Involvement of phospholipid signaling in plant growth and hormone effects
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Biochemical and genetics studies demonstrated the critical roles of phospholipid signaling and relevant molecules in regulating multiple processes of plant growth and development, signal transduction, mediating hormone effects and cell responses to environmental stimuli, through modulating protein subcellular localization, cross-talking with other signaling or metabolic pathways, or interacting with signaling molecules. The updated achievements of physiological effects and functional mechanisms of phospholipid signaling in higher plants were reviewed.

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Introduction
Phospholipid signaling, which was first reported in the 1950s, plays critical roles in both plants and animals. CDP-diglycerol and free cytoplasmic inositol are synthesized to phosphatidylinositol, which is then sequentially phosphorylated at the 4- and 5-positions of inositol ring by PI4K and PI5K. The resultant phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) is hydrolyzed, by phospholipase C (PLC), to inositol 1,4,5-trisphosphate (IP3) and DAG, the two second messenger molecules, which regulate the downstream signal cascades through stimulating Ca2+ release from internal stores or protein kinases [1]. In a tightly regulated manner, IP3 is further phosphorylated to inositol 1,4,5,6-tetraphosphate (in plants, inositol 1,3,4,5-tetraphosphate in animals), by IP3 kinase, or dephosphorylated to inositol 1,4-bisphosphate, by 5PTases, to balance the cycling of phospholipid molecules. To date, biochemical or genetics studies significantly helped to illuminate the characters and physiological functions of the members of phospholipid signaling in multiple developmental processes, and their relations with other signaling pathways [2–4]. In this review, we focus on the updated advances in the functions and relevant mechanisms of phospholipid signaling in plant growth, hormone effects, and responses to abiotic stresses.

Phospholipid signaling in plant growth and development
Many studies have shown that maintaining the normal levels of phospholipid molecules, including PI(4,5)P2 [5–9], PC [10,11], phosphatidylethanolamine (PE) [11,12], IP3 [13*,14–16], and phosphatidic acid (PA) [10,17**,18,19] is important for various aspects of plant growth and development.

Embryo development and seed germination
PE, the substrate of PLD, is crucial in embryo development. Deficiency of phosphorylethanolamine cytidylyltransferase (PECT), a rate-limiting enzyme in PE biosynthesis, resulted in embryo abortion before the octant stage, delayed embryo maturation and reduced seed fertility [12]. Arabidopsis PLDc1 is critical in the process of seed deterioration and aging, and suppressed expression of which will enhance the seed germination and oil stability after storage or exposure to stress. In addition, the loss of unsaturated fatty acids and accumulation of lipid peroxides were decreased as well [20**].

Besides, PLD also functions in the process of seed germination. PLD activities, as well as the amount of its product PA, are increased in the stage of early seed germination [21,22]; while treatment with PLD specific inhibitor 1-butanol inhibited seed germination. In addition, de novo PA biosynthesis is essential in embryo development and deficiency of lysophosphatidyl acyltransferase (LPAT), a pivotal enzyme in de novo PA biosynthesis in plants, caused embryo lethality, of which the embryo is arrested at the globular stage [23]. However, endoplasmic reticulum-located LPAT2 is essential for female but not male gametophyte development in Arabidopsis, and heterozygous mutant (LPAT2/Ipat2) showed altered vegetative growth and produced shorter siliques containing normal seeds and remnants of aborted ovules in a 1:1 ratio [24].

Root growth
Ablation of phosphatidylcholine (PC) (xipotl, mutant of S-adenosyl-l-methionine:phosphoethanolamine N-methyltransferase, a key enzyme catalyzing PE to PC) resulted in short root and induced epidermis cell death, which can be rescued by applied exogenous PC or PA [10]. Treated with PLD inhibitor 1-butanol or PLDc2 deficiency suppressed primary root elongation and inhibited the lateral...
roots formation; while overexpression of PLDζ2 showed longer primary root [17**].

Mutation of phosphoinositide phosphatase SAC9 over-accumulated PIP2 in plants and resulted in the short roots [5]. Studies on the gain of function mutant of Arabidopsis PIPK9 indicated that it interacted with a cytosolic inositol-tase to negatively regulate sugar-mediated root growth, although it is still unclear whether this is also due to the accumulated PI(4,5)P2 [25**]. In addition, phospholipid molecules or signaling related proteins including PC, PLD, phosphatidylinositol (PI) phosphatase, IP3 kinase and PIP kinase, have been proved to regulate root growth with differential mechanisms. Deficiency of PIP5Ks (Atsfh1, [8]), PLDζ1 [18], AtIPK2α [16], or double mutant of PI-4Kβ1 and PI-4Kβ2 [9] exhibited arrested root hair pattern formation, which are due to the disassembled actin and microtubule skeleton [8], altered calcium concentration [9,16,8] or vesicle trafficking [18].

Vein formation
There is still little known about the function of phospholipid signaling in vein formation, and only 5PTases have been detected to regulate the process. Deficiency of Arabidopsis ccp2 (5PTase6) exhibited various abnormal vein patterns, including increased free vein endings and a resultant open vein network, which was due to that the elevated IP3 level reduced the ground cell recruitment into vascular cell fate [14]. 5PTase13 deficiency resulted in incorrect vein orientation, with additional or altered loop, caused by altered auxin homeostasis and can be rescued by exogenous auxin or partially by brassinolide [13*].

Pollen germination and pollen tube growth
Phospholipid signaling involved in pollen germination and pollen tube growth possibly through regulating the cytosolic calcium ([Ca2+]c) and relevant signaling, or vesicle trafficking. Effects of PI(4,5)P2, IP3 and PA in pollen germination and pollen tube germination have been documented. Changes of intracellular PI(4,5)P2 and IP3 levels modify pollen tube growth rate and axis orientation, however, the effects of them on [Ca2+]c and apical secretion are different. PI(4,5)P2 release leads to increased [Ca2+]c, transient growth perturbation and inhibition of apical secretion; while applied with IP3 caused a transient [Ca2+]c increase at similar magnitude, stimulated apical secretion and severe growth perturbation [26]. In addition, IPK2α modulated pollen germination and pollen tube elongation through regulating the IP3 pool and Ca2+ signaling [16].

PA is important to maintain the ultrastructural polarity of the pollen tube, and inhibition of PA production dissipated the tip-focused gradient of [Ca2+]c, lost the tip polarity and inhibited membrane recycling [26]. In addition, DAG, which displayed a similar intracellular localization as PI(4,5)P2, was required in pollen tube growth as well. Treatment with PLC specific inhibitor induced lateral spreading of PI(4,5)P2 at the pollen tube tip and abolised the membrane accumulation of DAG [27].

Phospholipid signaling in hormone effects
Over the past years, studies have shown that phospholipid signaling plays important role in mediating plant hormone effects. Some key proteins of the phosphatidylinositol metabolic pathway (phospholipase, kinase, phosphatases) and lipid mediators (IP3, PA) are involved in plant hormone mediated processes, including ABA-regulated seed germination and stomatal closing, auxin-regulated root and shoot architecture.

ABA response
The roles of phospholipid signaling in ABA response have been well documented. Key enzymes including PLDα1 [28,29], PLC1 [30], 5PTases [13*,31,32], and lipid molecules including phosphatidylinositol 3-phosphate (PI3P) and phosphatidylinositol 4-phosphate (PI4P) [33,34], PA [28,29] and diacyl glycerol pyrophosphate (DGPP) [35] have been proved being crucial or involved in ABA responses. PA, produced by PLDα1, bond to ABH1 to inhibit its activity and to promote the ABA-induced stomatal closure; whereas PLDα1 and PA could interact with Ga subunit of heterotrimeric G protein to participate in ABA-inhibited stomatal opening [3,29]. In addition, PLC activity is stimulated by ABA and inhibition of PLC activity by treatment with PLC inhibitor U-73122 reduced ABA-induced changes of [Ca2+]c, stomatal closure, and proline accumulation upon ionic stress [36–38]. Ecopic expression of Arabidopsis 5PTase1 or deficiency of 5PTase13 decreased the ABA effects on stomatal closure or seed germination [13*,32], whereas deficiency of 5PTase1, 5PTase2, 5PTase11, and double mutant 5ptase-1,2-1 were hypersensitive to exogenous ABA, which is consistent with the increased IP3 levels [14,32,39] and suggests the involvement of At5PTase1 and 2 in ABA responses through modulating IP3 levels. Besides signaling, the ABA content in seed is increased under 5PTase1 deficiency [32], suggesting that phospholipid signaling might interacts with ABA signaling in a multi-lay and complex network.

Auxin response
Auxin involves in many developmental processes including root elongation [40], gravity response [41], shoot architecture [15], and vein pattern formation [42], and studies have shown that several PI related proteins, including PLA2 [43], 5PTases [13*], IPK2β [15] and PLDζ2 [17**], are involved in auxin responses.

Both 5PTase13 [13*] and IP3 kinase (IPK) are involved in plant growth through regulating auxin homeostasis or response. Deficiency of 5PTase13 resulted in decreased auxin content, and suppressed responses to exogenous
auxin [15]. Overexpression of AtIPK2β resulted in suppressed auxin responses including increased axillary branches, more elongated cotyledons under light and much longer hypocotyls under dark [15]. In addition, treatment with PLC specific inhibitor, U73122, resulted in suppressed gravity curvature and reduced the ratio for IAA levels in the lower halves versus in the upper halves, providing evidence that IAA transport might correlate with the changes of IP3 levels or distribution [44].

Besides PLC, other phospholipases including phospholipase A (PLA), phospholipase D (PLD) involve in auxin response as well. The activity of PLA could be induced by auxin within 2 min [45] and E7YA and HELSS, the inhibitors of PLD, inhibited elongation growth of etiolated Arabidopsis hypocotyls [46]. Indeed, AtsPLA2 regulates shoot gravitropism through regulating auxin-induced cell elongation [47**].

Recent study showed that PLDζ2, whose expression is induced by IAA, stimulated auxin response through regulating auxin transport and distribution [17**]. Deficiency of PLDζ2 or treated with PLD specific inhibitor 1-butanol resulted in reduced sensitivities to exogenous auxin. Detailed studies indicated that PLDζ2 and its product PA were required for the normal cycling of PIN2 in the vesicles, suggesting the effects of PLDζ2 on polar auxin transport through regulating vesicle trafficking. Most interestingly, PLDζ2 specifically expressed in the narrow band of the root cells, corresponding to the distal portion of the transition zone, where auxin mainly accumulated and functioned, providing another evidence to deduce that PLDζ2 effect on the transport of auxin [17**]. Recent research further confirmed that PLDζ2 regulated auxin transport especially in the narrow band and the auxin flux decreased about 40% in pldζ2 mutant and 1-butanol treated roots, while auxin flux increased about 30% in PLDζ2-overexpressing line and PA treated roots. Importantly, there is no change of auxin flux in the elongation region, indicating that PLDζ2, but not other PLDs, was specialized for vesicular regulation of the polar auxin transport in root apices [48*].

Phospholipid signaling in abiotic responses

Stress response

Over the past few years, the relationships between the phospholipid signaling and stress signaling have been well demonstrated, including those to osmotic, temperature and pathogen stresses. Besides the roles of PLD, inositol polyphosphate 5-phosphatase (5Pase), and PA, other members of phospholipid pathway have been detected to involve in responses to abiotic stress. PLC is essential for proline accumulation upon salt stress in Arabidopsis [38]. Analysis through microarray hybridization showed that PLD and PLC/diacylglycerol kinase-mediated pathways were involved in two different cold-related pathways leading to the activation of cold response [49]. Under low-temperature, PLDα1 deficiency induced freezing tolerance by modulating the cold-responsive genes and accumulation of osmolytes [50]. PLD mediated early drought response in Arabidopsis [51]. Liu et al. provided the primary evidence for the involvement of IP3 in heat-shock signal transduction in Arabidopsis [52].

Light response

Phospholipid pathway is associated with photosynthesis. In oat seedling, PLD activity was regulated by light, with the involvement of phytochrome photoreceptor. White and red lights irradiation inhibited PLD activity in etiolated seedlings [53]. Ins(1,3,4)P3 5/6 kinase (AtItpk-1) functioned as a protein kinase to involve in photomorphogenesis possibly via interacting with COP9 signaling-some under red light [54*]. In addition, PHOT1 and PHOT2 mediate blue light-induced transient increases of [Ca2+]c, and PLC-mediated phospholipid signaling is involved in the PHOT2-mediated increase of [Ca2+]c [55*], providing the evidence to connect with photomorphogenesis and phospholipid signaling.

Further, our studies show that 5Pase13 functions in PHOT1 signaling through altering [Ca2+]c, and PLC-mediated phospholipid signaling is involved in the PHOT2-mediated increase of [Ca2+]c [55*], providing evidence to reveal the relationship between phospholipid signaling and light response. However, the evidences of the phospholipid signaling in light response are quite few and detailed mechanisms remain unclear.

Sugar metabolism

Relationship between phospholipid signaling and sugar metabolism has been detected in animal cells. A hypothesis on insulin showed that increased concentrations of plasma free fatty acids induced insulin resistance in humans through inhibition of glucose transport activity, which appears to be a consequence of decreased insulin receptor substrate-1-associated PI3K activity [36]. PTEN (phosphatase and tensin homologue deleted on chromosome 10) and SHIP2 (SH2 domain-containing inositol polyphosphate 5-phosphatase-2), the two phosphoinositide phosphatases served as negative modulators of insulin signaling [57].

However, relations between phospholipid signaling and sugar metabolism in plants are less known. Myo-inositol participated in the synthesis of hemicellulose [6,58] and the secondary wall synthesis greatly associated with sugar metabolism, which might deduce the relationship between phospholipid signaling and sugar metabolism also exist in plants. Indeed, recent studies showed that FRA3 (5Pase11), a member of type II 5Pases, played an essential role in the secondary wall synthesis in fiber cells and xylem vessels [59]. In addition, PI(4,5)P2 plays a pivotal role in actin cytoskeleton which directly leads to the change of the secondary metabolism, and PIP5K9 could interact with a cytosolic invertase to negatively regulate sugar-mediated root growth [25**]. The PIP5K9 deficiency mutant was less sensitive to glucose and
sucrose due to the reduced activity of invertase, which resulted in the decreased concentrations of sucrose and total disaccharide [25**].

**Phosphorus starvation**

There is still little known about the utilization of endogenous phosphate of plant seeds until now. Various phospholipids such as PC, PE, and phosphatidylglycerol (PG) constitute ~30% of total phosphate storage in plants. Studies on agricultural ecosystems of phosphate utilization showed that PLDζ2 was involved in hydrolyzing PC and PE to produce DG for digalactosyldiacylglycerol (DGDG) synthesis and free phosphate to sustain other phosphate-requiring processes [60**]. Especially, during phosphate starvation, the hydrolysis of PC by PLDζs became the supply of inorganic phosphate for cell metabolism and galactolipid synthesis [61]. The PLD-derived PA serves as a key factor involving in the regulation of phosphate storage in plant seeds.

The phytate, IP₆, the main portion of seed’s phosphate (75.0–90%) [62], represents another major source of phosphate in plant growth. IP₆ also represented the major mineral storage for K⁺, Mg²⁺, Ca²⁺, Zn²⁺ and Fe³⁺ [62]. In wheat and barley, multiple inositol polyphosphate phosphatases (MINPPs) have been proved to be essential for the developing and germinating seeds [63]. Breeding of low-PA crops has recently been considered as a potential approach to increase nutritional quality of crop products. The non-lethal low-PA mutants containing lower PA content ranging from 34 to 64% have been constructed and provided as a good research model [64]. IP₃ kinases, at the later stages of phytate synthesis, also played important roles in phosphate cycling, and disruption of which, especially the double mutant atipk1-1/atipk2β-1 increased seed phosphate amount by 10-fold and the root hairs exhibited insensitive responses to lower concentration of phosphate [65].

However, which members of phospholipid signaling are involved in these important cellular pathways to balance the phosphate pool is not clear. The most likely candidates are SPTases, which are all downstream of IP₃ signaling. Several studies showed that SPTases altered IP₃ levels, which are important during germination and early seed development [32,66].

**Conclusions and perspectives**

Extensive evidences showed the involvement of phospholipid signaling in a wide variety of plant growth and development, hormone effects and stress responses, through different mechanisms (Figure 1). Besides the involvement in auxin and ABA responses, phospholipid signaling and relevant molecules have also been proved to mediate the effects of other plant hormones jasmonic acid [67,68], salicylic acid [69], cytokinin [70,71], gibberelline [72], and ethylene [73], although there are only few evidences at the moment and detailed mechanism is still unclear. Expressions of some key enzymes of phospholipid signaling are regulated by brassinosteroids [74], however, there is still no report on the roles of phospholipid signaling in BR response yet.

Recent studies indicate that trafficking of cell vesicles is closely link, sometimes critical, to the subcellular localizations, cycling, movement, secretion, and degradation of proteins, and indeed, phospholipid molecules including PI(4,5)P₂ and PA, key proteins including PI4P kinase, PI(4,5)P₂ kinase and PLD are associated with actin/microtube or regulate vesicle trafficking and endo-

**Figure 1**

Involvement of phospholipid signaling and related proteins/molecules in plant growth and development, hormone effects and stress responses. Interactions with various signal pathways including sugar (CINV1), Ca²⁺, or hormones (ABI1, CYP83B1, PIN) were highlighted.
cytosis. Detailed studies on these may help to further illustrate the functional mechanisms of phospholipid signaling in various aspects of plant growth.

In addition, currently most studies on phospholipids signaling functions were carried out using *Arabidopsis*, and those in monocots (such as rice, wheat, maize) are little known. Our recent analysis showed the presence of a secreted type PLD in rice, which contains a signal peptide in N terminus instead of a C2 or PXPH domain in types of C2-PLD or PXPH-PLD [75]. Such kind of PLD is not identified in *Arabidopsis* or other plants. In addition, the different isoform numbers of PI related proteins (PLC, PLD, 5PTase) and different domain structures, suggest the possibly differential roles and functional mechanisms of phospholipid signaling between dicots and monocots.

In sum, further systemic and comparative studies to illustrate the roles of PI related proteins and phospholipid molecules will come to the age. The genetic resources and establishment of metabolomics platform (or some new methods) will provide significant supports.

### Acknowledgement

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### References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest


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PISK9, links phospholipid signaling to sugar metabolism through its interactions with CIN1, and negatively regulates root cell elongation.


This study revealed gravitistimulation-induced differential lateral IAA transport is affected by gravity-induced asymmetric changes of IP3 levels in oat shoots.


This study shows AlsPLA(2β) regulates cell elongation and involves in shoot gravitropism by mediating auxin-induced cell elongation.


Through measuring the auxin flux in plt2 mutant and PLD(z)-overexpressing plants in root apex, the effects of PLD(z) on auxin flux contribution was validated.


Arabidopsis inositol 1,3,4-trisphosphate 5/6 kinase (Attplk-1) interacts with CSN and involves in photomorphogenesis under red light conditions.


Phot1 and phot2 mediate the BL-induced (Ca2+) increase and phot2 could trigger CICR from internal Ca2+ stores such as the ER or vacuoles, depending on PLG-mediated IP3 production.


Myo-inositol is known to be converted to α-glucuronic acid by an oxidase in rat kidney and yeast. The studies provided the evidence for the conversion of myo-inositol to α-Glucuronic acid, D-Xylose, L-gulonic acid, pectin, hemicellulose.


FRAGILE FIBER3 (FRA3), a type II 5PTase, plays an essential role in the secondary wall synthesis in fiber cells and xylem vessels.


Arabidopsis **pid** mutants are defective in the hydrolysis of phospholipids and has a reduced capacity to accumulate galactolipids under limiting phosphate conditions. PLD2 is involved in the eukaryotic galactolipid biosynthesis pathway to sustain other phosphate-requiring processes.


Hydrolysis of phosphatidyicholine by PLDzetas during phosphorus starvation contributes to the supply of inorganic phosphorus for cell metabolism and diacylglycerol moieties for galactolipid synthesis.


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