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Crosstalk between phospholipase D and sphingosine kinase in plant stress signaling

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The activation of phospholipase D (PLD) produces phosphatidic acid (PA), whereas plant sphingosine kinase (SPHK) phosphorylates long-chain bases to generate long-chain base-1-phosphates such as phytosphingosine-1-phosphate (phyto-S1P). PA and phyto-S1P have been identified as lipid messengers. Recent studies have shown that PA interacts directly with SPHKs in *Arabidopsis*, and that the interaction promotes SPHK activity. However, SPHK and phyto-S1P act upstream of PLDα1 and PA in the stomatal response to abscisic acid (ABA). These findings indicate that SPHK/phyto-S1P and PLD/PA are co-dependent in the amplification of lipid messengers, and that crosstalk between the sphingolipid- and phospholipid-mediated signaling pathways may play important roles in plant stress signaling.

Keywords: phospholipase D, phosphatidic acid, sphingosine kinase, phytosphingosine, lipid signaling, abscisic acid

INTRODUCTION

Different classes of lipids have been implicated as lipid messengers in plant growth, development, and stress responses, and recent results have begun to unveil complex interactions among different lipid signaling pathways (Peters et al., 2010; Guo et al., 2011). Under a given stress, more than one lipid mediators are often produced, with some being antagonistic and others having similar functions. Both phosphatidic acid (PA) and long-chain base-1-phosphate (LCBP) promote abscisic acid (ABA)-mediated stomatal closure and decrease reactive oxygen species (ROS)-induced cell death (Jacob et al., 1999; Zhang et al., 2003; Coursol et al., 2005; Shi et al., 2007). ABA and ROS are pivotal signals impacting various aspects of plant growth and stress responses. This raises intriguing questions of how these two lipid signaling processes interact to mediate plant stress responses. Recent results indicate a crosstalk between phospholipase D (PLD) and sphingosine kinase (SPHK) during the production of lipid messengers. These interactions of phospholipid- and sphingolipid-mediated signaling pathways may play important roles in plant response to various stresses.

DIFFERENT PLDS INVOLVED IN DIVERSE STRESS RESPONSES

Phospholipase D hydrolyzes phospholipids to produce PA and a free head group (**Figure 1**). This enzyme was first discovered in plants and has since been found to occur also in bacteria, fungi, and animals (Wang et al., 1994; Qin et al., 1997; Wang, 2001). The *Arabidopsis* genome has 12 genes encoding PLDs, which are grouped into six classes, PLDα(1–3), β(1, 2), γ(1–3), δ, ε, and ζ(1, 2) based on the gene sequences, protein domain structures, and enzymatic biochemical properties (Wang et al., 2006). PLDα, β, γ, δ, and ε contain a Ca^{2+}/phospholipids-binding C2 domain whereas PLDζ1 and ζ2 contain the pleckstrin homology (PH) and phox homology (PX) domain (Wang et al., 2006). All the PLDs have two conserved HxKxxxD (HKD) motifs that are involved in catalytic activities (Wang et al., 2006; Li et al., 2009). Some of the C2-containing PLDs contain a polyphosphoinositide-binding region (PBR1) located between two HKD domains, which binds phosphatidylinositol 4,5-bisphosphate (PIP_2; Zheng et al., 2002).

These sequence differences provide a structural basis for distinctively different biochemical properties for different PLDs. All the C2-containing PLDs require Ca^{2+} for activity, but PX and PH-containing PLDζs do not (Wang et al., 2006). In addition, the differences in the C2 sequences can explain in part, the different Ca^{2+} concentration requirements. PLDα1 is most active when assayed at millimolar [Ca^{2+}] whereas PLDβ1 and PLDγ1 require micromolar concentrations of Ca^{2+} for optimal activity and also require PIP_2 as a co-factor (Qin et al., 1997; Zheng et al., 2002; Pappan et al., 2004). PLDδ and PLDε both are active within a broad range of Ca^{2+} concentrations (μM–mM; Hong et al., 2008, 2009). PLDδ requires oleate and PIP_2 for its activity, but PLDε is active under the reaction conditions of PLDα1, β1, γ1, and δ (Wang and Wang, 2001; Qin et al., 2002; Hong et al., 2008). *Arabidopsis* PLDs also selectively hydrolyze common membrane phospholipids such as PC, PE, and PG (Li et al., 2009). The varied co-factor requirements and substrate preferences for different PLDs indicate that specific PLDs are activated differently in the cell, and may have unique cellular and physiological functions (Li et al., 2009).

Different PLDs are involved in various physiological processes, displaying unique and overlapping functions (**Figure 1**; Li et al., 2009). *PLDα1*-deficient plants have an altered plant response to several stresses, including water loss (Sang et al., 2001a), ROS production (Sang et al., 2001b; Zhang et al., 2009), and salt tolerance (Bargmann et al., 2009; Yu et al., 2010). PLDδ is involved in freezing tolerance (Li et al., 2004), dehydration (Katagiri et al., 2001), salt tolerance (Bargmann et al., 2009), H_2O_2-induced programmed cell death (PCD; Zhang et al., 2003), microtubule organization, and cytoskeletal rearrangement (Gardiner et al., 2001, 2003). PLDα3 is also involved in salt tolerance (Hong et al., 2008) whereas

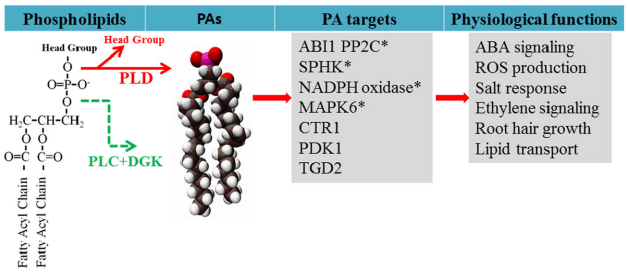

FIGURE 1 | Generation of PA from phospholipids and PA target proteins functionally characterized in plants. PA is generated via two pathways during stress responses: PLD hydrolyzes phospholipid to generate PA, and PLC hydrolyzes phospholipid to generate DAG which can be phosphorylated by DAG kinase (DGK). PA has been found to interact with target proteins to regulate cellular functions. Examples of PA regulation of target proteins are discussed in the text and the references are cited in the text. *Indicates that PA is generated from PLD while others are not determined.

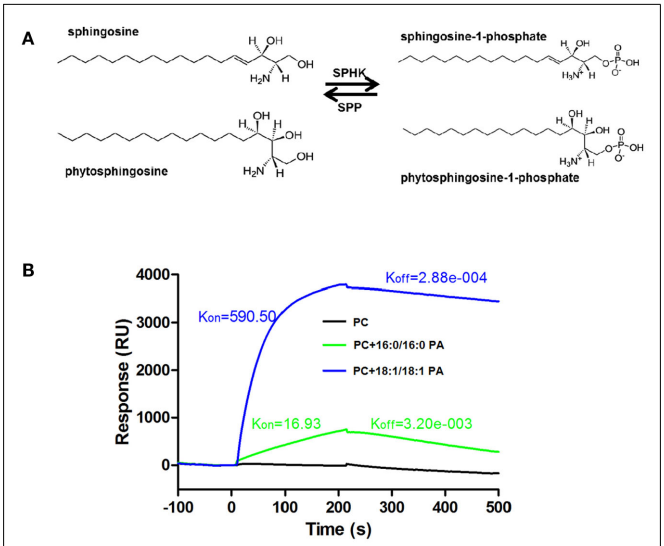

FIGURE 2 | Phosphorylation of sphingosine and phytosphingosine by SPHK and the interaction SPHK and PA. (A) SPHK catalyzes the formation of S1P or phyto-S1P from sphingosine or phytosphingosine. S1P or phyto-S1P can be degraded by S1P phosphatase (SPP) or S1P lyase (not shown). **(B)** Surface plasmon resonance (SPR) analysis of interaction of PA with SPHK1. Liposomes containing PC only or PC plus 16:0/16:0 or 18:1/18:1 PA were used to analyze the interaction. Liposome containing of PC did not bind to SPHK1. Liposomes containing both PC and PA (16:0/16:0 or 18:1/18:1) bound to SPHK1. **(B)** is based on data from Guo et al., 2011.

PLDε enhances *Arabidopsis* nitrogen signaling and growth (Hong et al., 2009). PLDζ1 and ζ2 are involved in lipid remodeling and root growth in plant responses to phosphate deprivation (Cruz-Ramirez et al., 2006; Li et al., 2006a,b). PLDζ1 is implicated in root-hair patterning (Ohashi et al., 2003), and PLDζ2 participates in vesicle trafficking to regulate auxin response (Li and Xue, 2007).

PA AS A PIVOTAL CLASS OF LIPID MESSENGERS

One mechanism by which PLDs affect plant stress responses is to produce PA, which has been identified as a class of lipid messengers in plants and animals (**Figure 1**). PA constitutes less than 1% of total phospholipids in most plant tissues, but the cellular level of PA changes dynamically in plants under abiotic and biotic stresses (Wang et al., 2006). The amount of PA in *Arabidopsis* leaves increased more than 60% within 10 min of application of ABA (Zhang et al., 2004). Other stresses, including wounding, freezing, various osmotic stresses, oxidative stress, and drought, induce accumulation of PA (Li et al., 2009). Manipulations of various PLDs in *Arabidopsis* have shed light on the regulatory functions of PA. Characterization of knockouts, knockdown, and overexpression lines of PLDs, has shown that PA produced from different PLDs has unique roles in plant response to different stresses, including water deficits, high salinity, freezing, phosphate deprivation, nitrogen availability, and plant-pathogen interactions (Sang et al., 2001b; Zhang et al., 2003; Hong et al., 2008, 2009; Bargmann et al., 2009; Peters et al., 2010).

One mode of PA action is its direct interaction with target proteins (**Figure 1**). In yeast and animal cells, PA binds to transcriptional factors, protein kinases, lipid kinases, protein phosphatases, and proteins involved in vesicular trafficking and cytoskeletal rearrangement (Wang et al., 2006; Gomez-Cambronero, 2010). In plants, PA has been found to interact with ABI1 PP2C phosphatase (Zhang et al., 2004), phosphoinositide-dependent protein kinase1 (Anthony et al., 2004), phosphoenolpyruvate carboxylase (Testerink et al., 2004), CTR1 protein kinase (Testerink et al., 2007), the actin capping protein AtCP (Huang et al., 2006), lipid transport protein TGD2 (Lu and Benning, 2009), NADPH oxidase (Zhang et al., 2009), mitogen-activated protein kinase 6 (Yu et al., 2010), and SPHK (Guo et al., 2011; **Figure 1**). Several potential PA-interacting proteins were identified by PA-affinity chromatography followed by mass spectrometric analyses in plants (Testerink et al., 2004). PA-protein interaction may modulate the function of a protein in two ways, tethering it to the membrane to change their localization, and/or increasing or decreasing the enzyme catalytic activity. For example, PLDα1-derived PA interacts with ABI1 and tethers ABI1 to the plasma membrane (Zhang et al., 2004). PA binds to *Arabidopsis* NADPH oxidase and SPHK to promote their activity (Zhang et al., 2009; Guo et al., 2011).

In addition to PLD, signaling PA can be produced by the diacylglycerol (DAG) kinase phosphorylation of DAG, which is often produced by the activation of phospholipase C (PLC; **Figure 1**). Two distinctively different PLC families have been described in plants, the phosphoinositol 4,5-bisphosphate-hydrolyzing PI-PLC (Munnik, 2001) and the non-specific PLC (NPC) that hydrolyze common membrane phospholipids such as PC and PE (Peters et al., 2010). It should be noted that DAG itself can serve as a lipid mediator; DAG promotes stomatal opening (Lee and Assmann, 1991; Peters et al., 2010), whereas PA promotes stomatal closure (Jacob et al., 1999; Zhang et al., 2004; Mishra et al., 2006).

SPHKs IN PLANTS

Sphingosine kinase is a member of the DAG kinase family (Strub et al., 2010), and phosphorylates long-chain bases (LCBs) to LCBPs, such as sphingosine-1-phopshate (S1P) and phyto-S1P (**Figure 2A**). SPHK activity and function have been well characterized in animals and yeast (Worrall et al., 2003). In mammals,

two SPHKs and their product S1P have important roles in regulation of many cellular processes including cell growth, suppression of apoptosis, and pathophysiology of various diseases (Strub et al., 2010). While sphingosine (d18:1$^{\Delta 4}$) is the predominant LCB in animal cells, it is only detected as a minor LCB in some plants or absent in other plants, such as *Arabidopsis* (Lynch et al., 2009; Michaelson et al., 2009). A recent survey of 21 species from different phylogenetic groups has found that d18:1$^{\Delta 4}$ is present in non-seed land plants and monocots (wheat, barley, maize, and ryegrass), but it is absent in *Arabidopsis* and soybean (Islam et al., 2012). Instead, 4-hydroxy-sphingenine (t18:0, commonly known as phytosphingosine), 4-hydroxy-8-sphingenine (t18:1Δ^8), and 8-sphingenine (d18:1Δ^8) are predominant LCBs in plants (Lynch et al., 2009). Plant extracts and purified SPHKs phosphorylate various LCBs to generate LCBPs (Coursol et al., 2005; Guo et al., 2011).

The *Arabidopsis* genome contains five genes with sequence similarities to mammalian SPHKs. At5g23450 encodes a LCB kinase AtLCBK1 (Nishiura et al., 2000; Imai and Nishiura, 2005) whereas At5g51290 is regarded as a ceramide kinase (Liang et al., 2003). At2g46090 did not have sphingosine-phosphorylating activity (Worrall et al., 2008). At4g21540 was originally annotated as one SPHK, and this sequence consists of two repeats that are most similar to mammalian SPHKs. A cDNA from the second repeat was reported to encode an active SPHK, designated SPHK1 (Worrall et al., 2008). A recent study has established that the At4g21540 locus is actually comprised of two separate *SPHK* genes, *SPHK1* and *SPHK2*. The conclusion is supported by molecular cloning, sequence analysis, and the distinguishable patterns of expression of *SPHK1* and *SPHK2* in *Arabidopsis* tissues (Guo et al., 2011). The stop codon of *SPHK2* is 788 bp upstream of the start codon of *SPHK1*. Both SPHK1 and SPHK2 were localized on tonoplasts (Worrall et al., 2008; Guo et al., 2011). SPHK1, SPHK2, and AtLCBK1 utilize various LCBs as substrates with different preference. Among the substrates tested, AtLCBK1 prefers D-*erythro*-dihydrosphingosine to sphingosine and phytosphingosine, whereas SPHK1 and SPHK2 are most active on sphingosine. AtLCBK1 cannot phosphorylate D-*threo*-dihydrosphingosine (Imai and Nishiura, 2005) but both SPHK1 and 2 can even though SPHK2 has much a lower activity than SPHK1 (Guo et al., 2011). Because of the low occurrence of sphingosine in plant tissues and the broad substrate specificity of SPHKs, it was suggested that plant SPHKs should be called LCB kinase (LCBK) in plants (Lynch et al., 2009). This change will require renaming some of the genes in the family. SPHK1 and SPHK2 are used here for consistency with published nomenclature on these enzymes (Worrall et al., 2008; Guo et al., 2011, 2012).

LCBs AS LIPID MEDIATORS

Like glycerophospholipids, sphingolipids serve not only as a main component of cell membranes, but also important signaling molecules (Lynch et al., 2009; Pata et al., 2010). S1P is produced in animal cells by two SPHKs and is degraded either by S1P lyase or S1P phosphatases (**Figure 2A**). S1P regulates a variety of developmental and disease processes in animals (Strub et al., 2010). Many lines of evidence indicate that S1P is an intracellular messenger acting directly on intracellular target proteins (Maceyka et al., 2012). In addition, S1P is exported out of cells to mediate signaling pathways through five specific G protein-coupled receptors (S1RP1–S1RP5) on the plasma membrane (Maceyka et al., 2012).

Sphingolipids are emerging as important mediators in plants and accumulating evidence indicates that sphingolipid metabolites, including LCBs, LCBPs, and ceramides, are involved in various signaling pathways in plants (Lynch et al., 2009; Pata et al., 2010). Characterization of *Arabidopsis* deficient in sphingolipid metabolism genes facilitates the understanding of signaling and physiological functions of sphingolipid in plants. The key roles of sphingolipids in PCD have been extensively investigated (Berkey et al., 2012). For example, characterization of ceramide kinase mutant (*acd5*) shows that ceramide induces plant PCD whereas phosphorylated ceramide partially attenuates PCD (Liang et al., 2003). Recent studies suggest that both LCB and LCBP are involved in PCD (Shi et al., 2007; Alden et al., 2011). Mutation of a LCB1 subunit of serine palmitoyltransferase blocks accumulation of LCBs in *Arabidopsis* and indicates that LCBs are involved in initiating PCD through induction of ROS production in *Arabidopsis* (Shi et al., 2007; Wang et al., 2008). LCBPs have been shown to decrease ROS-induced PCD whereas unphosphorylated LCBs promote ROS-mediated cell death (Shi et al., 2007). LCB-induced ROS production is also found to depend on NADPH oxidase Respiratory Burst Oxidase Homolog D (Peer et al., 2011). Recently, a study indicates that another subunit of serine palmitoyltransferase, LCB2a, is required for PCD, and MPK6 mediates downstream signal in LCB-induced PCD (Saucedo-Garcia et al., 2011). These results suggest that the balance between unphosphorylated and phosphorylated form of sphingolipids may function as a rheostat in regulation of PCD.

SPHK/PHYTO-S1P AND PLD/PA BOTH INVOLVED IN THE ABA SIGNALING PATHWAY

One of the functions that have been studied for SPHK and phyto-S1P is their roles in mediating ABA-promoted stomatal closure. ABA treatments increased SPHK activity in *Arabidopsis* and drought stress induced the production of LCBPs in *Commelina communis* (Ng et al., 2001; Coursol et al., 2003). Application of S1P induces stomatal closure and inhibits stomatal opening (Ng et al., 2001). Knockout of either *SPHK1 or SPHK2* decreased the sensitivity to ABA in *Arabidopsis*, whereas overexpression of *SPHK1* or *SPHK2* increased ABA sensitivity (Worrall et al., 2008; Guo et al., 2011). The involvement of LCBP in the ABA signaling in guard cells is further supported by analysis of the LCBP phosphatase AtSPP1 mutant *spp1* (**Figure 2A**). AtSPP1 is suggested to be involved in regulation of LCBP level during ABA response. The *spp1* plants displayed increased sensitivity to ABA in stomatal closure due to a defect in LCBP degradation in the mutant (Nakagawa et al., 2011). Thus, LCBP levels regulated by SPHKs and AtSPP1 may play an important role in the ABA signaling pathway.

Likewise, a number of studies have shown that PLD and PA play important roles in signaling ABA-mediated stomatal closure (Jacob et al., 1999; Zhang et al., 2004). PLD and PA promote open stomata to close and meanwhile prevent the closed stomata from opening (Jacob et al., 1999; Zhang et al., 2004). In *Arabidopsis*, *PLDα1*-deficient plants displayed insensitivity to ABA, whereas

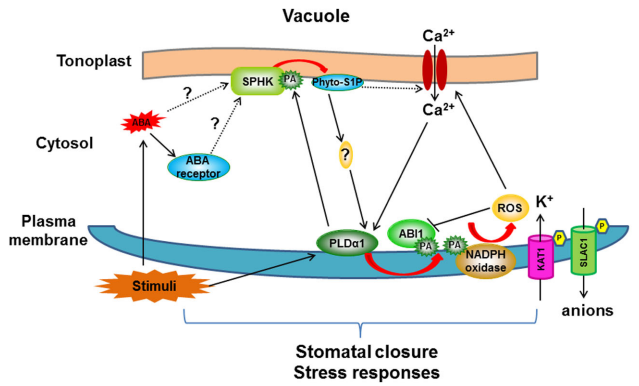

FIGURE 3 | Proposed model for crosstalk between PLDα1/PA and SPHK/phyto-S1P in ABA-mediated stomatal closure signaling pathway. ABA may be perceived by the receptor (PYR/PYL/RCAR) in the cytosol, leading to activation of SPHK to produce phyto-S1P which initiates a cascade to activate PLDα1. PLDα1 hydrolyzes phospholipids to increase PA level in membrane (plasma membrane and tonoplast). PLDα1-deprived PA promotes the ABA effect through three targets: (i) PA binds to ABI1 and tethers ABI1 to the membrane to inhibit its negative effect; (ii) PA stimulates plasma membrane-localized NADPH oxidase to form secondary messenger: ROS; (iii) Increased PA in tonoplast interacts with SPHK and promotes its activity to form a positive loop. PLDα1/PA- and SPHK/phyto-S1P-mediated signaling pathway activates ion channel activity, leading to ion flux in guard cell and finally stomatal closure. Note that this model summarizes the crosstalk between PLDα1/PA and SPHK/phyto-S1P and their roles in ABA-mediated stomatal closure, not all ABA signaling components are included in this model. Arrow indicates positive regulation, bar indicates repression. Red arrow represents reactions which produce secondary signaling molecules.

overexpression (OE) of *PLDα1* resulted in increased sensitivity to ABA (Sang et al., 2001a). PLDα1 regulates ABA signaling pathways through different interactions (**Figure 3**). PA binds to ABI1 phosphatase 2C, and this interaction inhibits the negative function of ABI1 in ABA response and mediates ABA-promoted stomatal closure (Zhang et al., 2004; Mishra et al., 2006). On the other hand, PLDα1 interacts with Gα to mediate the ABA inhibition of stomatal opening (Zhao and Wang, 2004; Mishra et al., 2006). In addition, PLDα1-derived PA binds to and increases NADPH oxidase activity to promote the production of ROS in ABA-mediated stomatal closure (**Figure 1**; Zhang et al., 2009).

PA INTERACTION WITH SPHK TO PROMOTE LCBP PRODUCTION

The findings that both PLD/PA and SPHK/phyto-S1P are involved in stomatal closure raise an intriguing question of whether the two lipid signaling processes interact to mediate plant responses to ABA and stress. A recent study investigated the direct interaction of PA with two *Arabidopsis* SPHKs (Guo et al., 2011). PA binds to both *Arabidopsis* SPHKs and the interaction stimulates SPHK activity. The interaction was demonstrated by different approaches, including lipid-filter binding, liposome binding, surface plasmon resonance (SPR), and validated using PA-SPHK co-precipitation from protoplasts (**Figure 2B**; Guo et al., 2011, 2012). PA has various molecular species which differ in acyl chain length and degree of saturation. PAs with 18:1/18:1, 16:0/18:1, and 16:0/18:2 acyl chains bind strongly to both SPHKs, whereas 16:0/16:0, 8:0/8:0, 18:0/18:0, and 18:2/18:2 PAs bind poorly to SPHKs (Guo et al., 2011).

The identification of SPHKs as molecular targets of PA indicates that PA may mediate the ABA activation of SPHK in plants. Indeed, in response to ABA, the LCBP level is lower in *pldα1*. In addition, the application of PA increased the LCBP production in protoplasts (Guo et al., 2012). These results are consistent with the hypothesis that SPHK activation by ABA is mediated by PA. 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., 2012). Taken together, these results indicate a co-dependence of PLD/PA and SPHK/phyto-S1P in the production of PA and phyto-S1P lipid messengers (**Figure 3**).

SPHK/LCBP ACTING UPSTREAM OF PLD/PA

To delineate the signaling steps of PLDα1 and SPHKs in the ABA signaling, PA and phyto-S1P were supplemented to the epidermal peels of *PLDα1* or *SPHK*-deficient plants. PA promoted stomatal closure in *PLDα1*-KO or *SPHK*-KO leaves, whereas phyto-S1P promoted stomatal closure in *SPHK*-KO but not in *PLDα1*-KO mutant. Furthermore, the addition of 1-butanol, which suppresses PA production by PLD, attenuated the effect of phyto-S1P-induced stomatal closure (Guo et al., 2012). These results suggest that phyto-S1P-mediated stomatal closure requires PLDα1, and that SPHK/phyto-S1P acts upstream of PLDα1.

These enzymatic, genetic, physiological, and lipid analyses indicate a positive interplay between the two lipid signaling processes, SPHK/phyto-S1P and PLD/PA, in plant response to stresses (**Figure 3**). ABA is produced under various stresses, such as drought and high salinity. ABA activates SPHKs to generate phyto-S1P which promotes the activation of PLDα1, possibly through increasing the cytoplasmic Ca^{2+} concentration (**Figure 3**). PA produced by activated PLDα1 binds to SPHK and promotes SPHK activity, forming a positive feedback loop in response to ABA. The resulting increase in PA regulates downstream proteins including ABI1 and NADPH oxidase in ABA-mediated stomatal closure (Zhang et al., 2004, 2009; Mishra et al., 2006; **Figure 3**).

The interplay between PLDα1 and SPHK provides insights to a mechanism by which stress signaling events are communicated between the plasma and vacuolar membranes (**Figure 3**). The subcellular localization of membrane-based lipid signaling is expected to play an important role in 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. In animals, acidic phospholipids including PA have been shown to stimulate SPHK activity (Olivera et al., 1996). PA has been implicated in promoting the intracellular translocation of cytosolic murine SPHK1 to membrane regions that are enriched in PA (Delon et al., 2004). By comparison, SPHKs in *Arabidopsis* are already associated with tonoplasts, and surface dilution kinetics analysis indicates that PA stimulates SPHK activity by promoting the substrate binding to the catalytic site of SPHK (Guo et al., 2011). At present, the source and level of free LCBs in the tonoplast are unknown. LCBs are synthesized in the ER

and the level of free LCBs is very low in *Arabidopsis*. It might be possible that LCBs are released from the catabolism of complex sphingolipids by ceramidase activity. In addition, the level of free LCBs may increase via *de novo* biosynthesis in response to stimuli. For example, infection of *Pseudomonas syringae* triggered *de novo* synthesis of phytosphingosine from sphinganine and phytosphingosine constitutes about 5–8% of total LCBs in *Arabidopsis* leaves (Lynch et al., 2009; Maceyka et al., 2012).

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., 2012). 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.

PERSPECTIVES

The progress in understanding the crosstalk of signaling events provides a functional arrangement of SPHK/phyto-S1P and PLD/PA in transducing ABA signals in guard cells. Meanwhile, the connections between the two lipid signaling processes raise many questions that warrant further investigations. ABA signaling involves multiple pathways and many regulatory elements. A core pathway of ABA signaling has been established: ABA binds to the receptor PYR/PYL/RCARs, leading to inhibition of negative regulator type 2C protein phosphatases such as ABI1, resulting in SNF1-related kinase 2 (SnRK2) activation involved in mediating downstream signaling (Klingler et al., 2010; Umezawa et al., 2010). How would the SPHK/phyto-S1P- and PLD/PA-mediated processes interact with the PYR/PYL/RCAR-ABI1-SnRK2 components? PA has been shown to bind to and inhibit ABI1 (Zhang et al., 2004). Would the PYR/PYL/RCAR-ABI1-SnRK2 components be involved in the activation of SPHK and/or PLD? ABA is a key stress hormone involved in plant response to various stresses. Is the crosstalk between PLDα1/PA and SPHK/phyto-S1P involved in other regulatory pathways in plant response to other stresses? Both PA and LCBP have been implicated in decreasing ROS-induced PCD, and different PLDs have been shown to promote ROS production and response (Zhang et al., 2003, 2009). PLDs are activated rapidly under various stress conditions. Would the activation of the plasma membrane-associated PLDs act upstream of SPHKs under different stresses? In addition, multiple LCB kinases, including AtLCBK1, SPHK1, and SPHK2, exist in *Arabidopsis*, and double and triple mutants deficient in two or more of these kinases can be made to help determine the role of these enzymes in the production and function of LCBPs in plant growth, development and response to stresses. Further study of the signaling events will lead to a better understanding of how plants adapt to stresses and changing environments.

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