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REVIEW ARTICLE

Function and regulation of phospholipid signalling in plants

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As an important metabolic pathway, phosphatidylinositol metabolism generates both constitutive and signalling molecules that are crucial for plant growth and development. Recent studies using genetic and molecular approaches reveal the important roles of phospholipid molecules and signalling in multiple processes of higher plants, including root growth, pollen and vascular development, hormone effects and cell responses to environmental stimuli plants. The present review summarizes the current progress in our understanding of the functional mechanism of phospholipid signalling, with an emphasis on the regulation of Ins(1,4,5)P_3–Ca^{2+} oscillation, the second messenger molecule phosphatidic acid and the cytoskeleton.

Key words: phospholipid, plant, signalling.

BACKGROUND

Membranes act as barriers to hydrophilic molecules and ions because of the hydrophobic core of the phospholipid bilayer [1]. Phospholipid molecules are one of the main structural components of membranes, and they have emerged as important second messengers [2] to regulate plant growth and development and cellular responses to environmental change or stress [3].

Phosphorylations of the inositol ring of phosphatidylinositol are carried out by specific phosphoinositide kinases, including PI3Ks (phosphoinositide 3-kinases) [4], PI4Ks (phosphoinositide 4-kinases) [5] and PI5Ks (phosphoinositide 5-kinases) [6] at the D-3, D-4 or D-5 positions to generate PtdIns3*P*, PtdIns4*P* or PtdIns5*P* respectively. Sequential phosphorylation by phosphoinositide 4-phosphate 5-kinase [7] or phosphoinositide 5-phosphate 4-kinase [8] will generate PtdIns(4,5)P_2, which is then hydrolysed by PLC (phospholipase C), resulting in the production of two important second messenger molecules: Ins(1,4,5)P_3 and DAG (diacylglycerol) [2]. The inositol polyphosphates can be phosphorylated to generate InsP_6 or dephosphorylated by inositol polyphosphate phosphatases, which are classified into four groups on the basis of the position of the phosphates [9]. In addition, PLD (phospholipase D) hydrolyses phospholipids at the terminal phosphodiester bond and generates PA (phosphatidic acid) [10] (Figure 1).

The presence of many isoforms of the key enzymes in phosphoinositide metabolism has been demonstrated. Some 12 PI3/4K-domain-containing proteins are predicted to be PI4K genes [11], and there are 15 isoforms of PIP5Ks (phosphoinositide phosphate 5-kinases) [11], 15 isoforms of 5PTases (inositol polyphosphate 5-phosphatases) [12], 12 PLD isoforms [13], 12 PI4K isoforms [10] and nine PLC isoforms [10,14] in *Arabidopsis*. In rice, a monocotyledon, the distribution and functions of relevant isoforms have been less studied. An analysis showed that there are 17 PLD members [15] and four PLC members [14] in rice. Our preliminary analysis using BLAST and CLUSTW shows that there are ten PIP5Ks, 11 PI4Ks and 20 5PTase members in rice (Figure 1).

In the present review, we focus on the functions and mechanisms of PIP5K, PLC, 5PTase, PLD and the phospholipid molecules PtdIns4*P*, PtdIns(4,5)P_2, Ins(1,4,5)P_3 and PA, as well as Ca^{2+}, in plant growth and development.

CRITICAL EFFECTS OF PHOSPHOLIPIDS IN PLANT GROWTH AND STRESS RESPONSES

Root growth and root hair patterning

Mutation in *XIPOTL1*, which encodes a PEAMT (S-adenosyl-L-methionine:phosphoethanolamine N-methyltransferase) and is critical in phosphatidylcholine biosynthesis, resulted in a short primary root and short epidermal cells due to the cell death induced by decreased phosphatidylcholine content [16]. *Arabidopsis* lines with a deficiency of *INT1* (*INOSITOL TRANSPORTER 1*) show increased intracellular *myo*-inositol concentrations and reduced root growth, which is possibly due to sequestration of inositol in vacuoles [17]. *Arabidopsis* PIP5K9 negatively regulates sugar-mediated root cell elongation through interaction with a cytosolic invertase, CINV1 [18]. Conversely, suppressed *AtIPK2α* (*Arabidopsis thaliana* inositol polyphosphate kinase 2α) expression and application of exogenous Ins(1,4,5)P_3 lead to enhanced root growth [19].

Seedlings treated with the PLD inhibitor butan-1-ol or deficient in *PLDζ2* display suppressed primary root elongation and inhibited lateral root formation [20], and PLD-derived PA production is an early signalling event during adventitious root formation induced by auxin and NO in cucumber explants [21]. In addition, accumulated PtdIns(4,5)P_2 and Ins(1,4,5)P_3 under *SAC9* deficiency result in shorter primary roots and fewer lateral roots

Abbreviations used: ABA, abscisic acid; AtCP, actin-capping protein; AtIPK2, *Arabidopsis thaliana* inositol polyphosphate kinase 2; AVR4, avirulence 4; CTR1, constitutive triple response 1; DAG, diacylglycerol; DGK, DAG kinase; F-actin, filamentous actin; IAA, indoleacetic acid; IP_3R, Ins(1,4,5)P_3 receptor; LOX2, lipoxygenase 2; LPP, lipid phosphate phosphatase; MAPK, mitogen-activated protein kinase; OsPIPK, *Oryza sativa* phosphoinositide phosphate kinase; PA, phosphatidic acid; PDK1, phosphoinositide-dependent kinase 1; PI3K, phosphoinositide 3-kinase; PI4K, phosphoinositide 4-kinase; PIPK, phosphoinositide phosphate kinase; PIP5K, phosphoinositide phosphate 5-kinase; PLC, phospholipase C; PLD, phospholipase D; PP2A, protein phosphatase 2A; 5PTase, inositol polyphosphate 5-phosphatase; RACK1, receptor for activated C-kinase 1; SNX1, sorting nexin 1; ZmPLC1, *Zea mays* PLC1.

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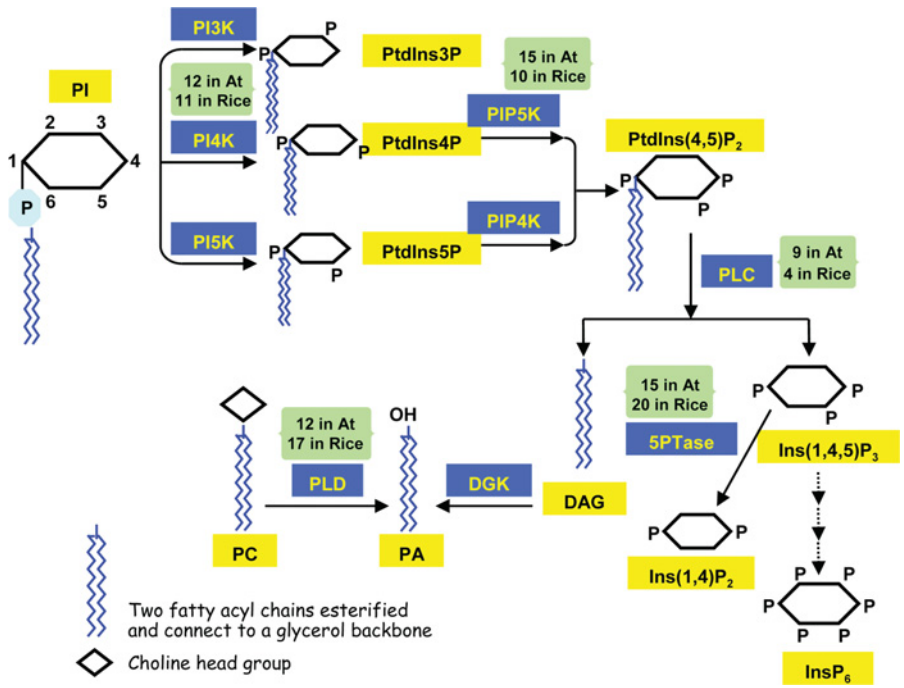

Figure 1 The phosphoinositide metabolic pathway in higher plants

The isoform numbers of the key enzymes in *Arabidopsis* (At) and rice are indicated. Abbreviations: PI, phosphoinositide; PI5K, phosphoinositide 5-kinase.

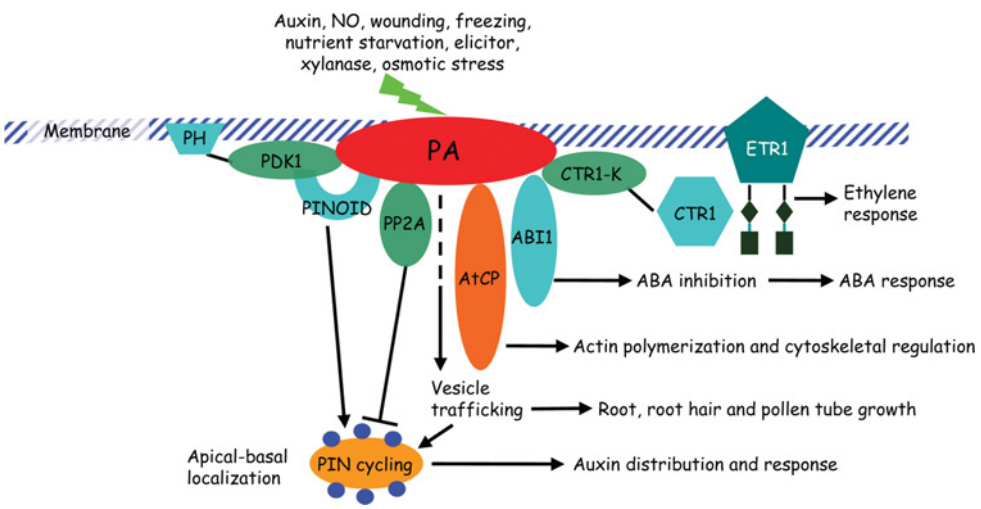

Figure 2 PA functions in hormone responses and growth of roots, root hairs and pollen tubes

PA accumulates in response to treatment with auxin and NO, wounding, freezing, nutrient starvation, elicitor, xylanase, and osmotic stress. PA could bind CTR1 and block the interaction with ethylene receptor ETR1 to regulate the ethylene response. PA binds and decreases the activity of ABI1, resulting in the insensitive response to ABA treatment. PA promotes actin polymerization by binding to and inhibiting the activity of AtCP. PA regulates cycling of PIN proteins through directly mediating vesicle trafficking, or binding and mediating the activity of auxin transport upstream proteins PDK1, PINOID and PP2A. Abbreviations: CTR1-K, CTR1 kinase domain; PH, pleckstrin homology domain.

[22]. PA takes part in root growth, root hair and pollen tube patterning by regulating cytoskeleton and vesicle trafficking [20,21,23] (Figure 2).

Regarding the root hair pattern, a deficiency of *AtSfh1*, which encodes a PITP (phosphatidylinositol transfer protein), compromises polarized root hair expansion in a manner that coincides with a loss of tip-directed PtdIns(4,5)P_2 [24]. Decreased AtIPK2α and PLDζ1 expression confers insensitivity of root hair growth to calcium depletion [19] and leads to random initiation of root hairs [25] respectively, whereas increased *PLDζ1* expression induces branched and swollen root hairs [25]. Treatment with a PI3K-specific inhibitor inhibits root hair growth by regulating PtdIns3*P*-mediated vesicle trafficking and ROS (reactive oxygen species) production [26]. The double mutant *PI4Kβ1*/*PI4Kβ2* displays aberrant root hair morphologies due to altered calcium signalling [27], whereas mutation in a phosphoinositide 4-phosphate phosphatase gene (*rhd4*) results in shorter and morphologically aberrant root hairs due to the

altered accumulation of PtdIns4*P* together with PI4Kβ1 in membrane compartments [28]. Studies by two independent groups have shown that *Arabidopsis* PIP5K3 is essential for root hair formation through its production of PtdIns(4,5)P_2 at the root hair apex. Furthermore, reduced expression of *PIP5K3* caused significantly shortened root hairs, whereas overexpression resulted in increasingly deformed root hairs [29,30]. In *Medicago truncatula*, inhibition of PI3K and PI-PLC (phosphatidylinositol-specific PLC) prevented root hair curling and the formation of infection threads [31].

Pollen development

Phospholipid signalling is involved in pollen development, including pollen viability, germination and pollen tube elongation, through regulation of the production of PtdIns3*P*, PtdIns4*P*, PtdIns(3,5)P_2, PtdIns(4,5)P_2, PA, Ins(1,4,5)P_3 and downstream $[Ca^{2+}]_{cyt}$ levels. Studies reveal that PI3K and PI4K are involved in regulation of pollen viability through different mechanisms. PI3K regulates vacuole reorganization and nuclear division in pollen grains, possibly by regulating the products PtdIns3*P* and PtdIns(3,5)P_2, which are essential for protein targeting to vacuoles and normal vacuole function and morphology respectively [32], whereas PI4Kγ1 performs crucial intracellular trafficking regulation for tapetum and microspore development [33]. In addition, PtdIns(4,5)P_2 leads to increased $[Ca^{2+}]_{cyt}$, transient growth perturbation and inhibition of apical secretion, whereas Ins(1,4,5)P_3 treatment causes a transient $[Ca^{2+}]_{cyt}$ increase of similar magnitude and stimulates apical secretion and severe growth perturbation, indicating the different targets of these two molecules [23]. Recent reports have shown that deficiency of two *Arabidopsis* pollen-expressed PIP5Ks, PIP5K4 and PIP5K5, reduced pollen germination and pollen tube elongation, which is consistent with the effect of their product, PtdIns(4,5)P_2 [34,35]. However, overexpression of either *PIP5K4* or *PIP5K5* results in multiple pollen tip branching, which is thought to occur by regulating membrane trafficking and apical pectin secretion [34,35].

PA plays an important role in pollen tube polarity by regulating microfilament polymerization and membrane transport. A reduced level of PA leads to inhibited apical plasma membrane recycling and formation of thick, but non-directional, microfilaments [23]. PLD-derived PA stimulates pollen germination and tube elongation [36] and expands the pollen tube apical region [37] (Figure 2).

In addition, because both PtdIns(4,5)P_2 and DAG are accumulated in an apical domain of the plasma membrane at the pollen tube tips, disruption of this domain causes defective pollen tube growth, which highlights the importance of maintaining an apical domain enriched in PtdIns(4,5)P_2 and DAG for polar cell growth [38].

Vascular development

Although several studies have demonstrated the role of phospholipid signalling in vascular growth and development, the mechanism is still unclear. *CVP2*, encoding a type I inositol polyphosphate 5-phosphatase 6 (*At5PTase6*), promotes vascular cell proliferation and prevents the termination of premature veins (the *cvp2*-knockout mutant shows an open cotyledon vein [39]). The type II *At5PTase13* regulates cotyledon vein formation by regulating auxin homoeostasis [40]. *FRA3*, a type II 5PTase gene, plays an essential role in secondary wall synthesis in fibre cells and xylem vessels, and the *fra3*-knockout mutant contains higher amounts of PtdIns(4,5)P_2 and Ins(1,4,5)P_3 and shows reduced thickness of the secondary wall and decreased stem strength owing to the disruption in actin organization [41]. AtSAC1 exhibits phosphatase activity toward PtdIns(3,5)P_2, and mutation of *AtSAC1* causes a dramatic decrease in the cell wall thickness of fibre cells and vessel elements, resulting in a weak stem [42], suggesting the important effect of the PtdIns(3,5)P_2 pool in the formation of aberrant cell shape.

Hormone effects

Altered phospholipid signalling shows changed responses to various hormones. Seedlings deficient in *5TPase1*, *5PTase2* [43] and *cvp2* [39] are hypersensitive to exogenous ABA (abscisic acid), whereas a deficiency in *5PTase13* causes insensitivity to ABA in seed germination [40]. PA, generated by PLDα1, binds to ABI1 and decreases the phosphatase activity of ABI1, resulting in the insensitivity of stomatal closure of PLDα1-null mutant leaves to ABA treatment [44] (Figure 2). In addition, PLDα1 directly interacts with and inactivates the G_α subunit of heterotrimeric G-proteins to mediate ABA inhibition of stomatal opening [45], and suppression of rice *PLDβ1* results in reduced sensitivity to ABA in seed germination [15].

Ins(1,4,5)P_3 oscillation mediates auxin transport and is involved in gravistimulation responses [46]. *Arabidopsis 5PTase13* and *PLDζ2* regulate the cotyledon vein [40] and root development [20] respectively by modulating auxin synthesis and transport. *PLA* activity is rapidly induced by auxin within 2 min [47], and *Arabidopsis sPLA2* regulates shoot gravitropism by regulating auxin-induced cell elongation [48].

Besides auxin and ABA, involvement of phospholipid signalling in other hormone cascades is seldom reported. Testerink et al. [49] demonstrated that PA regulates CTR1 activity to influence ethylene signalling. Lin et al. [50] pointed out that phospholipid signalling could be regulated by multiple hormones, hinting at the cross-talk between them.

Stress responses

The involvement of phospholipid signalling in the cell responses to stress stimuli, including salt, osmotic, temperature and pathogen stressors, has been well demonstrated, and multiple members of the phosphoinositide pathway are supposed to mediate the stress responses through different mechanisms.

Salt stress

Salinity is a major stress that threatens plant growth and crop production. *Arabidopsis* plants show rapidly increased PtdIns(4,5)P_2 synthesis in response to treatments with NaCl, KCl and sorbitol [51], suggesting an important role of PtdIns(4,5)P_2 in plant salt tolerance. PtdIns(4,5)P_2 and its derivative Ins(1,4,5)P_3 are accumulated in plants under salt, cold and osmotic stresses, and *sac* (suppressor of actin) mutants harbour accumulated PtdIns(4,5)P_2 and Ins(1,4,5)P_3 and show a constitutive stress response [22]. Consistent with this, pharmacological studies using U73122 (a specific inhibitor of PLC) have shown that, in salt-treated seedlings, PLC activity, as well as Ins(1,4,5)P_3 and calcium, are necessary for the expression of pyrroline-5-carboxylate synthetase and resultant pyrroline levels [52].

Recent studies revealed that salt-stress-induced association of PtdIns(4,5)P_2 with clathrin-coated vesicles [53] and PI3K-mediated endocytosis are necessary for plant salt tolerance [54], highlighting the importance of vesicle trafficking in the salt response. Multiple PLDs, including PLDα1, α3, δ and ε, are required for salt and water deficit tolerance in *Arabidopsis*, possibly by regulating PA production and membrane lipid compositions [55,56].

Drought stress

Drought directly leads to water deficiency and inhibits plant growth. Studies have shown that members of both PLC and PLD families are involved in drought tolerance. Expression of maize *ZmPLC1* (*Zea mays* PLC1) is up-regulated under dehydration and this enhanced expression improves the drought tolerance of maize [57]. Expression pattern analysis revealed that six of nine *Arabidopsis PLC* genes are stimulated by dehydration [14], suggesting a similar function of *Arabidopsis* PLCs to that of ZmPLC1. PLDs and the derived PA are also widely involved in plants' drought tolerance. Both *PLDα1* and *PLDα3* are necessary for drought tolerance in *Arabidopsis* [58]; however, the regulatory mechanisms are different. *PLDα1* mediates early drought response and ABA signalling [59], whereas *PLDα3* is involved in promoting root growth and stress avoidance under hyperosmotic conditions [55]. *PLDδ* is accumulated under dehydration and contributes to the dehydration-induced PA accumulation [60], whose knockout mutants display hypersensitivity to water deficit [56]. Recently, Perera et al. [61] showed that *Arabidopsis* transgenic plants overexpressing mammalian type I 5PTase present significantly enhanced drought tolerance.

Cold stress

A transcriptomic analysis of cold-treated *Arabidopsis* suspension cells in the presence of U73122 or ethanol, the inhibitors for PLC and PLD respectively, revealed that both PLC- and PLD-mediated signal pathways are activated simultaneously, which leads to the activation of different clusters of cold-response genes [62]. Genetic analysis reveals that different PLD isoforms have opposite effects in cold response. *Arabidopsis* plants that are deficient for *PLDα1* show improved tolerance to freezing through activating the cold-responsive genes and increasing osmolyte accumulation [63,64], whereas deficiency of the membrane-associated PLD*δ* rendered hypersensitivity to freezing (*PLDδ* overexpression resulted in increased freezing tolerance [65]). Suppression of *PLDα1* decreases phospholipid hydrolysis and PA production in both freezing and post-freezing phases, whereas ablation of *PLDδ* increases lipid hydrolysis and PA production in post-freezing recovery [66]. Chilling and freezing result in the increased Ins(1,4,5)P_3–Ca^{2+} influx [67] and transgenic plants overexpressing mammalian 5PTase display a ~30 % decrease in the rapid transient Ca^{2+} peak in response to cold or salt stimuli [61].

Oxidative stress

H_2O_2 treatment activates the PLD activity, and deficiency of *PLDδ* displays hypersensitivity to H_2O_2-induced cell death, suggesting a crucial role of PA in H_2O_2-induced cell death [68]. This is consistent with the fact that PA could trigger an oxidative burst through activating a MAPK (mitogen-activated protein kinase) cascade [69] and indicates PA as a possible important regulator of Rop-regulated ROS generation to mediate the process of cell death [70]. In addition, PI3K can stimulate plasma membrane endocytosis and produce ROS, subsequently inducing root hair curling [31].

Other stress

Wounding triggers a rapid activation of PLD-mediated phospholipid hydrolysis. The Ca^{2+} increase upon wounding mediates the translocation of PLD protein from cytosol to membrane [71]. Multiple members of PLD are activated by wounding, and suppression of *PLDα* by an antisense approach decreases the wound-induced expression of *LOX2* (lipoxygenase 2), suggesting that LOX2 is probably a downstream target through PLD-mediated production of jasmonic acid [72]. MAPK signalling is activated by wounding in soya bean seedlings, and wound-induced activation of MAPK signalling is suppressed when PA production is inhibited with n-butanol, which indicates that PA acts as a second messenger in wound-induced MAPK signalling [73]. In addition, *PLDζ2* plays a key role in phosphate cycling, and *pldζ2* mutants exhibit hypersensitivity to phosphate starvation [74,75]. *PLDε* regulates the root surface area to improve the nitrogen uptake and utilization [76].

Besides abiotic stress, phospholipid signalling is also regulated by biotic stress. The *AVR4* (avirulence 4) gene encodes an elicitor protein and displays resistance in tomato plants carrying the *Cf-4* resistance gene to the fungus *Cladosporium fulvum*. In response to the infection, PA is rapidly produced in a few minutes when AVR4 interacts with Cf-4, indicating that the PA accumulation is an early response in the Cf-4–AVR4 interaction [77].

Others

In addition to the above-mentioned developmental processes and stress responses, phospholipid signalling is involved in embryo development, stomata closure, and light and sugar signal transduction. Deficiency of PECT (phosphorylethanolamine cytidylyltransferase), a rate-limiting enzyme in phosphatidylethanolamine biosynthesis, results in embryo abortion before the octant stage, delays embryo maturation and reduces seed fertility [78]. Deficiency of LPAT (lysophosphatidyl acyltransferase), which is responsible for PA biosynthesis, caused embryo lethality (arrested embryo at the globular stage [79]).

The *Arabidopsis 5PTase13* deficiency mutant (*5pt13*) displays shorter hypocotyls under blue light [80]. Mutants *5pt1* and *5pt2* grow faster and have elongated hypocotyls in the dark [43]. The *ITPK-1* (inositol 1,3,4-trisphosphate 5/6-kinase) knockout mutant is hypersensitive to red light, similar to the *csn* (COP9 signalosome) mutant [81]. In addition, PtdIns(4,5)P_2 and Ins(1,4,5)P_3-mediated Ca^{2+} oscillation are important for stomatal opening [82,83]. *Arabidopsis* 5PTase13 regulates SnRK1 (sucrose non-fermenting-1-related kinase) activity, which differs according to nutrient availability, and deficiency of *5PTase13* results in a shortened root under no-nutrient or low-nutrient conditions and is less sensitive to sugar and insensitive to ABA [84].

The altered growth patterns of the loss-of-function knockout mutant and transgenic lines with overexpression or decreased expression of phospholipid signalling-related genes are summarized in Table 1.

REGULATION OF PHOSPHOLIPID SIGNALLING

Regulation of key enzymes and molecules in phosphoinositide signalling by hormones and environmental stimuli

Studies of expression patterns of genes encoding key enzymes in phosphoinositide signalling employing DNA chip technology and RT (reverse transcription)–PCR reveal that the expression of multiple enzymes is widely and differentially regulated by various hormones and abiotic stressors, especially in the families of PIPK (phosphoinositide phosphate kinase), PLD and 5PTase [50]. Of nine *Arabidopsis PLC* genes, four are induced by salt, three are induced by ABA, four respond to cold, and six are stimulated by dehydration [14]. *Arabidopsis* PLD is activated by various hyperosmotic stresses, including high salinity [93], dehydration [60] and freezing [63,65], as

Table 1 Altered growth of loss-of-function knockout mutants (KO) and transgenic lines with overexpression (OE) or decreased expression (DE) of phospholipid signalling-related genes

Unless indicated otherwise, results are from *Arabidopsis*.

Gene	Protein	Type	Phenotype	Reference(s)
VSP34	PI3K	KO	Short root hairs, and decreased pollen viability, germination and pollen tube growth	[26,32]
PI4Kβ1/*PI4Kβ2*	PI4Kβ1, PI4Kβ2	KO	(Double mutant) Aberrant root hair morphology	[27]
PI4Kγ1	PI4Kγ1	KO	Decreased pollen viability, abnormal tapetum and microspore development	[33]
RHD4	PI4P phosphatase	KO	Short and morphologically aberrant root hairs	[28]
PIP5K3	PIP5K3	DE	Shorter root hair	[29,30]
		OE	Deformed root hair	
PIP5K4	PIP5K4	KO	Reduced stomatal opening, and impaired pollen germination, tube growth and polarity	[34,35,82]
		OE	Perturbed pollen tube growth, multiple pollen tip branching	
PIP5K5	PIP5K5	OE	Multiple pollen tip branching	[34]
PIP5K9	PIP5K9	OE	Shortened primary root	[18]
SAC1 (*fra7*)	SAC domain phosphoinositide phosphatase 1	KO	Weak stem, decreased cell wall thickness	[42]
SAC9	SAC domain phosphoinositide phosphatase 9	KO	Shorter primary root and fewer lateral roots	[22]
DAD1	PLA1	KO	Defects in anther dehiscence, pollen maturation and flower opening	[85]
AtsPLA2β	AtsPLA2β	DE	Shortened leaf petioles and stems, delayed light-induced stomatal opening	[48,86]
		OE	Prolonged leaf petioles and inflorescence stems, faster stomatal opening	
AtPLC1	AtPLC1	DE	Insensitive to ABA in seed germination and growth	[87]
ZmPLC1	ZmPLC1	OE	Improved drought tolerance	[57]
NtPLC3	NtPLC3	OE	Inhibited pollen tube growth	[38]
OsPLDβ1	OsPLDβ1	KO	Reduced sensitivity to ABA during seed germination	[15]
PLDα1	PLDα1	KO	Decreased wound-induced synthesis of jasmonic acid, decreased drought tolerance, enhanced seed quality after storage, insensitive response of stomatal closure to ABA, enhanced sensitivity to high salinity, increased freezing tolerance	[56,58,64,65,72,88]
PLDα3	PLDα3	KO	Increased sensitivities to salinity and water deficiency, later flowering in drought conditions	[55]
		OE	Decreased sensitivities to salinity and water deficiency, earlier flowering in drought conditions	
PLDε	PLDε	KO	Decreased root growth and biomass accumulation, decreased lateral root elongation	[76]
		OE	Increased root growth and biomass accumulation, increased lateral root and root hair elongation	
PLDδ	PLDδ	KO	Increased sensitivity to H_2O_2-induced cell death, sensitive to freezing	[68,89]
		OE	Increased freezing tolerance	
PLDζ1	PLDζ1	OE	Branched and swollen root hairs	[25]
		DE	Random initiation of root hairs	
PLDζ1/*PLDζ2*	PLDζ1, PLDζ2	KO	(Double mutant) Hypersensitive to phosphate deficiency in root growth	[74,75]
PLDζ2	PLDζ2	KO	Suppressed primary root elongation and inhibited lateral root formation, less sensitive to auxin, reduced root gravitropism	[20]
		OE	Enhanced primary root growth, root gravitropism, hypersensitive to auxin.	
5PTase1/*5PTase2*	5PTase1, 5PTase2	KO	(Double mutant) Faster germination and longer hypocotyl in the dark, hypersensitive to ABA	[43]
5PTase5	5PTase5	KO	Disrupted root-hair tip growth	[90]
CVP2	5PTase6	KO	Open reticulum and increased free vein endings	[39]
5PTase11	5PTase11	KO	Slower germination and decreased hypocotyl growth when grown in the dark	[91]
FRA3	5PTase12	KO	Dramatic reduction in secondary wall thickness and a concomitant decrease in stem strength	[41]
5PTase13	5PTase13	KO	Defect in development of the cotyledon vein, shortened hypocotyls and expanded cotyledons under blue light, hypersensitive to sugar and ABA in seed germination	[40,80,84]
Itpk-1	ITPK-1	KO	Decreased hypocotyl length under red light	[81]
OsITL1	OsITPK1	OE	(In tobacco) Decreased tolerance to NaCl during germination and seedling development.	[92]
ATS2	LPAAT (lysophosphatidic acid acyltransferase β)	KO	Embryo lethality	[79]
XIPOTL1	PEAMT	KO	Short primary root and induced cell death	[16]
PECT	PECT	KO	Embryo abortion before the octant stage, delayed embryo maturation and reduced seed fertility	[78]
INT1	INT1	KO	Reduced root length	[17]
AtSfh1	PITP	KO	Short root hairs	[24]

well as ABA [44]. Similarly, many rice *PLD* genes are induced by salt and drought stress [15]. *PIP5K* family genes are reported to be stimulated by water stress and ABA [50,59,94] and repressed by cold [18]. In addition, the plant hormone auxin is broadly related to phosphoinositide signalling, inducing the expression of *AtIPK2β* [95] and *PLDζ2* [20], and suppressing the expression of *5PTase13* [40].

Some phospholipid molecules, such as PA, PtdIns*P* and PtdIns(4,5)P_2, are accumulated after treatment with auxin, NO [21], wounding [72,96], freezing [63], nutrient starvation [74,97], elicitor [98], xylanase [99] or under osmotic stress [100].

Ins(1,4,5)P_3-mediated Ca^{2+} oscillation

PtdIns(4,5)P_2 throughout the membrane establishes the basis for rapid and transient increases of Ins(1,4,5)P_3 in response to environmental stimuli, which leads to the rapid oscillation of Ca^{2+}. Many studies have shown that Ins(1,4,5)P_3-mediated Ca^{2+} influx is important for plant cell growth (Figure 3). In *cvp2* mutants, the Ins(1,4,5)P_3 levels increase approx. 3-fold compared with wild-type, and Ins(1,4,5)P_3 releases Ca^{2+} from internal stores to tightly control the number of ground-to-procambial cell transitions and to regulate the vein formation [39]. The *5pt13* mutant has a

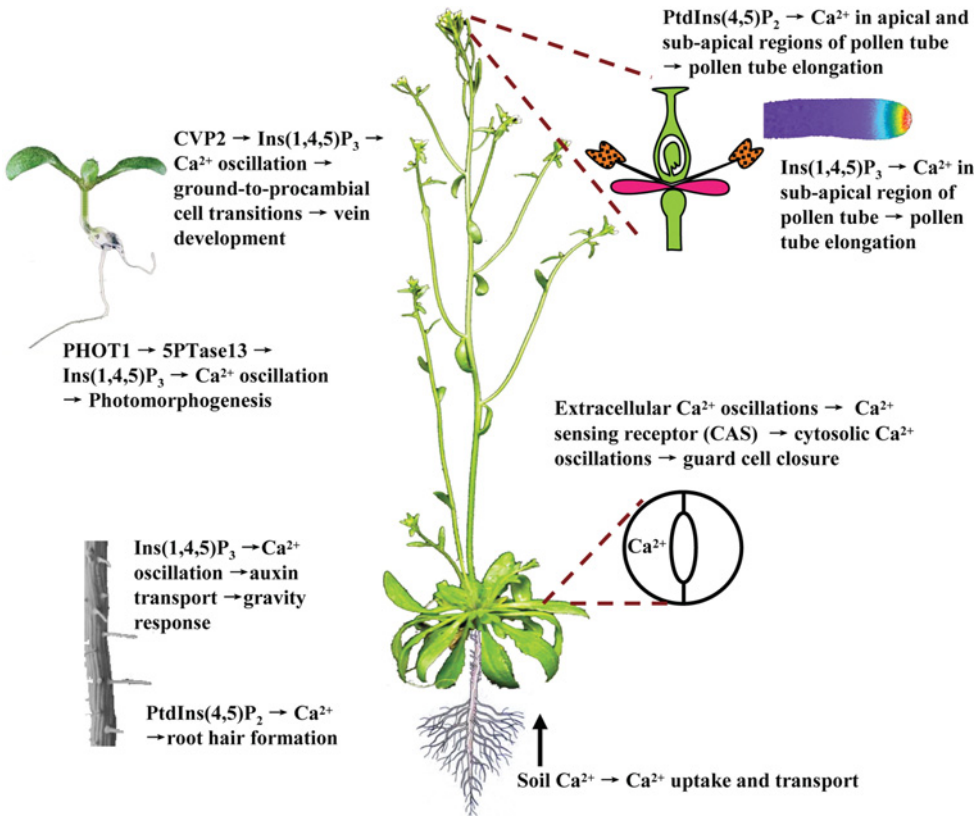

Figure 3 The Ca^{2+} oscillation, mediated by PtdIns(4,5)P_2 and Ins(1,4,5)P_3, is crucial for multiple processes of plant growth including root tropism, root hair formation, cotyledon vein development, photomorphogenesis, guard cell closure, pollen tube elongation and hormone effects

higher Ins(1,4,5)P_3 level compared with wild-type and mediates the effects of blue light through Ins(1,4,5)P_3-mediated Ca^{2+} oscillations [80].

Gravistimulation induced differential lateral IAA (indoleacetic acid) transport, which is consistent with the changed Ins(1,4,5)P_3 levels in maize and oat pulvini [46]. Previous studies have shown that the level of Ins(1,4,5)P_3 increases approx. 3-fold in the first 5 min of gravistimulation, and the second peak of Ins(1,4,5)P_3 appears by 15–20 min [101]. After 30 min, the Ins(1,4,5)P_3 level returns to baseline [101], indicating that the oscillation of Ins(1,4,5)P_3 is a key component of the response to gravity stimuli.

Environmental stimuli also evoke a transient increase of Ins(1,4,5)P_3 levels, leading to the following question: is the long-term increase or decrease of the Ins(1,4,5)P_3 levels associated with cell growth? The shorter hypocotyls of the *5pt13* mutant under blue light provide evidence that a higher Ins(1,4,5)P_3 level, and hence increased $[Ca^{2+}]_{cyt}$ under blue light irradiation, regulates cell growth [80]. Similarly, in maize pulvini, the inositol lipids' rate of turnover is associated with an increase in membrane biogenesis and Ins(1,4,5)P_3–Ca^{2+} release from internal stores to initiate cell growth upon gravistimulation [102].

The PtdIns(4,5)P_2–Ca^{2+} gradient plays a key role in pollen tube growth [103] and root hair formation [24]. Because Ins(1,4,5)P_3–Ca^{2+} influx across the plasma membrane is required to maintain the Ca^{2+} gradient in the pollen tube cell and Ins(1,4,5)P_3-sensitive Ca^{2+} channels have not been identified [10], it was speculated that the function of Ca^{2+} influx was a direct result of Ins(1,4,5)P_3 in pollen tube growth [38]. When PtdIns(4,5)P_2 and Ins(1,4,5)P_3 were loaded into an *Agapanthus* pollen tube, Ins(1,4,5)P_3 was more effective than PtdIns(4,5)P_2 [23], indicating that Ins(1,4,5)P_3–Ca^{2+} and PtdIns(4,5)P_2–Ca^{2+} are spatially different. PtdIns(4,5)P_2–Ca^{2+} is increased in both apical and sub-apical regions, whereas Ins(1,4,5)P_3–Ca^{2+} is increased mainly in sub-apical regions [23], indicating that both PtdIns(4,5)P_2 and Ins(1,4,5)P_3 cause transient increases in Ca^{2+} levels; however, the effects are markedly different.

Transient and rapid increase in intracellular Ca^{2+} have been demonstrated in response to abiotic stresses, including cold, salt and osmotic stress [104,105], and Ins(1,4,5)P_3-mediated Ca^{2+} oscillation is also involved in the response to these stimuli.

In addition, Ca^{2+} is important for the effects of phospholipids and activity of relevant enzymes. The interaction of the PLD C2 domain with Ca^{2+} is stimulated by phospholipids [106], and Ca^{2+} sensitivities of different PLD isoforms vary from the micromolar to the millimolar range [10,107,108]. Increased $[Ca^{2+}]_{cyt}$ evokes conformational changes in PLDs to assist with the recruitment of substrate PtdIns(4,5)P_2 [109]. Additionally, enzymatic activity of PLC is regulated by Ca^{2+} through binding with the EF-hand motif of PLC [110], and PLA2s are classified as Ca^{2+}-dependent or -independent members [2].

Cross-talk with hormone signalling

Auxin transporters is regulated by phosphorylation, and PINOID plays a fundamental role in the asymmetrical localization of membrane proteins during polar auxin transport by regulating the polarity of PIN's apical-basal localization [111]. Interestingly, PDK1 (phosphoinositide-dependent kinase 1) enhances PINOID activity and is involved in polar auxin transport regulation [111]. Both PDK1 and PINOID can bind to PA, PtdIns3*P*, PtdIns(3,4)P_2 and PtdIns(4,5)P_2 through the PH (pleckstrin homology) domain [111,112]. PP2A (protein phosphatase 2A) and PINOID act

antagonistically on mediating PIN apical-basal polar localization [113] and PP2A can bind to PA [114]. In addition, PLDζ2 and product PA are required for the normal cycling of PIN2-containing vesicles to maintain auxin transport, and the deficiency of *PLDζ2* results in suppressed sensitivity to auxin and reduced root gravitropism [20]. These findings suggest that membrane phospholipid turnover is important for regulating polar auxin transport (Figure 2).

Recently several studies revealed the crucial functions of phospholipids in vesicular trafficking. The trafficking of auxin efflux carrier PIN2 protein is dependent on SNX1 (sorting nexin 1)-containing endosomes which are sensitive to the PI3K inhibitor wortmannin [115]. SNX1 co-localizes with PtdIns3*P*-enriched membrane subdomains, and is a candidate downstream effector of PI3K because of the presence of phosphatidylinositol-binding PX (phox homology) domain of SNX1 [116]. During the regulation of turnover of auxin transporters, PIN2 is targeted to the vacuole for degradation, and the trafficking of PIN2-containing vacuolar depends on the phosphatidylinositol pathway [116]. Wortmannin affects the recycling of vacuolar sorting receptors between the PVC (prevacuolar compartment) and the TGN (*trans*-Golgi network) [116–118] and whose treatment inhibits the trafficking and degradation of PIN2, and increases the total PIN2 protein level in membrane [116]. Such cross-talk prompts us to ask questions. Do the membrane compositions affected by phospholipids influence the localization of membrane protein PINs? Do phospholipids serve as mediators to recruit the proteins to membrane sites and to promote signal transduction? Although the current studies suggest that the latter speculation may be the case, the relevant mechanisms are still unclear.

At5PTase13 regulates the expression of *CYP83B1*, which is important for auxin homoeostasis [40], and overexpression of *AtIPK2β* leads to more axillary shoot branching and an IAA-related phenotype by mediating the transcription of the IAA-related genes *CYP83B1*, *PIN4*, *MAX4* and *SPS* [95].

Only PA was reported to bind CTR1 (constitutive triple response 1), which negatively regulates ethylene responses in *Arabidopsis* [119] to disrupt the intramolecular interaction between the CTR1 kinase domain and the CTR1 N-terminal regulatory domain to inhibit its kinase activity [49] (Figure 2).

Regulation through PA

PA, the simplest membrane phospholipid, has emerged as a new class of lipid mediator involved in various cellular processes, such as signal transduction, membrane trafficking, secretion and cytoskeletal rearrangement [10]. Cellular PA is generated mainly via two routes: DGK (DAG kinase) phosphorylating DAG or PLD hydrolysing structural phospholipids. In plants, PA serves as a second messenger and is triggered in response to various biotic and abiotic stresses, including pathogen infection [77,120], drought, salinity, wounding, cold, cell death [68,70,98] and oxylipin production [71–73]. The PA signal production is fast (minutes) and transient.

PA plays a role in the ABA response during stomatal movement and seed germination. PLDα1-derived PA binds to ABI1, a negative regulator of the ABA response, and inhibits its activity to promote stomata closure [44]. T-DNA (transferred DNA) insertional mutants of PA catabolic enzyme LPP (lipid phosphate phosphatase), *lpp2-1* and *lpp2-2*, are hypersensitive to ABA during germination due to the increase in PA levels [121].

Regarding the roles of PA in the abiotic stress response, it is suggested that PA produced by high PLDα1 activity destabilizes the membrane and increases membrane leakage, whereas a regulated increase of plasma membrane PA by PLDδ may produce signalling PA species that mitigate stress damage [63,65]. The PA level increases during chilling and cold acclimatization, as well as in response to freezing [63,122], and the results of microarray analysis imply that PA produced from the PLD and PLC/DGK pathways are involved in two different pathways in cold responses [62]. Osmotic stress also induces the rapid accumulation of PA [60,123], which may be involved in regulation of proline biosynthesis [124]. Studies using soya bean cells show that PA can bind MAPK6-related protein, an important mediator in stress and ethylene signalling, suggesting a different mechanism of PA involvement in the stress response [125] (Figure 2). Furthermore, the PA-interacting/binding proteins should be studied in more detail to illustrate the downstream mechanisms.

Regulation through cytoskeleton

Both the actin and microtubular cytoskeletons can be regulated by phospholipids and participate in regulating the tip growth of the root hair and pollen tube. PtdIns(4,5)P_2, Ins(1,4,5)P_3, PA and the related PI3K, PI4K and PIP5K are involved in the organization of actin filaments; the microtubular cytoskeleton is mainly modulated by PLD.

Phospholipids play an integral role in regulating the structure and dynamics of the cytoskeleton through interaction between PtdIns(4,5)P_2 and many actin-binding proteins, including profilin, gelsolin, α-actin, cofilin, filamin and vinculin [126–130]. It has been shown that PtdIns(4,5)P_2 controls the dynamic organization of F-actin (filamentous actin) through regulating profilin, a G-actin (globular actin)-binding protein [131], and actin remodelling in root hairs and pollen tubes, sites of rapid growth in plants, is sensitive to alterations of PtdIns(4,5)P_2 biosynthesis. Expression of mutant *Arabidopsis* Rac2 in tobacco pollen tubes decreases the plasma membrane PtdIns(4,5)P_2 and disrupts the normal actin filament orientation [132]. *Arabidopsis* PIP5K1, which synthesizes PtdIns(4,5)P_2 *in vivo*, interacts directly with actin and recruits PI4Kβ1 to the actin cytoskeleton [133]. Although it has not been confirmed, Stenzel et al. [30] speculated that the regulation of membrane-associated PIP5K3 in root hair elongation might relate to the control of F-actin (Figure 4).

The PLC-mediated PtdIns(4,5)P_2 turnover also affects the actin structure and normal pollen growth [38,134]. Elevation of intracellular PtdIns(4,5)P_2 [through photolysis of caged PtdIns(4,5)P_2] leads to the perturbation of apical morphology and the appearance in this region of a fine cortical mesh of microfilaments [23].

PA promotes actin polymerization by binding to and inhibiting the activity of AtCP, an actin-capping protein [135]. *Arabidopsis* PLDβ1 can bind directly to actin, and the activity of PLD is, in turn, regulated by the actin cytoskeleton [136]. *AtSAC1* and *FRA3*, which encode PtdIns(3,5)P_2 phosphatase and 5PTase respectively, are both required for actin organization and cell wall synthesis [41,42]. Deficiency of *AtSfh1*, a PITP-encoding gene, results in arrested root hair pattern formation by altering both actin and the microtubular cytoskeleton [24]. A recent study shows that both PtdIns3*P* and PtdIns4*P* modulate actin filament organization in guard cells of day flowers [137].

The involvement of the microtubular cytoskeleton in phospholipid signalling is mediated by PLD. Treatment with the PLD inhibitor butan-1-ol disrupts cortical microtubule organization in soya bean cells and fucoid alga [138,139] and promotes microtubule depolymerization and release from the plasma membrane in *Arabidopsis* [140]. Unlike in mammalian cells, cortical microtubules lie beneath the plasma membrane in plant cells, and PLD is postulated to be the linker [141].

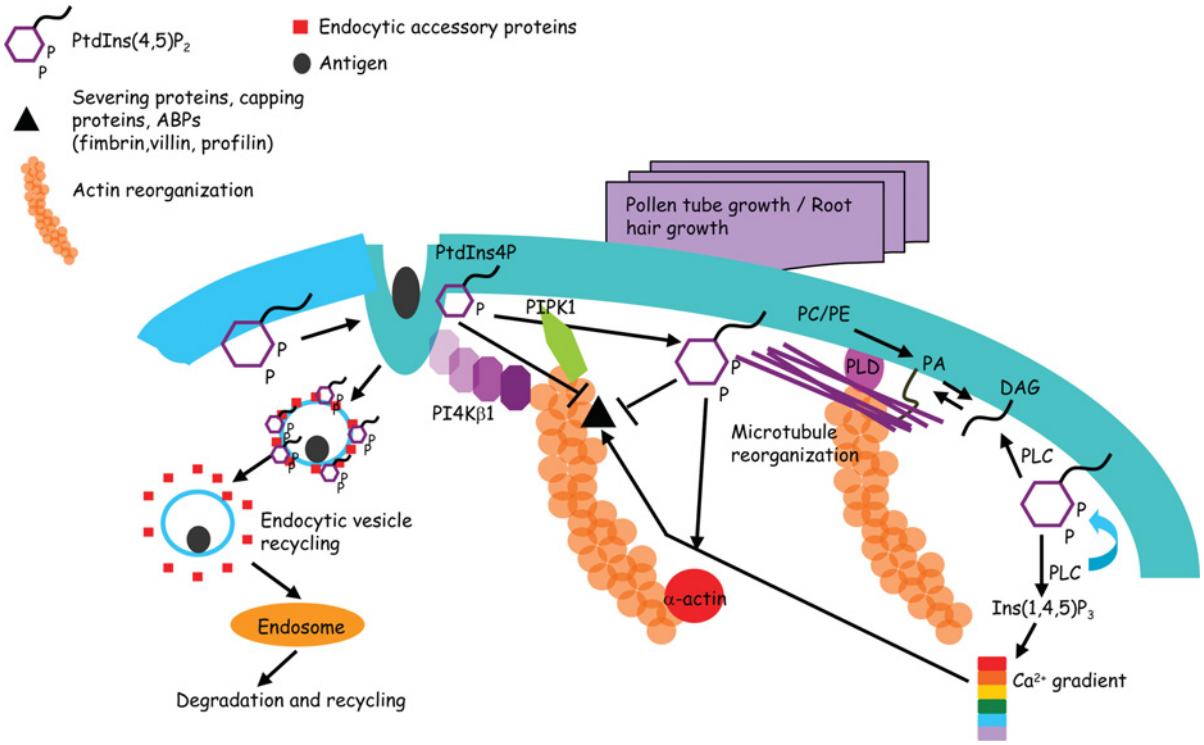

Figure 4 Phospholipids participate in the tip growth of the root hair and pollen tube through regulating actin and microtubule cytoskeletons

PtdIns(4,5)P_2 is synthesized on the plasma membrane and assembles a number of endocytic accessory proteins to induce the antigen-stimulated endocytosis. PIP5K1 and PtdIns(4,5)P_2 interact directly with actin and recruit PI4Kβ1 to actin. PtdIns(4,5)P_2 interacts and suppresses the activity of actin-binding proteins, including severing proteins, capping proteins, ABPs (actin-binding proteins: fimbrin, villin, profilin), and induces the activity of α-actin to regulate actin remodelling. PA turnover from DAG is the substrate for PLD which is involved in microtubule reorganization. Ins(1,4,5)P_3–Ca^{2+} oscillation regulates the activity of actin-binding proteins to participate in actin remodelling. Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine.

Others

Other kinds of regulation of phospholipids and relevant key proteins also exist. PLD activity, with the exception of ζ-class PLDs, is dependent on calcium, whereas β-, γ- and ζ-class PLDs require PtdIns(4,5)P_2 for *in vitro* activity [142]. A recent study shows that aluminium ions inhibit PLD in a microtubule-dependent manner [143]. The activity of AtPIP5K1 is inhibited by phosphorylation through protein kinase A [144]. In addition, the MORN motifs in OsPIPK1 (*Oryza sativa* PIPK1) and the highly charged region of OsPI4K2 are involved in the regulation of subcellular localization and substrate binding [145,146]. The WD40 domain of 5PTase13 interacts with SnRK1 and acts as a positive regulator of SnRK1 activity to stabilize the protein and suppress its degradation [84].

CONCLUSIONS AND PERSPECTIVES

Substantial evidence demonstrates that phospholipid signalling plays an important role in various aspects of plant growth and development, stress response and hormone signalling, and extensive progress has been made toward understanding the regulation and function of phospholipid molecules and related enzymes.

It has been noted that several gene families involved in phospholipid signalling contain more than ten isoforms in *Arabidopsis*, including PIP5K, PLD and 5PTase, and isoforms in the same family have distinct expression patterns and may have specific functions. The precise analysis of the expression patterns in specific tissues, at different developmental stages and under different stimulations of individual isoforms will facilitate the dissection of their specific functions.

Although post-transcriptional regulation is an important process in functional modulation, studies of the regulation of phospholipid-related enzymes and protein level are limited. PIP5K activity can be inhibited by phosphorylation and modulated by changes in subcellular localization and substrate binding [145,146], shedding light on the cellular regulation of phospholipid signalling.

The IP_3Rs [Ins(1,4,5)P_3 receptors] have been identified from animal and yeast cells; however, none are known in plants. Ins(1,4,5)P_3 activates IP_3R in the endoplasmic reticulum, resulting in the Ca^{2+} release and leading to the $[Ca^{2+}]_i$ oscillations. In plants, an external Ca^{2+}-sensing receptor (CAS) serves as a receptor to regulate Ins(1,4,5)P_3 levels and then in turn trigger the Ca^{2+} release. Although cell-surface receptors and IP_3Rs are unknown [2,83], it is reasonable to speculate that CAS may also act as a candidate receptor for Ins(1,4,5)P_3 after studies using biochemical and single-cell imaging analyses [83]. RACK1 (receptor for activated C-kinase 1), which has highly conserved WD40 repeats, acts as a scaffold protein through interacting with IP_3R and other proteins in animal nervous systems [147]. In *Arabidopsis*, the first crystal structure of RACK1 isoform A has been characterized [148]. Does the WD40 domain of 5PTase family also play a role as a scaffold protein for IP_3R in plants? In addition, the functions of Ins(1,4,5)P_3-mediated Ca^{2+} oscillation seems to be more important than Ins(1,4,5)P_3 itself in *Arabidopsis*; do Ins(1,4,5)P_3 and Ca^{2+} share the same receptor?

Until now, most studies have been performed using the dicotyledonous model plant *Arabidopsis*; few studies have been carried

out in rice [15,149]. Whether the function is conserved in monocotyledons (such as rice) is not well understood. Preliminary analysis reveals a difference in the domain structure and isoform numbers of key enzymes in the PLD family [15], suggesting a functional divergence between dicotyledons and monocotyledons. Understanding these differences may provide further information for agricultural improvement.

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REFERENCES

1 Kuypers, F. A. (2008) Red cell membrane lipids in hemoglobinopathies. Curr. Mol. Med. **8**, 633–638
2 Munnik, T., Irvine, R. F. and Musgrave, A. (1998) Phospholipid signalling in plants. Biochim. Biophys. Acta **1389**, 222–272
3 Xue, H., Chen, X. and Li, G. (2007) Involvement of phospholipid signaling in plant growth and hormone effects. Curr. Opin. Plant Biol. **10**, 483–489
4 Lingaraj, T., Donovan, J., Li, Z., Li, P., Doucette, A., Harrison, S., Ecsedy, J. A., Dang, L. and Zhang, W. (2008) A high-throughput liposome substrate assay with automated lipid extraction process for PI 3-kinase. J. Biomol. Screen. **13**, 906–911
5 Carricaburu, V., Lamia, K. A., Lo, E., Favereaux, L., Payrastre, B., Cantley, L. C. and Rameh, L. E. (2003) The phosphatidylinositol (PI)-5-phosphate 4-kinase type II enzyme controls insulin signaling by regulating PI-3,4,5-trisphosphate degradation. Proc. Natl. Acad. Sci. U.S.A. **100**, 9867–9872
6 Coburn, R. F., Labelle, E. F. and Baron, C. B. (2006) Polyamines, PI(4,5)P_2, and actin polymerization. J. Cell. Physiol. **209**, 405–412
7 D'Angelo, G., Vicinanza, M., Di Campli, A. and De Matteis, M. A. (2008) The multiple roles of PtdIns(4)*P*, not just the precursor of PtdIns(4,5)P_2. J. Cell Sci. **121**, 1955–1963
8 Coronas, S., Ramel, D., Pendaries, C., Gaits-Iacovoni, F., Tronchere, H. and Payrastre, B. (2007) PtdIns5*P*: a little phosphoinositide with big functions? Biochem. Soc. Symp. 117–128
9 Liu, L., Damen, J. E., Ware, M., Hughes, M. and Krystal, G. (1997) SHIP, a new player in cytokine-induced signalling. Leukemia **11**, 181–184
10 Meijer, H. J. and Munnik, T. (2003) Phospholipid-based signaling in plants. Annu. Rev. Plant Biol. **54**, 265–306
11 Mueller-Roeber, B. and Pical, C. (2002) Inositol phospholipid metabolism in *Arabidopsis*: characterized and putative isoforms of inositol phospholipid kinase and phosphoinositide-specific phospholipase C. Plant Physiol. **130**, 22–46
12 Berdy, S. E., Kudla, J., Gruissem, W. and Gillaspy, G. E. (2001) Molecular characterization of At5PTase1, an inositol phosphatase capable of terminating inositol trisphosphate signaling. Plant Physiol. **126**, 801–810
13 Qin, C. and Wang, X. (2002) The *Arabidopsis* phospholipase D family: characterization of a calcium-independent and phosphatidylcholine-selective PLDζ1 with distinct regulatory domains. Plant Physiol. **128**, 1057–1068
14 Tasma, I. M., Brendel, V., Whitham, S. A. and Bhattacharyya, M. K. (2008) Expression and evolution of the phosphoinositide-specific phospholipase C gene family in *Arabidopsis thaliana*. Plant Physiol. Biochem. **46**, 627–637
15 Li, G., Lin, F. and Xue, H.-W. (2007) Genome-wide analysis of the phospholipase D family in *Oryza sativa* and functional characterization of PLDβ1 in seed germination. Cell Res. **17**, 881–894
16 Cruz-Ramírez, A., López-Bucio, J., Ramírez-Pimentel, G., Zurita-Silva, A., Sánchez-Calderon, L., Ramírez-Chávez, E., González-Ortega, E. and Herrera-Estrella, L. (2004) The *xipotl* mutant of *Arabidopsis* reveals a critical role for phospholipid metabolism in root system development and epidermal cell integrity. Plant Cell **16**, 2020–2034
17 Schneider, S., Beyhl, D., Hedrich, R. and Sauer, N. (2008) Functional and physiological characterization of *Arabidopsis INOSITOL TRANSPORTER1*, a novel tonoplast-localized transporter for *myo*-inositol. Plant Cell **20**, 1073–1087
18 Lou, Y., Gou, J. Y. and Xue, H.-W. (2007) PIP5K9, an *Arabidopsis* phosphatidylinositol monophosphate kinase, interacts with a cytosolic invertase to negatively regulate sugar-mediated root growth. Plant Cell **19**, 163–181
19 Xu, J., Brearley, C. A., Lin, W. H., Wang, Y., Ye, R., Mueller-Roeber, B., Xu, Z. H. and Xue, H.-W. (2005) A role of *Arabidopsis* inositol polyphosphate kinase, AtIPK2α, in pollen germination and root growth. Plant Physiol. **137**, 94–103
20 Li, G. and Xue, H.-W. (2007) *Arabidopsis* PLDζ2 regulates vesicle trafficking and is required for auxin response. Plant Cell **19**, 281–295
21 Lanteri, M. L., Laxalt, A. M. and Lamattina, L. (2008) Nitric oxide triggers phosphatidic acid accumulation via phospholipase D during auxin-induced adventitious root formation in cucumber. Plant Physiol. **147**, 188–198
22 Williams, M. E., Torabinejad, J., Cohick, E., Parker, K., Drake, E. J., Thompson, J. E., Hortter, M. and Dewald, D. B. (2005) Mutations in the *Arabidopsis* phosphoinositide phosphatase gene *SAC9* lead to overaccumulation of PtdIns(4,5)P_2 and constitutive expression of the stress-response pathway. Plant Physiol. **138**, 686–700
23 Monteiro, D., Liu, Q., Lisboa, S., Scherer, G. E., Quader, H. and Malho, R. (2005) Phosphoinositides and phosphatidic acid regulate pollen tube growth and reorientation through modulation of $[Ca^{2+}]_c$ and membrane secretion. J. Exp. Bot. **56**, 1665–1674
24 Vincent, P., Chua, M., Nogue, F., Fairbrother, A., Mekeel, H., Xu, Y., Allen, N., Bibikova, T. N., Gilroy, S. and Bankaitis, V. A. (2005) A Sec14p-nodulin domain phosphatidylinositol transfer protein polarizes membrane growth of *Arabidopsis thaliana* root hairs. J. Cell Biol. **168**, 801–812
25 Ohashi, Y., Oka, A., Rodrigues-Pousada, R., Possenti, M., Ruberti, I., Morelli, G. and Aoyama, T. (2003) Modulation of phospholipid signaling by GLABRA2 in root-hair pattern formation. Science **300**, 1427–1430
26 Lee, Y., Bak, G., Choi, Y., Chuang, W. I. and Cho, H. T. (2008) Roles of phosphatidylinositol 3-kinase in root hair growth. Plant Physiol. **147**, 624–635
27 Preuss, M. L., Schmitz, A. J., Thole, J. M., Bonner, H. K., Otegui, M. S. and Nielsen, E. (2006) A role for the RabA4b effector protein PI-4Kβ1 in polarized expansion of root hair cells in *Arabidopsis thaliana*. J. Cell Biol. **172**, 991–998
28 Thole, J. M., Vermeer, J. E., Zhang, Y., Gadella, Jr, T. W. and Nielsen, E. (2008) *ROOT HAIR DEFECTIVE4* encodes a phosphatidylinositol-4-phosphate phosphatase required for proper root hair development in *Arabidopsis thaliana*. Plant Cell **20**, 381–395
29 Kusano, H., Testerink, C., Vermeer, J. E., Tsuge, T., Shimada, H., Oka, A., Munnik, T. and Aoyama, T. (2008) The *Arabidopsis* phosphatidylinositol phosphate 5-kinase PIP5K3 is a key regulator of root hair tip growth. Plant Cell **20**, 367–380
30 Stenzel, I., Ischebeck, T., Konig, S., Holubowska, A., Sporysz, M., Hause, B. and Heilmann, I. (2008) The type B phosphatidylinositol-4-phosphate 5-kinase 3 is essential for root hair formation in *Arabidopsis thaliana*. Plant Cell **20**, 124–141
31 Peleg-Grossman, S., Volpin, H. and Levine, A. (2007) Root hair curling and *Rhizobium* infection in *Medicago truncatula* are mediated by phosphatidylinositide-regulated endocytosis and reactive oxygen species. J. Exp. Bot. **58**, 1637–1649
32 Lee, Y., Kim, E. S., Choi, Y., Hwang, I., Staiger, C. J. and Chung, Y. Y. (2008) The *Arabidopsis* phosphatidylinositol 3-kinase is important for pollen development. Plant Physiol. **147**, 1886–1897
33 Alves-Ferreira, M., Wellmer, F., Banhara, A., Kumar, V., Riechmann, J. L. and Meyerowitz, E. M. (2007) Global expression profiling applied to the analysis of *Arabidopsis* stamen development. Plant Physiol. **145**, 747–762
34 Ischebeck, T., Stenzel, I. and Heilmann, I. (2008) Type B phosphatidylinositol-4-phosphate 5-kinases mediate *Arabidopsis* and *Nicotiana tabacum* pollen tube growth by regulating apical pectin secretion. Plant Cell **20**, 3312–3330
35 Sousa, E., Kost, B. and Malho, R. (2008) *Arabidopsis* phosphatidylinositol-4-monophosphate 5-kinase 4 regulates pollen tube growth and polarity by modulating membrane recycling. Plant Cell **20**, 3050–3064
36 Potocky, M., Elias, M., Profotova, B., Novotna, Z., Valentova, O. and Zarsky, V. (2003) Phosphatidic acid produced by phospholipase D is required for tobacco pollen tube growth. Planta **217**, 122–130
37 Zonia, L. and Munnik, T. (2004) Osmotically induced cell swelling versus cell shrinking elicits specific changes in phospholipid signals in tobacco pollen tubes. Plant Physiol. **134**, 813–823
38 Helling, D., Possart, A., Cottier, S., Klahre, U. and Kost, B. (2006) Pollen tube tip growth depends on plasma membrane polarization mediated by tobacco PLC3 activity and endocytic membrane recycling. Plant Cell **18**, 3519–3534
39 Carland, F. M. and Nelson, T. (2004) *COTYLEDON VASCULAR PATTERN2*-mediated inositol (1,4,5) triphosphate signal transduction is essential for closed venation patterns of *Arabidopsis* foliar organs. Plant Cell **16**, 1263–1275
40 Lin, W. H., Wang, Y., Mueller-Roeber, B., Brearley, C. A., Xu, Z. H. and Xue, H.-W. (2005) At5PTase13 modulates cotyledon vein development through regulating auxin homeostasis. Plant Physiol. **139**, 1677–1691
41 Zhong, R., Burk, D. H., Morrison, 3rd, W. H. and Ye, Z. H. (2004) *FRAGILE FIBER3*, an *Arabidopsis* gene encoding a type II inositol polyphosphate 5-phosphatase, is required for secondary wall synthesis and actin organization in fiber cells. Plant Cell **16**, 3242–3259
42 Zhong, R., Burk, D. H., Nairn, C. J., Wood-Jones, A., Morrison, W. H. and Ye, Z. H. (2005) Mutation of *SAC1*, an *Arabidopsis* SAC domain phosphoinositide phosphatase, causes alterations in cell morphogenesis, cell wall synthesis, and actin organization. Plant Cell **17**, 1449–1466

43 Gunesekera, B., Torabinejad, J., Robinson, J. and Gillaspy, G. E. (2007) Inositol polyphosphate 5-phosphatases 1 and 2 are required for regulating seedling growth. Plant Physiol. **143**, 1408–1417

44 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

45 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

46 Yun, H. S., Joo, S. H., Kaufman, P. B., Kim, T. W., Kirakosyan, A., Philosoph-Hadas, S., Kim, S. K. and Chang, S. C. (2006) Changes in starch and inositol 1,4,5-trisphosphate levels and auxin transport are interrelated in graviresponding oat (*Avena sativa*) shoots. Plant Cell Environ. **29**, 2100–2111

47 Scherer, G. F., Zahn, M., Callis, J. and Jones, A. M. (2007) A role for phospholipase A in auxin-regulated gene expression. FEBS Lett. **581**, 4205–4211

48 Lee, H. Y., Bahn, S. C., Kang, Y. M., Lee, K. H., Kim, H. J., Noh, E. K., Palta, J. P., Shin, J. S. and Ryu, S. B. (2003) Secretory low molecular weight phospholipase A2 plays important roles in cell elongation and shoot gravitropism in *Arabidopsis*. Plant Cell **15**, 1990–2002

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

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

51 DeWald, D. B., Torabinejad, J., Jones, C. A., Shope, J. C., Cangelosi, A. R., Thompson, J. E., Prestwich, G. D. and Hama, H. (2001) Rapid accumulation of phosphatidylinositol 4,5-bisphosphate and inositol 1,4,5-trisphosphate correlates with calcium mobilization in salt-stressed *Arabidopsis*. Plant Physiol. **126**, 759–769

52 Parre, E., Ghars, M. A., Leprince, A. S., Thiery, L., Lefebvre, D., Bordenave, M., Richard, L., Mazars, C., Abdelly, C. and Savoure, A. (2007) Calcium signaling via phospholipase C is essential for proline accumulation upon ionic but not nonionic hyperosmotic stresses in *Arabidopsis*. Plant Physiol. **144**, 503–512

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

54 Leshem, Y., Seri, L. and Levine, A. (2007) Induction of phosphatidylinositol 3-kinase-mediated endocytosis by salt stress leads to intracellular production of reactive oxygen species and salt tolerance. Plant J. **51**, 185–197

55 Hong, Y., Pan, X., Welti, R. and Wang, X. (2008) Phospholipase Dα3 is involved in the hyperosmotic response in *Arabidopsis*. Plant Cell **20**, 803–816

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

57 Wang, C. R., Yang, A. F., Yue, G. D., Gao, Q., Yin, H. Y. and Zhang, J. R. (2008) Enhanced expression of phospholipase C 1 (ZmPLC1) improves drought tolerance in transgenic maize. Planta **227**, 1127–1140

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

59 Mane, S. P., Vasquez-Robinet, C., Sioson, A. A., Heath, L. S. and Grene, R. (2007) Early PLDα-mediated events in response to progressive drought stress in *Arabidopsis*: a transcriptome analysis. J. Exp. Bot. **58**, 241–252

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

61 Perera, I. Y., Hung, C. Y., Moore, C. D., Stevenson-Paulik, J. and Boss, W. F. (2008) Transgenic *Arabidopsis* plants expressing the type 1 inositol 5-phosphatase exhibit increased drought tolerance and altered abscisic acid signaling. Plant Cell **20**, 2876–2893

62 Vergnolle, C., Vaultier, M. N., Taconnat, L., Renou, J. P., Kader, J. C., Zachowski, A. and Ruelland, E. (2005) The cold-induced early activation of phospholipase C and D pathways determines the response of two distinct clusters of genes in *Arabidopsis* cell suspensions. Plant Physiol. **139**, 1217–1233

63 Welti, R., Li, W., Li, M., Sang, Y., Biesiada, H., Zhou, H. E., Rajashekar, C. B., Williams, T. D. and Wang, X. (2002) Profiling membrane lipids in plant stress responses: role of phospholipase Dα in freezing-induced lipid changes in *Arabidopsis*. J. Biol. Chem. **277**, 31994–32002

64 Rajashekar, C. B., Zhou, H. E., Zhang, Y., Li, W. and Wang, X. (2006) Suppression of phospholipase Dα1 induces freezing tolerance in *Arabidopsis*: response of cold-responsive genes and osmolyte accumulation. J. Plant Physiol. **163**, 916–926

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

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

67 Viswanathan, C. and Zhu, J. K. (2002) Molecular genetic analysis of cold-regulated gene transcription. Philos. Trans. R. Soc. London Ser. B **357**, 877–886

68 Zhang, W., Wang, C., Qin, C., Wood, T., Olafsdottir, G., Welti, R. and Wang, X. (2003) The oleate-stimulated phospholipase D, PLDδ, and phosphatidic acid decrease H_2O_2-induced cell death in *Arabidopsis*. Plant Cell **15**, 2285–2295

69 Laxalt, A. M. and Munnik, T. (2002) Phospholipid signalling in plant defence. Curr. Opin. Plant Biol. **5**, 332–338

70 Park, J., Gu, Y., Lee, Y., Yang, Z. and Lee, Y. (2004) Phosphatidic acid induces leaf cell death in *Arabidopsis* by activating the Rho-related small G protein GTPase-mediated pathway of reactive oxygen species generation. Plant Physiol. **134**, 129–136

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

72 Wang, C., Zien, C. A., Afitlhile, M., Welti, R., Hildebrand, D. F. and Wang, X. (2000) Involvement of phospholipase D in wound-induced accumulation of jasmonic acid in *Arabidopsis*. Plant Cell **12**, 2237–2246

73 Lee, S., Hirt, H. and Lee, Y. (2001) Phosphatidic acid activates a wound-activated MAPK in *Glycine max*. Plant J. **26**, 479–486

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

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

76 Hong, Y., Devaiah, S. P., Bahn, S. C., Thamasandra, B. N., Li, M., Welti, R. and Wang, X. (2009) Phospholipase Dε and phosphatidic acid enhance *Arabidopsis* nitrogen signaling and growth. Plant J. **58**, 376–387

77 de Jong, C. F., Laxalt, A. M., Bargmann, B. O., de Wit, P. J., Joosten, M. H. and Munnik, T. (2004) Phosphatidic acid accumulation is an early response in the Cf-4/Avr4 interaction. Plant J. **39**, 1–12

78 Mizoi, J., Nakamura, M. and Nishida, I. (2006) Defects in CTP:PHOSPHORYLETHANOLAMINE CYTIDYLYLTRANSFERASE affect embryonic and postembryonic development in *Arabidopsis*. Plant Cell **18**, 3370–3385

79 Yu, B., Wakao, S., Fan, J. and Benning, C. (2004) Loss of plastidic lysophosphatidic acid acyltransferase causes embryo-lethality in *Arabidopsis*. Plant Cell Physiol. **45**, 503–510

80 Chen, X., Lin, W. H., Wang, Y., Luan, S. and Xue, H.-W. (2008) An inositol polyphosphate 5-phosphatase functions in PHOTOTROPIN1 signaling in *Arabidopsis* by altering cytosolic Ca^{2+}. Plant Cell **20**, 353–366

81 Qin, Z. X., Chen, Q. J., Tong, Z. and Wang, X. C. (2005) The *Arabidopsis* inositol 1,3,4-trisphosphate 5/6 kinase, Atltpk-1, is involved in plant photomorphogenesis under red light conditions, possibly via interaction with COP9 signalosome. Plant Physiol. Biochem. **43**, 947–954

82 Lee, Y., Kim, Y. W., Jeon, B. W., Park, K. Y., Suh, S. J., Seo, J., Kwak, J. M., Martinoia, E., Hwang, I. and Lee, Y. (2007) Phosphatidylinositol 4,5-bisphosphate is important for stomatal opening. Plant J. **52**, 803–816

83 Tang, R. H., Han, S., Zheng, H., Cook, C. W., Choi, C. S., Woerner, T. E., Jackson, R. B. and Pei, Z. M. (2007) Coupling diurnal cytosolic Ca^{2+} oscillations to the CAS-IP_3 pathway in *Arabidopsis*. Science **315**, 1423–1426

84 Ananieva, E. A., Gillaspy, G. E., Ely, A., Burnette, R. N. and Erickson, F. L. (2008) Interaction of the WD40 domain of a myoinositol polyphosphate 5-phosphatase with SnRK1 links inositol, sugar, and stress signaling. Plant Physiol. **148**, 1868–1882

85 Ishiguro, S., Kawai-Oda, A., Ueda, J., Nishida, I. and Okada, K. (2001) The *DEFECTIVE IN ANTHER DEHISCIENCE* gene encodes a novel phospholipase A_1 catalyzing the initial step of jasmonic acid biosynthesis, which synchronizes pollen maturation, anther dehiscence, and flower opening in *Arabidopsis*. Plant Cell **13**, 2191–2209

86 Seo, J., Lee, H. Y., Choi, H., Choi, Y., Lee, Y., Kim, Y. W., Ryu, S. B. and Lee, Y. (2008) Phospholipase A2β mediates light-induced stomatal opening in *Arabidopsis*. J. Exp. Bot. **59**, 3587–3594

87 Sanchez, J. P. and Chua, N. H. (2001) *Arabidopsis* PLC1 is required for secondary responses to abscisic acid signals. Plant Cell **13**, 1143–1154

88 Devaiah, S. P., Roth, M. R., Baughman, E., Li, M., Tamura, P., Jeannotte, R., Welti, R. and Wang, X. (2006) Quantitative profiling of polar glycerolipid species from organs of wild-type *Arabidopsis* and a phospholipase Dα1 knockout mutant. Phytochemistry **67**, 1907–1924.

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

90 Jones, M. A., Raymond, M. J. and Smirnoff, N. (2006) Analysis of the root-hair morphogenesis transcriptome reveals the molecular identity of six genes with roles in root-hair development in *Arabidopsis*. Plant J. **45**, 83–100

91 Ercetin, M. E., Ananieva, E. A., Safaee, N. M., Torabinejad, J., Robinson, J. Y. and Gillaspy, G. E. (2008) A phosphatidylinositol phosphate-specific *myo*-inositol polyphosphate 5-phosphatase required for seedling growth. Plant Mol. Biol. **67**, 375–388

92 Niu, X., Chen, Q. and Wang, X. (2008) *OsITL1* gene encoding an inositol 1,3,4-trisphosphate 5/6-kinase is a negative regulator of osmotic stress signaling. Biotechnol. Lett. **30**, 1687–1692

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

94 Mikami, K., Katagiri, T., Iuchi, S., Yamaguchi-Shinozaki, K. and Shinozaki, K. (1998) A gene encoding phosphatidylinositol-4-phosphate 5-kinase is induced by water stress and abscisic acid in *Arabidopsis thaliana*. Plant J. **15**, 563–568

95 Zhang, Z. B., Yang, G., Arana, F., Chen, Z., Li, Y. and Xia, H. J. (2007) *Arabidopsis* inositol polyphosphate 6-/3-kinase (AtIpk2β) is involved in axillary shoot branching via auxin signaling. Plant Physiol. **144**, 942–951

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

97 Lakin-Thomas, P. L. (1993) Effects of inositol starvation on the levels of inositol phosphates and inositol lipids in *Neurospora crassa*. Biochem. J. **292**, 805–811

98 Yamaguchi, T., Minami, E., Ueki, J. and Shibuya, N. (2005) Elicitor-induced activation of phospholipases plays an important role for the induction of defense responses in suspension-cultured rice cells. Plant Cell Physiol. **46**, 579–587

99 Gonorazky, G., Laxalt, A. M., Testerink, C., Munnik, T. and de la Canal, L. (2008) Phosphatidylinositol 4-phosphate accumulates extracellularly upon xylanase treatment in tomato cell suspensions. Plant Cell Environ. **31**, 1051–1062

100 Meijer, H. J., Berrie, C. P., Iurisci, C., Divecha, N., Musgrave, A. and Munnik, T. (2001) Identification of a new polyphosphoinositide in plants, phosphatidylinositol 5-monophosphate (PtdIns5*P*), and its accumulation upon osmotic stress. Biochem. J. **360**, 491–498

101 Perera, I. Y., Hung, C. Y., Brady, S., Muday, G. K. and Boss, W. F. (2006) A universal role for inositol 1,4,5-trisphosphate-mediated signaling in plant gravitropism. Plant Physiol. **140**, 746–760

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

103 Holdaway-Clarke, T. L., Weddle, N. M., Kim, S., Robi, A., Parris, C., Kunkel, J. G. and Hepler, P. K. (2003) Effect of extracellular calcium, pH and borate on growth oscillations in *Lilium formosanum* pollen tubes. J. Exp. Bot. **54**, 65–72

104 Knight, H., Trewavas, A. J. and Knight, M. R. (1996) Cold calcium signaling in *Arabidopsis* involves two cellular pools and a change in calcium signature after acclimation. Plant Cell **8**, 489–503

105 Knight, H. and Knight, M. R. (2001) Abiotic stress signalling pathways: specificity and cross-talk. Trends Plant Sci. **6**, 262–267

106 Pappan, K., Zheng, L., Krishnamoorthi, R. and Wang, X. (2004) Evidence for and characterization of Ca^{2+} binding to the catalytic region of *Arabidopsis thaliana* phospholipase Dβ. J. Biol. Chem. **279**, 47833–47839

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

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

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

110 Rebecchi, M. J. and Pentyala, S. N. (2000) Structure, function, and control of phosphoinositide-specific phospholipase C. Physiol. Rev. **80**, 1291–1335

111 Zegzouti, H., Anthony, R. G., Jahchan, N., Bogre, L. and Christensen, S. K. (2006) Phosphorylation and activation of PINOID by the phospholipid signaling kinase 3-phosphoinositide-dependent protein kinase 1 (PDK1) in *Arabidopsis*. Proc. Natl. Acad. Sci. U.S.A. **103**, 6404–6409

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

113 Michniewicz, M., Zago, M. K., Abas, L., Weijers, D., Schweighofer, A., Meskiene, I., Heisler, M. G., Ohno, C., Zhang, J., Huang, F. et al. (2007) Antagonistic regulation of PIN phosphorylation by PP2A and PINOID directs auxin flux. Cell **130**, 1044–1056

114 Testerink, C., Dekker, H. L., Lim, Z. Y., Johns, M. K., Holmes, A. B., Koster, C. G., Ktistakis, N. T. and Munnik, T. (2004) Isolation and identification of phosphatidic acid targets from plants. Plant J. **39**, 527–536

115 Jaillais, Y., Fobis-Loisy, I., Miege, C., Rollin, C. and Gaude, T. (2006) AtSNX1 defines an endosome for auxin-carrier trafficking in *Arabidopsis*. Nature **443**, 106–109

116 Kleine-Vehn, J., Leitner, J., Zwiewka, M., Sauer, M., Abas, L., Luschnig, C. and Friml, J. (2008) Differential degradation of PIN2 auxin efflux carrier by retromer-dependent vacuolar targeting. Proc. Natl. Acad. Sci. U.S.A. **105**, 17812–17817

117 Matsuoka, K., Bassham, D. C., Raikhel, N. V. and Nakamura, K. (1995) Different sensitivity to wortmannin of two vacuolar sorting signals indicates the presence of distinct sorting machineries in tobacco cells. J. Cell Biol. **130**, 1307–1318

118 daSilva, L. L., Taylor, J. P., Hadlington, J. L., Hanton, S. L., Snowden, C. J., Fox, S. J., Foresti, O., Brandizzi, F. and Denecke, J. (2005) Receptor salvage from the prevacuolar compartment is essential for efficient vacuolar protein targeting. Plant Cell **17**, 132–148

119 Kieber, J. J., Rothenberg, M., Roman, G., Feldmann, K. A. and Ecker, J. R. (1993) CTR1, a negative regulator of the ethylene response pathway in *Arabidopsis*, encodes a member of the raf family of protein kinases. Cell **72**, 427–441

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

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

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

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

124 Thiery, L., Leprince, A. S., Lefebvre, D., Ghars, M. A., Debarbieux, E. and Savoure, A. (2004) Phospholipase D is a negative regulator of proline biosynthesis in *Arabidopsis thaliana*. J. Biol. Chem. **279**, 14812–14818

125 Liu, Y. and Zhang, S. (2004) Phosphorylation of 1-aminocyclopropane-1-carboxylic acid synthase by MPK6, a stress-responsive mitogen-activated protein kinase, induces ethylene biosynthesis in *Arabidopsis*. Plant Cell **16**, 3386–3399

126 Janmey, P. A. (1994) Phosphoinositides and calcium as regulators of cellular actin assembly and disassembly. Annu. Rev. Physiol. **56**, 169–191

127 Simonsen, A., Wurmser, A. E., Emr, S. D. and Stenmark, H. (2001) The role of phosphoinositides in membrane transport. Curr. Opin. Cell Biol. **13**, 485–492

128 Takenawa, T. and Itoh, T. (2001) Phosphoinositides, key molecules for regulation of actin cytoskeletal organization and membrane traffic from the plasma membrane. Biochim. Biophys. Acta **1533**, 190–206

129 Yin, H. L. and Janmey, P. A. (2003) Phosphoinositide regulation of the actin cytoskeleton. Annu. Rev. Physiol. **65**, 761–789

130 Downes, C. P., Gray, A. and Lucocq, J. M. (2005) Probing phosphoinositide functions in signaling and membrane trafficking. Trends Cell Biol. **15**, 259–268

131 Meagher, R. B., McKinney, E. C. and Kandasamy, M. K. (1999) Isovariant dynamics expand and buffer the responses of complex systems: the diverse plant actin gene family. Plant Cell **11**, 995–1006

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

133 Davis, A. J., Im, Y. J., Dubin, J. S., Tomer, K. B. and Boss, W. F. (2007) *Arabidopsis* phosphatidylinositol phosphate kinase 1 binds F-actin and recruits phosphatidylinositol 4-kinase β1 to the actin cytoskeleton. J. Biol. Chem. **282**, 14121–14131

134 Dowd, P. E., Coursol, S., Skirpan, A. L., Kao, T. H. and Gilroy, S. (2006) Petunia phospholipase C_1 is involved in pollen tube growth. Plant Cell **18**, 1438–1453

135 Lee, S., Park, J. and Lee, Y. (2003) Phosphatidic acid induces actin polymerization by activating protein kinases in soybean cells. Mol. Cells **15**, 313–319

136 Kusner, D. J., Barton, J. A., Qin, C., Wang, X. and Iyer, S. S. (2003) Evolutionary conservation of physical and functional interactions between phospholipase D and actin. Arch. Biochem. Biophys. **412**, 231–241

137 Choi, Y., Lee, Y., Jeon, B., Staiger, C. J. and Lee, Y. (2008) Phosphatidylinositol 3- and 4-phosphate modulate actin filament reorganization in guard cells of day flower. Plant Cell Environ. **31**, 366–377

138 Gardiner, J. C., Collings, D. A., Harper, J. D. I. 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
139 Peters, N. T., Logan, K. O., Miller, A. C. and Kropf, D. L. (2007) Phospholipase D signaling regulates microtubule organization in the fucoid alga *Silvetia compressa*. Plant Cell Physiol. **48**, 1764–1774
140 Dhonukshe, P., Laxalt, A. M., Goedhart, J., Gadella, T. W. J. and Munnik, T. (2003) Phospholipase D activation correlates with microtubule reorganization in living plant cells. Plant Cell **15**, 2666–2679
141 Gardiner, J. C., Harper, J. D. I., Weerakoon, N. D., Collings, D. A., Ritchie, S., Gilroy, S. and Marc, J. (2001) A 90-kD phospholipase D from tobacco binds to microtubules and the plasma membrane. Plant Cell **13**, 2143–2158
142 Bargmann, B. O. and Munnik, T. (2006) The role of phospholipase D in plant stress responses. Curr. Opin. Plant Biol. **9**, 515–522
143 Pejchar, P., Pleskot, R., Schwarzerová, K., Martinec, J., Valentová, O. and Novotna, Z. (2008) Aluminum ions inhibit phospholipase D in a microtubule-dependent manner. Cell Biol. Int. **32**, 554–556
144 Westergren, T., Dove, S. T., Sommarin, M. and Pical, C. (2001) AtPIP5K1, an *Arabidopsis thaliana* phosphatidylinositol phosphate kinase, synthesizes PtdIns(3,4)P_2 and PtdIns(4,5)P_2 *in vitro* and is inhibited by phosphorylation. Biochem. J. **359**, 583–589
145 Lou, Y., Ma, H., Lin, W. H., Chu, Z. Q., Mueller-Roeber, B., Xu, Z. H. and Xue, H.-W. (2006) The highly charged region of plant β-type phosphatidylinositol 4-kinase is involved in membrane targeting and phospholipid binding. Plant Mol. Biol. **61**, 215–226
146 Ma, H., Lou, Y., Lin, W. H. and Xue, H.-W. (2006) MORN motifs in plant PIPKs are involved in the regulation of subcellular localization and phospholipid binding. Cell Res. **16**, 466–478
147 Sklan, E. H., Podoly, E. and Soreq, H. (2006) RACK1 has the nerve to act: structure meets function in the nervous system. Prog. Neurobiol. **78**, 117–134
148 Ullah, H., Scappini, E. L., Moon, A. F., Williams, L. V., Armstrong, D. L. and Pedersen, L. C. (2008) Structure of a signal transduction regulator, RACK1, from *Arabidopsis thaliana*. Protein Sci. **17**, 1771–1780
149 Ma, H., Xu, S. P., Luo, D., Xu, Z. H. and Xue, H.-W. (2004) OsPIPK1, a rice phosphatidylinositol monophosphate kinase, regulates rice heading by modifying the expression of floral induction genes. Plant Mol. Biol. **54**, 295–310

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