramirez et al. 2006; Li et al. 2006a, b) suggest that the PLDζ2 activity of hydrolyzing phospholipids to release Pi is critical for root tip cell survival under Pi deficiency.

The role of PLDζs in lipid remodeling in Pi starvation was also found in other plants species. A study on oat found that phospholipids such as PC and PE were decreased with a corresponding increase of DGDG in the plasma membrane and tonoplasts (Andersson et al. 2005). The remodeling was accompanied by the activation of PLD and PAH (Andersson et al. 2005; Nakamura et al. 2009). The involved PLD is Ca^{2+} independent, similar to PLDζ type activity. A subcellular study showed that PLDζ2 is localized in the tonoplasts as revealed by GFP tracking in vivo (Yamaryo et al. 2008). The results show that PLDζ2 was active on tonoplast phospholipids and was also involved in the plasma membrane and mitochondria membrane remodeling during Pi starvation. A study on bean seedlings treated with long-term Pi starvation also showed a significant decrease in PE, PG, and a marked increase in DGDG level in the plasma membrane in roots (Russo et al. 2007).

3.3 Nonspecific PLCs Are Involved in Lipid Remodeling Under Pi Starvation

Another pathway involved in lipid turnover from phospholipids to galactolipids is initiated by the activity of NPC that uses PC as primary substrate. Arabidopsis genome contains six *NPC* genes, *NPC1-6*. Transcript analysis revealed that *NPC4* and *NPC5* were also induced significantly by Pi limitation (Misson et al. 2005; Morcuende et al. 2007; Nakamura et al. 2005), whereas the expression levels of *NPC1*, *NPC2*, *NPC3*, and *NPC6* were not changed under Pi starvation (Wimalasekera et al. 2010). NPC4 and NPC5 share 84.7 % identity in their deduced amino acid sequence. Biochemical studies revealed that both NPC4 and NPC5 use PC and PE as substrates to produce DAG. NPC4 activity is independent of Ca^{2+}, Mg^{2+}, and Cu^{2+}, but is inhibited by Co^{2+}, Mn^{2+}, and Zn^{2+} (Nakamura et al. 2005). Further studies showed that the regulations of two NPCs, NPC4 and NPC5, are distinguishable in their expression level, activation, and subcellular localization. The transcript level and PC-hydrolyzing activity of NPC4 were highly induced in roots and shoots by Pi deprivation, whereas mRNA level and activity of NPC5 were less induced by Pi limitation and were much lower than that of NPC4. NPC4 is mainly localized at the plasma membrane, whereas NPC5 is predominantly present in the cytosol as revealed by GFP fusions and membrane fractionation (Nakamura et al. 2005; Gaude et al. 2008). NPC4 and NPC5 may function differently through temporal and spatial regulation. Cytosol-localized NPC5 may facilitate its hydrolysis toward various membrane phospholipids. PC-hydrolyzing activity was decreased by 80 % in *npc4* mutant, but was not changed in *npc5* mutants, when compared with WT plants under Pi limitation (Gaude et al. 2008). In vitro assays showed that NPC4 activity was 40-fold higher than NPC5 (Nakamura et al. 2005; Gaude et al. 2008).

Although the transcript level and activity of NPC4 were highly induced by Pi limitation, its contribution to lipid remodeling is rather small as shown by *npc4*

mutant accumulating similar level of DGDG to WT under the condition tested (Nakamura et al. 2005). Thus, the questions remain open on whether NPC4 contributes to lipid remodeling and in what process NPC4 is involved in plant response to Pi deprivation.

By comparison, DGDG accumulation in *npc5* leaves was 50 % of that in WT during Pi limitation (Gaude et al. 2008). However, the effect of NPC5 on DGDG accumulation in Pi-starved roots was rather small, indicating that NPC5 is specifically responsible for leaf DGDG accumulation during Pi limitation (Gaude et al. 2008; Eastmond et al. 2010). Lipid profiling showed that the eukaryotic pathway-derived fatty acid species 16:0 DGDG increased markedly in WT leaves, but was impaired in *npc5* mutant (Hartel et al. 2000; Gaude et al. 2008), suggesting that NPC5 is primarily involved in DGDG synthesis through eukaryotic pathways under Pi starvation. The impairments in lipid remodeling resulted in growth arrest in *npc5* mutant under Pi starvation. It was shown that the leaves of *pho1* mutant exhibit a permanently Pi-starved status that is independent of environmental Pi supply (Poirier et al. 1991). *npc5*/*pho1* double mutants displayed a more severe defect in plant growth than *pho1* single mutant (Hartel et al. 2000; Poirier et al. 1991; Gaude et al. 2008). In addition, SQDG accumulation in *npc5*/*pho1* double mutants was less than that of *npc5* single mutant, indicating that NPC5 also plays a positive role in sulfolipid synthesis under severe Pi limitation. In contrast, levels of phospholipids PE and PC in *npc5* were higher than in WT, and the magnitude of elevated PE and PC in *pho1*/*npc5* double mutant was greater than that of *pho1* single mutant (Gaude et al. 2008). These data suggest that NPC5 activity in Pi starvation is through the hydrolysis of phospholipids such as PE and PC to release Pi for cell activity and the production of DAG for DGDG accumulation in leaves via eukaryotic pathway. NPC5 is a major contributor to DGDG accumulation in leaves and is important for plant growth regulation during Pi limitation (Fig. 2).

As mentioned earlier, the DGDG accumulation by PLDζ2 occurred in roots but not in leaves (Cruz-Ramirez et al. 2006; Li et al. 2006a, b), whereas NPC5 is responsible for DGDG accumulation in leaves under Pi starvation. However, *npc5* mutants still had 50 % contribution to DGDG accumulation in leaves, suggesting that other potential phospholipases may be involved in DGDG synthesis during Pi starvation. A recent study showed that loss of NPC3 also conferred the inhibition on lateral root growth under low Pi in Arabidopsis (Wimalasekeraa et al. 2010), implicating that alternative NPCs or other phospholipases are also involved in DGDG synthesis under Pi limitation. Taken together, we suggest that PLD is primarily responsible for DGDG accumulation in roots, regulating root architecture, whereas NPC5 is mainly involved in DGDG accumulation in leaves, affecting plant growth. We also propose that NPC4 has a minor effect on lipid remodeling and may be involved in lipid signaling under Pi starvation (Figs. 1 and 2).

3.4 Dual Effects of Phospholipases on Pi-Derived Response

In addition to lipid remodeling, phospholipases are involved in cell signaling in response to Pi deprivation. The dual effect on lipid metabolism and signaling depends on the severity and stages of Pi starvation. PA is the intermediate substrate for phospholipid synthesis and is an important signaling molecule involved in a variety of physiological processes. PA is a minor lipid that accounts for 1–5 % of total glycerolipids in leaf and can be produced directly from PLD activation or from a two-step reaction of NPC to generate DGA, followed by phosphorylation to PA by DAG kinase (Fig. 1). In cells, PA is structurally unstable and could be rapidly transformed to other lipids such as PC, PE, PG, DAG, MGDG, and DGDG. It was found that the expression level of *PLDζ2* is very low, and the lipid composition in *pldζ2* mutant was not significantly different from that of WT under Pi-sufficient condition. Under severe Pi deprivation (0 μM Pi), *pldζ2* mutant exhibited higher content of PC and PE and a corresponding lower content of DGDG in roots than that of WT, while PA levels in *pldζ*s mutant were not significantly different from those of WT (Cruz-Ramirez et al. 2006; Li et al. 2006a, b), suggesting that PA produced from PLDζ2 is rapidly transformed into other lipids such as DGDG.

Under mild Pi-starved conditions (25 μM Pi), however, *pldζ2* or *pldζ1/2* double mutants had lower PA levels and displayed growth retardation, especially in primary root elongation. PLDζ2 contributes more to PA formation (Li et al. 2006a, b). It also was found that PLDζ is mainly involved in signaling in early stages or short term of Pi starvation (Russo et al. 2007). The results suggest that PLDζs involvement in low Pi response is through different mechanisms depending on the severity and stages of Pi starvation. In response to severe Pi starvation, PLDζ1/2 are involved in lipid metabolism by hydrolyzing PC and PE to release Pi and by providing DAG for DGDG synthesis to retain membrane integrity for plant survival. In response to moderate Pi limitation, PLDζ1/2 hydrolyze PC and PE to produce PA, which functions as a signaling molecule to regulate root growth and enhance Pi acquisition (Fig. 1). PA has been found to be very important for plant growth and nutrient use, probably through activation of several protein kinases such as PDK1 or S6K, involved in root growth (Anthony et al. 2004; Hong et al. 2009) (Fig. 1).

In addition, NPC4-produced DAG may function as a signal in low Pi response. NPC4-derived DAG could be phosphorylated to PA by DG kinase (Fig. 1). Both DAG and PA are important messengers in signal transduction. Recent reports show that NPC4 is involved in brassinolide signaling to regulate root growth and may have cross talk with Pi-starved response to regulate root architecture (Wimalasekeraa et al. 2010; Pokotylo et al. 2013) (Fig. 1).

3.5 Lipid Transport Between Chloroplast and Extraplastidic Membranes

Phospholipids are predominantly present in extraplastidic membranes, whereas galactolipids are synthesized and located primarily in plastidic membranes (Moellering and Benning 2011) (Fig. 2). It is still not clear how extraplastid-localized phospholipids and DAG are transported into plastidic membranes for MGDG, DGDG, and SQDG synthesis. How synthesized DGDG is exported to extraplastidic membranes to replace degraded phospholipids under Pi limitation has yet to be determined either. It was proposed that the lipid trafficking protein complex TGD1/TGD2/TGD3/TGD4 that connects the ER and chloroplasts may be responsible for DGDG accumulation under Pi-starved condition (Xu et al. 2010). Moreover, the TGD2 subunit was found to bind to PA, suggesting that PA is involved in TGD complex formation and may function as a transported phospholipid from the ER to the plastid (Xu et al. 2010).

In addition, genes encoding putative lipid-binding or transport proteins are potential candidates for lipid transport under Pi limitation. A recently identified protein, GlcT, is targeted to chloroplasts and is involved in GlcGalDG transport in response to Pi limitation (Holzl et al. 2009). Overexpression of GlcT in dgd1 mutant resulted in GlcGalDG accumulation to replace 90 % of DGDG (Holzl et al. 2009). The connection sites between different organelles or vesicle trafficking are also involved in DAG and DGDG transport (Jouhet et al. 2007). The PLDζ2-enriched domain in tonoplasts was close to mitochondria and chloroplasts, which may be responsible for galactolipid accumulation in extraplastidic membranes (Yamaryo et al. 2008).

3.6 Upstream Regulators of Phospholipases in Pi Starvation

The fact that *PLDζs* and *NPCs* were rapidly induced by Pi deprivation suggests that the expression of these phospholipase genes is tightly regulated by Pi deficiency. Pi itself may function as a signaling molecule as well as a nutrient, whereas phosphite (Phi, PO_3^{2-}) acts as a signaling but not a nutrient since it cannot be converted into Pi or further metabolized in cells (Carswell et al. 1996, 1997; Chiou and Lin 2011). It was shown that exogenous Phi application attenuated Pi starvation response such as root/shoot ratio, root hair elongation, anthocyanin accumulation, lipid remodeling, and expression of Pi starvation-induced (PSI) genes (Carswell et al. 1996, 1997; Kobayashi et al. 2006; Ticconi et al. 2001; Varadarajan et al. 2002). The repressed expression by Phi is specific and an early event, suggesting that Pi itself also functions in signaling during Pi starvation. Increased intracellular Pi concentrations could suppress Pi starvation response. In addition, *PLDζ2* was induced by sucrose in a concentration-dependent manner and was suppressed by cytokinin under Pi deprivation as tested by PLDζ2::GUS activity

(Martin et al. 2000; Karthikeyan et al. 2007; Oropeza-Aburto et al. 2012). The results suggest that multiple effectors control the transcriptional activation of *PLDζ2* or *NPCs* in response to Pi starvation.

Sequence analysis revealed the promoter region (1,232 bp) of *PLDζ2* contains five copies of the P1BS motif (GNATATNC), which is found in the promoters of many Pi-response genes (Bustos et al. 2010). P1BS, the cis-regulatory element, is important for the expression of Pi-response gene and is the consensus sequence for its binding to Pi-responsive transcript factor PHR1 (Rubio et al. 2001). When the construct containing *PLDζ2*::GUS was introduced into the *phr1* mutant background, the GUS expression controlled by *PLDζ2* promoter was significantly reduced (Oropeza-Aburto et al. 2012), indicating that *PLDζ2* transcript level is controlled by PHR1. Other factors may be also involved in *PLDζ2* inductions by Pi limitation because the inducible expression of *PLDζ2* was not completely abolished in the mutant background. Deletion analysis of *PLDζ2* promoter showed that the five motifs of P1BS are not equally important for *PLDζ2* transcript activation in Pi limitation. GUS reporter activity showed that there is an enhancer element located in the region between –782 and –717 containing two motifs, P1BS3 and P1BS4, that are necessary for *PLDζ2* response to Pi deficiency (Oropeza-Aburto et al. 2012). The enhancer response to low Pi is dependent on sucrose availability (Oropeza-Aburto et al. 2012). The results shed light on PLDζ2 upstream effectors, such as Pi, sucrose, and cytokinin, which modulate *PLDζ2* expression through the enhancer in the promoter region (Fig. 2).

3.7 *Downstream Processes Affected by Phospholipase in Response to Pi Limitation*

The activation of phospholipases is an early response to Pi limitation and is coupled with functions of related enzymes using PA and DAG as substrates for galactolipid synthesis (Fig. 2). All these enzymes are tightly regulated as demonstrated by a decrease in phospholipids with a concomitant increase in galcatolipids containing the same type of fatty acyl moieties of glycerol backbone in Pi-starved plant tissues (Cruz-Ramirez et al. 2006; Li et al. 2006a, b; Fritz et al. 2007). Recently, several enzymes, including PA phosphohydrolase (PAH), galactolipid synthases (MGD, DGD), and sulfolipid synthases (SQD) involved in lipid remodeling, were identified as downstream targets of phospholipases in Pi deprivation response. PA, a direct product of PLDζs, is unchanged in *pldζ1/pldζ2* mutants during Pi starvation (Cruz-Ramirez et al. 2006; Li et al. 2006a, b), indicating that PA is rapidly dephosphorylated to DAG for galactolipid synthesis. A recent study showed that PAH1/2 dephosphorylated PA to produce DAG (Fig. 2). Loss of PAH1/2 resulted in PA, PC, and PE elevation and reduced MGDG and DGDG accumulation in shoots, suggesting that PAH1/2 function in downstream of PA. PLDζ-produced PA may serve as substrate for PAH1/2 activity because *pah1/2* double mutants display a

similar manner in lipid remodeling under Pi starvation (Nakamura et al. 2009), suggesting that PAH1/PAH2 is involved in PLDζ2-mediated Pi starvation response.

Galactolipids are initiated by galactose-UDP synthesis, and then galactose is transferred to DAG to generate MGDG by MGDG synthase (MGD) and subsequent DGDG formation by DGDG synthase (DGD). Arabidopsis contains three MGDs including type A (MGD1) and type B (MGD2 and MGD3) responsible for MGDG synthesis and two DGDs, DGD1 and DGD2, for DGDG synthesis (Awai et al. 2001; Kelly and Dörmann 2004). MGDG, a major lipid in chloroplasts representing 60 % of total lipids, is synthesized mostly by the activity of MGD1 localized in inner envelope membrane of the chloroplast. DGDG is synthesized mainly by DGD1, localized in the outer envelope membrane of the chloroplast (Fig. 2) (Kelly and Dörmann 2004). In response to Pi starvation, the induction of phospholipase genes such as *PLDζ1/2* and *NPC4/5* was temporally correlated with genes involved in galactolipid synthesis such as *PAH1/2*, *MGD2/3*, and *DGD1/2* (Narise et al. 2010). The inner envelope membrane-localized MGD1 was activated by PA (Dubots et al. 2010, 2012). This evidence implies that activation of enzymes that are responsible for phospholipid degradation and galactolipid synthesis is coordinately regulated. It was shown that MGD2, MGD3, and DGD2 play a predominant role in galactolipid synthesis through a phospholipid recycling pathway during Pi starvation (Hartel et al. 2000; Benning and Otha 2005; Kelly and Dörmann 2004) (Fig. 2). This is consistent with DGDG species being derived from the eukaryotic pathway as revealed in the lipid profiling of *pldζ1/2* or *npc5* mutants (Li et al. 2006a, b; Cruz-Ramirez et al. 2006; Gaude et al. 2008). Likewise, DAG can also serve as a substrate for sulfolipid (SQDG) synthesis to compensate for degraded PG in the chloroplast (Essigmann et al. 1998; Hartel et al. 2000; Yu et al. 2002). Arabidopsis SQD1 and SQD2 are involved in sulfolipid biosynthesis. A knockout mutant by T-DNA insertion *SQD2* gene displayed a lower SQDG level and showed reduced growth compared to WT under Pi starvation (Yu et al. 2002). Taken together, in response to Pi limitation, phospholipases PLDζs and NPCs function as a bridge, linking the sensing of upstream regulators such as Pi, auxin, and sucrose signaling to enhance nutrient use efficiency, via downstream enzymes including PAH1/2, MGD2/3, DGD1/2, and SQD1/2 to regulate lipid metabolism and preserve membrane function and integrity (Figs. 1 and 2).

4 Perspectives

Phospholipases are involved in N and Pi deprivation via different mechanisms (Figs. 1 and 2). Recent studies have increased our understanding of nitrogen uptake, transport, and assimilation in plants. How plants sense nitrogen signals remains unclear. PLDε is almost exclusively localized at the plasma membrane and has a positive effect on plant growth in response to N availability. This raises intriguing questions as to whether PLDε and its PA signaling may play a role in linking the sensing of external N stimuli at the plasma membrane to intracellular translational

and metabolic regulation, thus enhancing growth. It would be of interest to investigate the molecular linkage between PLD/PA and cellular regulators to determine how plants integrate nutrient usage and growth regulation.

The involvement of phospholipases in lipid remodeling under Pi starvation is through an integral system with phospholipid hydrolysis and concomitant galactolipid biosynthesis. Phospholipases, such as PLDζ1/2 and NPC4/5, have been characterized in Pi-starved response. Several questions concerning lipid remodeling remain unanswered. First, some genes involved in lipid remodeling are unidentified. For example, PLDζ2 activity only accounts for partial DGDG accumulation in roots, considering *pldζ2* mutant still had 40 % DGDG accumulated in roots when compared to WT under Pi starvation. Meanwhile, the growth effect of PAH1/2 was much stronger than that of PLDζs (Nakamura et al. 2009), suggesting that PA, as substrate for PAH1/2, may be derived from PLD activation and also from de novo synthesis. Second, instead of lipid remodeling by PLDζs in response to severe Pi starvation, under moderate Pi-starved condition, PLDζ1/2 may function in signaling. PA has been implicated in root and root hair growth regulation. Whether Pi-starved signaling mediated by PLDζ-derived PA shares the same pathway with nitrogen signaling mediated by PLDε-derived PA is yet to be determined. It will be of great of interest to identify PA targets involved in growth regulation in response to nutrient availability. Third, it was shown that the expression of *PLDζ2* and *NPC4* is rapidly induced by Pi deprivation, auxin, and sucrose, which are known cues for the transcriptional upregulation (Nakamura et al. 2005; Li et al. 2006a, b; Cruz-Ramirez et al. 2006; Karthikeyan et al. 2007; Oropeza-Aburto et al. 2012). However, transcriptional regulators directly controlling PLDζ or NPC activation have yet to be identified. Finally, little is known about lipid transport between chloroplast membranes and extraplastidic membranes. Several proteins such as TGD complex are proposed to function as lipid transport under Pi starvation. Organelle contact may be also involved in lipid transport in response to Pi-limited conditions, but direct evidence is still lacking. Future studies will be required to unravel these issues and increase our understanding of plant Pi-starved response, which enhances internal Pi use and recycling.

Acknowledgments The work is supported by grants from National Natural Science Foundation of China (30871303, 30971852, 31271514), the Chinese National Key Basic Research Project (2012CB114200), and Specialized Research Fund for the Doctoral Program of Higher Education (20100146110025, 2012146110004).

References

Andersson M, Larsson K, Tjellstrom H, Liljenberg C, Sandelius AS (2005) Phosphate-limited oat: the plasma membrane and the tonoplast as major targets for phospholipid-to-glycolipid replacement and stimulation of phospholipases in the plasma membrane. J Biol Chem 280:27578–27586

Anthony RG, Henriques R, Helfer A, Meszaros T, Rois 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

Awai K, Marechal E, Block MA, Brun D, Masuda T, Shimada H, Takamiya K, Ohta H, Joyard J (2001) Two types of MGDG synthase genes, found widely in both 16:3 and 18:3 plants, differentially mediate galactolipid syntheses in photosynthetic and nonphotosynthetic tissues in Arabidopsis thaliana. Proc Natl Acad Sci USA 98:10960–10965

Benning C (1998) Biosynthesis and function of the sulfolipid sulfoquinovosyl diacylglycerol. Annu Rev Plant Physiol Plant Mol Biol 49:53–75

Benning C, Otha H (2005) Three enzyme systems for galactoglycerolipid biosynthesis are coordinately regulated in plants. J Biol Chem 280:2397–2400

Bustos R, Castrillo G, Linhares F, Puga MI, Rubio V, Perez-Perez J, Solano R, Leyva A, Paz-Ares J (2010) A central regulatory system largely controls transcriptional activation and repression responses to phosphate starvation in Arabidopsis. PLoS Genet 6:e1001102

Carswell C, Grant BR, Theodorou ME, Harris J, Niere JO, Plaxton WC (1996) The fungicide phosphonate disrupts the phosphate-starvation response in Brassica nigra seedlings. Plant Physiol 110:105–110

Carswell MC, Grant BR, Plaxton WC (1997) Disruption of the phosphate-starvation response of oilseed rape suspension cells by the fungicide phosphonate. Planta 203:67–74

Cerezo M, Tillard P, Filleur S, Munos S, Daniel-Vedele F, Gojon A (2001) Majoralterations of the regulation of root NO3 uptake are associated with the mutation of Nrt2.1 and Nrt2.2 genes in Arabidopsis. Plant Physiol 127:262–271

Chiou TJ, Lin SI (2011) Signaling network in sensing phosphate availability in plants. Annu Rev Plant Biol 62:185–206

Crawford NM (1995) Nitrate: nutrient and signal for plant growth. Plant Cell 7:859–868

Cruz-Ramirez A, Oropeza-Aburto A, Razo-Hernandez F, Ramirez-Chavez E, Herrera-Estrella L (2006) Phospholipase DZ2 plays an important role in extraplastidic galactolipid biosynthesis and phosphate recycling in Arabidopsis roots. Proc Natl Acad Sci USA 103:6765–6770

Desnos T (2008) Root branching responses to phosphate and nitrate. Curr Opin Plant Biol 11:82–87

Dubots EM, Audry MY, Yamaryo YO, Bastien OH, Ohta HC, Breton CE, Marechal E, Block MA (2010) Activation of the chloroplast monogalactosyldiacylglycerol synthase MGD1 by phosphatidic acid and phosphatidylglycerol. J Biol Chem 285:6003–6011

Dubots E, Botte C, Boudiere L, Yamaryo-Botte Y, Jouhet J, Marechal E, Block MA (2012) Role of phosphatidic acid in plant galactolipid synthesis. Biochimie 94:86–93

Eastmond PJ, Quettier AL, Kroon JT, Craddock C, Adams N, Slabas AR (2010) Phosphatidic acid phosphohydrolase 1 and 2 regulate phospholipid synthesis at the endoplasmic reticulum in Arabidopsis. Plant Cell 22:2796–2811

Essigmann B, Güler S, Narang RA, Linke D, Benning C (1988) Phosphate availability affects the thylakoid lipid composition and the expression of SQD1, a gene required for sulfolipid biosynthesis in Arabidopsis thaliana. Proc Natl Acad Sci U S A 95:1950–1955

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

Fang Y, Park IH, Wu AL, Du G, Huang P, Frohman MA, Walker SJ, Brown HA, Chen J (2003) PLD1 regulates mTOR signaling and mediates Cdc42 activation of S6K1. Curr Biol 13:2037–2044

Foyer CH, Valadier MH, Migge A, Becker TW (1998) Drought-induced effects on nitrate reductase activity and mRNA and on the coordination of nitrogen and carbon metabolism in maize leaves. Plant Physiol 117:283–292

Fritz M, Lokstein H, Hackenberg H, Welti R, Roth M, Zahringer U, Fulda M, Hellmeyer W, Ott C, Wolter FP, Heinz E (2007) Channeling of eukaryotic diacylglycerol into the biosynthesis of plastidial phosphatidylglycerol. J Biol Chem 282:4613–4625

Gaude N, Nakamura Y, Scheible WR, Ohta H, Dörmann P (2008) Phospholipase C5 (NPC5) is involved in galactolipid accumulation during phosphate limitation in leaves of Arabidopsis. Plant J 56:28–39

Guo L, Devaiah SP, Narasimhan R, Pan X, Zhang Y, Zhang W, Wang X (2012) Cytosolic glyceraldehyde-3-phosphate dehydrogenases interact with phospholipase Dδ to transduce hydrogen peroxide signals in the Arabidopsis response to stress. Plant Cell 24:2200–2212

Hartel H, Dormann P, Benning C (2000) DGD1-independent biosynthesis of extraplastidic galactolipids after phosphate deprivation in Arabidopsis. Proc Natl Acad Sci USA 97:10649–10654

Heckathorn SA, De Lucia EH, Zielinski RE (1997) The contribution of drought-related decreases in foliar nitrogen concentration to decreases in photosynthetic capacity during and after drought in prairie grasses. Physiol Plant 101:173–182

Hirel B, Le Gouis J, Ney B, Gallais A (2007) The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. J Exp Bot 58:2369–2387

Holzl G, Witt S, Gaude N, Melzer M, Schöttler MA, Dörmann P (2009) The role of diglycosyl lipids in photosynthesis and membrane lipid homeostasis in Arabidopsis. Plant Physiol 150:1147–1159

Hong Y, Pan X, Welti R, Wang X (2008) Phospholipase Da3 is involved in the hyperosmotic response in Arabidopsis. Plant Cell 20:803–816

Hong Y, Devaiah SP, Bahn SC, Thamasandra BN, Li M, Welti R, Wang X (2009) Phospholipase Dε and phosphatidic acid enhance Arabidopsis nitrogen signaling and growth. Plant J 58:376–387

Hong Y, Zhang W, Wang X (2010) Phospholipase D and phosphatidic acid signaling in plant response to drought and salinity. Plant Cell Environ 33:627–635

Huang NC, Chiang CS, Crawford NM, Tsay YF (1996) CHL1 encodes a component of the low-affinity nitrate uptake system in Arabidopsis and shows cell type-specific expression in roots. Plant Cell 8:2183–2191

Jouhet J, Maréchal E, Block MA (2007) Glycerolipid transfer for the building of membranes in plant cells. Prog Lipid Res 46:37–55

Karthikeyan A, Varadarajan D, Jain A, Held M, Carpita N, Raghothama K (2007) Phosphate starvation responses are mediated by sugar signaling in Arabidopsis. Planta 225:907–918

Kelly AA, Dörmann P (2004) Green light for galactolipid trafficking. Curr Opin Plant Biol 7:262–269

Kobayashi K, Masuda T, Takamiya K, Ohta H (2006) Membrane lipid alteration during phosphate starvation is regulated by phosphate signaling and auxin/cytokinin cross-talk. Plant J 47:238–248

Lai F, Thacker J, Li Y, Doerner P (2007) Cell division activity determines the magnitude of phosphate starvation responses in Arabidopsis. Plant J 50:545–556

Lehman N, Ledford B, Di Fulvio M, Frondorf K, McPhail LC, Gomez-Cambronero J (2007) Phospholipase D2-derived phosphatidic acid binds to and activates ribosomal p70 S6 kinase independently of mTOR. FASEB J 21:1075–1087

Li M, Qin C, Welti R, Wang X (2006a) Double knockouts of phospholipases Dz1 and Dz2 in Arabidopsis affect root elongation during phosphate limited growth but do not affect root hair patterning. Plant Physiol 140:761–770

Li M, Welti R, Wang X (2006b) Quantitative profiling of Arabidopsis polar glycerolipids in response to phosphorus starvation. Roles of phospholipases D zeta1 and D zeta2 in phosphatidylcholine hydrolysis and digalactosyldiacylglycerol accumulation in phosphorus-starved plants. Plant Physiol 142:750–761

Linkohr BI, Williamson LC, Fitter AH, Leyser HM (2002) Nitrate and phosphate availability and distribution have different effects on root system architecture of Arabidopsis. Plant J 29:751–760

Mahfouz MM, Kim S, Delauney AJ, Verma DP (2006) Arabidopsis TARGET OF RAPAMYCIN interacts with RAPTOR, which regulates the activity of S6 kinase in response to osmotic stress signals. Plant Cell 18:477–490

Martın AC, Del Pozo JC, Iglesias J, Rubio V, Solano R, De La Pena A, Leyva A, Paz-Ares J (2000) Influence of cytokinins on the expression of phosphate starvation responsive genes in Arabidopsis. Plant J 24:559–567

Misson J, Raghothama K, Jain A, Jouhet J, Block M, Bligny R, Ortet P, Creff A, Somerville S, Rolland N, Doumas P, Nacry P, Herrerra-Estrella L, Nussaume L, Thibaud MC (2005) A genome-wide transcriptional analysis using Arabidopsis thaliana affymetrix gene chips determined plant responses to phosphate deprivation. Proc Natl Acad Sci USA 102:11934–11939

Moellering ER, Benning C (2011) Galactoglycerolipid metabolism under stress: a time for remodeling. Trends Plant Sci 16:98–107

Morcuende R, Bari R, Gibon Y, Zheng W, Pant BD, Blasing O, Usadel B, Czechowski T, Udvardi MK, Stitt M, Scheible WR (2007) Genomewide reprogramming of metabolism and regulatory networks of Arabidopsis in response to phosphorus. Plant Cell Environ 30:85–112

Nakamura Y, Awai K, Masuda T, Yoshioka Y, Takamiya K, Ohta H (2005) A novel phosphatidylcholine-hydrolyzing phospholipase C induced by phosphate starvation in Arabidopsis. J Biol Chem 280:7469–7476

Nakamura Y, Koizumi R, Shui G, Shimojima M, Wenk MR, Ito T, Ohta H (2009) Arabidopsis lipins mediate eukaryotic pathway of lipid metabolism and cope critically with phosphate starvation. Proc Natl Acad Sci USA 106:20978–20983

Narise T, Kobayashi K, Baba S, Shimojima M, Masuda S, Fukaki H, Ohta H (2010) Involvement of auxin signaling mediated by IAA14 and ARF7/19 in membrane lipid remodeling during phosphate starvation. Plant Mol Biol 72:533–544

Oropeza-Aburto A, Cruz-Ramırez A, Acevedo-Hernandez GJ, Perez-Torres CA, Caballero-Perez J, Herrera-Estrella L (2012) Functional analysis of the Arabidopsis PLDZ2 promoter reveals an evolutionarily conserved low-Pi-responsive transcriptional enhancer element. J Exp Bot 63:2189–2202

Poirier Y, Thoma S, Somerville C, Schiefelbein J (1991) Mutant of Arabidopsis deficient in xylem loading of phosphate. Plant Physiol 97:1087–1093

Poirier Y, Bucher M (2002) Phosphate transport and homeostasis in Arabidopsis. In: Somerville C, Meyerowitz EM (eds) The Arabidopsis book. American Society of Plant Biologists, Rockville, MD

Pokotylo I, Pejchar P, Potocky M, Kocourkova D, Kova Z, Ruelland E, Kravets V, Martinec J (2013) The plant non-specific phospholipase C gene family. Novel competitors in lipid signaling. Prog Lipid Res 52:62–79

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 calcium-independent and phosphatidylcholineselective PLDζ1 with distinct regulatory domains. Plant Physiol 128:1057–1068

Qin W, Pappan K, Wang X (1997) Molecular heterogeneity of phospholipase D (PLD). Cloning of PLDγ and regulation of plant PLDγ, -β, and -α by polyphosphoinositides and calcium. J Biol Chem 272:28267–28273

Raghothama KG (1999) Phosphate acquisition. Annu Rev Plant Physiol Plant Mol Biol 50:665–693

Reymond M, Svistoonoff S, Loudet O, Nussaume L, Desnos T (2006) Identification of QTL controlling root growth response to phosphate starvation in Arabidopsis thaliana. Plant Cell Environ 29:115–125

Rubio V, Linhares F, Solano R, Martin AC, Iglesias J, Leyva A, Paz-Ares J (2001) A conserved MYB transcription factor involved in phosphate starvation signaling both in vascular plants and in unicellular algae. Genes Dev 15:2122–2133

Russo MA, Quartacci MF, Izzo R, Belligno A, Navari-Izzo F (2007) Long- and short-term phosphate deprivation in bean roots: plasma membrane lipid alterations and transient stimulation of phospholipases. Phytochemistry 68:1564–1571

Sanchez-Calderon L, Lopez-Bucio J, Chacon-Lopez A, Cruz- Ramirez A, Nieto-Jacobo F, Dubrovsky JG, Herrera-Estrella L (2005) Phosphate starvation induces a determinate developmental program in the roots of Arabidopsis thaliana. Plant Cell Physiol 46:174–184

Svistoonoff S, Creff A, Reymond M, Sigoillot-Claude C, Ricaud L, Blanchet A, Nussaume L, Desnos T (2007) Root tip contact with low phosphate media reprograms plant root architecture. Nat Genet 39:792–796

Takahashi M, Sasaki Y, Ida S, Morikawa H (2001) Nitrite reductase gene enrichment improves assimilation of NO2 in Arabidopsis. Plant Physiol 126:731–741

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

Theodorou ME, Plaxton WC (1993) Metabolic adaptations of plant respiration to nutritional phosphate deprivation. Plant Physiol 101:339–344

Ticconi CA, Delatorre CA, Abel S (2001) Attenuation of phosphate starvation responses by phosphate in Arabidopsis. Plant Physiol 127:963–972

Tjellstrom H, Andersson MX, Larsson KE, Sandelius AS (2008) Membrane phospholipids as a phosphate reserve: the dynamic nature of phospholipid-to-digalactosyl diacylglycerol exchange in higher plants. Plant Cell Environ 31:1388–1398

Vance CP, Uhde-StoneC C, Allan DL (2003) Phosphorus acquisition and use: critical adaptations by plants for securing a non-renewable resource. New Phytol 157:423–447

Varadarajan DK, Karthikeyan AS, Matilda PD, Raghothama KG (2002) Phosphite, ananalog of phosphate, suppresses the coordinated expression of genes under phosphate starvation. Plant Physiol 129:1232–1240

Walch-Liu P, Ivanov II, Filleur S, Gan Y, Remans T, Forde BG (2006) Nitrogen regulation of root branching. Ann Bot 97:875–881

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

Wang X, Devaiah SP, Zhang W, Welti R (2006) Signaling functions of phosphatidic acid. Prog Lipid Res 45:250–278

Williamson LC, Ribrioux SP, Fitter AH, Leyser HM (2001) Phosphate availability regulates root system architecture in Arabidopsis. Plant Physiol 126:875–882

Wimalasekera R, Pejcharb P, Holka A, Martinecb J, Scherera G (2010) Plant phosphatidylcholine-hydrolyzing phospholipases C NPC3 and NPC4 with roles in root development and brassinolide signaling in Arabidopsis thaliana. Mol Plant 3:610–625

Wullschleger S, Loewith R, Hall M (2006) TOR signaling in growth and metabolism. Cell 124:471–484

Xu C, Moellering ER, Muthan B, Fan J, Benning C (2010) Lipid transport mediated by Arabidopsis TGD proteins is unidirectional from the endoplasmic reticulum to the plastid. Plant Cell Physiol 51:1019–1028

Yamaryo Y, Dubots E, Albrieux C, Baldan B, Block M (2008) Phosphate availability affects the tonoplast localization of PLDZ2, an Arabidopsis thaliana phospholipase D. FEBS Lett 582:685–690

Yu B, Xu C, Benning C (2002) Arabidopsis disrupted in SQD2 encoding sulfolipid synthase is impaired in phosphate-limited growth. Proc Natl Acad Sci USA 99:5732–5737

Zhang H, Jennings A, Barlow PW, Forde BG (1999) Dual pathways for regulation of root branching by nitrate. Proc Natl Acad Sci U S A 96:6529–6534

Zhang H, Rong H, Pilbeam D (2007) Signaling mechanisms underlying the morphological responses of the root system to nitrogen in Arabidopsis thaliana. J Exp Bot 58:2329–2338

Part III
Phospholipases in Plant Biotic Interactions

Phospholipase A in Plant Immunity

Susana Rivas and Thierry Heitz

Abstract In addition to their structural role as major components of cellular membranes, lipids and lipid-derived molecules act as important regulators of plant cell signaling. The roles of lipid deacylating enzymes in the control of plant growth, development, and responses to stress are becoming increasingly evident. Following perception of attack by pathogenic microbes or herbivorous insects, plant phospholipase A (PLA) activity has been linked to the activation of defense signaling and appears to play crucial roles during the establishment of plant immune responses through, for example, the production of defense signals such as jasmonates and other oxylipins. Evidence for nonenzymatic modes of action of PLA is also emerging. In this chapter, we review current data on plant PLA proteins, and their involvement in plant defense responses to microbes and insects, before discussing new ways to decipher PLA-dependent processes.

Keywords Lipid acyl hydrolase • Galactolipase • Oxylipin • Lipid profiling • Plant immunity • Phospholipase A

S. Rivas (✉)
Laboratoire des Interactions Plantes-Microorganismes (LIPM), INRA, UMR441, F-31326 Castanet-Tolosan, France

Laboratoire des Interactions Plantes-Microorganismes (LIPM), CNRS, UMR2594, F-31326 Castanet-Tolosan, France
e-mail: Susana.Rivas@toulouse.inra.fr

T. Heitz
Institut de Biologie Moleculaire des plantes (IBMP), UPR 2357 du CNRS, Université de Strasbourg, 67084 Strasbourg, France

X. Wang (ed.), *Phospholipases in Plant Signaling*, Signaling and Communication in Plants 20, DOI 10.1007/978-3-642-42011-5_10,

1 Introduction

In plants, recognition of pathogenic microbes triggers a signal transduction cascade that leads to the transcriptional reprogramming of the plant cell. In the case of resistant plants, this signaling cascade directs the activation of plant immune responses that are frequently associated with the development of the so-called hypersensitive response (HR), a form of programmed cell death that contributes to plant resistance by restricting pathogen growth to the inoculation site (Mur et al. 2008; Coll et al. 2010).

Rapid accumulation of reactive oxygen species (ROS), changes in ion fluxes, activation of protein kinase cascades, and production of defense molecules, such as phytolexins, are some of the early signaling events that have been described following pathogen perception by plant cells. In addition, a growing body of evidence indicates that phospholipases and phospholipid-related molecules, such as the secondary signal messanger molecule phosphatidic acid (PA), play essential roles during the establishment of plant disease resistance (Andersson et al. 2006a; Testerink and Munnik 2011). This suggests that phospholipids are much more than just structural components of biological membranes. Moreover, a great variety of lipid-related components including glycerolipids, sphingolipids, fatty acids, oxylipins, jasmonates, and sterols are also increasingly involved in the regulation of defense-related signaling (Shah 2005; Kachroo and Kachroo 2009).

In contrast to phospholipases C and D that act on the polar head of phospholipids, phospholipase A catalyzes the hydrolysis of phospholipids into lysophospholipids and free fatty acids, either at the *sn*-1 (PLA_1) and/or *sn*-2 position (PLA_2) of glycerolipids (Matos and Pham-Thi 2009). Free fatty acids may be oxidized by lipoxygenases (LOX) or an α-dioxygenase (α-DOX) resulting in the biosynthesis of oxylipins and jasmonic acid (JA), which play important roles during plant defense signaling as mentioned above (Blee 2002; Browse 2009; La Camera et al. 2009), and lysophospholipids are also bioactive molecules. In addition, plant PLA_2 activity has been linked to ROS production (Chandra and Low 1995) as well as efflux of vacuolar protons, which triggers a pH-dependent signal for the biosynthesis of phytoalexins (Viehweger et al. 2006). Galactolipids being the major components of plant cell membranes, plant lipid acyl hydrolases (LAHs) hydrolyze phospholipids but are also able to act on galactolipids, several characterized LAHs even showing preferential galactolipase (GLA) activity in vitro. Numerous LAH (bearing PLA, GLA, or triglyceride lipase, TGL activities) have been identified in plants but, in many cases, knowledge of their real in vivo substrates and cleavage specificities awaits deeper characterization.

In Arabidopsis, PLA proteins have been grouped in three main subfamilies (Table 1): small secretory PLAs with PLA_2 activity (*At*sPLA_2s), patatin-like proteins with both PLA_1 and PLA_2 activities (pPLAs), and lipase-like PLA_1s (DAD1-like, for Defective in Anther Dehiscence1-like) (Grienenberger et al. 2010). In recent years, the extensive use of molecular approaches related to lipid signaling has led to the characterization of new enzymes and to the identification of new proteins with putative lipase/esterase signatures (Matos and Pham-Thi 2009).

Table 1 Examples of LAH proteins with reported roles in responses to microbial infections or wounding

LAH family[a]	Member name	AGI	Subcellular localization	Stress inducing transcriptional regulation	Activity	Effect on lipid profile during defense	In vivo function in immunity	References
Secretory PLA_2 (4)	$AtsPLA_2$-α	At2g19690	Golgi, apoplast, nucleus	Bacterial infection	PLA_2, not required for AtMYB30 regulation	ND	Interacts with and represses AtMYB30-mediated HR	Froidure et al. (2010), Lee et al. (2010), Jung et al. (2012)
α/β hydrolase	SOBER1	At4g22300	ND	ND	PLA, carboxylesterase	Reduced PA levels	Suppression of HR	Cunnac et al. (2007), Kirik and Mudgett (2009)
Patatin (10)	pPLA-IIα	At2g26560	Cytosol	Fungal and bacterial infection	GLA > PLA	Increased 2OH-FA levels	Host cell death execution	La Camera et al. (2005, 2009)
	pPLA-IIIß	At3g54950	Membrane-bound	Fungal and bacterial infection	PLA, GLA, AcylCoA thioesterase	ND	ND	La Camera et al. (2005), Li et al. (2011)
	pPLA-I	At1g61850	Cytosol, chloroplast	ND	GLA > PLA	Basal JA production	Antifungal resistance	Yang et al. (2007)
	NtPAT1, NtPAT3		Cytosol, chloroplast	Viral, fungal, and bacterial infection	GLA > PLA	ND	Host cell death execution, resistance to TMV	Dhondt et al. (2000)

(continued)

Table 1 (continued)

LAH family[a]	Member name	AGI	Subcellular localization	Stress inducing transcriptional regulation	Activity	Effect on lipid profile during defense	In vivo function in immunity	References
DAD1 (12)	DAD1	At2g44810	Chloroplast	Expressed in anthers; wound-induced in leaves	PLA > GLA > TGL	JA production in anthers	Late JA production in wounded leaves	Ishiguro et al. (2001)
	DGL (PLA1-Iα-1)	At1g05800	Lipid bodies	Wound-induced in leaves	GLA, PLA		Minor role in wound-induced JA biosynthesis ?	Hyun et al. (2008), Ellinger et al. (2010)
	PLA1-Iγ1	At1g06800	Chloroplast	Fungal and bacterial infection	GLA, PLA, TGL	Contributes to wound-induced JA accumulation	ND	Seo et al. (2009), T. Heitz, unpublished
	PLA1-Iγ2	At2g30550	Chloroplast	Fungal and bacterial infection	GLA, PLA, TGL	ND	ND	Seo et al. (2009), T. Heitz, unpublished
	NaGLA1		Chloroplast	Repressed by wounding and infection	ND	Supplies FAs for JA and divinyl-ether synthesis	ND	Kallenbach et al. (2010), Bonaventure et al. (2011)
Related to fungal lipases	PAD4	At3g52430	Nucleo-cytoplasmic shuttling	Bacterial infection, SA treatment	ND	ND	Immune regulator	Wiermer et al. (2005), Feys et al. (2001)
	EDS1	At3g48090	Nucleo-cytoplasmic shuttling	Bacterial infection, SA treatment	ND	ND	Immune regulator	Wiermer et al. (2005), Feys et al. (2001)

GDSL (108)	GLIP1	At5g40990	Extracellular	*A. brassicicola*, ethylene	Esterase	ND	Signaling; antifungal activity	Oh et al. (2005), Kwon et al. (2009)
	GLIP2	At1g53940	ND	SA	Esterase	ND	Antifungal, antibacterial resistance	Lee et al. (2009)

FA fatty acid, *GLA* galactolipase, *HR* hypersensitive response, *JA* jasmonic acid, *PA* phosphatidic acid, *PLA* phospholipase A, *SA* salicylic acid, *TGL* triacylglycerolipase, *TMV* Tobacco Mosaic Virus, *ND* not determined. All examples are from Arabidopsis except NaGLA from *Nicotiana attenuata* and NtPAT1 and NtPAT3 from *Nicotiana tabacum*

[a]Number of genes in Arabidopsis

Here we summarize our current knowledge about plant LAH proteins that have been found to play a role in the regulation of plant immunity.

2 Secretory PLAs

Plant sPLA$_2$ genes encode proteins with N terminal signal peptides that are of low molecular weight (13–18 kDa) after secretion. sPLA$_2$s present a highly conserved Ca^{2+}-binding loop and an active site motif with a conserved His/Asp dyad. The mature proteins contain 12 cysteine residues that can form six disulfide bonds to stabilize the protein's three-dimensional structure (Ryu 2004).

In Arabidopsis, the secretory PLA$_2$ family (*At*sPLA$_2$) consists of four isoforms named *At*sPLA$_2$-α, *At*sPLA$_2$-β, *At*sPLA$_2$-γ, and *At*sPLA$_2$-δ (Ryu 2004). *AtsPLA$_2$-γ* and *AtsPLA$_2$-δ*, which are exclusively expressed in pollen and localize to the endoplasmic reticulum (ER) and Golgi apparatus, are involved in pollen germination and tube growth (Kim et al. 2011b). *AtsPLA$_2$-β*, which is expressed in different tissues and localizes to the ER, mediates cell elongation, shoot gravitropism, stomatal opening, and pollen development and likely acts as a downstream component of auxin signaling (Lee et al. 2003; Seo et al. 2008; Kim et al. 2011b). Interestingly, the amino acid sequence, enzymatic properties, and expression pattern of *At*sPLA$_2$-α are distinguishable from those of the other three *At*sPLA$_2$ isoforms, suggesting that *At*sPLA$_2$-α may play a distinct role that differs from that of the other *At*sPLA$_2$s (Ryu 2004).

AtsPLA$_2$-α is expressed in diverse plant organs (Kim et al. 2011b) and is localized to Golgi-associated vesicles (Lee et al. 2010). *At*sPLA$_2$-α was reported to modulate PIN protein trafficking to the plasma membrane (and therefore regulation of auxin transport) in Arabidopsis root cells (Lee et al. 2010). A recent report showed that *AtsPLA$_2$-α* gene expression is controlled in a developmental stage- and tissue-specific manner (Jung et al. 2012). The subcellular localization of *At*sPLA$_2$-α appears to be dependent on the leaf developmental stage. Indeed, in premature young leaves of transgenic Arabidopsis plants expressing an *At*sPLA$_2$-α-RFP fusion protein, *At*sPLA$_2$-α was localized at the Golgi apparatus, whereas in mature leaves, it was primarily detected in the apoplast (Jung et al. 2012). Apoplasts are important sites for the interaction between plant cells and invading bacteria. In agreement with this notion, translocation of *At*sPLA$_2$-α to the apoplast was found to be enhanced after plant inoculation with avirulent bacteria, suggesting that *At*sPLA$_2$-α may participate to the plant defense response that is mounted in the apoplast.

A role for intracellular *At*sPLA$_2$-α in the regulation of plant defense has been reported. Golgi-associated *At*sPLA$_2$-α partially relocalized to the plant cell nucleus after co-expression with the Arabidopsis MYB transcription factor AtMYB30 (Froidure et al. 2010). AtMYB30 is a positive regulator of defense- and cell death-associated responses through the activation of the lipid biosynthesis pathway that leads to the production of very long chain fatty acids [VLCFAs; (Raffaele et al. 2008)]. AtMYB30 and *At*sPLA$_2$-α physically interact in the plant cell nucleus, leading to repression of AtMYB30-mediated transcriptional activity. Physical or

functional interactions have been previously suggested between cytoplasmic PLA_2s ($cPLA_2$) and proteins involved in transcriptional regulation in animals (Flati et al. 1996; Pawliczak et al. 2002; Tashiro et al. 2004b). In agreement with the finding that transcriptional activation of VLCFA-related genes by AtMYB30 is required to mount an efficient defense response during bacterial infection (Raffaele et al. 2008), the protein interaction between AtMYB30 and *At*sPLA$_2$-α correlates with the negative regulation of Arabidopsis HR and defense responses (Froidure et al. 2010). Together, these data highlight the importance of dynamic nucleocytoplasmic protein trafficking for the regulation of the transcriptional activation related to defense (Rivas 2012).

Interestingly, *At*sPLA$_2$-α nuclear targeting, interaction with AtMYB30, repression of AtMYB30 transcriptional activity, and repression of HR development appeared to be independent of *At*sPLA$_2$-α enzymatic activity (Froidure et al. 2010). Therefore, it was proposed that *At*sPLA$_2$-α may control AtMYB30-mediated response through the physical interaction between the two proteins (preventing AtMYB30 from activating its targets) rather than through a lipid signal produced by *At*sPLA$_2$-α. This assumption is in agreement with the hypothesis formulated for animal $cPLA_2$, which is able to interact not only with B-Myb but also with additional nuclear proteins that stimulate apoptosis, strongly supporting the notion that $cPLA_2$ plays additional roles in the nucleus other than its enzymatic activity at the nuclear membrane (Sheridan et al. 2001; Tashiro et al. 2004a). Moreover, new functions have been attributed to $sPLA_2$s that do not require enzymatic activity in animal cells. For example, it has been reported that $sPLA_2$s bind to and possibly signal through cell surface molecules (Lambeau and Lazdunski 1999; Granata et al. 2005; Triggiani et al. 2006; Boyanovsky and Webb 2009). In addition to acting as lipolytic enzymes, $sPLA_2$s may serve as high-affinity ligands for cell surface receptors (Cupillard et al. 1997; Kundu and Mukherjee 1997; Lambeau and Lazdunski 1999; Higashino et al. 2002). $sPLA_2$s are also able to bind proteoglycans with high affinity due to their overall positive charge (Rosengren et al. 2006). Although our knowledge of the cellular functions of plant PLAs is limited, it is tempting to speculate that, similar to animal PLAs, functions of plant PLAs may extend beyond those related to their catalytic activities.

3 The Arabidopsis PLA_2-Type Protein SOBER1

A well-characterized example of a PLA_2-type protein that is involved in plant defense responses is the Arabidopsis *α/β* hydrolase SOBER1 (*S*uppressor *O*f Avr*B*sT-*E*licited *R*esistance*1*). AvrBsT is a type III effector from *Xanthomonas campestris* pv. *vesicatoria* that is predicted to encode a Cys protease. In an attempt to identify lines resistant to *Xanthomonas* expressing AvrBsT, the natural variation existing among Arabidopsis ecotypes was surveyed. Only the ecotype Pi-0 was found to develop an HR in response to AvrBsT (Cunnac et al. 2007). In addition, Pi-0 is resistant to *Pseudomonas syringae* pathovar *tomato* strain DC3000 expressing AvrBsT [*Pst* DC3000 (AvrBsT)] and resistance was found to be due

to a loss-of-function mutation in SOBER1, suggesting that SOBER1 inhibits AvrBsT-triggered phenotypes in Arabidopsis (Cunnac et al. 2007). Members of the SOBER1 protein family possess phospholipase and carboxylesterase activity with diverse substrate specificities. SOBER1-dependent suppression of the HR in Pi-0 suggested that it might hydrolyze a plant lipid or precursor required for HR induction. Indeed, Pi-0 leaves infected with *Pst* DC3000 (AvrBsT) accumulated higher levels of PA compared to leaves infected with virulent *Pst* DC3000, in a Phospholipase D (PLD)-dependent manner (Kirik and Mudgett 2009). Overexpression of SOBER1 in Pi-0 led to reduction of PA levels and inhibition of the HR. Moreover, SOBER1 was found to hydrolyze PC (phosphatidylcholine) but not PA or lysoPC in vitro, indicating that the enzyme displays PLA_2 activity (Kirik and Mudgett 2009). Chemical inhibition of PLA_2 activity in leaves expressing SOBER1 resulted in HR in response to *Pst* DC3000 AvrBsT, suggesting that SOBER1 PLA_2 activity suppresses PLD-dependent production of PA in response to AvrBsT elicitation. Although the mechanism by which AvrBsT leads to PA accumulation in Arabidopsis Pi-0 remains to be determined, this work indicated an important role for SOBER1 in the regulation of PA levels generated in plants in response to bacterial inoculation.

4 Patatin-Related Phospholipases (pPLA)

Patatins were identified and then isolated some decades ago as abundant storage proteins in potato tubers (Park et al. 1983), which are at the origin of the generic name given to this group of closely related proteins (Hendriks et al. 1991). These proteins are responsible for most of the soluble LAH activity extracted from this organ, acting in vitro on galactolipids or phospholipids (with low or no positional specificity) but not on triglycerides (Racusen 1984; Andrews et al. 1988). Later on, LAH activities purified from plant leaves were found to be related to patatins, and patatin-encoding genes appeared to be inducible by various environmental stresses such as microbial infection, drought, or senescence. The nomenclature of plant patatins was recently updated, namely by introducing the acronym "pPLA" (Scherer et al. 2010).

While patatins are suspected to accumulate in potato tubers for defensive functions in addition to their presumed nitrogen storage function, the formal demonstration of such a hypothesis is still missing. Two reports have described direct activities on insect and microbial aggressors. An early study established that potato tuber patatin inhibits Southern corn rootworm larval growth and that this activity is sensitive to serine hydrolase inhibitors (Strickland et al. 1995). Similarly, a patatin-related protein was purified from a somatic hybrid between potato and a wild relative and displayed inhibitory activity in *Phytophthora* spore germination assays (Sharma et al. 2004).

The first in-depth study of a non-tuber patatin in a context of induced innate immunity was initiated by purification of LHA activity in soluble extracts of

tobacco leaves reacting hypersensitively to *Tobacco Mosaic Virus* (TMV) (Dhondt et al. 2000). Two hypersensitive response (HR)-induced genes (*NtPAT*) were cloned that encode highly active LAH with preferential galactolipase over phospholipase A activity. Their early expression was correlated with appearance of cell death symptoms, upregulation of oxylipin biosynthetic genes, and jasmonic acid (JA) accumulation. Further investigation using ß-megaspermin, a necrotizing protein elicitor from *Phytophthora megasperma*, to spatially dissect the LAH response showed that *NtPAT*-encoded LAH activity was transiently stimulated in elicitor-treated tissues that undergo programmed cell death and later on in living tissue immediately surrounding the collapsed zone (Dhondt et al. 2002). However, JA accumulation only occurred in tissue committed to cell death and therefore uncoupling NtPAT activity from JA biosynthesis. *NtPAT* expression was induced in tobacco leaves by viral, bacterial, and fungal pathogens, all triggering different forms of host cell death. Transgenic tobacco plants overexpressing NtPAT3 exhibited an altered response to TMV infection manifested by decreased necrotic lesion areas. In an independent study, using elicitin- and bacteria-induced HR, Cacas et al. (2005) proposed that the combined action of 9-lipoxygenase and *NtPAT*-encoded galactolipase is sufficient to bring about programmed cell death during HR by converting plastid-released fatty acids into toxic hydroperoxides. A similar situation was described in cotton leaves where co-expression of a patatin galactolipase with a 9-LOX precedes *Xanthomonas campestris*-mediated HR symptoms, pointing to a possible conservation of an oxylipin-based cell death pathway across the plant kingdom. The coupling of different LAHs with oxylipin biosynthetic pathways seems to be evolutionary ancient, as lipolytic activities trigger oxylipin defense upon wounding or epiphytic growth in red algae (Lion et al. 2006).

Patatin genes are ubiquitous in plant genomes and although they have only been investigated thoroughly in a few species, stress-inducible patatins can be identified in most plant species where large-scale transcriptional data are available, including rice (Singh et al. 2012), wheat (Desmond et al. 2008), tomato, or maize. Inducible patatin genes and LAH activity have been associated with other physiological processes. For example, in cowpea, the extent of galactolipid breakdown upon drought stress correlates with drought sensitivity, galactolipase activity, and patatin gene induction (Matos et al. 2001).

The patatin family has been characterized in recent years in Arabidopsis, where a family of ten *pPLA* gene members has been defined and classified into three subclasses, according to gene structure and overall similarity (Holk et al. 2002; La Camera et al. 2005; Scherer et al. 2010). There is evidence that at least one member of each subclass is involved in plant defense mechanisms. pPLAI, the single member of subclass I, is an atypical patatin as its sequence resembles $iPLA_2\alpha$, a member of the animal so-called PNPLA protein family displaying a patatin domain (Kienesberger et al. 2009). pPLAI also possesses leucine-rich and Armadillo repeats that suggest regulatory interactions with protein partners. When assayed in vitro, pPLAI exhibits preferential galactolipase over phospholipase activity and hydrolyzes both oxylipin-containing and unoxidized galactolipids (Yang et al. 2007). However, the in planta relevance of this observation is unknown, as

lipid profiling of *Botrytis*-infected leaves did not evidence alterations in mono- or di-galactosyldiacylglycerol hydrolysis between wild-type and *pplaI*-deficient plants. Despite *pPLAI* transcription being stable upon biotic stress, *pplaI* mutants were more sensitive to *Botrytis* infection, but their JA levels were not altered. Instead, basal JA levels were reduced compared to WT, although how this change conditions antifungal resistance is unclear (Yang et al. 2007). Several studies showed that upon infection or wounding, Arabidopsis plants accumulate large amounts of bound oxylipins, namely, the JA-precursor 12-oxo-phytodienoic acid, that are esterified to galactolipids (Stelmach et al. 2001; Andersson et al. 2006b; Buseman et al. 2006). Also, the induction by bacterial infection of novel and abundant galactolipid accumulation bearing fragmented and/or peroxidized fatty acids was reported (Zoeller et al. 2012).

Subclass II of patatin-related PLAs (pPLAII) comprises five genes in Arabidopsis that are the closest by organization and sequence to classical potato tuber patatins and also generally comprise the isoforms known so far in other plant species (Scherer et al. 2010). Subclass I and II contain the canonical GTSTG esterase box that is part of the catalytic center. Transcript profiling identified pPLAIIα/PLP2 as the only isoform of subclass II whose expression was upregulated in response to necrotizing bacteria or fungi (La Camera et al. 2005), and this induction is dependent on JA signaling in response to *Botrytis*. Overexpression of Arabidopsis pPLAIIα in petunia has independently conducted to similar observations (Zahn 2005). *pPLAIIα* was also reported to be responsive to numerous stimuli in Arabidopsis (Narusaka et al. 2003), including drought stress (Matos et al. 2008) where it may contribute to galactolipid breakdown. Functional analysis of pPLAIIα in various pathosystems has shown that its expression increases the severity of symptoms and favors colonization of leaves by fungal and bacterial pathogens. pPLAIIα-deficient plants are more resistant to *Botrytis* or avirulent *Pseudomonas syringae* infection whereas overexpressing plants display enhanced cell death symptoms and elevated pathogen multiplication (La Camera et al. 2005). These results were supported by altered sensitivity of engineered plants to the cell death-inducing herbicide paraquat and point a primary role of pPLAIIα in promoting host cell death execution. Conversely, a positive impact of pPLAIIα expression on resistance was recorded when plants were inoculated with the obligate pathogen *Cucumber Mosaic Virus* whose multiplication is counteracted by plant cell death (La Camera et al. 2009). Finally, pPLAIIα effect on developmentally triggered cell death was examined, eliminating the variable contribution of pathogen virulence factors to the plant response. Silencing or overepression of pPLAIIα in the lesion mimick mutant background *vascular associated disease* (*vad1*) reduced or enhanced, respectively, the extent and kinetics of leaf damage. Collectively, these results illustrate the multifaceted contribution of the pPLAIIα to the execution of leaf cell death triggered by various microbial attackers, or by the plant itself.

To gain insight into the metabolic changes related to *pPLAIIα* expression, broad-spectrum oxylipin profiling was performed in pPLAIIα-modifed, *Botrytis*-infected plants. The results indicated that pPLAIIα manipulation does not significantly alter fungus-induced accumulation of free C18 fatty acids and jasmonates, but that it

modulated α-dioxygenase-derived oxylipin abundance, which was previously linked to cell death promotion (La Camera et al. 2009). This analysis suggests that in response to microbes, pPLAIIα does not seem to act as a signaling enzyme, but rather catalyzes late, nonspecific hydrolysis of membrane lipids that commits tissue to cell death. The exact mechanism by which pPLAIIα affects the cell death process is still uncertain, as is the precise nature of endogenous substrate(s), because no extensive phospho- and galacto-lipid profiling of pathogen-challenged modified plants is available for this pPLA. Blocking genetically branches of oxylipin pathways in plants displaying pPLAIIα-enhanced cell death may inform if cellular collapse relies on specific oxylipin synthesis.

The subclass III of patatins contains four members in Arabidopsis that share less overall similarity to potato tuber patatin (Holk et al. 2002; La Camera et al. 2005; Scherer et al. 2010) and are distinguished by a variant esterase motif where the central reactive serine typical of serine hydrolases is lacking, resulting in a GXGXG motif. Until recently, there was no formal evidence of enzymatic activity for this class of patatins, but characterization of pPLAIIIß clearly revealed LAH (with a preference for *sn*-2 position) and acyl-CoA thioesterase activities, whereas misexpression of pPLAIIIß affects plant growth and morphology (Li et al. 2011). Transcript profiling identified pPLAIIIß as the only subclass III isoform that is upregulated upon *Botrytis* or *Pseudomonas* infection (La Camera et al. 2005). However, pPLAIIIß mutant or overexpressing plants appeared unaffected in their resistance to these pathogens, and there is therefore no clear evidence of pPLAIIIß involvement in plant innate immunity.

Interestingly, lipolytic proteins with limited patatin-resembling domains evolved in animals with important functions in fat storage mobilization (Kienesberger et al. 2009) or in pathogenic bacteria as virulence factors (Banerji and Flieger 2004; Lang and Flieger 2011). Three Arabidopsis loci encode proteins with a minimal patatin domain. Two of them (SDP1 and SDP1-like) are related to the yeast triacylglycerol lipase TGL4, but with very limited resemblance to canonical pPLA, and are responsible for most triglyceride breakdown upon post-germinative seedling growth (Scherer et al. 2010).

When relatively stable and usually soluble patatin-encoded LAH activity is detectable in total protein extracts from plant tissues, it is typically very difficult to biochemically monitor other PLAs, because of their weaker or less stable/soluble activity. Consequently, other LAHs have been poorly accessible to direct purification in tissues/organs, such as leaves, that are not specialized in lipolysis. Alternative approaches, including genetics, are then suitable, although its has been difficult to rationally design phenotypic screens that specifically target the isolation of PLA mutants.

5 DAD1-Like LAHs

This LAH gene family was identified by forward genetics when Arabidopsis T-DNA mutant collections were screened for male sterility phenotypes. One of the lines, *deficient in anther dehiscence 1* (*dad1*), is impaired in flower and anther opening and pollen maturation and hence its sterility (Ishiguro et al. 2001). The inactivated gene was identified as encoding a plastidial PLA_1 that initiates JA synthesis in stamens to regulate water transport and late stage development processes in anthers. This finding was the first description of a specific LAH that meets the properties expected from a LAH feeding fatty acids to the plastid-localized early steps of JA synthesis. *DAD1* is expressed in filaments rather than in anthers of wild-type stamens, suggesting that water flux is controlled by JA in filaments. Phylogeny searches showed that DAD1 is one of a 12-member gene family encoding putative lipolytic enzymes. Their catalytic region contains an esterase (GXSXG) motif and these proteins are somehow related to fungal triacylglycerolipases, with which they share *sn-1*-specific PLA and galactolipase activities. These predicted proteins were grouped into three subclasses according to the structure of their N-termini and overall similarity. Subclass I contains seven members including DAD1 that bear predicted transit peptides for targeting to plastids. Subclass II members lack this feature and may reside in the cytosol. The single subclass III member has an N terminal peptide typical for mitochondrial targeting. A nomenclature for this *PLA_1* gene family has been defined (Ryu 2004; Ellinger et al. 2010). Organelle targeting has been experimentally confirmed for some class I members in chloroplasts (Padham et al. 2007; Seo et al. 2009; Ellinger et al. 2010; Grienenberger et al. 2010) and for subclass III AtDLAH (or $AtPLA_1$-III) in mitochondria (Seo et al. 2011). Lipolytic activities of the seven subclass I proteins have also been reported, and in vitro assays of recombinant proteins allowed distinguishing three patterns of substrate preference for these enzymes. The first one displays PLA-only activity, the second PLA and galactolipase activities, and the third deacylates phosphatidylcholine, galactolipids, and triglycerides (Seo et al. 2009). The catalytic diversification in this structural family is consistent with distinct patterns of chloroplast labeling when expressing LAH-GFP fusions (Grienenberger et al. 2010). This differential distribution may reflect association of the enzymes with subchloroplastic compartments such as the envelope, thylakoids, and plastoglobules that are enriched in phospholipids, galactolipids, and triglycerides, respectively. These LAH thus seem to play distinct roles in plastidial lipid homeostasis.

The conclusive characterization of $DAD1/PLA_1$-Iβ1 in JA biosynthesis for anther and pollen development fostered additional functional studies to explore the possibility that other family members may initiate JA synthesis in other organs/situations, for example, under stress conditions. The initial enzymatic steps of JA synthesis from linolenic acid up to its precursor 12-oxo-phytodienoic acid are localized in the chloroplast (Schaller and Stintzi 2009), making the seven subclass I enzymes good candidates to feed fatty acid precursors into the JA pathway in

leaves. DAD1 deficiency was initially reported not to affect JA accumulation after mechanical leaf wounding mimicking attacks by herbivorous insects (Ishiguro et al. 2001). Later on, the sequential cooperation for JA biosynthesis between DAD1 and a closely related galactolipase, DGL (also named PLA_1-Iα-1), was described (Hyun et al. 2008). An Arabidopsis activation line that overexpresses DGL displays stunted growth, accumulates high levels of JA, and displays strong resistance to *Alternaria brassicicola*, a necrotrophic fungus for which resistance is mediated through the JA/ethylene defense pathway. No data on fungal resistance are available for DGL-deficient lines. According to the authors, ectopic expression of other Arabidopsis *PLA_1* genes did not result into elevated JA levels. DGL was proposed to play a role in maintaining basal JA levels and for early accumulation while DAD1 would participate in the later phases. These conclusions that DAD1/PLA_1-Iß1 and DGL/PLA_1-Iα-1 are necessary and sufficient for JA accumulation were debated in another study (Ellinger et al. 2010) that failed to confirm diminished basal or early-induced JA levels in DGL-deficient or *dad1* plants upon wounding or *Pseudomonas* infection. Also, detailed localization studies showed that DGL is associated with lipid bodies rather than with chloroplasts. A reduction by half in the wound-induced JA levels was evidenced in the single mutant *PLA_1-γ 1*. This impairement could be accentuated to about 30 % of the wild-type basal and injury-induced JA accumulation in a quadruple mutant in four *DAD1*-like genes (including *PLA_1-γ1*), showing that in Arabidopsis, different lipases are partially redundant and may act during different phases of the response to account for the full JA burst. In search of novel players to catalyze this step, 14 additional genes encoding putative lipolytic enzymes with predicted plastidial transit peptides were identified in the Arabidopsis genome, but analysis of mutant lines in 11 of these genes showed normal basal and wound-induced JA levels (Ellinger et al. 2010). This suggests that in chloroplasts, many lipases do not present the adequate activity, association with lipids, or enzymatic partners to channel linolenic acid into JA biosynthesis. Three other *PLA_1-I* genes, *PLA_1-γ1*, *PLA_1-Iγ2*, and *PLAIII*, were examined in the context of antimicrobial defense and found to be induced by *Pseudomonas* or *Botrytis* attack (Grienenberger et al. 2010). However, mutant lines in these genes did not exhibit a clear defect in microbe-induced JA levels or in pathogen resistance. Therefore, strong evidence from PLA_1-deficient Arabidopsis lines demonstrating the role of these enzymes in antimicrobial defense is still lacking. Other Arabidopsis genes in the PLA_1 family have been linked to roles in leaf senescence [PLA_1-α2 (Padham et al. 2007)], seed viability [mitochondria-targeted PLA_1-III (Seo et al. 2011)], or negative regulation of seed storage oil mobilization [PLA_1-IIγ (Kim et al. 2011a)].

Interestingly, the functional analysis of *PLA_1-I* genes in inducible defense in the wild tobacco relative *Nicotiana attenuata* provided more clear data. Three DGL-DAD1 homologs named GLA (for glycerolipase A) have been identified in *N. attenuata*. Although *GLA1* was the only isoform whose mRNA abundance was downregulated upon wounding (Kallenbach et al. 2010), its silencing clearly and specifically attenuated the accumulation dynamics of jasmonates and some other oxylipins. *GLA1*-silenced plants were reduced by 50–65 % in their content in JA

and JA-Ile in leaves or roots that were wounded or treated with insect elicitor (Bonaventure et al. 2011). In the same material, *sn-1*- and *sn-2*-specific lysophospho- and lysogalacto-lipid levels showed a similar reduction, in line with the in vitro *sn-1*-specific activity on phospholipids, galactolipids, and triglycerides determined for recombinant GLA1 (Kallenbach et al. 2010). In contrast, *GLA1*-silenced plants display normal levels of JA and JA-Ile in flowers and are fully fertile. Similarly, JA levels of leaves infected by the fungus *Fusarium oxysporum* or by the oomycete *Phytophthora parasitica* var. *nicotianae* were unaffected as compared to the different plant genotypes. Impact on other branches of the lipoxygenase pathway (Feussner and Wasternack 2002; La Camera et al. 2004) was also investigated. Green leaf volatiles that are hydroperoxide lyase cleavage products released upon wounding were not modified. However, *GLA1* silencing was found to affect the early metabolism of divinyl ethers upon infection of leaves by *P. parasitica* (Bonaventure et al. 2011). These results indicate that in vivo, the plastidial lipase GLA1 is post-transcriptionally recruited to act on different lipid classes and provides C18 fatty acid substrate to initiate JA synthesis or modulate other oxylipins in some specific situations. This reinforces the idea that biosynthesis of a given oxylipin may require different lipases depending on the stimulus and the plant organ. Conversely, a given lipase can affect the abundance of different types of oxylipins, pointing to a growing complexity of lipid hydrolysis networks. Understanding how these critical events are regulated and interconnected is a major challenge for future biochemical/cellular studies.

6 Other Predicted Lipases

6.1 The EDS1/PAD4/SAG101 Regulatory Node

In Arabidopsis, EDS1 (*E*nhanced *D*isease *S*usceptibility*1*) has emerged as a central immune regulator (Aarts et al. 1998; Wiermer et al. 2005; Garcia and Parker 2009). In association with its sequence-related partners PAD4 (*P*hyto*A*lexin *D*eficient*4*) and SAG101 (*S*enescence-*A*ssociated *G*ene*101*), EDS1 forms an essential regulatory node that controls both low-level basal immunity to virulent pathogens and effector-triggered activation of intracellular nucleotide-binding leucine-rich repeat (NB-LRR) proteins, triggering the HR (Feys et al. 2001, 2005). EDS1 presents different molecular and spatial in planta associations with PAD4 and SAG101, and these complexes have distinct signaling activities (Feys et al. 2001, 2005). Indeed, EDS1 homodimers are restricted to the cytoplasm whereas EDS1-PAD4 heterodimers accumulate in both the cytoplasm and the nucleus, and EDS1-SAG101 complexes are confined to nuclei. Importantly, dynamic interactions between nuclear and cytoplasmic pools of EDS1 are important in directing defense signaling (Feys et al. 2005; Garcia et al. 2010).

Based on primary-sequence analysis, the N terminal domain of EDS1 has homology to proteins of the α/β-hydrolase fold family, including three residues that comprise a serine hydrolase catalytic triad (Ser-Asp-His) (Ollis et al. 1992). EDS1 shares this overall homology and a unique C terminal "EP" (EDS1-PAD4-specific) domain with PAD4 and SAG101 (He and Gan 2002; Wiermer et al. 2005). Although SAG101 lacks the canonical catalytic triad, weak acyl esterase activity was detected for recombinant SAG101 expressed in *Escherichia coli* (He and Gan 2002). However, no enzymatic activity has been detected in the case of EDS1 or PAD4 and their biochemical mode of action remains unknown. Although the lipase homologies of these proteins might be misleading in terms of catalytic activity, these domains appear to be conserved in all plant EDS1 and PAD4 orthologs examined, and it has been suggested that they may play a structural rather than an enzymatic role during plant immunity (Wiermer et al. 2005). EDS1 and PAD4 are required to transduce reactive oxygen species-derived signals leading to cell death during photooxidative stress and immune responses, suggesting the involvement of a redox component necessary for EDS1 signal transduction (Muhlenbock et al. 2008; Straus et al. 2010). Based on these observations and the lack of enzymatic activity, it has been also proposed that that EDS1 and its partners may help traffick rather than hydrolyze oxygenated lipids inside the cell (Wiermer et al. 2005).

6.2 Other Structural Families

There are at least two more large gene families structurally related to lipases, with members that have been clearly linked to innate immunity, but whose glycerolipase activity remains unclear. The first one is the so-called GDSL family that displays a conserved motif (PFAM 00657) present in the catalytic site and that is distinct from the widespread GxSxG serine hydrolase motif discussed earlier. Structural analysis suggests a flexible active site that can accommodate various substrates and characterized members display lipase, protease, esterase, or even acyltransferase activities (Volokita et al. 2011; Kikuta et al. 2012). Therefore, it is not known to which extent some isoforms are true lipolytic enzymes or even if some act on glycerolipids (Li-Beisson et al. 2010). Arabidopsis has 108 GDSL members, but none has been described as displaying PLA activity. In most described studies, poor enzyme characterization was conducted, using synthetic short-chain *p*-nitro-phenyl esters as substrates. Still, two genes have been identified with functions in plant defense. GLIP1 was purified as a protein secreted by salicylic acid-treated cultured cells (Oh et al. 2005). *GLIP1* gene expression is induced by fungal infection and *glip1* mutant plants are susceptible to *Alternaria* infection. GLIP1 has direct fungitoxic activity but may also act in systemic signaling as its expression conditions the presence in phloem exudates of a defense-inducing activity (Kwon et al. 2009). A closely related member called GLIP2 seems to affect both auxin signaling and resistance to the necrotrophic bacterium *Erwinia carotovora* (Lee et al. 2009).

Finally, a pepper GDSL ortholog is a positive effector of defense against biotrophic microbial infections (Hong et al. 2008), suggesting that this class of enzymes may affect a conserved step in defense against pathogens with different colonization strategies.

The second family called PRLIP was first identified in Arabidopsis (Jakab et al. 2003) and similar sequences were found in rice and grapevine (Szalontai et al. 2012). Predicted PRLIP proteins present features of serine hydrolases/esterases and class 3 lipases but again no true lipidic substrate is known. These sequences are grouped into two clades, one of which with constitutive expression is found in all angiosperms with available sequences, the second being taxon-specific and comprising defense signal- and pathogen-induced members.

7 Conclusions and Perspectives

Although interesting advances have been recently seen in the description of the properties of diverse phospho- or galacto-lipases, our understanding of their roles in innate immunity remains largely fragmentary. We are still far from understanding how and where different lipid hydrolysis events are orchestrated by the recruitment of the numerous players at specific subcellular sites.

Two main problems whose resolution will need the implementation of more powerful strategies based on novel technologies are behind the slow progress in the functional characterization of LAHs in recent studies. First, most reverse genetic studies involve pre-identified gene families in plants or in other organisms where candidate proteins exhibit recognizable features of esterases/lipases, such as in rice (Singh et al. 2012). Gene annotation can sometimes be misleading and has conducted many investigators to regard as lipolytic some protein candidates that likely use non-acyl-lipidic substrates. Conversely, many true lipolytic enzymes have likely been missed because of their "noncanonical" primary structures and remain to be discovered. This functional gap between insufficient gene annotation and in-depth characterization could be partly bridged by screening candidates at the protein rather than transcript level, for example, by applying activity-based proteomics. This approach allows to visualize enzymes in complex proteomes by incubation with fluorescent chemical probes that covalently label active sites (Birner-Gruenberger and Hermetter 2007). Mass spectrometry-based identification of labeled proteins then provides access to functional studies. This strategy proved highly successful in the global analysis in animal models of storage lipid hydrolysis such as adipocytes (Schittmayer and Birner-Gruenberger 2009) and has started to be used in plant pathology. For example, this technique was applied to the *Arabidopsis–Botrytis* interaction and led to the identification of 45 Arabidopsis serine hydrolases of which only 9 had been characterized previously and also of fungus-derived cutinases and lipases (Kaschani et al. 2009). Similar experiments repeated with probes specific for PLA/GLA activities have the potential to reveal

unknown lipolytic enzymes, including some that are activated to establish immunity independently of their transcriptional upregulation.

A second challenge is to determine where a given PLA acts in the cell and on which substrate. Images of fluorescently tagged PLA provide a first indication as to where hydrolysis may take place, but they are generally acquired in heterologous expression systems and do not account for the cellular changes and damage that occur when immune reponses are being deployed in infected tissue. In this context, the ultimate aim would be to visualize kinetically and with high-resolution individual lipolytic events in living cells. Before this is feasible, we must rely on global lipid profiling approaches where impressive analytical progress has been recently made. Early studies attempted to link the activation or deficiency of a given LAH with changes in lipid or presumed hydrolysis product abundance, but the analytic resolution was limited to the separation of global lipid classes and frequently did not correlate with the modification of lipid composition. A major pitfall is that many phospholipids are present in several membrane systems across the cell, potentially masking the discrete action of individual LAHs at a defined subcellular location. In vitro-determined lipolytic activities can also be misinterpreted as to the real substrate that is hydrolyzed in vivo. Improved protocols largely based on electrospray ionization-mass spectrometry, either by direct infusion of coupled to liquid chromatography, allowed the separation, identification, and in some cases quantification of many lipid molecular species (Welti and Wang 2004). In further recent methodological developments, hundreds of glycerolipid species differing by their polar head and acyl chains were characterized (Ibrahim et al. 2011; Vu et al. 2012; Zoeller et al. 2012). Furthermore, in response to wounding or bacterial infection, the appearance of dozens of novel oxidized or fragmented galactolipid species was reported. This enhanced analytic resolution will allow a much deeper metabolic examination of LAH-modified lines with increased possibility of identifying subtle changes in their lipid contents. Alterations in organelle- or membrane-specific (lyso)-lipid species can even provide precise information on real in vivo substrate(s). Finally, the emergence of MS-imaging techniques on tissues or isolated organelles opens another door to the visualization of PLA product formation (Horn and Chapman 2012). The combination of expanded genetic resources with more systematic biochemical and analytical tools should significantly contribute to increase our understanding about how PLAs impact the adaptation of plants to their ever changing environment.

References

Aarts N, Metz M, Holub E, Staskawicz BJ, Daniels MJ, Parker JE (1998) Different requirements for *EDS1* and *NDR1* by disease resistance genes define at least two *R* gene-mediated signaling pathways in *Arabidopsis*. Proc Natl Acad Sci U S A 95:10306–10311

Andersson MX, Kourtchenko O, Dangl JL, Mackey D, Ellerstrom M (2006a) Phospholipase-dependent signalling during the AvrRpm1- and AvrRpt2-induced disease resistance responses in *Arabidopsis thaliana*. Plant J 47:947–959

Andersson MX, Hamberg M, Kourtchenko O, Brunnstrom A, McPhail KL, Gerwick WH, Gobel C, Feussner I, Ellerstrom M (2006b) Oxylipin profiling of the hypersensitive response in Arabidopsis thaliana. Formation of a novel oxo-phytodienoic acid-containing galactolipid, arabidopside E. J Biol Chem 281:31528–31537

Andrews DL, Beames B, Summers MD, Park WD (1988) Characterization of the lipid acyl hydrolase activity of the major potato (Solanum tuberosum) tuber protein, patatin, by cloning and abundant expression in a baculovirus vector. Biochem J 252:199–206

Banerji S, Flieger A (2004) Patatin-like proteins: a new family of lipolytic enzymes present in bacteria? Microbiology 150:522–525

Birner-Gruenberger R, Hermetter A (2007) Activity-based proteomics of lipolytic enzymes. Curr Drug Discov Technol 4:1–11

Blee E (2002) Impact of phyto-oxylipins in plant defense. Trends Plant Sci 7:315–322

Bonaventure G, Schuck S, Baldwin IT (2011) Revealing complexity and specificity in the activation of lipase-mediated oxylipin biosynthesis: a specific role of the Nicotiana attenuata GLA1 lipase in the activation of jasmonic acid biosynthesis in leaves and roots. Plant Cell Environ 34:1507–1520

Boyanovsky BB, Webb NR (2009) Biology of secretory phospholipase A2. Cardiovasc Drugs Ther 23:61–72

Browse J (2009) Jasmonate passes muster: a receptor and targets for the defense hormone. Annu Rev Plant Biol 60:183–205

Buseman CM, Tamura P, Sparks AA, Baughman EJ, Maatta S, Zhao J, Roth MR, Esch SW, Shah J, Williams TD, Welti R (2006) Wounding stimulates the accumulation of glycerolipids containing oxophytodienoic acid and dinor-oxophytodienoic acid in Arabidopsis leaves. Plant Physiol 142:28–39

Cacas JL, Vailleau F, Davoine C, Ennar N, Agnel JP, Tronchet M, Ponchet M, Blein JP, Roby D, Triantaphylidès C (2005) The combined action of 9 lipoxygenase and galactolipase is sufficient to bring about programmed cell death during tobacco hypersensitive response. Plant Cell Environ 33:1453–1473

Chandra S, Low PS (1995) Role of phosphorylation in elicitation of the oxidative burst in cultured soybean cells. Proc Natl Acad Sci U S A 92:4120–4123

Coll NS, Vercammen D, Smidler A, Clover C, Van Breusegem F, Dangl JL, Epple P (2010) Arabidopsis type I metacaspases control cell death. Science 330:1393–1397

Cunnac S, Wilson A, Nuwer J, Kirik A, Baranage G, Mudgett MB (2007) A conserved carboxylesterase is a SUPPRESSOR OF AVRBST-ELICITED RESISTANCE in Arabidopsis. Plant Cell 19:688–705

Cupillard L, Koumanov K, Mattei MG, Lazdunski M, Lambeau G (1997) Cloning, chromosomal mapping, and expression of a novel human secretory phospholipase A2. J Biol Chem 272:15745–15752

Desmond OJ, Manners JM, Schenk PM, Maclean DJ, Kazan K (2008) Gene expression analysis of the wheat response to infection by Fusarium pseudograminearum. Physiol Mol Plant Pathol 73:40–47

Dhondt S, Geoffroy P, Stelmach BA, Legrand M, Heitz T (2000) Soluble phospholipase A2 activity is induced before oxylipin accumulation in tobacco mosaic virus-infected tobacco leaves and is contributed by patatin-like enzymes. Plant J 23:431–440

Dhondt S, Gouzerh G, Muller A, Legrand M, Heitz T (2002) Spatio-temporal expression of patatin-like lipid acyl hydrolases and accumulation of jasmonates in elicitor-treated tobacco leaves are not affected by endogenous levels of salicylic acid. Plant J 32:749–762

Ellinger D, Stingl N, Kubigsteltig II, Bals T, Juenger M, Pollmann S, Berger S, Schuenemann D, Mueller MJ (2010) DONGLE and DEFECTIVE IN ANTHER DEHISCENCE1 lipases are not essential for wound- and pathogen-induced jasmonate biosynthesis: redundant lipases contribute to jasmonate formation. Plant Physiol 153:114–127

Feussner I, Wasternack C (2002) The lipoxygenase pathway. Annu Rev Plant Biol 53:275–297

Feys BJ, Moisan LJ, Newman MA, Parker JE (2001) Direct interaction between the Arabidopsis disease resistance signaling proteins, EDS1 and PAD4. EMBO J 20:5400–5511

Feys BJ, Wiermer M, Bhat RA, Moisan LJ, Medina-Escobar N, Neu C, Cabral A, Parker JE (2005) Arabidopsis SENESCENCE-ASSOCIATED GENE101 stabilizes and signals within an ENHANCED DISEASE SUSCEPTIBILITY1 complex in plant innate immunity. Plant Cell 17:2601–2613

Flati V, Haque SJ, Williams BR (1996) Interferon-alpha-induced phosphorylation and activation of cytosolic phospholipase A2 is required for the formation of interferon-stimulated gene factor three. EMBO J 15:1566–1571

Froidure S, Canonne J, Daniel X, Jauneau A, Briere C, Roby D, Rivas S (2010) *At*sPLA2-alpha nuclear relocalization by the Arabidopsis transcription factor AtMYB30 leads to repression of the plant defense response. Proc Natl Acad Sci U S A 107:15281–15286

Garcia AV, Blanvillain-Baufume S, Huibers RP, Wiermer M, Li G, Gobbato E, Rietz S, Parker JE (2010) Balanced nuclear and cytoplasmic activities of EDS1 are required for a complete plant innate immune response. PLoS Pathog 6:e1000970

Garcia AV, Parker JE (2009) Heaven's gate: nuclear accessibility and activities of plant immune regulators. Trends Plant Sci 14:479–487

Granata F, Petraroli A, Boilard E, Bezzine S, Bollinger J, Del Vecchio L, Gelb MH, Lambeau G, Marone G, Triggiani M (2005) Activation of cytokine production by secreted phospholipase A2 in human lung macrophages expressing the M-type receptor. J Immunol 174:464–474

Grienenberger E, Geoffroy P, Mutterer J, Legrand M, Heitz T (2010) The interplay of lipid acyl hydrolases in inducible plant defense. Plant Signal Behav 5:1181–1186

He Y, Gan S (2002) A gene encoding an acyl hydrolase is involved in leaf senescence in Arabidopsis. Plant Cell 14:805–815

Hendriks T, Vreugdenhil D, Stiekema WJ (1991) Patatin and four serine proteinase inhibitor genes are differentially expressed during potato tuber development. Plant Mol Biol 17:385–394

Higashino K, Yokota Y, Ono T, Kamitani S, Arita H, Hanasaki K (2002) Identification and purification of a soluble form phospholipase A2 receptor as a circulating endogenous inhibitor for secretory phospholipase A2. J Biol Chem 277:13583–13858

Holk A, Rietz S, Zahn M, Quader H, Scherer GF (2002) Molecular identification of cytosolic, patatin-related phospholipases A from Arabidopsis with potential functions in plant signal transduction. Plant Physiol 130:90–101

Hong JK, Choi HW, Hwang IS, Kim DS, Kim NH, du Choi S, Kim YJ, Hwang BK (2008) Function of a novel GDSL-type pepper lipase gene, CaGLIP1, in disease susceptibility and abiotic stress tolerance. Planta 227:539–558

Horn PJ, Chapman KD (2012) Lipidomics in tissues, cells and subcellular compartments. Plant J 70:69–80

Hyun Y, Choi S, Hwang HJ, Yu J, Nam SJ, Ko J, Park JY, Seo YS, Kim EY, Ryu SB, Kim WT, Lee YH, Kang H, Lee I (2008) Cooperation and functional diversification of two closely related galactolipase genes for jasmonate biosynthesis. Dev Cell 14:183–192

Ibrahim A, Schutz AL, Galano JM, Herrfurth C, Feussner K, Durand T, Brodhun F, Feussner I (2011) The alphabet of galactolipids in Arabidopsis thaliana. Front Plant Sci 2:95

Ishiguro S, Kawai-Oda A, Ueda J, Nishida I, Okada K (2001) The DEFECTIVE IN ANTHER DEHISCIENCE gene encodes a novel phospholipase A1 catalyzing the initial step of jasmonic acid biosynthesis, which synchronizes pollen maturation, anther dehiscence, and flower opening in Arabidopsis. Plant Cell 13:2191–2209

Jakab G, Manrique A, Zimmerli L, Metraux JP, Mauch-Mani B (2003) Molecular characterization of a novel lipase-like pathogen-inducible gene family of Arabidopsis. Plant Physiol 132:2230–2239

Jung J, Kumar K, Lee HY, Park YI, Cho HT, Ryu SB (2012) Translocation of phospholipase A2alpha to apoplasts is modulated by developmental stages and bacterial infection in Arabidopsis. Front Plant Sci 3:126

Kachroo A, Kachroo P (2009) Fatty Acid-derived signals in plant defense. Annu Rev Phytopathol 47:153–176

Kallenbach M, Alagna F, Baldwin IT, Bonaventure G (2010) Nicotiana attenuata SIPK, WIPK, NPR1, and fatty acid-amino acid conjugates participate in the induction of jasmonic acid biosynthesis by affecting early enzymatic steps in the pathway. Plant Physiol 152:96–106

Kaschani F, Gu C, Niessen S, Hoover H, Cravatt BF, van der Hoorn RA (2009) Diversity of serine hydrolase activities of unchallenged and botrytis-infected Arabidopsis thaliana. Mol Cell Proteomics 8:1082–1093

Kienesberger PC, Oberer M, Lass A, Zechner R (2009) Mammalian patatin domain containing proteins: a family with diverse lipolytic activities involved in multiple biological functions. J Lipid Res 50(Suppl):S63–S68

Kikuta Y, Ueda H, Takahashi M, Mitsumori T, Yamada G, Sakamori K, Takeda K, Furutani S, Nakayama K, Katsuda Y, Hatanaka A, Matsuda K (2012) Identification and characterization of a GDSL lipase-like protein that catalyzes the ester-forming reaction for pyrethrin biosynthesis in Tanacetum cinerariifolium—a new target for plant protection. Plant J 71:183–193

Kim EY, Seo YS, Kim WT (2011a) AtDSEL, an Arabidopsis cytosolic DAD1-like acylhydrolase, is involved in negative regulation of storage oil mobilization during seedling establishment. J Plant Physiol 168:1705–1709

Kim HJ, Ok SH, Bahn SC, Jang J, Oh SA, Park SK, Twell D, Ryu SB, Shin JS (2011b) Endoplasmic reticulum- and Golgi-localized phospholipase A2 plays critical roles in Arabidopsis pollen development and germination. Plant Cell 23:94–110

Kirik A, Mudgett MB (2009) SOBER1 phospholipase activity suppresses phosphatidic acid accumulation and plant immunity in response to bacterial effector AvrBsT. Proc Natl Acad Sci U S A 106:20532–20537

Kundu GC, Mukherjee AB (1997) Evidence that porcine pancreatic phospholipase A2 via its high affinity receptor stimulates extracellular matrix invasion by normal and cancer cells. J Biol Chem 272:2346–2353

Kwon SJ, Jin HC, Lee S, Nam MH, Chung JH, Kwon SI, Ryu CM, Park OK (2009) GDSL lipase-like 1 regulates systemic resistance associated with ethylene signaling in Arabidopsis. Plant J 58:235–245

La Camera S, Gouzerh G, Dhondt S, Hoffmann L, Fritig B, Legrand M, Heitz T (2004) Metabolic reprogramming in plant innate immunity: the contributions of phenylpropanoid and oxylipin pathways. Immunol Rev 198:267–284

La Camera S, Balague C, Gobel C, Geoffroy P, Legrand M, Feussner I, Roby D, Heitz T (2009) The Arabidopsis patatin-like protein 2 (PLP2) plays an essential role in cell death execution and differentially affects biosynthesis of oxylipins and resistance to pathogens. Mol Plant Microbe Interact 22:469–481

La Camera S, Geoffroy P, Samaha H, Ndiaye A, Rahim G, Legrand M, Heitz T (2005) A pathogen-inducible patatin-like lipid acyl hydrolase facilitates fungal and bacterial host colonization in Arabidopsis. Plant J 44:810–825

Lambeau G, Lazdunski M (1999) Receptors for a growing family of secreted phospholipases A2. Trends Pharmacol Sci 20:162–170

Lang C, Flieger A (2011) Characterisation of Legionella pneumophila phospholipases and their impact on host cells. Eur J Cell Biol 90:903–912

Lee DS, Kim BK, Kwon SJ, Jin HC, Park OK (2009) Arabidopsis GDSL lipase 2 plays a role in pathogen defense via negative regulation of auxin signaling. Biochem Biophys Res Commun 379:1038–1042

Lee HY, Bahn SC, Kang YM, Lee KH, Kim HJ, Noh EK, Palta JP, Shin JS, Ryu SB (2003) Secretory low molecular weight phospholipase A2 plays important roles in cell elongation and shoot gravitropism in Arabidopsis. Plant Cell 15:1990–2002

Lee OR, Kim SJ, Kim HJ, Hong JK, Ryu SB, Lee SH, Ganguly A, Cho HT (2010) Phospholipase A(2) is required for PIN-FORMED protein trafficking to the plasma membrane in the Arabidopsis root. Plant Cell 22:1812–1825

Li-Beisson Y, Shorrosh B, Beisson F, Andersson MX, Arondel V, Bates PD, Baud S, Bird D, Debono A, Durrett TP, Franke RB, Graham IA, Katayama K, Kelly AA, Larson T, Markham JE, Miquel M, Molina I, Nishida I, Rowland O, Samuels L, Schmid KM, Wada H, Welti R, Xu C, Zallot R, Ohlrogge J (2010) Acyl-lipid metabolism. In: Arabidopsis book, vol 8, p e0133

Li M, Bahn SC, Guo L, Musgrave W, Berg H, Welti R, Wang X (2011) Patatin-related phospholipase pPLAIIIbeta-induced changes in lipid metabolism alter cellulose content and cell elongation in Arabidopsis. Plant Cell 23:1107–1123

Lion U, Wiesemeier T, Weinberger F, Beltran J, Flores V, Faugeron S, Correa J, Pohnert G (2006) Phospholipases and galactolipases trigger oxylipin-mediated wound-activated defence in the red alga Gracilaria chilensis against epiphytes. Chembiochem 7:457–462

Matos AR, d'Arcy-Lameta A, Franca M, Petres S, Edelman L, Kader J, Zuily-Fodil Y, Pham-Thi AT (2001) A novel patatin-like gene stimulated by drought stress encodes a galactolipid acyl hydrolase. FEBS Lett 491:188–192

Matos AR, Gigon A, Laffray D, Petres S, Zuily-Fodil Y, Pham-Thi AT (2008) Effects of progressive drought stress on the expression of patatin-like lipid acyl hydrolase genes in Arabidopsis leaves. Physiol Plant 134:110–120

Matos AR, Pham-Thi AT (2009) Lipid deacylating enzymes in plants: old activities, new genes. Plant Physiol Biochem 47:491–503

Muhlenbock P, Szechynska-Hebda M, Plaszczyca M, Baudo M, Mateo A, Mullineaux PM, Parker JE, Karpinska B, Karpinski S (2008) Chloroplast signaling and LESION SIMULATING DISEASE1 regulate crosstalk between light acclimation and immunity in Arabidopsis. Plant Cell 20:2339–2356

Mur LA, Kenton P, Lloyd AJ, Ougham H, Prats E (2008) The hypersensitive response; the centenary is upon us but how much do we know? J Exp Bot 59:501–520

Narusaka Y, Narusaka M, Seki M, Fujita M, Shida J, Nakashima M, Enju A, Sakurai T, Satou M, Kamiya A, Park P, Kobayashi M, Shinozaki K (2003) Expression profiles of Arabidopsis phospholipase A IIA gene in response to biotic and abiotic stresses. Plant Cell Physiol 44:1246–1252

Oh IS, Park AR, Bae MS, Kwon SJ, Kim YS, Lee JE, Kang NY, Lee S, Cheong H, Park OK (2005) Secretome analysis reveals an Arabidopsis lipase involved in defense against Alternaria brassicicola. Plant Cell 17:2832–2847

Ollis DL, Cheah E, Cygler M, Dijkstra B, Frolow F, Franken SM, Harel M, Remington SJ, Silman I, Schrag J (1992) The alpha/beta hydrolase fold. Protein Eng 5:197–211

Padham AK, Hopkins MT, Wang TW, McNamara LM, Lo M, Richardson LG, Smith MD, Taylor CA, Thompson JE (2007) Characterization of a plastid triacylglycerol lipase from Arabidopsis. Plant Physiol 143:1372–1384

Park WD, Blackwood C, Mignery GA, Hermodson MA, Lister RM (1983) Analysis of the heterogeneity of the 40,000 molecular weight tuber glycoprotein of potatoes by immunological methods and by NH(2)-terminal sequence analysis. Plant Physiol 71:156–160

Pawliczak R, Han C, Huang XL, Demetris AJ, Shelhamer JH, Wu T (2002) 85-kDa cytosolic phospholipase A2 mediates peroxisome proliferator-activated receptor gamma activation in human lung epithelial cells. J Biol Chem 277:33153–33163

Racusen D (1984) Lipid acyl hydrolase activity of patatin. Can J Bot 62:1640–1644

Raffaele S, Vailleau F, Leger A, Joubes J, Miersch O, Huard C, Blee E, Mongrand S, Domergue F, Roby D (2008) A MYB transcription factor regulates very-long-chain fatty acid biosynthesis for activation of the hypersensitive cell death response in Arabidopsis. Plant Cell 20:752–767

Rivas S (2012) Nuclear dynamics during plant innate immunity. Plant Physiol 158:87–94

Rosengren B, Peilot H, Umaerus M, Jonsson-Rylander AC, Mattsson-Hulten L, Hallberg C, Cronet P, Rodriguez-Lee M, Hurt-Camejo E (2006) Secretory phospholipase A2 group V: lesion distribution, activation by arterial proteoglycans, and induction in aorta by a western diet. Arterioscler Thromb Vasc Biol 26:1579–1585

Ryu SB (2004) Phospholipid-derived signaling mediated by phospholipase A in plants. Trends Plant Sci 9:229–235
Schaller A, Stintzi A (2009) Enzymes in jasmonate biosynthesis—structure, function, regulation. Phytochemistry 70:1532–1538
Scherer GF, Ryu SB, Wang X, Matos AR, Heitz T (2010) Patatin-related phospholipase A: nomenclature, subfamilies and functions in plants. Trends Plant Sci 15:693–700
Schittmayer M, Birner-Gruenberger R (2009) Functional proteomics in lipid research: lipases, lipid droplets and lipoproteins. J Proteomics 72:1006–1018
Seo J, Lee HY, Choi H, Choi Y, Lee Y, Kim YW, Ryu SB (2008) Phospholipase A2beta mediates light-induced stomatal opening in Arabidopsis. J Exp Bot 59:3587–3594
Seo YS, Kim EY, Kim JH, Kim WT (2009) Enzymatic characterization of class I DAD1-like acylhydrolase members targeted to chloroplast in Arabidopsis. FEBS Lett 583:2301–2307
Seo YS, Kim EY, Kim WT (2011) The Arabidopsis sn-1-specific mitochondrial acylhydrolase AtDLAH is positively correlated with seed viability. J Exp Bot 62:5683–5698
Shah J (2005) Lipids, lipases, and lipid-modifying enzymes in plant disease resistance. Annu Rev Phytopathol 43:229–260
Sharma N, Gruszewski HA, Park SW, Holm DG, Vivanco JM (2004) Purification of an isoform of patatin with antimicrobial activity against Phytophthora infestans. Plant Physiol Biochem 42:647–655
Sheridan AM, Sapirstein A, Lemieux N, Martin BD, Kim DK, Bonventre JV (2001) Nuclear translocation of cytosolic phospholipase A2 is induced by ATP depletion. J Biol Chem 276:29899–29905
Singh A, Baranwal V, Shankar A, Kanwar P, Ranjan R, Yadav S, Pandey A, Kapoor S, Pandey GK (2012) Rice phospholipase A superfamily: organization, phylogenetic and expression analysis during abiotic stresses and development. PLoS One 7:e30947
Stelmach BA, Muller A, Hennig P, Gebhardt S, Schubert-Zsilavecz M, Weiler EW (2001) A novel class of oxylipins, sn1-O-(12-oxophytodienoyl)-sn2-O-(hexadecatrienoyl)-monogalactosyl Diglyceride, from Arabidopsis thaliana. J Biol Chem 276:12832–12838
Straus MR, Rietz S, Loren V, van Themaat E, Bartsch M, Parker JE (2010) Salicylic acid antagonism of EDS1-driven cell death is important for immune and oxidative stress responses in Arabidopsis. Plant J 62:628–640
Strickland JA, Orr GL, Walsh TA (1995) Inhibition of diabrotica larval growth by patatin, the lipid acyl hydrolase from potato tubers. Plant Physiol 109:667–674
Szalontai B, Stranczinger S, Palfalvi G, Mauch-Mani B, Jakab G (2012) The taxon-specific paralogs of grapevine PRLIP genes are highly induced upon powdery mildew infection. J Plant Physiol 17:1767–1775
Tashiro S, Muto A, Tanimoto K, Tsuchiya H, Suzuki H, Hoshino H, Yoshida M, Walter J, Igarashi K (2004a) Repression of PML nuclear body-associated transcription by oxidative stress-activated Bach2. Mol Cell Biol 24:3473–3484
Tashiro S, Sumi T, Uozumi N, Shimizu T, Nakamura T (2004b) B-Myb-dependent regulation of c-Myc expression by cytosolic phospholipase A2. J Biol Chem 279:17715–17722
Testerink C, Munnik T (2011) Molecular, cellular, and physiological responses to phosphatidic acid formation in plants. J Exp Bot 62:2349–2361
Triggiani M, Granata F, Frattini A, Marone G (2006) Activation of human inflammatory cells by secreted phospholipases A2. Biochim Biophys Acta 1761:1289–1300
Viehweger K, Schwartze W, Schumann B, Lein W, Roos W (2006) The Galpha protein controls a pH-dependent signal path to the induction of phytoalexin biosynthesis in Eschscholzia californica. Plant Cell 18:1510–1523
Volokita M, Rosilio-Brami T, Rivkin N, Zik M (2011) Combining comparative sequence and genomic data to ascertain phylogenetic relationships and explore the evolution of the large GDSL-lipase family in land plants. Mol Biol Evol 28:551–565

Vu HS, Tamura P, Galeva NA, Chaturvedi R, Roth MR, Williams TD, Wang X, Shah J, Welti R (2012) Direct infusion mass spectrometry of oxylipin-containing Arabidopsis membrane lipids reveals varied patterns in different stress responses. Plant Physiol 158:324–339

Welti R, Wang X (2004) Lipid species profiling: a high-throughput approach to identify lipid compositional changes and determine the function of genes involved in lipid metabolism and signaling. Curr Opin Plant Biol 7:337–344

Wiermer M, Feys BJ, Parker JE (2005) Plant immunity: the EDS1 regulatory node. Curr Opin Plant Biol 8:383–389

Yang W, Devaiah SP, Pan X, Isaac G, Welti R, Wang X (2007) AtPLAI is an acyl hydrolase involved in basal jasmonic acid production and Arabidopsis resistance to *Botrytis cinerea*. J Biol Chem 282:18116–18128

Zoeller M, Stingl N, Krischke M, Fekete A, Waller F, Berger S, Mueller MJ (2012) Lipid profiling of the Arabidopsis hypersensitive response reveals specific lipid peroxidation and fragmentation processes: biogenesis of pimelic and azelaic acid. Plant Physiol 160:365–378

Zahn M, Wimalasekara R, Göbel C, Feussner I, Holk A. and Scherer G (2005) Expression of Arabidopis phospholipase A genes in Petunia x hybrida. Increased hypersensitive-like response after infection with Botrytis cinerea and Pseudomonas syringae pv. tomato DC3000 demonstrates a function for phospholipase A in pathogen defence. Physiol Mol Plant Pathol 67:2–14

Lipases in Signaling Plant Defense Responses

Jyoti Shah

Abstract Cellular membranes are important reservoirs for signaling lipids and their precursors. Plant lipases have important functions in the release/synthesis of signaling lipids that contribute to plant defense against pests, including effector- and MAMP-triggered immunity and the hypersensitive response. However, some pests have evolved a way to hijack host lipases and use them to counter and/or suppress host defenses. Other pests actively secrete lipases to breakdown host membranes, releasing molecular signals that benefit growth and development of the pest. This chapter focuses on progress made in recent years toward the identification and characterization of lipases that have important roles in the outcome of plant interaction with pests.

Keywords Effector-triggered immunity • Hypersensitive response • Biotrophy • Necrotroph • Phospholipase • Elicitor • Plant–pathogen interaction

1 Introduction

In their natural environment, plants encounter a variety of stressors, including pathogens and insects. Based on their life style, phytopathogens can be broadly classified as biotrophs (e.g., *Hyaloperonospora arabidopsidis* and *Erysiphe orontii*) and necrotrophs (*Erwinia carotovora*, *Botrytis cinerea*, and *Alternaria brassicicola*). Biotrophic pathogens derive nutrients from live host cells. In contrast, necrotrophs release toxins and enzymes that kill the host tissue releasing nutrients required by the pathogen (Glazebrook 2005; Mengiste 2012). Some pathogens exhibit a life cycle that combines biotrophy and necrotrophy. These hemi-biotrophs (e.g., *Pseudomonas syringae* and *Fusarium graminearum*) exhibit a

J. Shah (✉)
Department of Biological Sciences, University of North Texas, Denton, TX 76203, USA
e-mail: shah@unt.edu

X. Wang (ed.), *Phospholipases in Plant Signaling*, Signaling and Communication in Plants 20, DOI 10.1007/978-3-642-42011-5_11,

biotrophic phase during the early stages of infection when the pathogen establishes itself on the host plant, followed by a more aggressive necrotrophic phase, which in case of *F. graminearum* coincides with the spreading of the pathogen through the host. Plants utilize contrasting strategies to counter biotrophic and necrotrophic pathogens. For example, programmed cell death is one mechanism utilized by plants to control biotrophic pathogens. Biotrophs actively attempt to suppress the activation of cell death by the host. In agreement with a role for cell death in controlling infection by biotrophs, plant mutants exhibiting enhanced cell death are in general more resistant to biotrophic pathogens (Mengiste 2012). In contrast, these mutants exhibit heightened susceptibility to necrotrophic pathogens.

Plants have evolved surveillance mechanisms that recognize pathogen-derived ligands, resulting in the activation of defenses (Jones and Dangl 2006; Thomma et al. 2011; Spoel and Dong 2012). Recognition of conserved pathogen-associated molecular patterns (PAMPs) by immune receptors in the plant results in activation of pathogen-triggered immunity (PTI), which confers basal resistance against virulent pathogens, and controls the extent of pathogen growth in this compatible interaction. In comparison, immune receptor-mediated recognition of effectors released by some avirulent races of a pathogen results in the activation of effector-triggered immunity (ETI), which confers more robust resistance to this incompatible interaction than PTI does. In most cases ETI is accompanied by the hypersensitive response (HR), which is characterized by the activation of cell death and accumulation of defense-associated metabolites (Mur et al. 2008). In response to the localized activation of PTI and/or ETI, defenses can also be activated systemically through the plant, a mechanism termed systemic-acquired resistance (SAR). SAR confers resistance against a broad spectrum of pathogens (Durrant and Dong 2004; Spoel and Dong 2012; Shah and Zeier 2013). Salicylic acid (SA) signaling is a major contributor to PTI, ETI, and SAR. PTI and ETI have not been reported for necrotrophic pathogens. The oxylipin jasmonic acid (JA) has an important role in plant defense against necrotrophic pathogens (Glazebrook 2005; Antico et al. 2011). JA and its derivatives also have important signaling functions in plant defense against herbivores and physical wounding (Wasternack 2007). Cross talk between SA and JA signaling fine tunes defense responses in plants (Koornneef and Pieterse 2008).

Lipids are major structural constituents of prokaryotic and eukaryotic membranes that also function as energy stores. In addition, membrane lipids are precursors for signaling molecules that regulate growth, development, and stress response (Laxalt and Munnik 2002; Wang 2004; Ryu 2004; Shah 2005; Gillaspy 2011). Lipids are increasingly being recognized as important factors in plant interaction with other organisms, including pathogens and insects (Laxalt and Munnik 2002; Shah 2005; Kachroo and Kachroo 2009; Grienenberger et al. 2010; Canonne et al. 2011; Gillaspy 2011; Berkey et al. 2012; Dave and Graham 2012; Yan et al. 2013). In many cases of plant interaction with pathogens, the cuticular matrix, which contains cutin (composed mainly of C16 and C18 ω-hydroxylated esterified fatty acids), waxes, fatty acids, and terpenoids, provides the first line of defense against pests (Chassot and Métraux 2005; Shah 2005; Nawrath 2006;

Kachroo and Kachroo 2009). The cuticle is also a source of signaling molecules that can promote resistance against some pathogens and in other cases promote disease. Membrane lipids provide precursors for lipids that have signaling functions in plant response to pests, including phosphatidic acid (PA), *N*-acylethanolamines (NAEs), oxylipins like 12-oxo-phytodienoic acid (OPDA), and JA (Fig. 1). Oxidized membrane lipids (e.g., arabidopsides) (Fig. 1), some of which have antimicrobial activities, can also provide a source of oxylipins like OPDA that can be rapidly released in response to stress (Andersson et al. 2006a; Buseman et al. 2006; Kourtchenko et al. 2007). Other lipids that have important function in plant stress response include polyphosphoinositides, sphingolipids, lysophospholipids, and phytosterols. Plant lipases have key roles in the synthesis/release of many of these signaling lipids. Lipases produced by pests also influence plant–pest interaction (Voigt et al. 2005; Devescovi et al. 2007; Schäfer et al. 2011; Salomon et al. 2012). Here, I summarize advances made in recent years on the involvement of lipases and lipase-like proteins in plant–pest interactions, in particular plant defense against pathogens (Table 1).

2 Lipases in Plant Defense

As described below, lipid hydrolysis has been widely reported in plant–microbe interactions. The availability of genome sequences and molecular-genetic tools has expedited the identification of defense associated lipases, their corresponding genes, and their role in plant defense. Phospholipases A, C, and D, patatin-like acyl hydrolases, as well as other lipid acyl hydrolases have been linked to plant defense against diseases and insects. This section summarizes the involvement of lipases in different aspects of plant defense against pests.

2.1 Role of Lipases in Effector-Triggered Immunity

Specific recognition by a plant immune receptor of a race-specific effector released by a pathogen triggers a cascade of defense signaling that is associated with ETI, thus resulting in strong resistance. PA produced by both phospholipase C and D activities has been shown to participate in ETI-associated defenses.

In tomato (*Solanum lycopersicum*), the *Cf-4* resistance gene is involved in the recognition of Avr4, an elicitor produced by some races of *Cladosporium fulvum*, a biotrophic fungus that causes leaf molding of tomato (Thomas et al. 1997). *Cf-4*-conferred resistance against *C. fulvum* races that express Avr4 is accompanied by a HR and activation of Ca^{2+}-dependent kinases (Romeis et al. 2000). In experiments conducted with tobacco cell cultures that stably expressed *Cf-4*, de Jong et al. (2004) observed that addition of Avr4 rapidly induced PA production that was dependent of the sequential action of a phospholipase C (PLC) and

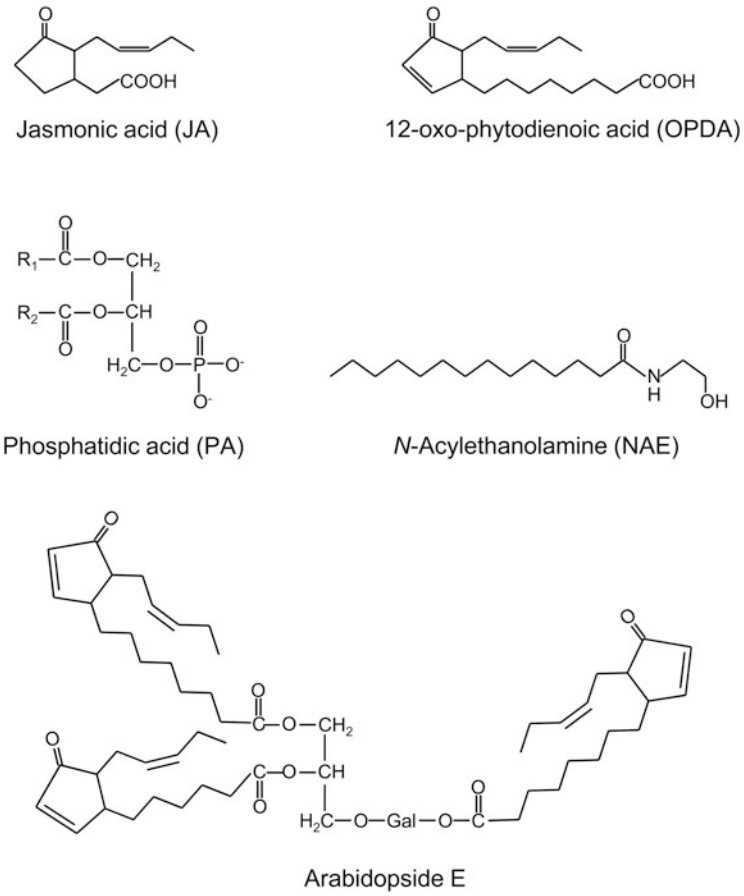

Fig. 1 Biologically active lipids in plant defense. Jasmonic acid, 12-oxo-phytodienoic acid, phosphatidic acid, and *N*-acylethanolamine are signaling molecules in plant defense. Arabidopsides are suggested to have antimicrobial properties and potentially serve as a reservoir for OPDA and dinor-OPDA. *R1 and R2* acyl chains, *Gal* galactose

diacylglycerol kinase (DGK). This accumulation of PA was associated with an increase in the production of reactive oxygen species by an NADPH oxidase complex. More recently, *SlPLC4* and *SlPLC6* were identified as two PLC-encoding genes in tomato that are involved in Cf-4/Avr4-mediated signaling (Vossen et al. 2010). Expression of *SlPLC4* and *SlPLC6* was induced during this incompatible interaction as well as in compatible interactions involving *C. fulvum* and tomato. Virus-induced gene silencing of *SlPLC4* expression in tomato resulted in loss of *Cf-4*-mediated HR and resistance against Avr4 expressing *C. fulvum*. In comparison, silencing of *SlPLC6*, resulted in loss of *Cf-4*-mediated resistance but had no impact on HR development. ETI against *Verticillium dahliae* conferred by the *Ve1* resistance gene and against *Pseudomonas syringae* conferred by the *Pto*/*Prf*

Table 1 *Arabidopsis* lipases involved in plant–pest interaction

Name (gene id)	Description	Biological function	Reference
pPLA-I (At1g61850)	Patatin family lipase	Resistance against *Botrytis cinerea*; JA production (basal)	Yang et al. (2007)
pPLA-IIα (At2g26560)	Patatin family lipase	Plant cell death; hypersensitive response	La Camera et al. (2005, 2009)
$sPLA_2$-α (At2g06925)	Secretory phospholipase	Suppress hypersensitive response	Froidure et al. (2010), Jung et al. (2012)
DAD1 (At2g44810)	Plastidyl PLA1	Wounding-induced JA	Hyun et al. (2008), Ellinger et al. (2010)
PLA-Iα1; DONGLE (At1g05800)	Galactolipase and phospholipase activity	Basal and wounding-induced JA? Resistance to *Alternaria brassicicola*?	Hyun et al. (2008), Ellinger et al. (2010)
PLA1-Iβ2 (At4g16820)	DAD1 family lipase	OPDA, dnOPDA, JA production in response to wounding	Ellinger et al. (2010)
PLA1-Iγ1 (At1g06800)	DAD1 family lipase	OPDA, dnOPDA, JA production in response to wounding; Interaction with *Botrytis cinerea*	Ellinger et al. (2010), Grienenberger et al. (2010)
PLA1-Iγ2 (At2g30550)	DAD1 family lipase	Interaction with *Botrytis cinerea*	Grienenberger et al. (2010)
SOBER1 (At4g22300)	Phospholipase A2	Suppression of plant response to *Xanthomonas campestris* Avr*Bst* effector	Cunnac et al. (2007), Kirik and Mudgett (2009)
GLIP1 (At5g40990)	GDSL lipase 1	Resistance to necrotrophic and hemi-biotrophic pathogens; ethylene signaling	Oh et al. (2005), Kwon et al. (2009)
GLIP2 (At1g53940)	GDSL lipase 2	Negative regulation of auxin signaling; Resistance against *Erwinia carotovora*	Lee et al. (2009)
MPL1 (At5g14180)	Lipid acyl hydrolase	Resistance against green peach aphid	Louis et al. (2010)

gene pair was also compromised in the *SlPLC6*-silenced plants, thus suggesting differential requirement of these two PLCs in ETI in tomato. *SlPLC4* is specifically involved in *Cf-4* mediated resistance, while *SlPLC6* seems to have a more general function in ETI (Vossen et al. 2010). Although pharmacological experiments conducted by de Jong et al. (2004) implicate the potential involvement of PA in *Cf4*-mediated signaling, the simultaneous involvement of polyphosphoinositides cannot be ruled out.

In addition to the PLC pathway, PA can also be synthesized by PLD. In *Arabidopsis* interaction with the bacterial pathogen *Pseudomonas syringae*, both PLC and PLD were shown to be involved in PA production in response to activation of ETI. The *RPM1* and *RPS2* resistance loci in *Arabidopsis* confer ETI against

P. syringae expressing the AvrRpm1 and AvrRpt2 effectors, respectively. Using a pathogen-free system in which these effectors were transiently expressed in *Arabidopsis*, Andersson et al. (2006b) demonstrated a biphasic accumulation of PA. Using selective inhibitors of PLC and PLD activity, they demonstrated that the first burst of PA was dependent on PLC/DGK activity. They suggested that in this pathogen-free system, polyphosphoinositides produced by PLC action likely stimulate Ca^{2+} fluxes that promote DGK activity, thus contributing to the first burst of PA. Pharmacological experiments further suggested that the second and stronger PA peak was linked to PLD activity and that PA functions upstream of reactive oxygen species (ROS) accumulation in this incompatible interaction.

In rice the expression and intracellular distribution of PLD was altered during an incompatible interaction between rice expressing the *Xa10* resistance gene and the bacterial blight pathogen *Xanthomonas oryzae* pv. *oryzae* expressing the cognate AvrXa10 effector (Young et al. 1996). PLD expression was induced during compatible and incompatible interactions involving *X. oryzae*. However, the induction of PLD expression was faster in the incompatible interaction compared to the compatible interaction. Furthermore, in contrast to the uniform distribution of PLD at the plasma membrane during a compatible interaction, the PLD protein was found to cluster on the plasma membrane at the site of pathogen contact. The data suggest that PLD-promoted changes in plasma membrane, at the site of pathogen contact, likely contribute to ETI-mediated resistance against bacterial blight in rice.

2.2 Contribution of Lipases to the Hypersensitive Response

As mentioned above, ETI is accompanied by an HR, which is characterized by localized death of cells at the site of pathogen ingress, accumulation of ROSs and SA, and increased expression of various *PATHOGENESIS-RELATED* (*PR*) genes (Mur et al. 2008). Cell death has also been observed in plant cells treated with various elicitors derived from pathogens, as well as in the pathogen-free distal leaves during the activation of SAR. While cell death is likely beneficial for plants when responding to a biotroph, it could be detrimental against necrotrophs, which thrive on dead cells and in fact secrete factors that promote cell death.

Phospholipases have been documented to have a role in the HR. As mentioned above in case of the incompatible interaction between *Cf-4* expressing tomato and Avr4 expressing *C. fulvum*, *StPLC4* is required for the activation of the HR (Vossen et al. 2010). In contrast, AtsPLA$_2$-α, a secretory phospholipase A2 encoded by the *Arabidopsis* At2g06925 (*AtsPLA$_2$-α*) locus has been suggested to be a negative regulator of HR. In cells inoculated with an AvrRpm1 expressing avirulent strain of *P. syringae* pv. *tomato* DC3000, AtsPLA$_2$-α protein is rapidly translocated to the apoplast (Jung et al. 2012). However, in experiments conducted with a transient expression system in *Nicotiana benthamiana*, it was observed that AtsPLA$_2$-α physically associates with the transcription factor AtMYB30 (Froidure

et al. 2010). In this system, transient overexpression of AtMYB30 promotes HR induced by AvrRpt2-expressing bacteria. However, when AtMYB30 and AtsPLA$_2$-α were coexpressed, the interaction between AtMYB30 and AtsPLA$_2$-α resulted in the translocation of AtsPLA$_2$-α associated with AtMYB30 into the nucleus, where AtsPLA$_2$-α suppressed AtMYB30 promoted HR (Froidure et al. 2010). Consistent with a negative role of AtsPLA$_2$-α in the activation of the HR, AvrRpm1 expressing *P. syringae*, when inoculated at a low dose that does not induce HR in wild-type plants, resulted in HR in the *atsPLA$_2$-α* mutant plant. Furthermore, the mutant line exhibited enhanced resistance to the avirulent pathogen (Froidure et al. 2010).

A negative role in HR has also been reported for another phospholipase encoded by the *Arabidopsis SUPPRESSOR OF AVRBST-ELICITED RESISTANCE1* (*SOBER1*) gene. AvrBsT is an effector from *Xanthomonas campestris* pv. *vesicatoria*, a pathogen of pepper plants. When expressed in *P. syringae* pv. *tomato*, this effector conferred avirulence on the *Arabidopsis* accession Pi-0 resulting in the activation of the HR (Cunnac et al. 2007). In contrast, AvrBsT-expressing strains were virulent on all other *Arabidopsis* accessions. Resistance in Pi-0 was due to a *sober1-1* mutation in a phospholipase A2-encoding gene. Lipid profiling of Pi-0 plants inoculated with AvrBsT-expressing bacteria indicated that loss of SOBER1 function in Pi-0 results in increased accumulation of PA, which required PLD activity (Kirik and Mudgett 2009). In agreement with a role for PLD in this interaction, PLD inhibitors blocked AvrBsT-induced HR in Pi-0. Recombinant SOBER1 protein utilized phosphatidylcholine (PC), but not PA or LsyoPC as a substrate, thus suggesting that SOBER1 has PLA$_2$ activity. Indeed, chemical inhibition of PLA$_2$ in *Arabidopsis* leaves expressing SOBER1 resulted in HR in response to AvrBsT expression. The authors have proposed a model according to which the PLA$_2$ activity of SOBER1 suppresses HR by competing/suppressing PLD-dependent production of PA in response to AvrBsT. How SOBER1's PLA activity inhibits HR is unclear. Furthermore, since SOBER1 does not impact HR associated with ETI elicited by AvrRpt2, which as discussed above is mediated by PA produced by PLC and PLD (Andersson et al. 2006b), SOBER1 is not a general suppressor of HR.

The activation of HR-like cell death in tobacco leaves by the oomycete *Phytophthora megasperma* elicitor β-megaspermin was also accompanied by the induction of PLA$_2$ activity (Dhondt et al. 2002). Similarly, a gene encoding a patatin-like protein was induced in tobacco leaves treated with cryptogein, another elicitor of HR-like cell death (Cacas et al. 2005). Thus, depending on the pathosystem, different lipase activities seem to be associated with the HR.

2.3 Lipases in Basal and Elicitor-Induced Resistance

As opposed to ETI, basal resistance in many cases is conferred by recognition of elicitors that are common to a group of microbes. Examples of these elicitors, also

known as MAMPs, include the bacterial flg22 peptide derived from flagellin, the bacterial flagellar protein, oligosaccharides derived from chitin in fungal cell walls, and lipopolysaccharide derived from gram negative bacteria. Purified elicitors when applied to plant cells induce changes in ion fluxes, ROS production, and defense gene expression. In many cases, elicitors have been shown to stimulate expression/activity of lipases. For example, in rice suspension cells treated with *N*-acetylchitooligosaccharide, a chitin-derived elicitor, a biphasic increase in ROS was observed (Yamaguchi et al. 2003, 2005). The first ROS burst was associated with the activation of PLC and PLD, while the second burst was as a result of PLD and was associated with the recruitment of PLD to the membrane. Similarly, the general elicitors *N*,*N*′,*N*″,*N*‴-tetraacetylchitotetraose, xylanase, and flg22 promoted PA accumulation in tomato suspension cells (van der Luit et al. 2000). Inhibitor studies indicated that DGK activity was required for PA accumulation in response to elicitor treatment, suggesting that PLC is likely involved in providing DAG for PA synthesis. A significant contribution of PLD, in addition to PLC/DGK was also observed in the case of xylanase-induced PA accumulation (van der Luit et al. 2000).

In *Arabidopsis*, expression of several PLD genes was observed to be upregulated in response to infection with *P. syringae* (de Torres Zabela et al. 2002), and in tomato, expression of a *PLDβ1* was upregulated in response to xylanase treatment (Laxalt et al. 2001). However, *PLDβ1* expression was not induced when the tomato cells were elicited with chitotetraose, suggesting that *PLDβ1* involvement is not a general elicitor-induced response. Xylanase-induced H_2O_2 accumulation was enhanced in tomato suspension cells in which expression of *PLDβ1* was silenced (Bargmann et al. 2006). These results suggest that PLD may protect elicitor-treated cells against damage caused by H_2O_2. Similarly, in *Arabidopsis* H_2O_2-induced cell was antagonized by a PLD (Zhang et al. 2003). These results suggest that different PA molecular species produced by different PLDs likely have different effects on ROS and cell death.

The PLC pathway, which yields phosphoinositides, has also been suggested to participate in elicitor-induced responses (Legendre et al. 1993). In soybean suspension cells, the elicitor polygalacturonic acid induced a rapid transient increase of inositol 1,4,5-trisphosphate (IP_3), which was accompanied by a decrease in the IP_3 precursors PIP and PIP_2. This increase of IP_3 preceded the accumulation of H_2O_2. Neomycin sulfate, an inhibitor of IP hydrolysis, blocked the polygalacturonic acid-induced oxidative burst, confirming that IP_3 accumulation promotes the polygalacturonic acid-induced oxidative burst.

The *Arabidopsis ssi2* mutant, which is deficient in a chloroplastic stearoyl-acyl carrier protein desaturase, exhibits a spontaneous cell death phenotype and heightened resistance to biotrophic and hemi-biotrophic pathogens (Shah et al. 2001). In comparison to the wild-type plant, expression of the *MYZUS PERSICE-INDUCED LIPASE1* (*MPL1*) gene was constitutively elevated in the *ssi2* mutant (Morton 2007; S. Sarowar and J. Shah, unpublished results). Analysis of the *ssi2 mpl1* double mutant indicated that *MPL1* was required for the full extent of *ssi2*-conferred resistance to *P. syringae*. In contrast, overexpression of *MPL1* enhanced

resistance against *P. syringae* (S. Sarowar and J. Shah, unpublished). As indicated below, MPL1 is also required for defense against insects. *MPL1* encodes a α/β fold acyl hydrolase that exhibits lipase activity against a variety of phospholipids in vitro (Louis et al. 2010).

2.4 Involvement of Lipases in Plant Interaction with Necrotrophic Pathogens

As mentioned above, necrotrophic pathogens derive nutrients from dead cells and actively engage mechanisms that result in the death of plant cells at the infection site. Since cell death involves membrane lipid turnover, it is anticipated that lipases will be involved in promoting cell death, but are also a part of plant defense to control spread of cell death. Indeed, infection with the necrotrophic pathogens *E. carotovora* and *B. cinerea* induced a patatin-like PLA_2 activity in tobacco leaves (Dhondt et al. 2002). Patatins are lipid acyl hydrolases that are related to the potato tuber storage protein patatin. *Arabidopsis* contains ten genes, which encode patatin-like proteins. The *AtPLAI* (*At1g61850*) (new nomenclature *pPLA-I*)-encoded patatin was found to be required for controlling severity of *B. cinerea* infection (Yang et al. 2007). The *plaI* mutant exhibited increased severity of disease compared to wild type. As mentioned later, *pPLA-I* has been linked to basal JA content and it has been suggested that *pPLA-I*-dependent basal JA is critical for defense against *B. cinerea* (Yang et al. 2007).

Expression of another *Arabidopsis* gene (At2g26560) that encodes a patatin family enzyme PLA-IIA (new nomenclature *pPLA-IIα*) was induced in leaves inoculated with the necrotrophic fungi *Alternaria alternata* and *A. brassicicola* (Narusaka et al. 2003). La Camera et al. (2005) observed that expression of *pPLA-IIα* was also induced in response to infection by *B. cinerea* and an avirulent strain of the hemi-biotroph *P. syringae* (La Camera et al. 2005). However, suppressing expression of *pPLA-IIα* resulted in enhanced resistance against *B. cinerea* and an avirulent strain of *P. syringae*. In contrast, overexpression of *pPLA-IIα* resulted in increased growth of these pathogens, thus suggesting that this gene is involved in conferring susceptibility to *B. cinerea* and avirulent *P. syringae* strains (La Camera et al. 2005). Since JA contributes to resistance against *B. cinerea*, La Camera et al. (2005) suggested that *pPLA-IIα* is unlikely to be involved in pathogen infection-associated jasmonate synthesis. Quite to the contrary, they found that JA and ethylene signaling were required for accumulation of pPLA-IIα protein in pathogen-infected leaves. They further observed that *pPLA-IIα* -potentiated *B. cinerea* inflicted plant cell death and also lowered the efficacy of HR in limiting colonization by avirulent *P. syringae*. Thus, these pathogens have likely evolved to target expression of *pPLA-IIα* to facilitate infection. Fatty acids provided by *pPLA-IIα* likely contribute to this process, either by serving as precursors of products that impact HR/cell death, or alternatively, influence the fungal physiology resulting in

increased aggressiveness. In a subsequent study it was shown that PLA-IIα promotes the accumulation of α-dioxygenase (α-DOX)-derived oxylipins, which likely limit HR spread (La Camera et al. 2009). Thus, PLA-IIα could promote cell death through hydrolysis of cell membranes and simultaneously control the spread of cell death through the action of oxylipins.

GDSL lipases represent a distinct family of esterases/lipases that have a conserved catalytic triad consisting of Ser, Asp, and His. However, unlike most other lipases in which the Ser is contained within a conserved GXSXG motif, the Ser is contained within a $GXSX_4G$ motif in GDSL lipases. The GLIP1 (GDSL Lipase 1; At5g40990) protein was identified as a secretory protein from *Arabidopsis* cell cultures (Oh et al. 2005). GLIP1, which has lipase activity, is required for controlling *A. brassicicola* infection on *Arabidopsis*. The fungus spread more rapidly on the *glip1* mutant than the wild-type plant (Oh et al. 2005). In contrast, overexpression of GLIP1 resulted in enhanced resistance against *A. brassicicola*, *E. carotovora*, and *P. syringae* (Kwon et al. 2009). The recombinant GLIP1 protein had a detrimental effect on germination of fungal spores (Oh et al. 2005). In addition, when locally applied to *Arabidopsis* plants, GLIP1 induced disease resistance throughout the plant (Kwon et al. 2009), thus suggesting that GLIP1 activity promotes a systemic defense promoting signal. Ethylene, but not SA signaling, was required for GLIP1-induced resistance (Kwon et al. 2009). Mutations in the active site of GLIP1 that resulted in loss of lipase activity also resulted in loss of its antifungal activity (Oh et al. 2005), thus indicating that GLIP1's lipolytic activity is likely important for its toxicity toward fungi.

GLIP2 (At1g53940) is another member of the GDSL family of lipases that is required for controlling growth of necrotrophic pathogens. The *GLIP2* gene was expressed at elevated levels in plants treated with defense hormones such as JA, ethylene, and SA. T-DNA insertion knockout of GLIP2 function resulted in enhanced susceptibility to *E. carotovora* in the *glip2* mutant plants (Lee et al. 2009). GLIP2 also possesses antifungal activity. When applied to spores of *A. brassicicola*, GLIP2 inhibited spore germination. The *glip2* mutant showed increased proliferation of lateral roots, which was accompanied by elevated expression of auxin-responsive genes, thus suggesting that auxin signaling is deregulated in *glip2* mutants. Considering that many pathogens synthesize auxins, and that auxin signaling negatively impacts plant defenses (Fu and Wang 2011), Lee et al. (2009) have suggested that GLIP2 promotes disease resistance by inhibiting auxin signaling in plants.

3 Pathogens Target Plant Lipases to Facilitate Colonization

As discussed above, the patatin-like acyl-hydrolase *pPLA-IIα* is targeted by some necrotrophic pathogens to facilitate colonization (La Camera et al. 2005). Pathogens stimulate expression of the *pPLA-IIα* gene in the host plant, leading to

increased colonization (Narusaka et al. 2003; La Camera et al. 2005), which is likely due to the cell death-promoting activity of *pPLA-IIα*. Similarly, the pepper GDSL lipase CaGLIP1 also contributes to host susceptibility to pathogens. *CaGLIP1* was identified as a gene that was upregulated in pepper leaves in response to infection by *Xanthomonas campestris* pv. *vesicatoria*, the bacterial leaf spot pathogen (Hong et al. 2008). *CaGLIP1* expression is induced primarily during compatible interactions with the pathogen. In addition, *CaGLIP1* expression was also induced by other stressors. Resistance against *X. campestris* pv. *vesicatoria* was enhanced when *CaGLIP1* expression was silenced in pepper plants. In contrast, overexpression of *CaGLIP1* in *Arabidopsis* resulted in enhanced susceptibility to *P. syringae* pv. *tomato* and the oomycete *H. arabidopsidis*. Induction of defense genes was less sensitive to SA in the *CaGLIP1*-overexpressing plants. It is likely that a product of CaGLIP1 action on lipids is responsible for reducing plant sensitivity to SA and thus contributing to suppression of defenses.

4 Lipase and Plant–Insect Interaction

Similar to plant interactions with pathogens, lipases from plants and insects influence the colonization of plants by insects. In *Arabidopsis*, the *MPL1* gene, which encodes a lipase, is expressed at elevated levels in response to infestation by the phloem sap-feeding insect *Myzus persicae*, more commonly known as the green peach aphid (GPA) (Louis et al. 2010). The insect population grew larger on the *mpl1* mutant compared to the wild-type plant. In contrast, GPA population was smaller on transgenic plants that overexpress MPL1. Vascular sap-enriched petiole exudates of wild-type plants contain an antibiosis activity that normally controls insect population size on *Arabidopsis*. However, this antibiosis activity was reduced in *mpl1* mutant, and in contrast, higher in the *MPL1*-overexpressing plants (Louis et al. 2010). In agreement with a role for MPL1 in the vascular tissues, MPL1 promoter when fused to the GUS reporter gene was found to express GUS around the insect-feeding site (H. Mondal and J. Shah, unpublished). A signal peptide at the N terminus likely targets MPL1 to the endomembranes. Although the identity of the lipid product responsible for MPL1's impact on GPA is not known, it is possible that a MPL1-dependent lipid metabolite present in the phloem sap is responsible for the antibiosis activity. The phloem sap contains variety of lipids including oxylipins (Benning et al. 2012). Besides the GXSXG motif that is present in many lipases, MPL1 also contains an acyltransferase HX_4D motif, suggesting that it might possibly also be involved in membrane lipid transacylation.

Insects chewing on plants cause extensive physical wounding to the plant. In addition, the plant tissues come in contact with oral secretions from the insect, which could impact plant physiology. In case of *Arabidopsis* leaves damaged by the generalist grasshopper *Schistocerca gregaria* (Caelifera), the levels of oxylipins like 13HPOD, OPDA, and JA increase (Schäfer et al. 2011). This increase in oxylipins is paralleled by an increase in expression of various OPDA-responsive

genes. These responses could be mimicked when oral secretions from the grasshopper were applied to wounded leaves. OPDA accumulation could also be promoted when a greater than 10 kDa fraction derived from grasshopper oral secretions was applied to wounded leaves. This OPDA inducing factor was sensitive to boiling and hot isopropanol suggesting that it is a lipase. Indeed, oral secretions from the insect when mixed with plant lipids released OPDA, presumably from OPDA-esterified lipids (Schäfer et al. 2011). A lipase identified in this 10 kDa fraction was active in promoting OPDA accumulation when applied to wounded leaves. Similarly, oral secretions from other herbivores also promoted OPDA accumulation when applied to wounded leaves, indicating that this is a common phenomenon in plant–herbivore interactions. The OPDA that is released at the site of insect chewing likely elicits plant defenses in the neighboring undamaged cells. Other studies have shown that OPDA is a signaling molecule that has functions independent of its relationship with JA (Stintzi et al. 2001; Taki et al. 2005; Ribot et al. 2008) Thus, these lipases in insect oral secretions function as elicitors of plant defenses.

5 Pathogens Produce Lipases to Promote Colonization

As discussed above, many pathogens produce effectors that target host enzymes associated with lipid metabolism/signaling to promote colonization. This section highlights how some pathogens themselves release lipases that act on host lipids, thereby promoting colonization. Many fungi are known to depend on host lipids to complete their life cycles (Tsitsigiannis and Keller 2007; Christensen and Kolomiets 2011). Others like *F. graminearum*, an important pathogen of wheat and barley, require lipid cues from the host to promote pathogenicity. *F. graminearum* secretes a lipase, FGL1, when growing on the host, but not on synthetic medium (Voigt et al. 2005). On synthetic medium, *FGL1* expression and secretion can be induced by addition of wheat germ oil. *fgl1* mutant fungi could initiate infection on wheat. However, unlike the wild-type fungi, the *fgl1* mutant fungi were unable to spread through the host, suggesting that a FGL1-derived lipid is required for facilitating spread of the fungus through the host plant.

Burkholderia glumae causes seedling blight, seedling rot, and grain rot disease in rice. It is also an opportunistic human pathogen. Quorum sensing is important for successful infection. A secreted lipase that correlated with quorum sensing was identified and shown to be required for pathogenicity (Devescovi et al. 2007). The *lipA* mutant fungi that lack this lipase had lost the ability to cause disease on rice. Similarly, the biotrophic fungus *Blumeria graminis*, which causes powdery mildew disease in small grain cereals, secretes a lipase, Lip1, which is expressed at elevated levels during early stages of the infection (Feng et al. 2005, 2009). Lip1 action on epicuticular waxes likely releases metabolites that promote appressorium formation, thus facilitating infection. Secreted lipases have also been shown to be important for infection by *B. cinerea* and *A. brassicicola* (Berto et al. 1997, 1999;

Comménil et al. 1998, 1999). Disease development was reduced when fungal conidia were incubated in the presence of lipase-specific antibodies prior to inoculation on host, thus confirming an important function of fungal lipases in facilitating infection (Comménil et al. 1998; Berto et al. 1999).

6 Lipid-Derived Defense Signaling Molecules: Role of Lipases

PA is a phospholipid that also doubles up as a signaling molecule via its ability to regulate activity of various proteins. The involvement in plant defense of PA, PLD, and PLC that contribute to PA production has been discussed above. Highlighted in this section is the involvement of lipases in the metabolism of other lipid-derived molecules that have important signaling functions in plant defense.

6.1 Role of Lipases in Jasmonate Metabolism Associated with Plant Defense

Jasmonates include a family oxylipins that are derived from oxygenation of 18- and 16-carbon polyunsaturated fatty acids (Wasternack and Kombrink 2010; Acosta and Farmer 2010). JA (Fig. 1) and its derivatives methyljasmonate (MeJA) and jasmonoyl-isoleucine (JA-Ile) are among the better studied jasmonates that are associated with signaling in plant defense against foliar and root pathogens and insects (Acosta and Farmer 2010). In addition, they also influence plant interaction with detritivores and beneficial microbes. Increasing evidence suggests that OPDA (Fig. 1), which is a precursor for JA and its derivatives, also has important functions in plant stress response that are independent of JA (Dave and Graham 2012).

The first step in the biosynthesis of jasmonates, which occurs in the plastids, is the oxidation of polyunsaturated fatty acids (e.g., α-linolenic acid) by 13-lipoxygenases to yield 13-hydroperoxy fatty acids that are sequentially acted upon by allene oxide synthase and allene oxide cyclase to yield OPDA (Feussner and Wasternack 2002). Similarly, dinor-OPDA (dnOPDA) is synthesized from the 16-carbon hexadecatrienoic acid. OPDA and dnOPDA are then exported from plastids to the peroxisomes, where they are further processed to yield JA. JA can be further converted into MeJA and JA-Ile in the cytosol (Feussner and Wasternack 2002; Acosta and Farmer 2010). The identification of arabidopsides (Fig. 1), which can accumulate to high levels in wounded and pathogen-infected tissues, has led to the suggestion that the early steps of oxylipin synthesis could potentially occur on esterified fatty acids (Buseman et al. 2006; Kourtchenko et al. 2007). Irrespective of whether free or esterified fatty acids are the source of OPDA/dnOPDA, a lipase is

required to release the free fatty acid from glycerolipids or OPDA/dnOPDA from arabidopsides for the synthesis of JA and its derivatives.

6.1.1 Contribution of DAD-Like Lipid Acyl Hydrolases to Jasmonate Metabolism

In *Arabidopsis thaliana* flowers, DAD1 (DEFECTIVE IN ANTHER DEHISCENCE1 encoded by *At2g44810*), a phospholipase A_1 (PLA-Iβ1), is required for JA accumulation. Compared to the wild-type (WT) plants, the *Arabidopsis dad1* mutant had reduced levels of JA and was male sterile due to defects in pollen development and anther dehiscence (Ishiguro et al. 2001). Expression of *DAD1* and *DONGLE* (*DGL*; At1g05800), which encodes a DAD1-related PLA-Iα1 protein with strong galactolipase compared to phospholipase activity, was induced in response to wounding (Hyun et al. 2008; Ellinger et al. 2010). While *DGL* expression was transiently induced, peaking at 1 h after wounding, *DAD1* expression was more sustained and remained at elevated levels through 4 h after wounding (Hyun et al. 2008). Hyun et al. (2008) provided a combination of genetic, biochemical, and molecular evidence that DGL partakes in basal- and wounding-induced JA accumulation in *Arabidopsis* leaves. RNAi silencing of *DGL* resulted in lower (undetectable) basal levels of JA in leaves of the *dgl-i* plants. Furthermore, the increase in JA content that was observed 1 h after wounding leaves of WT plants was significantly lower in the *dgl-i* plants. However, at 4 h post-wounding JA levels were comparably high between the *dgl-i* and WT plants. Hyun et al. (2008) have suggested that DGL-dependent basal JA promotes the rapid accumulation of JA during the early phases of wounding. In comparison to the WT plant, the wounding-induced accumulation of JA was not observed in the *dgl-i dad1* plant suggesting that the combined activities of DGL and DAD1 function are required for wounding-induced JA accumulation in *Arabidopsis* leaves. Overexpression of DGL in the activation-tagged *dgl-D* plant resulted in elevated JA accumulation and expression of JA-responsive genes, which was accompanied by enhanced resistance to *A. brassicicola* (Hyun et al. 2008). However, whether DGL is required for JA accumulation in response to fungal infection was not reported.

Ellinger et al. (2010) noted that JA levels were not as dramatically lower in wounded *dgl-i* line as reported by Hyun et al. (2008). Furthermore, the wounding-induced levels of dnOPDA, which also contributes to JA synthesis and is synthesized from hexadecatrienoic acid released from the *sn-2* position of galactolipids, were comparably high in the WT and the *dgl-i* and *dad1* plants, suggesting that these genes by themselves do not contribute to the full extent of JA in wounded plants. Ellinger et al. (2010) studied the involvement in wounding of several genes predicted to encode chloroplast-localized lipases. While mutations in most of these genes did not impact jasmonate composition, compared to the WT plant, wounding-induced OPDA and dnOPDA levels were 75–80 % lower and JA levels 50 % lower in the *pla1-Iγ1* mutant that contains a T-DNA insertion in At1g06800. Similarly, wounding induced OPDA, JA, and dnOPDA content were approximately 40–50 %

lower in the *pla1-Iβ2* mutant at the At4g16820 locus. However, accumulation of these oxylipins was not completely abolished in any of the single mutant lines, suggesting that multiple lipases contribute to the overall content of jasmonates in wounded *Arabidopsis* leaves. The lowered content of 18:3-derived OPDA and 16:3-derived dnOPDA in the *pla1-Iγ1* and *pla1-Iβ2* knockout lines indicates that the corresponding enzymes likely have activity toward both the *sn-1* and *sn-2* acyl chains in glycerolipids. Expression of *PLA1-Iγ1* and *PLA1-Iγ2* (At2g30550) were induced in *Arabidopsis* challenged with *B. cinerea* and *P. syringae* (Grienenberger et al. 2010). However, according to these authors (data was not shown) individual knockdown of either of these genes did not adversely impact JA profile or resistance to these pathogens.

6.1.2 Contribution of Patatin-Like Lipases to Jasmonate Metabolism

Infection with the necrotrophic fungus *B. cinerea* resulted in a biphasic increase in JA content in *Arabidopsis*, which was accompanied by a reduction in phosphatidylglycerol and digalactosyldiacylglycerol levels, suggesting that plastidic polar lipids likely provide precursors for JA accumulation in response to infection by this necrotrophic pathogen (Yang et al. 2007). As mentioned above, the patatin family enzyme pPLA-I encoded by the *Arabidopsis* At1g61850 loci is required for controlling the severity of *B. cinerea* infection (Yang et al. 2007). The enhanced disease severity of the *plaI* mutant plants was paralleled by a reduction in the extent of induction of genes encoding lipoxygenase (*LOX2* and *LOX3*) and 12-oxo-phytodienoate reductase (*OPR3*) which are involved in jasmonate metabolism. Basal JA content was lower in the *plaI* mutant and resistance to *B. cinerea* was restored by MeJA application. However, fungal infection-induced JA accumulation was not compromised in this mutant (Yang et al. 2007). These results suggest that *pPLA-I*-dependent basal JA is critical for defense against *B. cinerea*. However, how basal JA impacts resistance against *B. cinerea* is unclear. Expression of a patatin-like PLA_2 encoding gene was also observed to be upregulated in tobacco leaves inoculated with β-megaspermin a cell death elicitor from *Phytophthora* (Dhondt et al. 2002). This increase in PLA_2 expression correlated with the accumulation of OPDA and JA. However, whether this PLA_2 is indeed required for jasmonate accumulation was not determined in the above study.

6.1.3 PLDα1 Modulates Stress-Associated Jasmonate Accumulation

PLDα1 has also been suggested to have a role in promoting JA biosynthesis in wounded plants (Wang et al. 2000). Compared to the WT plant, levels of JA, PA, and JA-responsive genes were lower in wounded leaves of a transgenic line in which PLDα1 expression was silenced. However, expression of *LOX2*, a 13-lipoxygenase-encoding gene that is involved in JA synthesis was also lower in the PLDα1-silenced line, suggesting that the JA defect in the silenced line is likely

due to lowered *LOX2* expression. Hyun et al. (2008) have further suggested that PA produced by PLDα1 also likely acts as a signaling molecule that promotes wounding-induced upregulation of *DGL* and *DAD1* expression. They observed that the application of 1-butanol, an inhibitor of PLD activity, significantly reduced the wounding-induced expression of *DGL* and *DAD1*. Furthermore, wounding-induced expression of *DGL* and *DAD1* were also attenuated in the *pld1α* mutant plant. By contrast, wounded *35S:PLDα1* plants, in which *PLDα1* is overexpressed from the *Cauliflower mosaic virus 35S* gene promoter, expressed higher levels of *DGL*, *DAD1*, and the JA-responsive *VSP* gene, than did the wounded WT plants. In addition, *35S:PLDα1*-promoted hyperactivation of *VSP* in response to wounding was attenuated in the absence of functional *DGL* and *DAD1* activity in the *35S:PLDα1 dgl-i dad1* plants, thus further indicating that *PLD1α*-promoted wounding responses likely function through *DGL* and *DAD1*.

6.2 *Polyphosphoinositides and* N-*Acylethanolamines*

Polyphosphoinositide and *N*-acylethanolamines (NAEs) (Fig. 1) are phospholipid-derived signaling molecules that influence plant defense against pathogens. Accumulation of NAEs, which are fatty acid ethanolamides, accompanied the activation of defense responses by the fungal elicitor xylanase (Tripathy et al. 1999). Furthermore, expression of defense genes that are induced by xylanase, was also induced by NAE application. Signaling mediated by abscisic acid, which is an important regulator of plant defense against abiotic and biotic stress, was also impacted by NAE metabolism (Teaster et al. 2007). Polyphosphoinositides are potent signaling molecules that have been extensively studied for their impact on Ca^{2+} fluxes. IPs also have important roles in plant–pathogen interaction. Expression of genes involved in IP metabolism are induced by pathogen infection, and microbial elicitors stimulate accumulation of IPs (Vasconsuelo et al. 2005; Vasconsuelo and Boland 2007; Gillaspy 2011). Phytoalexin accumulation in response to elicitor treatment was also associated with changes in levels of IPs (Zhao et al. 2004). IP metabolism is also important for SA signaling (Krinke et al. 2007) and for wounding-induced JA accumulation (Mosblech et al. 2008). Recently, IPs were shown to be involved in JA perception by COI1 (Mosblech et al. 2011). IP metabolism also modulates β-aminobutyric acid (BABA)-induced defense responses (Ton et al. 2005). Lipases are required for the synthesis of IPs and NAEs, which are derived from phosphatidylinositol and phosphatidylethanolamine, respectively. PLC function is involved in the synthesis of IPs, while NAE synthesis requires PLD action (Pappan et al. 1998; Kilaru et al. 2007; Gillaspy 2011).

7 Conclusion

Significant strides have been made during the last decade in identifying lipases that generate signaling lipids and their role in plant defense. However, the products of many lipases involved in defense remain to be determined. Furthermore, how the expression/activity of these genes is regulated in response to pathogen infection is largely unclear. Likewise, little is known about the downstream targets of these lipid signals and how they integrate into the defense-signaling grid. Finally, how pests utilize lipases to target host lipid metabolism to facilitate colonization is also poorly understood. The future challenge will be to exploit the recent improvements in lipidomics and genomics to address these and other questions concerning the involvement of lipids and lipid-metabolizing enzymes in plant defense and susceptibility to pests.

Acknowledgments This work was supported by grants to Jyoti Shah from the National Science Foundation (IOS-1121570 and MCB-0920600) and the U.S. Department of Agriculture as a cooperative project with the U.S. Wheat & Barley Scab Initiative (Agreement No. 59-0790-8-060)

References

Acosta IF, Farmer EE (2010) Jasmonates. *Arabidopsis* Book 8:e0129. doi:10.1199/tab.0129

Andersson MX, Hamberg M, Kortchenko O, Brunnström A, McPhail KL, Gerwick WH, Göbel C, Feussner I, Ellerström M (2006a) Oxylipin profiling of the hypersensitive response in *Arabidopsis thaliana*. Formation of a novel oxo-phytodienoic acid-containing galactolipid arabidopside E. J Biol Chem 281:31528–31537

Andersson MX, Kortchenko O, Dangl JL, Mackey D, Ellestgöm M (2006b) Phospholipase-dependent signaling during the AvrRpm1- and AvrRpt2-induced disease resistance responses in *Arabidopsis thaliana*. Plant J 281:31528–31537

Antico CJ, Colon C, Banks T, Ramonell KM (2011) Insights into the role of jasmonic acid-mediated defense against necrotrophic and biotrophic fungal pathogens. Front Biol 7:48–56

Bargmann BOR, Laxalt AM, ter Riet B, Schouten E, van Leeuwen W, Dekker HL, de Koster CG, Haring MA, Munnik T (2006) LePLDβ1 activation and localization in suspension-cultured tomato cells treated with xylanase. Plant J 45:358–368

Benning UF, Tamot B, Guelette BS, Hoffmann-Benning S (2012) New aspects of phloem-mediated long-distance lipid signaling in plants. Front Plant Sci 3:53

Berkey R, Bendigeri D, Xiao S (2012) Sphingolipids and plant defense/disease: the "death" connection and beyond. Front Plant Sci 3:68

Berto P, Belingheri L, Dehorter B (1997) Production and purification of a novel extracellular lipase from *Alternaria brassicicola*. Biotechnol Lett 19:533–536

Berto P, Comménil P, Belingheri L, Dehorter B (1999) Occurrence of a lipase in spores of *Alternaria brassicicola* with a crucial role in the infection of cauliflower leaves. FEMS Microbiol Lett 180:183–189

Buseman CM, Tamura P, Sparks AA, Baughman EJ, Maatta S, Zhao J, Roth MR, Esch SW, Shah J, Williams TD, Welti R (2006) Wounding stimulates the accumulation of glycerolipids containing oxophytodienoic acid and dinor-oxophytodienoic acid in *Arabidopsis* leaves. Plant Physiol 142:28–39

Cacas JL, Vailleau F, Davoine C, Ennar N, Agnel JP, Tronchet M, Ponchet M, Blein J-P, Roby D, Triantaphylides C, Montillet J-L (2005) The combined action of 9 lipoxygenase and galactolipase is sufficient to bring about programmed cell death during tobacco hypersensitive response. Plant Cell Environ 28:1367–1378

Canonne J, Froidure-Nicolas S, Rivas S (2011) Phospholipases in action during plant defense signaling. Plant Signal Behav 6:13–18

Chassot C, Métraux J-P (2005) The cuticle as source of signals for plant defense. Plant Biosyst 139:28–31

Christensen SA, Kolomiets MV (2011) The lipid language of plant-fungal interactions. Fungal Genet Biol 48:4–14

Comménil P, Belingheri L, Dehorter B (1998) Antilipase antibodies prevent infection of tomato leaves by *Botrytis cinerea*. Physiol Mol Plant Pathol 52:1–14

Comménil P, Belingheri L, Bauw G, Dehorter B (1999) Molecular characterization of a lipase induced in *Botrytis cinerea* by components of grape berry cuticle. Physiol Mol Plant Pathol 55:37–43

Cunnac S, Wilson A, Nuwer J, Kirik A, Baranage G, Mudgett MR (2007) A conserved carboxylesterase is a SUPPRESSOR OF AVRBST-ELICITED RESISTANCE in *Arabidopsis*. Plant Cell 19:688–705

Dave A, Graham IA (2012) Oxylipin signaling: a distinct role for the jasmonic acid precursor *cis*-(+)-12-oxo-phytodienoic acid (*cis*-OPDA). Front Plant Sci 3:42

de Jong C, Laxalt A, Bargmann B, De Wit P, Joosten M, Munnik T (2004) Phosphatidic acid accumulation is an early response in the Cf-4/Avr4 interaction. Plant J 39:1–12

de Torres Zabela M, Fernandez-Delmond I, Niittyla T, Sanchez P, Grant M (2002) Differential expression of genes encoding *Arabidopsis* phospholipases after challenge with virulent or avirulent *Pseudomonas* isolates. Mol Plant Microbe Interact 15:808–816

Devescovi G, Bigirimana J, Degrassi G, Cabrio L, LiPuma JJ, Kim J, Hwang I, Venturi V (2007) Involvement of a quorum-sensing-regulated lipase secreted by a clinical isolate of *Burkholderia glumae* in severe disease symptoms in rice. Appl Environ Microbiol 73:4950–4958

Dhondt S, Gouzerh G, Muller A, Legrand M, Heitz T (2002) Spatio-temporal expression of patatin-like lipid acyl hydrolases and accumulation of jasmonates in elicitor treated tobacco leaves are not affected by endogenous levels of salicylic acid. Plant J 32:749–762

Durrant WE, Dong X (2004) Systemic acquired resistance. Annu Rev Phytopathol 42:185–209

Ellinger D, Stingl N, Kubigsteltig II, Bals T, Juenger M, Pollmann S, Berger S, Scheunemann D, Mueller MJ (2010) DONGLE and DEFECTIVE IN ANTHER DEHISCENCE1 lipases are not essential for wound- and pathogen-induced jasmonate biosynthesis: redundant lipases contribute to jasmonate formation. Plant Physiol 153:114–127

Feng J, Liu G, Selvaraj G, Hughes GR, Wei Y (2005) A secreted lipase encoded by *LIP1* is necessary for efficient use of saturated triglyceride lipids in *Fusarium graminearum*. Microbiology 151:3911–3921

Feng J, Wang F, Liu G, Greenshields D, Shen W, Kaminskyj S, Hughes GR, Peng Y, Selvaraj G, Zou J, Wei Y (2009) Analysis of a *Blumeria graminis*-secreted lipase reveals the importance of host epicuticular wax components for fungal adhesion and development. Mol Plant Microbe Interact 22:1601–1610

Feussner I, Wasternack C (2002) The lipoxygenase pathway. Annu Rev Plant Biol 53:275–297

Froidure S, Canonne J, Daniel X, Jauneau A, Briere C, Roby D, Rivas S (2010) AtsPLA2-α nuclear relocalization by the *Arabidopsis* transcription factor AtMYB30 leads to repression of the plant defense response. Proc Natl Acad Sci U S A 107:15281–15286

Fu J, Wang S (2011) Insights into auxin signaling in plant-pathogen interactions. Front Plant Sci 2:74

Gillaspy G (2011) The cellular language of *myo*-inositol signaling. New Phytol 192:823–839

Glazebrook J (2005) Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annu Rev Phytopathol 43:205–227

Grienenberger E, Geoffroy P, Mutterer J, Legrand M, Heitz T (2010) The interplay of lipid acyl hydrolases in inducible plant defense. Plant Signal Behav 5:1181–1186

Hong JK, Choi HW, Hwang IS, Kim DS, Kim NH, Choi DS, Kim YJ, Hwang BK (2008) Function of a novel GDSL-type pepper lipase gene, CaGLIP1, in disease susceptibility and abiotic stress tolerance. Planta 227:539–558

Hyun Y, Choi S, Hwang HJ, Yu J, Nam SJ, Ko J, Park JY, Seo YS, Kim EY, Ryu SB, Kim WT, Lee Y-H, Kang H, Lee I (2008) Cooperation and functional diversification of two closely related galactolipase genes for jasmonic acid (JA) biosynthesis. Dev Cell 14:183–192

Ishiguro S, Kawai-Oda A, Ueda J, Nishida I, Okada K (2001) The *DEFECTIVE IN ANTHER DEHISCIENCE* gene encodes a novel phospholipase A1 catalyzing the initial step of jasmonic acid biosynthesis, which synchronizes pollen maturation, anther dehiscence and flower opening in *Arabidopsis*. Plant Cell 13:2191–2209

Jones JDG, Dangl JL (2006) The plant immune system. Nature 444:323–329

Jung J, Kumar K, Lee HY, Park Y-II, Cho H-T, Ryu SB (2012) Translocation of phospholipase A2a to apoplasts is modulated by developmental stages and bacterial infection in *Arabidopsis*. Front Plant Sci 3:126

Kachroo A, Kachroo P (2009) Fatty acid-derived signals in plant defense. Annu Rev Phytopathol 27:153–176

Kilaru A, Blancaflor EB, Venables BJ, Tripathy S, Mysore KS, Chapman KD (2007) The N-acylethanolamine-mediated regulatory pathway in plants. Chem Biodivers 4,:1933–1955

Kirik A, Mudgett MR (2009) SOBER1 phospholipase activity suppresses phosphatidic acid accumulation and plant immunity in response to bacterial effector AvrBsT. Proc Natl Acad Sci U S A 106:20532–20537

Koornneef A, Pieterse CMJ (2008) Cross talk in defense signaling. Plant Physiol 146:839–844

Kourtchenko O, Andersson MX, Hamberg M, Brunnström A, Göbel C, McPhail KL, Gerwick WH, Feussner I, Ellerström M (2007) Oxophytodienoic acid-containing galactolipids in *Arabidopsis*: jasmonate signaling dependence. Plant Physiol 145:1658–1669

Krinke O, Ruelland E, Valentová O, Vergnolle C, Renou JP, Taconnat L, Flemr M, Burketová L, Zachowski A (2007) Phosphatidylinositol 4-kinase activation is an early response to salicylic acid in *Arabidopsis* suspension cells. Plant Physiol 144:1347–1359

Kwon SJ, Jin HC, Lee S, Nam MH, Chung JH, Kwon SII, Ryu C-M (2009) GDSL lipase-like 1 regulates systemic resistance associated with ethylene signaling in *Arabidopsis*. Plant J 58:235–245

La Camera S, Geoffroy P, Samaha H, Ndiaye A, Rahim G, Legrand M, Heitz T (2005) A pathogen-inducible patatin-like lipid acyl hydrolase facilitates fungal and bacterial host colonization in *Arabidopsis*. Plant J 44:810–825

La Camera S, Balague C, Gobel C, Geoffroy P, Legrand M, Feussner I, Roby D, Heitz T (2009) The *Arabidopsis* patatin-like protein 2 (PLP2) plays an essential role in cell death execution and differentially affects biosynthesis of oxylipins and resistance to pathogens. Mol Plant Microbe Interact 22:469–481

Laxalt A, Munnik T (2002) Phospholipid signaling in plant defence. Curr Opin Plant Biol 5:332–338

Laxalt A, Ter Riet B, Verdonk JC, Parigi L, Tameling WIL, Vossen J, Haring M, Musgrave A, Munnik T (2001) Characterization of five tomato phospholipase D cDNAs: rapid and specific expression of *LePLDβ*1 on elicitation with xylanase. Plant J 26:237–247

Lee DS, Kim BK, Kwon SJ, Jin HC, Park OK (2009) *Arabidopsis* GDSL lipase 2 plays a role in pathogen defense via negative regulation of auxin signaling. Biochem Biophy Res Comm 379:1036–1042

Legendre L, Yueh YG, Crain R, Haddock N, Heinstein PF, Low PS (1993) Phospholipase C activation during elicitation of the oxidative burst in cultured plant cells. J Biol Chem 268:24559–24563

Louis J, Kukula K-L, Singh V, Reese JC, Jander G, Shah J (2010) Antibiosis against the green peach aphid requires the *Arabidopsis thaliana MYZUS PERSICAE-INDUCED LIPASE1* gene. Plant J 64:800–811

Mengiste T (2012) Plant immunity to necrotrophs. Annu Rev Phytopathol 50:267–294

Morton J (2007) Characterization of a lipase in *Arabidopsis* defense. MS thesis, Kansas State University. http://hdl.handle.net/2097/392

Mosblech A, König S, Stenzel I, Grezeganek P, Feussner I, Heilmann I (2008) Phosphoinositide and inositolpolyphosphate signalling in defense responses of *Arabidopsis thaliana* challenged by mechanical wounding. Mol Plant 1:249–261

Mosblech A, Thurow C, Gatz C, Feussner I, Heilmann I (2011) Jasmonic acid perception by COI1 involves inositol polyphosphates in *Arabidopsis thaliana*. Plant J 65:949–957

Mur LAJ, Kenton P, Lloyd AJ, Ougham H, Prats E (2008) The hypersensitive response; the centenary is upon us but how much do we know? J Exp Bot 59:501–520

Narusaka Y, Narusaka M, Seki M, Fujita M, Ishida J, Nakashima M, Enju A, Sakuai T, Satou M, Kamiya A, Park P, Kobayashi M, Shinozaki K (2003) Expression profiles of *Arabidopsis Phospholipase A IIA* gene in response to biotic and abiotic stresses. Plant Cell Physiol 44:1246–1252

Nawrath C (2006) Unraveling the complex network of cuticular structure and function. Curr Opin Plant Biol 9:281–287

Oh IS, Park AR, Bae MS, Kwon SJ, Kim YS, Lee JE, Kang NY, Lee S, Cheong H, Park OK (2005) Secretome analysis reveals an *Arabidopsis* lipase involved in defense against *Alternaria brassicicola*. Plant Cell 17:2832–2847

Pappan K, Austin-Brown S, Chapman KD, Wang X (1998) Substrate selectivities and lipid modulation of plant phospholipase Dα, β and γ. Arch Biochem Biophys 353:131–140

Ribot C, Zimmerli C, Farmer EE, Reymond P, Poirier Y (2008) Induction of the *Arabidopsis PHO1;H10* gene by 12-oxo-phytodienoic acid but not jasmonic acid via a CORONATINE INSENSITIVE1-dependent pathway. Plant Physiol 147:696–706

Romeis T, Piedras P, Jones JDG (2000) Resistance gene-dependent activation of a calcium-dependent protein kinase in the plant defense response. Plant Cell 12:803–815

Ryu SB (2004) Phospholipid-derived signaling mediated by phospholipase A in plants. Trends Plant Sci 9:229–235

Salomon S, Gácser A, Frerichmann S, Kröger C, Scäfer W, Voigt CA (2012) The secreted lipase FGL1 is sufficient to restore the initial infection step to the apathogenic *Fusarium graminearum* MAP kinase disruption mutant *Δgpmk1*. Eur J Plant Pathol 134:23–37

Schäfer M, Fischer C, Meldau S, Seebald E, Oelmüller R, Baldwin IT (2011) Lipase activity in insect oral secretions mediates defense responses in *Arabidopsis*. Plant Physiol 156:1520–1534

Shah J (2005) Lipids, lipases and lipid-modifying enzymes in plant disease resistance. Annu Rev Phytopathol 43:229–260

Shah J, Zeier J (2013) Long-distance communication and signal amplification in systemic acquired resistance. Front Plant Sci 4:30

Shah J, Kachroo PK, Nandi A, Klessig DF (2001) A recessive mutation in the *Arabidopsis SSI2* gene confers SA- and NPR1-independent expression of *PR* genes and resistance against bacterial and oomycete pathogens. Plant J 25:563–574

Spoel SH, Dong X (2012) How do plants achieve immunity? Defence without specialized immune cells. Nat Rev Immunol 12:89–100

Stintzi A, Weber H, Reymond P, Browse J, Farmer EE (2001) Plant defense in the absence of jasmonic acid: the role of cyclopentenones. Proc Natl Acad Sci U S A 98:12837–12842

Taki N, Sasaki-Sekimoto Y, Obayashi T, Kikuta A, Kobayashi K, Ainai T, Yagi K, Sakurai N, Suzuki H, Masuda T, Takamiya K, Shibata D, Kobayashi Y, Ohta H (2005) 12-Oxo-phytodienoic acid triggers expression of a distinct set of genes and plays a role in wound-induced gene expression in *Arabidopsis*. Plant Physiol 139:1268–1283

Teaster ND, Motes CM, Tang Y, Wiant WC, Cotter MQ, Wang Y-S, Kilaru A, Venables BJ, Hasenstein KH, Gonzalez G, Blancaflor EB, Chapman KD (2007) *N*-acylethanolamine

metabolism interacts with abscisic acid signaling in *Arabidopsis thaliana* seedlings. Plant Cell 19:2454–2469

Thomas CM, Jones DA, Parniske M, Harrison K, Balint-Kurti PJ, Hatzixanthis K, Jones JDG (1997) Characterization of the tomato *Cf-4* gene for resistance to *Cladosporium fulvum* identifies sequences that determine recognitional specificity in *Cf-4* and *Cf-9*. Plant Cell 9:2209–2224

Thomma BPHJ, Nürnberg T, Jossten MHAJ (2011) On PAMPs and effectors: the blurred PTI-ETI dichotomy. Plant Cell 23:4–15

Ton J, Jakab G, Toquin V, Flors V, Iavicoli A, Maeder MN, Metraux JP, Mauch-Mani B (2005) Dissecting the beta-aminobutyric acid-induced priming phenomenon in *Arabidopsis*. Plant Cell 17:987–999

Tripathy S, Venables BJ, Chapman KD (1999) *N*-acylethanolamines in signal transduction of elicitor perception. Attenuation of alkalinization response and activation of defense gene expression. Plant Physiol 121:1299–1308

Tsitsigiannis DI, Keller NP (2007) Oxylipins as developmental and host-fungal communication signals. Trends Microbiol 15:109–118

van der Luit AH, Piatti T, Van Doom A, Musgrave A, Felix G, Boller T, Munnik T (2000) Elicitation of suspension-cultured tomato cells triggers the formation of phosphatidic acid and diacylglycerol pyrophosphate. Plant Physiol 123:1507–1515

Vasconsuelo A, Boland R (2007) Molecular aspects of the early stages of elicitation of secondary metabolites in plants. Plant Sci 172:861–875

Vasconsuelo AA, Morelli S, Picotto G, Giuletti AM, Boland R (2005) Intracellular calcium mobilization: a key step for chitosan-induced anthraquinone production in *Rubia tinctorum* L. Plant Sci 169:712–720

Voigt CA, Schafer W, Salomon S (2005) A secreted lipase of *Fusarium graminearum* is a virulence factor required for infection of cereals. Plant J 42:364–375

Vossen JH, Abd-El-Haliem A, Fradin EF, van den Berg GC, Ekengren SK, Meijer HJ, Seifi A, Bai Y, ten Have A, Munnik T, Thomma BP, Joosten MH (2010) Identification of tomato phosphatidylinositol-specific phospholipase-C (PI-PLC) family members and the role of PLC4 and PLC6 in HR and disease resistance. Plant J 62:224–239

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

Wang C, Zien CA, Afitlhile M, Welti R, Hildebrand DF, Wang X (2000) Involvement of phospholipase D in wound-induced accumulation of jasmonic acid in *Arabidopsis*. Plant Cell 12:2237–2246

Wasternack C (2007) Jasmonates: an update on biosynthesis, signal transduction and action in plant stress response, growth and development. Ann Bot 100:681–697

Wasternack C, Kombrink E (2010) Jasmonates: structural requirements for lipid-derived signals active in plant stress responses and development. ACS Chem Biol. doi:10.1021/cb900269u

Yamaguchi T, Minami E, Shibuya N (2003) Activation of phospholipases by *N*-acetlychitooligosaccharide elicitor in suspension-cultured rice cells mediates reactive oxygen generation. Physiol Plant 118:361–370

Yamaguchi T, Minami E, Ueki J, Shibuya N (2005) Elicitor-induced activation of phospholipases plays an important role for the induction of defense responses in suspension-cultured rice cells. Plant Cell Physiol 46:579–587

Yan Y, Borrego E, Kolomiets MV (2013) Jasmonate biosynthesis, perception and function in plant development and stress response. In: Baez RV (ed) Lipid metabolism. InTech, Rijeka, pp 393–442, Chapter 16, http://dx.doi.org/10.5772/52675

Yang W, Devaiah SP, Pan X, Isaac G, Welti R, Wang X (2007) AtPLAI is an acyl hydrolase involved in basal Jasmonic acid production and *Arabidopsis* resistance to *Botrytis cinerea*. J Biol Chem 282:18116–18128

Young SA, Wang X, Leach JE (1996) Changes in the plasma membrane distribution of rice phospholipase D during resistant interactions with *Xanthomonas oryzae* pv *oryzae*. Plant Cell 8:1079–1090

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, Guo Y, Kosaihira A, Sakai K (2004) Rapid accumulation and metabolism of polyphosphoinositol and its possible role in phytoalexin biosynthesis in yeast elicitor-treated *Cupressus lusitanica* cell cultures. Planta 219:121–131