Crosstalk between phospholipase D and sphingosine kinase in plant stress signaling

Liang Guo1,2 and Xuemin Wang1,2*

1Department of Biology, University of Missouri, St. Louis, MO, USA
2Donald Danforth Plant Science Center, University of Missouri, St. Louis, MO, USA

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, PLDα1(1–3), β1(1, 2), γ1(1–3), δ, ε, and ζ1 based on the gene sequences, protein domain structures, and enzymatic biochemical properties (Wang et al., 2006). PLDα, β, γ, δ, ε, and ζ contain a Ca2+ /phospholipids-binding C2 domain whereas PLDε1 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 (PIP2; 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 Ca2+ for activity, but PX and PH-containing PLDεs do not (Wang et al., 2006). In addition, the differences in the C2 sequences can explain in part, the different Ca2+ concentration requirements. PLDα1 is most active when assayed at millimolar [Ca2+] whereas PLDβ1 and PLDγ1 require micromolar concentrations of Ca2+ for optimal activity and also require PIP2 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 Ca2+ concentrations (μM–mM; Hong et al., 2008, 2009). PLDδ requires oleate and PIP2 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), H2O2-induced programmed cell death (PCD; Zhang et al., 2003), microtubule organization, and cytoskeletal rearrangement (Gardiner et al., 2001, 2003). PLDε3 is also involved in salt tolerance (Hong et al., 2008) whereas...
PLDs enhances Arabidopsis nitrogen signaling and growth (Hong et al., 2009). PLDα1 and ε2 are involved in lipid remodeling and root growth in plant responses to phosphate deprivation (Cruz-Ramírez et al., 2006; Li et al., 2006a,b). PLDα1 is implicated in root-hair patterning (Ohashi et al., 2003), and PLDα2 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 transcription 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 protein phosphatase1 (Anthony et al., 2004), phosphoenolpyruvate carboxylase (Testeink et al., 2004), CTRL protein kinase (Testeink 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 (Testeink 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 phosphoinositide 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-phosphate (SIP) and phyto-SIP (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 \((d18:1^\Delta4)\) 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 \(d18:1^\Delta4\) 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-sphingosine \((t18:0,\text{ commonly known as phytosphingosine})\), 4-hydroxy-8-sphingenine \((t18:1^\Delta8)\), and 8-sphingenine \((d18:1^\Delta8)\) 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 (Nishiyama et al., 2000; Imai and Nishiyama, 2005) whereas At5g51290 is regarded as a ceramide kinase (Liang et al., 2003). At2g46090 did not have sphingosine-phosphorylating activity enzymes (Worrall et al., 2008; Guo et al., 2011, 2012). used here for consistency with published nomenclature on these renaming some of the genes in the family. SPHK1 and SPHK2 are similar to mammalian SPHKs. At4g21540 locus is actually comprised of two separate 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 \(\alpha\)-erythro-dihydrosphingosine to sphingosine and phytosphingosine, whereas SPHK1 and SPHK2 are most active on sphingosine. AtLCBK1 cannot phosphorylate D-threo-dihydrosphingosine (Imai and Nishiyama, 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 \((\text{LCBK})\) 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 \((\text{S1RP1–S1RP5})\) 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 \((\text{acd5})\) 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*, PLD*α1*-deficient plants displayed insensitivity to ABA, whereas
ABA (Sang et al., 2001a). PLD and SPHKs and the interaction stimulates SPHK activity to promote the production of ROS in ABA-mediated stomatal closure (Zhao and Wang, 2004; Mishra et al., 2006). In addition, PLD closure (Zhang et al., 2004; Mishra et al., 2006). 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 lipid messengers (Guo et al., 2011). PA binds to ABI1 and SPHK/phyto-S1P-mediated signaling pathway activates ion channel 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 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 Gu 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 plda1. 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).
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 sphingosine-1-P (possibly along with other LCBPs) on the vacuolar membrane. Phytosphingosine does not activate PLDα1 directly in vitro (Guo et al., 2012). It was shown that SIP causes an increase in Ca^{2+} in response to ABA (Ng et al., 2001), and thus sphingosine-1-P 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.

**REFERENCES**

Alden, K. P., Dhondt-Cordelier, S., Mcdonald, K. L., Reape, T. J., Ng, C. K., Mccabe, P. F., and Leaver, C. J. (2011). Sphingolipid long chain base phosphates can regulate apoptotic-like programmed cell death in plants. Biochem. Biophys. Res. Commun. 410, 574–580.

Anthony, R. G., Henriques, R., Helfer, A., Meszaros, T., Rios, G., Testerink, C., Mumik, T., Deak, M., Koncz, C., and Bogre, L. (2004). A protein kinase target of a PD13 signalling pathway is involved in root hair growth in Arabidopsis. EMBO J. 23, 572–581.

Bargmann, B. O., Lacalt, A. M., Ter Riet, B., Van Schooten, B., Merquel, E., Testerink, C., Haring, M. A., Barrels, D., and Mumik, T. (2009). Multiple PLDαs required for high salinity and water deficit tolerance in plants. Plant Cell Physiol. 50, 78–89.

Berkey, R., Bendigeri, D., and Xiao, S. (2012). Sphingolipids and plant disease/disease: the “death” connection and beyond. Front. Plant Physiol. (in press).

Cousrot, S., Fan, L. M., Le Stunff, H., Spiegel, S., Gilroy, S., and Assmann, S. M. (2003). Sphingolipid signalling in Arabidopsis guard cells involves heterotrimeric G proteins. Nature 423, 651–654.

Cousrot, S., Le Stunff, H., Lynch, D. V., Gilroy, S., Assmann, S. M., and Spiegel, S. (2005). Arabidopsis sphingosine kinase and the effects of sphingosine-1-phosphate on stomatal aperture. Plant Physiol. 137, 724–737.

Cruz-Ramirez, A., Oropéza-Aburto, A., Razo-Hernandez, F., Ramírez-Chavez, E., and Herrera-Estrella, L. (2006). Phospholipase D2 plays an important role in extraplasmatic galactolipid biosynthesis and phosphatidic acid recycling in Arabidopsis roots. Proc. Natl. Acad. Sci. U.S.A. 103, 6765–6770.

Delon, C., Manifava, M., Wood, E., Thompson, D., Krugmann, S., Pyne, S., and Kitakis, N. T. (2004). Sphingosine kinase 1 is an intracellular effector of phosphatidic acid. J. Biol. Chem. 279, 44763-44774.

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

Gardiner, J., Collins, D. A., Harper, J. D., and 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.

Gardiner, J. C., Harper, J. D., WeeraKoon, N. D., Collins, D. A., Ritchie, S., Gilroy, S., Cyr, R. J., and Marc, J. (2001). A 90-kD phospholipase D from tobacco binds to microtubules and the plasma membrane. Plant Cell 13, 2143–2158.

Gomez-Cambronero, J. (2010). New concepts in phospholipase D signaling in inflammation and cancer. ScientificWorldJournal 10, 1356–1369.

Guo, L., Mishra, G., Markham, J. E., Li, M., Tawfall, A., Welti, R., and Wang, X. (2012). Connections between sphingosine kinase and phospholipase D in the abscisic acid signaling pathway in Arabidopsis. J. Biol. Chem. doi: 10.1074/jbc.M111.274274

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

Hog, Y., Deviaia, S. P., Bahn, S. C., Thamasandra, B. N., Li, M., Welti, R., and Wang, X. (2009). Phosphatidic acid biosynthesis and regulation in Arabidopsis. Plant Physiol. 150, 869–906.

Hog, Y., Pan, X., Welti, R., and Wang, X. (2008). Phosphatidic acid D3 is involved in the hypersensitive response in Arabidopsis. Plant Cell 20, 803–816.

Huang, S., Gao, L., Blanchon, L., and Staber, C. J. (2006). Heterodimeric capping protein from Arabidopsis is regulated by phosphatidic acid. Mol. Biol. Cell 17, 1946–1958.

**ACKNOWLEDGMENTS**

Work from Xuemin Wang lab was supported by grants from the National Science Foundation (IOS-0818740; MCB-0922879) and the US Department of Energy (DE-SC0001295).
Ceramides modulate programmed cell death in plants. *Genes Dev.* 17, 3636–3641.

Lu, B., and Benning, C. (2009). A 23-amino acid sequence of the Arabidopsis TGD2 protein is sufficient for specific binding of phosphatic acid. *J. Biol. Chem.* 284, 17420–17427.

Lynch, D. V., Chen, M., and Cai, H. (2003). Lipid signaling in Arabidopsis: no sphingosine! No problem! *Trends Plant Sci.* 8, 463–466.

Maceyka, M., Hirakumar, K. B., Milstien, S., and Spiegel, S. (2012). Phosphatidic acid–phosphatase signaling and its role in disease. *Trends Cell Biol.* 22, 50–60.

Michaelsson, L. V., Zauer, S., Markham, J. E., Haslam, R. P., Desikan, R., Maceyka, M., Heinz, E., and Napier, J. A. (2009). Functional characterization of a higher plant sphingolipid Δ4-desaturase: defining the role of sphingosine and sphingosine-1-phosphate in Arabidopsis. *Plant Physiol.* 149, 487–498.

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

Munnik, T. (2001). Phosphatidic acid: an emerging plant lipid second messenger. *Plants Cell Physiol.* 42, 835–842.

Li, G., and Xue, H. W. (2007). Arabidopsis PLD2 regulates vesicle trafficking and is required for auxin response. *Plant Cell* 19, 281–295.

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

Li, M., Qin, C., Welti, R., and Wang, X. (2006a). Double knockouts of phospholipases D1 and D2 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., and Wang, X. (2006b). Quantitative profiling of Arabidopsis polar glycerolipids in response to phosphorus starvation. Roles of phospholipases D1 and D2 in Arabidopsis affect root elongation during phosphate-limited growth but do not affect root hair patterning. *Plant Physiol.* 142, 750–761.

Li, W., Li, M., Zhang, W., Welti, R., and Wang, X. (2004). The plasma membrane-bound phospholipase D3 enhances freezing tolerance in Arabidopsis thaliana. *Nat. Biotechnol.* 22, 427–433.

Liang, H., Yao, N., Song, J. T., Luo, S., Lu, H., and Greenberg, J. T. (2003).
Guo and Wang  Crosstalk between lipid-mediated signaling pathways

Worrall, D., Ng, C. K., and Hertherington, A. M. (2003). Sphingolipids, new players in plant signaling. *Trends Plant Sci.* 8, 317–320.

Yu, L., Nie, J., Cao, C., Jin, Y., Yan, M., Wang, F., Liu, J., Xiao, Y., Liang, Y., and 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., and 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, W., Wang, C., Qin, C., Wood, T., Olafsdottir, G., Welth, R., and Wang, X. (2003). The oleate-stimulated phospholipase D, PLD8, and phosphatidic acid decrease H₂O₂-induced cell death in *Arabidopsis*. *Plant Cell* 15, 2285–2295.

Zhang, Y., Zhu, H., Zhang, Q., Li, M., Yan, M., Wang, R., Wang, L., Welth, R., Zhang, W., and 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.

Zhao, J., and 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.

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

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 13 December 2011; accepted: 28 February 2012; published online: 19 March 2012.

Citation: Guo L. and Wang X. (2012) Crosstalk between phospholipase D and sphingosine kinase in plant stress signaling. *Front. Plant Sci.* 3:51. doi: 10.3389/fpls.2012.00051

This article was submitted to Frontiers in Plant Physiology, a specialty of Frontiers in Plant Science.

Copyright © 2012 Guo and Wang. This is an open-access article distributed under the terms of the Creative Commons Attribution Non Commercial License, which permits non-commercial use, distribution, and reproduction in other forums, provided the original authors and source are credited.