# Phospholipase Ds in Plant Response to Hyperosmotic Stresses

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

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

**Keywords** Phospholipase D • Hyperosmotic stress • PA targets

## 1 Introduction

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

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

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

## 2 Multiple PLD Isoforms are Involved in Hyperosmotic Stress Response

### *2.1 Hyperosmotic Stress Induces PLD Activation*

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

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

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

## 2.2 Some PLDs Mutually Interact in Response to Stresses

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

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

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

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

### 2.3 Manipulation of PLD to Improve Stress Tolerance

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

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

## 3 The Mechanisms of Stress Signaling by PLDs

### 3.1 Protein Phosphorylation

#### 3.1.1 MAP Kinase Cascades

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

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

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

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

### 3.1.2 SnRKs and SPHKs

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

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

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

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

## 3.2 Protein Dephosphorylation

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

## 3.3 ROS

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

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

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

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

## *3.4 Cytoskeleton*

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

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

mastoparan, xylanase, NaCl, and hypo-osmotic stress can induce the release of microtubules from plasma membrane, resulting in their reorganization. However, direct evidence was lacking for the direct involvement of PLD in the regulation of microtubule organization.

A more recent study has shown that PA derived from PLDα1 regulates microtubule organization by interacting with a microtubule-associated protein 65-1 (MAP65-1) (Zhang et al. 2012). The cortical microtubules in *pldα1* mutant are sensitive to NaCl treatment, being depolymerized into dot-like structures in plants exposed to NaCl. In addition, application of PA restores the sensitive phenotype of *pldα1* mutant (Zhang et al. 2012). PA binds to a microtubule-associated protein, MAP65-1, which can then bind to and bundle microtubules stabilizing them (Chang-Jie and Sonobe 1993; Smertenko et al. 2004; Mao et al. 2005; Lucas et al. 2011). PA promotes this polymerization and bundle activity of MAP65-1 both in vitro and in vivo, and mutations of the PA-binding amino acids disrupted the binding of PA and MAP65-1, and the organization of microtubules in response to salt stress (Zhang et al. 2012). PLDα1 itself does not bind to MAP65-1 or microtubules, and PA does not bind directly to microtubules either. Furthermore, unlike PLDα1, PLDδ is not involved directly in microtubule organization under salt stress response. Knockout of PLDδ does not change microtubule patterns or salt tolerance when compared with wild type (Zhang et al. 2012). Therefore, PA from PLDα1 hydrolysis specifically regulates microtubule organization in response to salt stress. It is unclear whether other PLDs, for example, PLDα3 which is involved in salt and osmotic stress (Hong et al. 2008), regulate microtubule organization during salt or other stress responses.

In contrast to the regulation of microtubule organization by PA–MAP65-1 interaction during salt stress response, MAP65-1-bundling activity is negatively regulated by MAPK cascades during cell division and development. The phosphorylation of MAP65-1 by MAPKs reduces its microtubule-bundling activity, thereby enhancing destabilization of microtubules and promoting mitosis (Sasabe et al. 2006; Smertenko et al. 2006). The deficiency of a MAPKKK (ANP2 or ANP3) induces a lesser phosphorylation status of MAP65-1, resulting in heavy bundling of microtubules and abnormal root growth (Beck et al. 2010). These results indicate that the dynamic organization of microtubules is controlled by complex mechanisms of the cellular response to developmental and environmental signals.

It is well known that plant cells remodel their actin cytoskeleton in response to biotic and abiotic stresses. PA has been recently reported to bind a capping protein (CP) and dissociate it from the ends of actin, thus enhancing actin filament–filament annealing (Huang et al. 2006; Li et al. 2012). However, a direct link between PA–actin filaments and any stress signal is yet to be established.

### *3.5 Proline*

Proline has been thought of as a compatible osmolyte which preserves protein activity, maintains pH, and prevents oxidative damage (Delauney and Verma 1993; Amtmann 2009). The significance of proline accumulation as an osmolyte has recently been debated because the proline metabolism intermediate pyrroline-5-carboxylate (P5C) is highly toxic to the cell, directly or indirectly triggering apoptosis (Thiery et al. 2004). PLD and PLC have been found to be related to the regulation of proline accumulation. The effect of PLD and PLC on proline accumulation is dependent on both the degree of stress and the plant species (Szabados and Savouré 2009). Using 1-butanol to inhibit PA generation, Thiery et al. (2004) found that PLD functions as a negative regulator of proline accumulation in *Arabidopsis* plants under non-stressed conditions. Treatments of plants with 200 mM NaCl or 400 mM mannitol induced an increase in proline, of which PLD was probably not involved (Thiery et al. 2004), whereas PLC triggered P5CS transcription and proline accumulation during salt stress (Parre et al. 2007).

In model halophyte, *Thellungiella halophila/salsuginea*, plants accumulate high levels of proline even in the absence of stress. Pharmacological evidence shows that PLDs positively control the proline accumulation under severe stress (400 mM NaCl or 400 mM mannitol) but have no effect on its accumulation in non-stressed conditions. Inhibition of PLC by the inhibitor U73122 leads to more proline accumulation under unstressed or moderate salt stress (200 mM NaCl) conditions, suggesting that PLC is a negative regulator (Ghars et al. 2012).

## 4 Conclusions and Perspectives

Plant PLDs are a family of multifarious enzymes with different biochemical, regulatory, and structural properties. Different PLDs show unique functions in response to hyperosmotic stresses and other stresses (e.g., PLDε for nutrition deficiency) and development signals (e.g., PLDζ for root hair development, which are described in other chapters in this book). With the extensive investigation in recent years, increasing numbers of components in PLD/PA-regulated signaling pathways are characterized. As outlined in Fig. 1, PLDs transmit osmotic stress signals by regulating of the activity of protein kinases and phosphatases, NADPH oxidase, as well as cytoskeleton organization. Genetic evidence indicates that the activation of SPHKs by ABA produces phyto-S1P which in turn activates PLDα1 to produce PA (Guo et al. 2012a). Early findings indicate that phyto-S1P activate G protein (GPA1) (Coursol et al. 2003), while activated GPA1 probably release and stimulate PLDα1 (Fig. 1). However, whether this loop exists in a cell in response to a specific stimulus still needs to be investigated.

Aside from phospholipase activity, mammalian PLD (PLD2) has recently been reported to bear an additional and novel catalytic function. The PLD2 acts as a

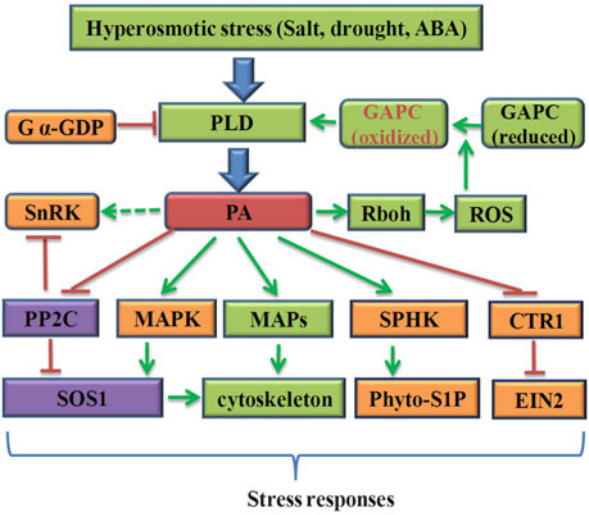


**Fig. 1** Proposed model depicting the functions of PLD/PA in response to hyperosmotic stresses. When plant cells sense hyperosmotic or other stresses, PLDs are regulated (activated) by upstream factors. For example, PLDα1 is activated with the stimulation of G protein by the conversation of Gα-GDP to Gα-GTP and release of PLDα1 from Gα-GDP binding. Different stresses may stimulate the appropriate PLDs. For example, ABA-promoted PA binds to and regulates ABI1 and NADPH oxidase in plasma membrane and interacts with SPHK in tonoplast, respectively. In response to NaCl stress, PA may bind to and activate MAPK6 to regulate SOS1antiporter and interact with MAP65-1 to stabilize microtubule, improving salt tolerance. Note that this model is not comprehensive and only includes some of the signaling components implicated in the hyperosmotic stress and ABA responses. *Arrows with solid lines* indicate established links and *arrows with dashed lines* denote putative links

guanine nucleotide exchange factor (GEF) for the small GTPase Rac2 by turnover of the inactive GDP-bound GTPase to the active GTP-bound GTPase (Mahankali et al. 2012). In *Arabidopsis*, the sequences of PLDζ1 and ζ2 are most similar to those of mammalian PLDs, raising intriguing questions of whether plant PLDζs also have GEF activity.

Therefore, in future work, it is necessary to further clarify PLD properties, including biochemical characteristics, localization, and cellular functions. Compared with identified PLD/PA targets, we know much less about the upstream effectors of PLDs such as receptors and the components connecting receptors and PLDs. More detailed and precise networks among PLDs and other signal molecules should be established using combined genetic, molecular, cellular, and physiological methods.

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