Phospholipase D and phosphatidic acid in plant defence response: from protein–protein and lipid–protein interactions to hormone signalling

Jian Zhao*
National Key Laboratory for Crop Genetic Improvement, College of Plant Science & Technology, Huazhong Agricultural University, Wuhan 430070, PR China

* To whom correspondence should be addressed. E-mail: jianzhao@mail.hzau.edu.cn

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Abstract
Phospholipase Ds (PLDs) and PLD-derived phosphatidic acids (PAs) play vital roles in plant hormonal and environmental responses and various cellular dynamics. Recent studies have further expanded the functions of PLDs and PAs into plant–microbe interaction. The molecular diversities and redundant functions make PLD–PA an important signalling complex regulating lipid metabolism, cytoskeleton dynamics, vesicle trafficking, and hormonal signalling in plant defence through protein–protein and protein–lipid interactions or hormone signalling. Different PLD–PA signalling complexes and their targets have emerged as fast-growing research topics for understanding their numerous but not yet established roles in modifying pathogen perception, signal transduction, and downstream defence responses. Meanwhile, advanced lipidomics tools have allowed researchers to reveal further the mechanisms of PLD–PA signalling complexes in regulating lipid metabolism and signalling, and their impacts on jasmonic acid/oxylipins, salicylic acid, and other hormone signalling pathways that essentially mediate plant defence responses. This review attempts to summarize the progress made in spatial and temporal PLD/PA signalling as well as PLD/PA-mediated modification of plant defence. It presents an in-depth discussion on the functions and potential mechanisms of PLD–PA complexes in regulating actin filament/microtubule cytoskeleton, vesicle trafficking, and hormonal signalling, and in influencing lipid metabolism-derived metabolites as critical signalling components in plant defence responses. The discussion puts PLD–PA in a broader context in order to guide future research.

Key words: Cytoskeleton, hormone signalling, phospholipase D, phosphatidic acid, plant–microbe interaction, protein/lipid–protein interaction.

Introduction
Phospholipase D (PLD) hydrolyses different membrane phospholipids, producing phosphatidic acid (PA) and various head groups, such as choline or ethanolamine. PLDs have been characterized as having diverse roles in lipid metabolism and cellular regulation, ranging from hormone signalling (abscisic acid, jasmonate, auxin, and gibberellic acid) and environmental stress responses (drought, freezing, wounding, heavy metal toxicity, and phosphorus starvation) to involvement in various types of cellular or subcellular dynamics (Wang et al., 2006). Different PLDs appear to have distinct but somewhat overlapping functions in cellular processes (Uraji et al., 2012). PLD functions are often carried out through its...
enzymatic product, PA, which is now regarded as a universal lipid signalling molecule regulating numerous physiological processes (Wang et al., 2006; Pleskot et al., 2013). PLD-derived PA often directly binds to proteins to alter protein localization, enzyme activity, membrane–cytoskeleton interaction, and other processes (Testerink and Munnik, 2011). More than 30 proteins from diverse physiological pathways have been identified as PA targets (See Supplementary Table S1) and the list is still being extended, although the roles of PLD–PA in many stress responses remain ambiguous (Wang et al., 2006; Testerink and Munnik, 2011; Jang et al., 2012). Emerging evidence is expanding our understanding of the physiological functions and biological significance of PLD–PA signalling complexes and thus has recently drawn considerable attention (Wang et al., 2006; Pleskot et al., 2013).

Plasma membrane-localized pattern recognition receptors such as FLAGELLIN-SENSING 2 (FLS2), RPM1, and RPS2 recognize conserved pathogen-associated molecular pattern (PAMP) molecules that are released during pathogen invasion through secretory systems (Boller and Felix, 2009; Dodds and Rathjen, 2010). PAMP recognition activates signalling networks that can be propagated within the cell, modulate cellular physiology, and ultimately result in changes in expression of defence-associated genes that contribute to an effective immune response (Dodds and Rathjen, 2010; Elmore et al., 2012). These pattern-triggered immunity (PTI) responses are thought to render plants resistant to most non-adapted pathogens (Boller and Felix, 2009). On the other hand, plants also use intracellular immune receptors to monitor pathogen-derived and secreted effector-induced changes. Effectors are recognized primarily by the nucleotide-binding leucine-rich repeat family of intracellular resistance (R) proteins through direct interactions. Pathogen-secreted effectors, such as AvrB, AvrRpm1, and AvrRpt2, interact with corresponding R proteins and result in effector-triggered immunity (ETI) (Dodds and Rathjen, 2010). Although both ETI and PTI share similar signalling requirements and defence outputs and are closely related through downstream defence responses, ETI induces a stronger response than PTI, as seen in the hypersensitive response, hormonal signalling, accumulation of defensive secondary metabolites, and activation of other defence responses in host cells (Fig. 1) (Zhao et al., 2005; Dodds and Rathjen, 2010; Tsuda and Katagiri, 2010; Kazan and Lyons, 2014).

While plant PLD/PA play roles in the cellular and physiological processes in response to hormonal and abiotic cues, they are also implicated in plant–microbial interaction and the plant defence response against bacterial and fungal pathogens (Andersson et al., 2006a; Bargmann et al., 2006; Kirik and Mudgett, 2009; Yamaguchi et al., 2009; Zhao et al., 2013). As well as PLD proteins themselves being recruited as players, PA also rapidly accumulates in pathogen-infected plants to regulate defence responses, although its roles remain unknown. As the primary barriers of cells, membrane phospholipids are first responders to pathogen attack, and their metabolic product by PLD, PA, is potentially one of the early signalling molecules transducing outside information to the inside of cells. An increasing body of evidence indicates that actin filaments and tubulin microfibres, membrane–cytoskeleton interfaces, hormone signalling pathways, and chloroplastic lipid metabolism are the direct targets of both plant pathogen effectors and PLD–PA signalling complexes (Pleskot et al., 2013; Fu et al., 2014; Henty-Ridilla et al., 2014). This review aims to update recent findings in these areas and to discuss the potential functions and mechanisms of PLD–PA signalling in modifying plant defence response through protein–protein and protein–lipid interactions and lipid metabolite signalling. The review also tries to dissect the possible connections between PLD–PAs and other well-known signalling pathways and metabolic networks during the plant defence response in order to guide our future research.

**PLD–PAs are involved in plant defence responses**

Plant PLDs comprise a large family in the genome of every plant species. The similar genetic, molecular, and biochemical properties, and overlapping functions in many physiological processes, make it hard to strictly distinguish the role of an individual PLD from others (Camelli et al., 2011; Uraj et al., 2012). Several PLD genes, including PLDα, PLDβ, PLDδ, and PLDγ, can be induced in different plant species challenged by a pathogen or elicitor, and these PLDs can modify plant defence responses against various pathogens (Young et al., 1996; van der Luit et al., 2000; Laxalt et al., 2001; de Torres Zabel et al., 2002; McGee et al. 2003). Although most Arabidopsis PLDs have been characterized in genetic or biochemical aspects, the in planta substrate specificity, the variety of PA species generated, and the specificity of PLD–PA targeting remain to be determined (Wang et al., 2006). Therefore, the redundancy of PLDs makes functional characterization of each individual PLD in plant defence a rather difficult task, and only null mutants and advanced transcriptomic and metabolomics tools may be employed to overcome these obstacles.

**PLDβ–PA modifies plant defence responses by regulating actin cytoskeleton dynamics**

PLDβ from different plant species is specifically correlated with plant defence responses. Suppression of PLDβ1 expression in tomato resulted in increased defence responses, as indicated by an enhanced oxidative burst and polyphenolic oxidase activity in response to elicitors (Bargmann et al., 2006). OsPLDβ1-knockdown rice plants spontaneously activated defence responses in the absence of pathogen infection, as indicated by overproduction of reactive oxygen species (ROS) and expression of PR genes (Yamaguchi et al., 2009). OsPLDβ1-knockdown rice exhibited increased resistance to infection by major pathogens such as Pyricularia grisea and Xanthomonas oryzae pv. oryzae, suggesting that OsPLDβ1 functions as a negative regulator of defence responses and disease resistance (Yamaguchi et al., 2009). Arabidopsis PLDβ1 (AtPLDβ1)-deficient mutants showed an enhanced resistance
Pseudomonas syringae pv. tomato DC3000 (Pst DC3000) accompanied by increased accumulation of ROS and salicylic acid (SA) and increased SA-inducible defence gene expression, but decreased Botrytis cinerea infection-induced jasmonic acid (JA) and PA production and JA-inducible gene expression (Zhao et al., 2013). AtPLDβ1 is responsible for most of the PA production in response to the necrotrophic fungal pathogen B. cinerea and virulent Pst DC3000 infection. Therefore, PLDβ1 is a negative regulator of defence responses in several plant species, by negatively affecting SA-mediated and positively regulating JA-mediated defence signalling pathways. Although SA-dependent signalling may mediate the disease resistance of the AtPLDβ1-deficient mutants against biotrophic Pst DC3000, and the JA-dependent pathway may mediate the susceptibility of the AtPLDβ1-deficient mutants against necrotrophic fungal pathogen B. cinerea, other unknown mechanisms were believed to underlie the complex phenotypes (Zhao et al., 2013).

One of these is the interaction between AtPLDβ1 and actin. Previous studies have clearly shown that different physical forms of actin have bidirectional effects on mammalian PLD activity. PLD1 binds both G-actin and F-actin filaments; monomeric G-actin inhibits PLD1 activity, whereas polymerized F-actin augments PLD1 activity, while β, γ-, and α-actins can specifically bind to PLD and regulate tubulins or ACETYLATED INTERACTING PROTEIN1 (ACIP1) to modify microtubule cytoskeletons. Vesicle trafficking related to the import and trafficking of pathogen-derived effectors is also dependent on cytoskeleton rearrangements. PLD1–PA binds to and regulates AGD7 to modulate vesicle trafficking, and PLD2–PA also regulates actin dynamics, through which they regulate auxin and PAMP signal perception and downstream defence responses.

Fig. 1. Schematic of the involvement of PLD–PA in plant defence response through regulating cytoskeleton and vesicle trafficking. Pathogen-associated molecular patterns (PAMPs) from virulent pathogens are recognized by pattern recognition receptors (PRRs), or pathogen-secreted effectors are recognized by cellular resistance (R) proteins, which then transfer downstream signalling to defence responses, such as activation of various PLDs and cytoskeleton dynamics. PAs generated from PLDs then target various proteins such as CP and MAP65-1 to form membrane–cytoskeleton interfaces, or to regulate actin filament and microtubule cytoskeleton dynamics during plant defence. PLDs (e.g. α, β, δ, ζ) may be activated and translocated to the different membranes to generate PA species of various molecular features, which may regulate specific pathways. PLDα1 may interact with GTP-binding protein α-subunit that is activated by pathogen infection to mediate a defence response. PLDα1-derived PAs can bind and activate NADPH oxidase to produce reactive oxygen species (ROS), activate mitogen-activated protein kinase (MPK6), and phosphoinositide-dependent kinase 1 (PDK1) to initiate defence signalling cascades, activate 14-3-3 to regulate plasma membrane H+-ATPase, which is also involved in plant pathogen defence by altering membrane potentials and nutrient transport, or interact with microtubule-associated proteins (MAP65-1) to modify cytoskeleton dynamics in response to pathogen infections. PLDβ1 and PLDζ can interact with actin filaments to modify actin cytoskeleton dynamics. PLDα can interact with tubulins to modify microtubule cytoskeleton dynamics. These cytoskeleton dynamics are involved in the defence response against pathogens through actin-depolymerizing factor 4 or 7 (ADF4 or -7) or other tubulin-associating proteins. Pathogen effectors HopZ1a and AvrBsT can interact directly with and regulate tubulins or ACIP1 to modify microtubule cytoskeletons. Vesicle trafficking related to the import and trafficking of pathogen-derived effectors is also dependent on cytoskeleton rearrangements. PLDζ1–PA binds to and regulates AGD7 to modulate vesicle trafficking, and PLDζ2–PA also regulates actin dynamics, through which they regulate auxin and PAMP signal perception and downstream defence responses.
layer of regulatory roles for PLDβ1 in plant defence responses to disease (Fig. 1). PLDβ1 interaction with actin may regulate actin cytoskeleton dynamics or bridge the membrane and the cytoskeleton interfaces that affect both pathogen effectors’ secretary system and plant defence responses against pathogen attacks.

More interestingly, studies have shown that PLD-derived PA binds to actin and promotes actin polymerization (Lee et al., 2003). Recently, the mechanism underlying these phenomena was revealed by demonstrating that PA binds to an actin-binding protein. Arabidopsis heterodimeric capping protein (AtCP), a key regulator of actin filament polymerization that binds to the barbed ends of actin filaments, was demonstrated to be a PA-binding protein and was negatively regulated by PA in vitro (Huang et al., 2006). Exogenous PA application increased filamentous actin levels in Arabidopsis suspension cells and poppy pollen grains by binding to AtCP. The binding of PA to AtCP inhibits the actin-binding activity of AtCP and disables the AtCP function, which is to block the growth of actin filaments, thereby stimulating actin polymerization from a large pool of profiling-actin complexes (Huang et al., 2006). In vivo studies, using live-cell imaging of cytoskeleton dynamics and reverse genetics analyses, provide further compelling evidence that AtCP is inhibited from binding filament ends by PA in planta; this allows rapid actin polymerization and increases in filament abundance following stimulation (Li et al., 2012). As a membrane component, PA interaction with AtCP may also mediate membrane–cytoskeleton interactions, which can have broad impacts on plant–microbe interaction and defence response (Fig. 1).

The plant cell initiates cytoskeletal array changes to respond to the perception of PAMPs during PTI and perturbations by ETI (Day et al., 2011; Guan et al., 2013; Henty-Ridilla et al., 2013). An immediate increase in actin filament abundance resulting from actin polymerization is also one of the conserved components of PTI, and the actin polymerization response requires the host-cell PAMP receptor kinase complex (Staiger, 2000; Takemoto and Hardham, 2004; Henty-Ridilla et al., 2013). Blocking the actin filament density increase causes an enhanced susceptibility of host plants to pathogenic and non-pathogenic bacteria (Henty-Ridilla et al., 2013). A few actin-modification proteins including actin depolymerization factors 4 and 7 (ADF4 and -7) and mitogen-activated protein kinase have been identified, playing essential roles in the plant innate immunity (Tian et al., 2009; Wang et al., 2013; Fu et al., 2014) (Fig. 1).

PLDβ1–PA and its targeted actin cytoskeleton dynamics may thus play key roles in plant–microbe interactions and plant defence responses. This adds another layer of regulatory mechanisms for PLDβ1–PA modification of plant defence. AtCP may act as a PA biosensor and a key transducer of membrane phospholipid remodelling signals in the changes in actin cytoskeleton dynamics, which is critical for pathogen effectors’ secretion into plant cells and moving to subcellular targets. Thus, it is plausible that PLDβ1–PA binding of actins or actin-modifying protein may modify pathogen-induced plant defence responses. Elimination of PLDβ1 protein and thereby reduction of pathogen-induced PA production in PLDβ1-deficient mutants may thus altered actin cytoskeleton dynamics during the plant defence response, which may substantially contribute to its resistance to biotrophic bacteria or susceptibility to necrotrophic fungi (Zhao et al., 2013) (Fig. 1).

PLDα1 and PLDδ participate in the plant defence response to pathogens by changing microtubule cytoskeletons

PLDα1 and PLDδ are two major PLD forms in Arabidopsis and have a strong activity distinguishable from other PLDs in terms of Ca2+, SDS, PIP2 and oleic acid requirement and PA production in Arabidopsis (Wang et al., 2006). A rice PLDα1 is suggested to play a role in resistance against penetration by fungal pathogens (Young et al., 1996). Similar functions of PLDα1 and PLDδ in symbiotic plant–endophytic fungus interaction have been implicated recently by phenotyping PLDα1- and PLDδ mutants. A critical role for both PLDα1-PA and PLDδ-PA signalling in the endophytic fungus Piriformospora indica-induced Arabidopsis root growth was suggested via an identified PA target, 3-PHOSPHOINOSITIDE-DEPENDENT PROTEIN KINASE1(PDK1), signalling pathway (Camehl et al., 2011). The effects of these PLDs were interpreted through PA effectors, since PA was shown to bind to NADPH oxidase and to regulate H2O2 production and oxidative stress (Zhang et al., 2009; Camehl et al., 2011). PLDα1 has also been shown to interact with a G-protein α-subunit to regulate some signalling pathways (Zhao and Wang, 2004, 2013; Mishra et al., 2006; Zhao et al., 2013). This may be a plausible alternative mechanism for PLDα1 involvement in the plant defence response. PLDα1 is translocated to the plasma membrane during ETI responses, whereas Gα and Gβ proteins are decreased on the plasma membrane during ETI responses (Elmore et al., 2012). The negative correlation between expression of PLDα1 and Gα and Gβ during ETI responses seems consistent with reports regarding their functions in plant disease resistance: both Gα and Gβ are involved in the plant disease resistance (Trusov et al., 2009; Liu et al., 2013; Torres et al., 2013). PLDδ is also involved in preventing the non-adapted pathogen barley powdery mildew fungus Blumeria graminis f. sp. hordei and pea powdery mildew fungus Erysiphe pisi from penetration into the Arabidopsis epidermal cell wall (Pinosa et al., 2013) (Fig. 1).

The mechanisms underlying the disease resistance phenotypes of PLDα1 and PLDδ mutants are not yet understood. However, PLDα1 and PLDδ may regulate the plant defence by modifying microtubules. PLDα1-derived PA binds to MAP65-1 to regulate microtubule polymerization and bundling under salt stress, but its role in plant–pathogen interaction has not yet been tested (Zhang et al., 2012). PLDδ has been identified as a cortical microtubule-binding protein (Gardiner et al., 2001, 2003; Dhonukshe et al., 2003), and both actin 7 and β-tubulin are co-sedimented with GFP–PLDδ in a pull-down assay from transgenic Arabidopsis suspension cells, suggesting that PLDδ is involved in interactions with both actin filaments and microtubule cytoskeletons, which
form a cytoskeletal network (Andreeva et al., 2009; Ho et al., 2009; Petrasek and Schwarzerova, 2009) (Fig. 1). Tubulin binding in turn may activate PLD activity (Chae et al., 2005). The capability of PLDδ–PA to regulate microtubule and actin filament cytoskeleton dynamics might contribute to the penetration resistance against non-adapted powdery mildew fungi, since PLDδ is translocated to the attack site (Pinosa et al., 2013).

The cytoskeleton is a major target of type III effectors for pathogen virulence (Takemoto and Hardham, 2004). A study showed that pathogenic bacteria-secreted effector acetyltransferase HopZ1a targets monomer tubulin and polymerizes microtubules to destroy the host microtubule network and disrupts secretory pathways for building up cell-wall defence (Lee et al., 2012). The virulence effector AvrBsT possesses acetyltransferase activity and acetylates Arabidopsis ACETYLATED INTERACTING PROTEIN1 (ACIP1), which localizes to punctae on the cell cortex and co-localizes with cortical microtubules during infection (Cheong et al., 2014). The identification of ACIP1, tubulin, and ADF4/7 as host targets of virulent effectors (such as HopZ1a and AvrBsT) strongly suggests that actin/tubulin cytoskeleton dynamic and vesicle trafficking are required in the plant defence response for antibacterial immunity (Tian et al., 2009; Cheong et al., 2014; Fu et al., 2014). These clearly connect PLDδ–PA signalling with cytoskeletons in the plant defence response. Collectively, PLD–PA has essential roles in actin filaments and microtubule cytoskeletons, which may form a potential mechanism for PLD–PA in modifying the plant defence response (Fig. 1).

PLDζs–PA participate in PAMP perception and downstream signalling during defence response

PLDζ1 and-2 and PLDζ–produced PAs are involved in vesicle trafficking of the auxin transporter PIN and in regulating root hair patterning (Li and Xue, 2007; Yao et al., 2013). Although the implication of PLDζ–PA in the pathogen defence response remains lacking, it was shown that PLDζ1 interferes with actin cytoskeleton formation in hair root cells and that PLDζ2 interferes with vesicles trafficking, most likely through PAs (Ohashi et al., 2003; Li and Xue, 2007). Recent studies suggest that PLDζ–PA may regulated brassinosteroid (BR) signalling and plant immunity via the PA effectors protein phosphate type 2A (PP2A) and transcription factor BRASSINAZOLE RESISTANT1 (BZR1) (Gao et al., 2013; Wu et al., 2014). In bacterial flagellin-triggered immunity, the PAMP receptor kinase FLS2 or BR receptor BR insensitive1 (BR11) forms a complex with BR11-ASSOCIATED KINASE 1 (BAK1) to transphosphorylate the respective kinase domains of FLS2/BR11 and BAK1 (Chinchilla et al., 2007). BOTRYTIS-INDUCED KINASE 1 (BIK1), a receptor-like cytoplasmic kinase, is phosphorylated by BAK1 and associates with the FLS2/BAK1 complex to transduce flagellin signalling to downstream defence responses, including increased actin filament density and cytoskeleton dynamics (Lin et al., 2013; Hentty-Ridilla et al., 2014; Segonzac et al., 2014). PP2A controls the activation of pattern recognition receptor (FLS2) complexes by modulating the phospho-status of the co-receptor and BAK1 (Segonzac et al., 2014). A potential PP2A-holoenzyme inhibits immune responses triggered by several PAMPs and antibacterial immunity, since PP2A constitutively associates with BAK1 in planta (Segonzac et al., 2014). Recent studies have further shown that PLD-derived PA binds PROTEIN PHOSPHATASE 2A subunit A1 (PP2AA1) and activates membrane PP2A activity, and thus may further negatively regulate FLS2/BR11–AK1–BZR1 and antibacterial immunity (Gao et al., 2013). BZR1, a transcription factor regulating BR signalling, is dephosphorylated by PP2A to regulate expression of BR target genes (Tang et al., 2011). PA binding to PP2AA1 decreases cytosolic PP2A activity, suppressing BZR1-mediated signalling (Gao et al., 2013). Moreover, PA binds to BZR1 directly and inhibits the dephosphorylation of BZR1, suggesting that PA binding of BZR1 acts as a negative regulator in the plant defence response (Wu et al., 2014) (Fig. 2). Further investigation of the details about how PLDζ-derived PAs interact and regulate PP2AA1 and BZR1 to modify the PAMP signal perception will shed more light on the mechanisms by which plants deliberately balance BR signalling and plant immunity for plant growth and defence responses.

Other PLDs participate in plant defence responses to pathogens

Unlike PLDβ1, PLDβ2 appears not to be induced by pathogen attack and contributes little to the defence phenotypes of PLDβ1-knockdown mutants (Zhao et al., 2013). PLDγ1 has been implicated in tolerance to aluminium stress (Zhao et al., 2011) but has also been shown to be induced by pathogen attack (van der Luit et al., 2000; Laxalt et al., 2001). PLDγ1 is recruited to the plasma membrane during ETI responses (Elmore et al., 2012), further suggesting the possible involvement of these PLDγs in plant defence responses. However, little is known about whether and how PLDγ1 is involved in the defence response and pathogen resistance. Also, whether three other PLDs (PLDδ2, -3, and -4) are involved in plant response to pathogen attack has not been investigated.

PLD–PA regulates ROS production and interacts with other lipid signal molecules

PLD-derived PAs accumulate and regulate ROS in plant–microbe interactions

Plant cells challenged with P. syringae with or without AvrRpm1, AvrBst or AvrRpt2, rhizobium, fungal pathogens, or elicitors produce a rapid or biphasic accumulation of PA (van der Luit et al., 2000; de Jong et al., 2004; Bargmann et al., 2006; Andersson et al., 2006a; Kirik and Mudgett, 2009; Yamaguchi et al., 2009; Zhao et al., 2013). Although the biological significance of PA production and physiological meanings that PA level changes encode remain elusive, it is recognized that PA production is downstream of nitric oxide and is essential for the plant defence response (van der Luit et al., 2000; den Hartog et al., 2001; de Jong.
et al., 2004; Bargmann et al., 2006; Laxalt et al., 2007; Kirik and Mudgett, 2009; Yamaguchi et al., 2009; Testerink and Munnik, 2011). A Nicotiana benthamiana mutant with silenced PA phosphatase 2 (PAP2) showed increased PA content and enhanced resistance to Ralstonia solanacearum (Nakano et al., 2013). This mutant also showed accelerated cell death and ROS overaccumulation and increased defense gene expression, which were partially dependent on JA and ROS via COI1 and NADPH oxidase RbohB (Nakano et al., 2013). Pathogen-triggered PA can be generated by either PLD or phospholipase C (PLC)-diacylglycerol kinase (DGK) (Arisz and Munnik, 2013). However, the majority of pathogen-induced PA is synthesized through PLD activity (de Jong et al., 2004; Andersson et al., 2006a; Bargmann et al., 2006). In pathogen-challenged Arabidopsis, the PLD that contributes most to pathogen-induced PA production has been confirmed as PLDβ1 (Zhao et al., 2013).

It is reported that PLDα1-PA promotes superoxide production and cell death, and possibly provides a link to disease resistance (Sang et al., 2001; Park et al., 2004; Camehl et al., 2011; Nakano et al., 2013; Pinosa et al., 2013). PLDα1-derived PA has been shown to interact directly with NADPH oxidase on the plasma membrane, or to modulate abscisic acid (ABA)-mediated signalling through AB1 under drought stress (Zhang et al., 2004, 2009). A PLDγ1 mutant and PLDγ1-knockdown mutants showed reduced ROS generation and oxidative stress under aluminium stress (Zhao et al., 2011). PA may also trigger ROS generation in a similar way during pathogen infection, since pathogens/elicitors activate both a rapid PA production and NADPH oxidase gene expression...
PLD–PA has been considered a signalling molecule downstream of nitric oxide, which is a well-known regulator of plant immunity and is required for PA generation via both the PLD and PLC/DGK pathway during plant defence response (Laxalt et al., 2007; Raho et al., 2011; Bellin et al., 2013). However, specific PLDs may regulate the temporal and spatial production of specific PA, which may have a particular function (Wang et al., 2006). For example, an earlier study showed that PLDβ and its derived PA were required in the Arabidopsis response to ROS, and elimination of PLDβ renders plants more sensitive to H$_2$O$_2$-promoted cell death (Zhang et al., 2003). In addition, PA is required for resistance against penetration of powdery mildew fungus, since inhibition of PA production by a PLD inhibitor reduced the penetration resistance (Pinosa et al., 2013).

Also, both PLDα1- and PLDδ-derived PAs are involved in the ROS response during plant–endophytic fungus interaction (Camehl et al., 2011). The counterintuitive observations in PLDβ1-deficient plants regarding the relationship between PA and ROS may reflect the complexity and flexibility of PLD–PA. PLDβ1–PA molecules negatively regulate ROS production through an unknown mechanism (Bargmann et al., 2006; Yamaguchi et al., 2009; Zhao et al., 2013).

Other lipid signalling molecules in PLD–PA modified plant defence

Except for PAs, other small lipid molecules, such as lysophospholipids, sphingolipids, and N-acylethanolamines (NAEs), were recently identified as important signalling components mediating plant defence responses (Coulona et al., 2012; Jang et al., 2012, Kimberlin et al., 2013). These lipid molecules interact with PLDs/PAs in regulating enzyme activity, biosynthesis, and signalling. Therefore, their roles in mediating defence responses should not be neglected. Lysophospholipids are minor components of plant membranes and are subject to tight regulation. However, lysophosphatidylcholine (lysoPC), lysophosphatidylethanolamine (lysoPE), and lysophosphatidylglycerol overaccumulated in PLDβ1-deficient mutants and may have significant physiological functions (Zhao et al., 2013). Except for the inhibitory effect of lysoPC on PLDα1 or possibly PLDβ1 activity, lysoPC often accumulates during plant–microbe interactions and may have additional biological activity. Yeast elicitor-induced PLA$_2$ produces lysoPC, which activates a tonoplast H$^+$/Na$^+$ antiporter to trigger cytosolic alkalization, ROS, and defence responses in suspension cells (Viehweger et al., 2002) and in arbuscular mycorrhizal symbiosis (Drissern et al., 2007). Plant PLA$_2$ and its products lysoPC and lysoPE are involved in systemic responses in wounded plants (Jang et al., 2012) (Fig. 1). LysoPE and lysophosphatidylinositol can inhibit, but lysophosphatidic acid stimulates plant phospholipase D (Ryu et al., 1997). Therefore, it is plausible that lysoPC could play a positive role in initiation of plant defence responses in PLDβ1 mutants. Another putative lipid signalling molecules that might be involved in the PLD-mediated plant defence response is sphingolipids. Sphingolipids have been shown to play essential roles in triggering programmed cell death, ROS generation, defence responses, and disease resistance in both animals and plants (König et al., 2012; Berkey et al., 2012; Kimberlin et al., 2013). Arabidopsis mutants with reduced ceramide and glucosylceramide levels accumulate higher level of SA and show enhanced disease resistance against obligate biotrophic pathogens (König et al., 2012). Recent studies have shown that PLDα1-derived PA binds to sphingosine kinases and stimulates sphingosine 1-phosphate, which mediates the effects of PLDs on ABA signalling (Guo et al., 2011, 2012). It is still unclear how sphingolipids interact with PLD–PA during plant defence response. NAEs are fatty acid amides derived from the hydrolysis of the membrane component N-acylphosphatidylethanolamine by PLDβ or PLDγ. It was shown that elicitor-activated PLDβ1 or PLDγ could generate NAEs (mainly NAE 14:0 and NAE 12:0) in tobacco leaves (Tripathy et al., 2003). Application of NAE 14:0 is sufficient to activate defence gene expression (Tripathy et al., 2003). Applied NAE 12:0 interfered with root development and primary root growth in Arabidopsis seedlings, probably by destroying cortical microtubules and F-actin cytoskeleton dynamics and interfering with endomembrane organization and membrane trafficking (Motes et al., 2005). Although it is still unclear how NAEs exert effects on cytoskeleton modification, an enhanced ROS production and lipid peroxidation by NAEs was observed (Coulona et al., 2012). NAEs are regarded as a potent inhibitor of PLDα1 (Austin-Brown and Chapman, 2002). It is still not known whether PLDβ1-deficient mutants produce normal levels of NAEs for regulating defence responses. However, NAEs play important roles in pathogenesis in mammalian cells (Coulona et al., 2012).

PLD–PA impacts on hormone signalling in plant defence response

To date, almost every known hormone is directly or indirectly related to plant defence response. As a classic defensive hormone, SA is well known as an essential hormone for initiation of plant systematic acquired resistance (SAR), playing critical roles in plant resistance against biotrophic bacterial pathogens such as Pst DC3000. JA/ethylene (ET) is known as an inducer of induced systemic resistance (ISR), playing important roles in plant resistance against necrotrophic fungal pathogens such as B. cinerea. The SAR and ISR signalling pathways do not function independently but rather affect each other through a complex network of regulatory interactions (Fig. 2). For instance, SA and JA do not only counteract in biosynthesis and signalling defence responses (Thaler et al., 2012; Gimenez-Ibanez and Solano, 2013). Recently other plant hormones, such as auxins, BRs, ABA, ET, and gibberellins (GAs), have also been reported as regulators of plant–microbe interactions, although their significance is not yet fully understood (Antolin-Llovers et al., 2012). Mounting evidence suggests that these hormones affect plant defence responses by feeding into the SA–JA/ET signalling circuitry through different mechanisms (Fig. 2). Meanwhile, a plenty of evidence shows that PLD–PA signalling has a fundamental impact on almost all hormone signalling pathways.
PLD–PA is involved in SA signalling

SA is synthesized in the chloroplast and transported into the cytosol and the nucleus, where SA regulates SAR through NONEXPRESSOR OF PATHOGENESIS-RELATED PROTEINS1 (NPR1) and other signalling pathways to initiate the plant innate immunity. Although there is no direct evidence supporting the interaction between lipid metabolism and SA biosynthesis and signalling, several lipase-like genes involved in SA signalling have been identified, including SA-binding protein 2 (SABP2), ENHANCED DISEASE SUSCEPTIBILITY1 (EDS1), Phytalexin Deficient 4 (PAD4), and PLDb1 (Feys et al., 2005; Shah, 2005; Zhu et al., 2011). Mutations of EDS1 and PAD4 impair SA levels and/or responsiveness and thereby enhance susceptibility to pathogen infection. SA application induces PA accumulation, followed by the induction of a subset of defence genes in Arabidopsis through activation of PLDs. PLDs could act both positively and negatively on defence gene induction or repression (Krinke et al., 2009). SA-induced PLDε1–PA signalling mediates NADPH oxidase RbohD activation and ROS production, suggesting that PLDε1 activation is an important component downstream of SA (Krinke et al., 2009; Kalachova et al., 2013). However, PLDb1 expression is repressed by SA and mutation of PLDb1 enhanced SA production and signalling pathways upon pathogen infection (Yamaguchi et al., 2009; Zhao et al., 2013). These data suggest that PLDs may be differentially regulated by SA. The pathogen induction of expression of PLDs, and production of SA, PA, ROS, and JA/oxylinpins in the chloroplast are likely to be cross-linked with each other in more complex ways (Fig. 2).

PLD–PA regulates JA/oxylinpin/ET signalling

Phospholipase A (PLA) hydrolyses galactolipids or phospholipids at the sn-1 or sn-2 position to yield lyso phospholipids and α-linolenic acid (18:3), which is regarded as a precursor of JA biosynthesis. PLAs are induced by pathogen attack and are involved in disease resistance (Yang et al., 2007; Kirik and Mudgett, 2009; Jang et al., 2012). Emerging evidence suggests that PLA and PLD interact in PA and JA production during the plant defence response. Arabidopsis PLA2 SOBER1 is a negative regulator of resistance against Pst DC3000 (avrBsT) by suppressing PLD-dependent production of PA during ETI in response to the effector AvrBsT (Kirik and Mudgett, 2009). Both PLP2/PLA2α and SOBER1 proteins accumulate to significant levels on the plasma membrane during ETI responses (Elmore et al., 2012). They also impact on ET signalling. GDSL LIPASE1 was shown to modulate plant immunity through positive and negative feedback regulation of ET signalling and fine-tuning of ET/SA (Kim et al., 2013b). ET signalling is also involved in the plant defence response against plant pathogens (Chen et al., 2013). PA binds and inhibits CTR1, a receptor kinase-like negative ET signalling factor (Testerink et al., 2007). JA/oxylinpins are the oxidation products of unsaturated fatty acids generated by the collaborative actions of PLA, PLD, and other enzymes (Andersson et al., 2006b; Vu et al., 2012). They actively function as signalling molecules in plant development processes and defence responses (Andersson et al., 2006b; Buseman et al., 2006; Zoeller et al., 2012). Recently, a study further indicated that, under different stresses, including challenge by Pst DC3000 or Pst 3000 (AvrRpt2), membrane lipids, including digalactosyldiacylglycerol (DGDG) and monogalactosyldiacylglycerol (MGDG), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), and acylated MGDG, contained one or more oxidized acyl chains, which could release oxylinpins upon the action of PLAs and PLDs (Vu et al., 2012). These data suggest that JA biosynthesis is positively affected or regulated by PLD–PA in the chloroplast. Thus, it is easy to understand why Arabidopsis PLDε1 and PLDb1 mutation impairs JA biosynthesis (Wang et al., 2001; Zhao et al., 2013).

PLD–PA modulates other hormonal signalling

Auxins also negatively regulate plant defence by interfering with the classical defence hormones SA, JA, and ET, or with PTI (Robert-Seilaniantz et al., 2011). The bacterial PAMP flg22 triggers suppression of auxin signalling and leads to enhanced resistance to Pst DC3000 and the oomycete Hyaloperonospora arabidopsis (Robert-Seilaniantz et al., 2011). SA stabilizes auxin/indole-3-acetic acid proteins and represses auxin signalling pathway, which is a part of the SA-induced resistance mechanism. The PLDζ–PA is involved in vesicle trafficking and regulating auxin transport (Li and Xue, 2007) (Fig. 2). How PLDζ–PA and SA cross-talk with auxin signalling during plant defence is unclear and is worth investigating.

BRs are also involved in plant defence responses against pathogens. BRs are bound by a receptor-like kinase, BR11. BR11-associated kinase 1 (BAK1) acts as a co-receptor for both BR1 and FLS2. In addition to the function as a BR receptor for regulating plant growth and development, BR11 is also involved in the perception of PAMPs in symbiosis and disease resistance. BR/Flg22 binds to receptor BR11/FLS2 and initiates the interaction between BAK1 and BR11/FLS2, following distinct BR hormone signalling, defence, or symbiotic response (Antolin-Llovera et al., 2012; Shi et al., 2013) (Fig. 2). In Arabidopsis, the FLS2/BR11–BAK1 complex plays an essential role in response to bacterial PAMP flg22 (Antolin-Llovera et al., 2012). PLDζ–PA targets both BZR1 and PP2AA1 to regulate plant immunity (Tang et al., 2011; Gao et al., 2013; Segonzac et al., 2014, Wu et al., 2014) (Fig. 2).

ABA acts as either a positive or a negative regulator of plant defence, depending on the plant–pathogen interactions (Ton et al., 2009). ABA biosynthesis- or signalling-defective mutants in Arabidopsis and tomato overexpressed defensive-signalling pathways and showed enhanced resistance to pathogens such as B. cinerea and P. syringae via negative interaction with SA and JA/ET signalling (Garcia-Andrade et al., 2011; Sánchez-Valso et al., 2012). PLDε1–PA regulates ABA signalling: PA binds ABI1, a negative regulator in ABA signalling, and inhibits the ABI1 repression effect
on ABA signalling (Zhang et al., 2004); PA also binds to
SHPK, a sphingosine kinase for generation of phytosphingosine-1-phosphate, to mediate ABA signalling. Therefore,
PLD–PA is regarded as a mediator complex of ABA signalling (Fig. 2).

Diversity and specificity of PLD–PA species and membrane–cytoskeleton dynamics

PA species vary in propensity to bind targets for various functions

One of the most significant features of the PLD–PA signalling complex is that PA often binds to and regulates a wide range of proteins, through which PLD–PA exerts biological functions. Physicochemical properties of PA species with different lengths and type of fatty acyl chain can affect lipid–protein interaction (Wang et al., 2006). The distinct roles of PA molecular species in regulating the target protein, such as actin and microtubule cytoskeletons, are reflected in biochemical assays (Testerink et al., 2004; Testerink and Munnik, 2011). For example, palmitoyl-linoleoyl PA (16:0/18:2 PA) increases under salt stress and alleviates microtubule depolymerization triggered by salt (Zhang et al., 2012). Among various PA species with different fatty acyl chains assayed for protein binding, 16:0/18:2 PA, but not dielaidoyl PA (di18:0 PA), showed specific and higher binding affinity to MAP65-1 (Zhang et al., 2012). Another PA effector, ABI1, prefers to bind dioleoyl PA (di18:1 PA) over dipalmitoyl PA (di16:0 PA), di18:0 PA, or dilinoleoyl PA (di18:2 PA) (Zhang et al. 2004). Dioleoyl PA can bind to TGD2 and -4 and then may be transported into the chloroplast where it may trigger chloroplast death (Zhang et al., 2003; Awai et al., 2006). Palmitoyl-linoleoyl PA (16:0/18:2 PA) has been shown to specifically bind NADPH oxidase (Zhang et al., 2009). SPHK1 and SPHK2 exhibit strong binding to 18:1/18:1, 16:0/18:1, and 16:0/18:2 PA but poor binding to 16:0/16:0, 8:0/8:0, 18:0/18:0, and 18:2/18:2 PA (Guo et al., 2011). 16:0/18:1 PA associates with 14-3-3 proteins and regulates the activity of the plasma membrane H+-ATPase (Camoni et al., 2012). Furthermore, the hydrophobic residues at the C terminus of AtCP have been found to be inserted into the lipid bilayer containing PA, and this interaction is likely to be regulated by the length of the PA acyl chains, although this has yet not been tested (Huang et al., 2006; Pleskot et al., 2012). PA molecular species with different lengths and types of acyl chain generated by PLD may also affect the interaction of PA with other lipids in the membrane and thus influence PA localization and biomembrane properties. This is also one of the reasons that PA binding can recruit a specific target protein to the membrane to alter membrane properties, cellular processes, and defence responses, and why PA binding to cytoskeleton components (actin- or tubulin-associating proteins AtTCP and MAP65-1) can regulate the membrane–cytoskeleton interfaces and cellular dynamics that are critical to pathogenesis (Nakanishi et al., 2006; Kooijman et al., 2007; Yang et al., 2008).

PA species from different PLDs function differently in defence responses

It was shown that PA derived from the DGK pathway can be distinguished from PLD-derived PA, based on its fatty acid lengths and biosynthesis characteristics (Arisz and Munnik, 2013). It is likely that different PLDs may produce distinct PA molecular species, which have specific physiological functions. Even produced by the same PLD, PA species derived from different membranes under different physiological conditions can be different. This could be due to varying PLD substrate preferences, different membrane structures, and different subcellular localizations, which are still not fully understood (Wang et al., 2006; Testerink and Munnik, 2011). For example, an increased PA level accompanied by a decrease in lysophospholipids is associated with enhanced defence responses and resistance in the SOBER1 mutant, but a decreased PA level accompanied by an increase in lysophospholipids is also associated with enhanced defence responses and resistance in PLDβ1 mutants (Kirik and Mudgett, 2009; Zhao et al., 2013). The PA species affected by PLDβ1 are the same in virulent bacterial infection but are different from the PA pool that SOBER1 inhibits upon avirulent infection. The key for different responses to virulent or avirulent infection might be different PA molecular species. Almost all PA species, such as the diacyl (total acyl carbons: total C=C double bonds) 34:2, 34:3, 34:4, 36:4, 36:5, and 36:6 PA species are significantly lower in PLDβ1 mutants than in the Columbia ecotype (Col-0) upon both Pst DC3000 and B. cinerea infections. SOBER1 mutant leaves infected with Pst DC3000 or Pst DC3000 only show decreases in 34:2 and 36:4 PA species, especially in response to Pst DC3000 (vector) infection. The SOBER1 mutant accumulates more total PAs in response to Pst DC3000 (avrBst) than Pst DC3000 after 16 h of infection (Kirik and Mudgett, 2009). The PLDβ1 mutants or Col-0 synthesized more PAs in response to Pst DC3000 (avrRpt2) than to Pst DC3000. The 34:3, 34:4, 36:5, and 36:6 PA species are the most increased PA species upon pathogen infection (Zhao et al., 2013). These data suggest that different PA species accumulate in response to avirulent and virulent pathogens. An AtPLA1-deficient mutant challenged by a necrotrophic fungal pathogen showed a significant decrease in 34:4 phosphatidylglycerol and 34:3, 34:4, and 36:6 DGDG, which primarily consist of 18:3/16:1, 18:3/16:0, 18:3/16:1, and 18:3/18:3 acyl groups, respectively (Yang et al., 2007). The AtPLA1-deficient mutant accumulated more lysoPE and lysoPC than the wild type, suggesting another acyl hydrolysing activity, except for AtPLA1, contributing to membrane lipid loss in disease-damaged tissues (Yang et al., 2007). However, a recent study failed to show PAs generation by PLD upon SA activation but showed over-representation of 16:0/18:2 and 16:0/18:3 PAs in PLD transphosphatidylation activity on 1-butanol. The results showed that these PAs mismatched with the acyl chain compositions of membrane phospholipid substrates (Rainteau et al., 2012). Further investigation on the in planta substrate preference of PLDs may throw light on the specificity of PLD-PA signalling.
PLD–PA regulates vesicle trafficking and membrane–cytoskeleton interfaces

The special properties of PAs, such as being negatively charged and cone-shaped, may profoundly influence membrane curvature, surface charge, vesicle formation from the endoplasmic reticulum, and membrane fission or fusion (Young et al., 2010; Pleskot et al., 2013). It is proposed that membrane–cytoskeleton interactions and endocytosis play essential roles in effector transport into plant cells and delivery to target sites in the cytoplasm, nucleus, mitochondria, and chloroplast (Fig. 2). The movement of these effectors in plant cells also involves PLD-ζ, PLA-α, or other lipase-mediated membrane remodelling, lipid turnover, and vesicle trafficking (Shah, 2005; Pleskot et al., 2013; Zhao et al., 2013). The mammalian PLD–PA signalling complex mediates protein–membrane interaction and membrane–cytoskeleton interfaces and is involved in vesicle trafficking-related physiological and cellular processes, such as exocytosis, endocytosis, membrane delivery, and vesicle budding (McMahon and Gallop, 2005; Donaldson, 2009).

Similar situations may be applied in plants during pathogen attack, effector import, defensive compound delivery, and exobiotics export processes. Vesicle membrane coat proteins, ARF and Rho GTPases in the Golgi complex and soluble N-ethylmaleimide-sensitive factor attachment receptor (SNARE) proteins, facilitate vesicle formation, transport, and fusion with the target organelle through PLD activators or as PA-binding or recruiting proteins; PA promotes negative membrane curvature both for recruiting other proteins and for membrane fission and fusion (Chernomordik and Kozlov, 2003; Donaldson, 2009). The yeast SNARE protein Spo20p and a key transcriptional repressor in PA signalling, Opi1p, are recognized as PA-binding proteins (Nakanishi et al., 2006; Testerink and Munnik, 2011). Similarly, Arabidopsis ARF GTPase-activating protein 7 (AGD7) regulates AtARF1 in the Golgi complex in a PA-dependent manner (Min et al., 2007) (Fig. 1). PLDζ1- and PLDζ2- PAs participate in actin cytoskeleton dynamics and vesicle trafficking (Ohashi et al., 2003; Li and Xue 2007) (Fig. 1).

Membrane phosphatidylinositol phosphates (PIPs) and their interconversion by various kinases also essentially participate in the interactions between PLD–PA, cytoskeleton dynamics, and vesicle trafficking. PIP2 is the enzymatic product of phosphatidylinositol-4-phosphate 5-kinase (PIPK). PIP2 has critical functions in cellular processes, including cytoskeletal reorganization, membrane trafficking, and signal transduction. AtTCP can bind to and be regulated by both PA and PIP2 (Delage et al., 2013; Pleskot et al., 2013). While plasma membrane PIP2 levels control the adhesion of the membrane bilayer to the underlying cytoskeleton, elevated PIP2 levels also affect the cortical plasma membrane–cytoskeleton structure and regulate numerous actin-binding proteins, such as PLDβ1 (Wang et al., 2006). PLD inhibitors block the membrane localization of PIPKγ and thereby its ability to induce actin cytoskeletal reorganization (Cockcroft, 2009; Roach et al., 2012). Arabidopsis PIPK1 activated by PA (Perera et al., 2005; Delage et al., 2013) and interacts directly with F-actin through a predicted linker region in the lipid kinase; this interaction also recruits AtPI4Kβ1 to the cytoskeleton (Davis et al., 2007). AtPIPK associates with ROP (Rho of plants) GTPases, which are regulators of secretion, microtubules, and actin (Pleskot et al., 2012). Coordinated actin/tubulin cytoskeleton dynamics and vesicle trafficking are not only employed for import of effectors but are also required for establishing plant defence responses, such as delivery of antibacterial or antifungal compounds to the sites of infection (McMahon and Gallop, 2005; Donaldson, 2009). Therefore, the PLD–PA signalling complex may act as a critical determining factor and/or as a coordinator of signal dynamics, membrane trafficking, and cytoskeleton reorganization during plant–pathogen interaction and defence responses.

PLD/PA localization and function in lipid metabolism for plant defence

Temporal and spatial changes of PLD activities and accumulation of PA species are the key for understanding the cellular functions of PLD–PA complexes, which are profoundly involved in lipid metabolism, signal transduction, and downstream defence responses during pathogen attack. The pathogen- and elicitor-induced activation of PLDs involves the recruitment of these PLDs to various membranes to hydrolyse phospholipids or to interact with other target proteins (Young et al., 1996; Bargmann et al., 2006; Yamaguchi et al., 2009; Elmore et al., 2012; Pinosa et al., 2013). Thus, the subcellular localization of PLDs is important with respect to their physiological functions and the creation of the corresponding PA pools during plant–pathogen interactions (Wang et al., 2006; Testerink and Munnik, 2011). Fractionation of soluble and membrane fractions, green fluorescent protein fusion imaging, and protein fingerprint identification using various mass spectrometry techniques have shown multiple localization sites of most Arabidopsis PLDs; for example, two major PLDs, PLDα1 and PLDα2, are either cytosolic or membrane-associated or both under certain conditions (Fan et al., 1999; Qi et al., 2011; Elmore et al., 2012; Pinosa et al., 2013). Most PLDs, as mentioned above, can be recruited to the membrane in response to environmental and hormonal cues to carry out their function (Bargmann et al., 2006). PLDα is relocalized to the plasma membrane at the fungal attack site, where it surrounds the cell-wall reinforcement (Pinosa et al., 2013). Both PLDα1 and PLDγ1 are relocated to the plasma membrane during ETI responses (Elmore et al., 2012). Unlike PLDα1 and PLDγ1, no PLDβ1 was detected on the plasma membrane (Elmore et al., 2012). Since PLDα1 and PLDβ1 affect JA biosynthesis, and two rice PLDs, PLDα4 and -α5, that are associated with herbivore-induced oxylipin (including JA) production are chloroplast localized (Qi et al., 2011), it is likely that PLDβ1 translocates to the plastids upon pathogen infection. PLDs interact with two cytosolic glyceraldehyde-3-phosphate dehydrogenases (GAPC1 and GAPC2) to regulate lipid metabolism and mediate H₂O₂ stress (Guo et al., 2012). GAPCs have also been found to be PA effectors; PA binding to GAPCs inhibits GAPC activity and causes a
feedback inhibition of PA biosynthesis (Kim et al., 2013c). These observations suggest that cytosolic PLDδ–PA–GAPCs form an interacting complex and feedback loops to coordinate carbohydrate and lipid metabolism (Kim et al., 2013c; Guo et al., 2012) (Fig. 3).

The diverse molecular variation and biological functions of PAs make the site of their generation in different subcellular compartments or membranes extremely important. PA molecules can be integrated into the plasma membrane, chloroplast envelope, endoplasmic reticulum, or nuclear envelope. The location of PAs directly affects the localization, activity, and biological functions of PA-targeted proteins (Testerink and Munnik, 2011). PA is not only an important signalling molecule and a membrane structural component but also an essential metabolic precursor for the biosynthesis of phospholipids and storage neutral lipids in the endoplasmic reticulum and galactolipids in the chloroplast (Eastmond et al., 2010; Loewen, 2012; Sembongi et al., 2013). Therefore, the complex roles of PAs in cellular signalling and lipid metabolism are one of the most interesting research frontiers of plant science (Loewen, 2012) (Fig. 3). From the metabolic point of view, as essential metabolic precursors for biosynthesis of neutral lipids, phospholipids, and galactolipids, PAs are in the centre of the lipid metabolic network. Changes in genes/enzymes involved in PA biosynthesis (such as LPAAT, ATS1, ATS2, PLD, and PLC/DGK), conversation (such as

![Fig. 3. Comprehensive schematic of PLD–PA involvement in lipid metabolism and signalling and impact on SA biosynthesis and signalling in plant defence. In the chloroplast, many enzymes such as stearoyl acyl carrier protein desaturase (SSAT2) are involved in the biosynthesis of C16:0 to 18:1 fatty acids. Many plasticid enzymes such as allene oxide synthase (AOS), α-dioxygenase (α-DOX), ENHANCED DISEASE SUSCEPTIBILITY1 (EDS1), fatty acid hydroxylase (FAH), hydroperoxide lyase (HPL), hydroperoxide reductase (HPR), lipoxygenase (LOX), and peroxygenase (POX) are involved in the biosynthesis of oxylipins including jasmonic acid (JA) and azelaic acid from oxidation of polyunsaturated fatty acids. JA is also generated from the action of phospholipase A (PLA) hydrolysis of plasticid membrane galactolipids such as monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), and phosphatidylglycerol (PG). JA and other oxylipins could activate induced system resistance (ISR) through CORONATINE INSENSITIVE 1 (COI1) receptor, SCFCOIA/JAZ/MYC complex signal transduction. Plasidic synthesis of salicylic acid (SA) through shikimate pathway is transported into the cytosol, where SA activates NPR1 to trigger NPR1 translocation into the nucleus to activate systemic acquired resistance (SAP). The fatty acid desaturases (FADs) such as FAD6 and FAD7/FAD8 modifying the fatty acyl chains (18:1 to 18:2 and 18:3) of galactolipids (GLs) also affect plant disease resistance by modifying JA- and SA-dependent or independent signalling pathways. The prokaryotic pathway of glycerolipid biosynthesis in the plastid is through acylation of glycerol-3-phosphate (G3P) by G3P acyltransferase (ATS1) to give lysophosphatidic acid (LPA). PA, MGDG, and DGDG. G3P can be transported into the plastid and the endoplasmic reticulum (ER) from the cytosol. The cytosolic G3P synthesis by glycerokinase (GK), glycerol-3-phosphate dehydrogenase (GPDH), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is also implicated in plant responses to pathogen attack. GAPDH is one of the targets of phospholipase D (PLDδ) and PA, PLA, PLD, or phospholipase C (PLC/diglycerol kinase (DGK)) could hydrolyse the oxidized acyl chain- or oxylipin-containing glycerolipids to release JA/oxylipins, which could induce JA-dependent defence signalling and responses. The fatty acyl CoAs synthesized in the plastid are transported out to form a cytosolic pool, supplying precursors for biosynthesis of neutral lipids and phospholipids in the ER. As the important precursors, PAs are involved in biosynthesis and turnover of phospholipids and the neutral lipids diacylglycerol (DAG) and triacylglycerol (TAG). PA synthesis and metabolism through lysophosphatidic acid transerase (PLAAT), phosphatidic acid phosphatase (PAP), ATS1, PLD, PLC, and DGK also affect plant defence. PA from the ER can also be transported into the plastid through trigalactosyldiacylglycerols (TGDs) and the TGD4 transport complex to provide precursors for biosynthesis of chloroplast glycerolipids. PA can affect JA biosynthesis in multiple ways. SA biosynthesis from the shikimate pathways may interact with JA and PA signalling by an unknown mechanism.
PAP and MGD), or degradation (PAP and PLA) will widely affect plant lipid metabolism and triacylglycerol biosynthesis (Figs 2 and 3). The endoplasmic reticulum is believed to contain the largest PA pool in plant cells, followed by the chloroplast, cytoplasm, and mitochondria (Testerink and Munnik, 2011). PA can be transported from the endoplasmic reticulum to the chloroplast in plant tissues, with the help of proteins such as PLDs or other phospholipids on the chloroplast envelope (Benning, 2009). In the chloroplast, lipid biosynthesis and catabolism of PAs and fatty acids, and turnover of galactolipids and phospholipids are closely related to JA and SA synthesis; all of them essentially participate in the plant defence response (Shah, 2005; Kachroo and Kachroo, 2009; Kim et al., 2013a). Fatty acid metabolism in the chloroplast plays a critical role in plant disease resistance (Kachroo and Kachroo, 2009). Reduced levels of 18:1 fatty acid induced a broad-spectrum SA-independent resistance against biotrophic pathogens, and C18:1 can stimulate PLDb activity to protect plants from oxidative stress-induced cell death (Zhang et al., 2003; Kachroo and Kachroo, 2009). C18:3-deficient plants are defective in ROS generation and subsequent signalling in R-mediated resistance since these C18:3 fatty acids are the major polyunsaturated fatty acid species in chloroplast membrane lipids that can translocate to extracellular spaces and activate NADPH oxidase to generate ROS against avirulent bacterial pathogens (Kachroo and Kachroo, 2009) (Figs 2 and 3). Galactolipids, primarily DGDG and MGDG, are the sources for wound- or pathogen-induced biosynthesis of JA/oxylipins, under the actions of PLA, PLD, and other enzymes. Application of various high-sensitivity and high-throughput mass spectrometry technologies have allowed scientists to identify and profile various lipid metabolites, such as PA and other small lipid signalling molecules, which now are regarded as important signalling molecules of various defence responses under abiotic and biotic stresses (Návarová et al., 2012; Vu et al., 2012; Zoeller et al., 2012).

Conclusions and perspectives

A window has opened for looking into the functions and mechanisms of PLD and PA and signalling complexes in plant defence responses. The combinations of recently developed technologies such as high-sensitivity and high-throughput mass spectrometry for identifying and profiling novel lipid metabolites, high-resolution cellular imaging systems to view in vivo dynamic interactions of lipid molecules, proteins, membranes, and cytoskeleton elements in plant cells, and high-throughput screening systems for lipid–protein, protein–protein, and membrane–protein interactions have greatly empowered scientists to explore cutting-edge research topics related to lipid metabolism and signalling. Plant scientists are showing greater interest and placing more research efforts on lipids, a class of the most important but also the most complex biomolecules that support the basic structure of the cell and every function of life. Plant lipid research now brings PLDs, PLAs, glycerolipids, particularly PAs, fatty acids and their derived oxylipins, sphingolipids, actin filament and microtubule cytoskeletons, and various types of PA-targeted proteins together to the plant defence response stage. This significantly broadens our view and understanding of protein–protein, lipid–protein, and membrane–cytoskeleton interactions, as well as their interaction with PTI or ETI during pathogen attack and plant defence responses. However, unlike the extensive studies of PLD and PAs in abiotic stresses, PLD–PA functions in the plant defence response against microbial pathogens are emerging. Our understanding of the functions of PLDs/PAs and their complex signalling in plant–microbial interactions is very limited. Their metabolic regulatory connections with fatty acids and neutral lipids are also full of mysteries. In particular, the following questions are of extreme importance:

1. What are the relationships between PLDs and other lipases such as PLAs when they are involved in JA/oxylipin and PA production and signalling in plant defence?
2. Why are PLDs involved in the plant defence response and interaction with actin and microtubule cytoskeletons? How does each PLD’s overlapping function and subcellular localization change during plant–pathogen interactions?
3. What is the exact role of PA production during plant–microbe interactions and the defence response?
5. How do PLD and PA affect galactolipid and phospholipid metabolism, JA and SA biosynthesis, and signalling in the chloroplast during plant response to disease?
6. How do plant cells employ PAs as both essential lipid signalling molecules and major metabolic precursors? Are the subcellular membrane sites and time that PAs are generated the key points?
7. How is PLD–PA involved in the ETI- or PTI-related signal perception, signal transduction, and cytoskeleton dynamics?
8. After chloroplast targeting by pathogen-derived effectors, what happens to plastidic fatty acid and PA metabolism, and how do they affect SA and JA biosynthesis and signalling?

Answering these questions should lead to an in-depth understanding of the roles of PLD–PA complex signalling during plant defence responses.

Supplementary data

Supplementary data are available at JXB online.

Supplementary Table S1. List of PA target proteins and detailed characteristics about them.

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