Networking of phospholipases in plant signal transduction

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Phospholipases are activated in response to various cellular and environmental cues. Their activation can affect many cellular processes through their roles in signal transduction. Recent advances in the biochemical and molecular understanding of phospholipase D (PLD) have provided insights into potential networks of PLDs and other phospholipases in plants. PLDs are a family of heterogeneous enzymes, and the activities of the multiple types of PLDs are regulated in distinctly different manners. Phosphoinositides, free fatty acids, lysophospholipids, and calcium are differential modulators of PLDs. Since these modulators are substrates, products, or targets of the various PLDs and other phospholipases, there are many potential regulatory and metabolic interrelationships among the various PLDs and other phospholipases.

Introduction

Phospholipids provide not only the structural base of cell membranes, but also rich resources for generating cellular regulators. Hydrolysis of phospholipids by phospholipases is often the first step in generating lipid and lipid-derived messengers. Activation of phospholipase D (PLD), C (PLC), and A2 (PLA2) has been linked to various signalling processes in plants, such as hormonal and stress responses (reviewed in Chapman 1998, Munnik et al. 1998, Wáng 2001). Often, more than one of these phospholipases are involved in a cellular response (Fan et al. 1997, Jacob et al. 1999, Staxen et al. 1999, Den Hartog et al. 2001). Indeed, even the production of lipid messengers within a given class, such as phosphatidic acid (PA), diacylglycerol (DAG), lysophospholipids, or free fatty acids, may be attributed to the action of multiple phospholipases (Lee et al. 1997, Ryu and Wáng 1998, van der Luit et al. 2000, Wáng 2000). These findings raise the possibility that crosstalk may occur among various phospholipases in mediating cellular functions.

Recent advances in the biochemical and molecular understanding of the PLD family have shed light on the potential networks of phospholipases in plants.

Diverse families of phospholipases

Phospholipases are grouped into families of PLD, PLC, PLA2, PLA1, and PLB according to their sites of hydrolysis on phospholipids (Fig. 1). PLD, PLC, and PLB are better understood than the others; each of these families is divided further into subfamilies based on sequences, biochemical properties, or a combination of both. For example, the Arabidopsis genome has 12 genes for PLD. This prominent family of phospholipases was first discovered and cloned in plants (Wáng 2000). They are grouped into five types, PLDα, β, γ, δ, and ζ, based on their sequences and biochemical properties (Qin and Wáng 2002). PLDα is the conventional plant PLD that is most active at millimolar levels of Ca2⁺. PLDβ and γ require phosphoinositides (PI) and are most active at micromolar levels of Ca2⁺, but PLDβ differs from PLDγ in substrate preference (Pappan et al. 1997, 1998, Qin et al. 1997). PLDδ is activated by unsaturated fatty acids and is associated primarily with the plasma membrane (Wang and Wáng 2001) and binds to microtubules (Gardiner et al. 2001). PLDα, β, γ, and ζ all contain the Ca2⁺-dependent phospholipid binding C2 domain and require Ca2⁺ for activity. In contrast to the others, PLDζ has a phox homology (PX) domain and a pleckstrin homology (PH) domain, but not the C2 domain, and it is Ca2⁺-independent (Qin and Wáng 2002). A rabidopsis lines deficient in some of these PLDs have been generated by gene knockout or antisense suppression (Fan et al. 1997, C. Wang and X. Wang, unpublished data). The varied properties of the PLD gene prod-

Abbreviations – DAG, diacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphoinositide; PtdIns (4,5)P2, phosphatidylinositol 4,5-bisphosphate; PLD, phospholipase D; PLA, phospholipase A; PLC, phospholipase C.
ucts indicate that individual members of the PLD family may be subject to unique controls in the cell and that the activation and function of individual PLD isoforms may be interwoven differently with PLC and PLA₂.

Intracellular PLCs can be divided into two groups based on substrate specificity. The better-characterized group are the PI-PLCs that hydrolyse phosphatidylinositol 4,5-bisphosphate [PtdIns (4,5)P₂]. Ten PI-PLC isoforms have been characterized in mammals and classified into three groups, PLCβ, PLCγ, and PLCδ (reviewed in Williams 1999). Multiple PLCs have been cloned from plants, and they all display similarity to PI-PLCδ (Kopka et al. 1998). The other group is the phosphatidylcholine (PC)-PLCs that hydrolyse the common membrane phospholipid, PC, as well as some other phospholipids. PC-PLC has been reported in various plant species and tissues (reviewed in Wang 2001). However, understanding of PC-PLC in plants, as well as in animals, has been hindered by the lack of molecular information on this enzyme.

PLA₂s from animals have been classified, based on sequences, into 10 groups. The classification can be simplified by combining the groups into three major types: (1) the secretory, low molecular weight PLA₂, sPLA₂; (2) the cytosolic Ca²⁺-dependent PLA₂, cPLA₂; and (3) the intracellular Ca²⁺-independent PLA₂, iPLA₂ (Balsinde et al. 1999). Recently, sPLA₂-like PLA₂s have been purified and cloned in plants (Stahl et al. 1998, 1999). Intracellular, iPLA₂-like PLA₂s have been reported (May et al. 1998, Jung and Kim 2000); the characterized enzymes show sequence similarities to patatin, a group of closely related, potato tuber vacuolar storage proteins that possess acyl hydrolase activity.

**Phospholipase-derived messengers; their interconversions and/or attenuation**

PLD hydrolyses various phospholipids, such as PC, phosphatidylethanolamine, and phosphatidylglycerol, to PA and water-soluble free head groups (Fig. 1). PI-PLC uses phosphoinositides as substrates to generate DAG and phosphorylated head groups such as inositol 1,4,5-trisphosphate (IP₃). PLA₂ cleaves phospholipids to lysophospholipids and free fatty acids (Fig. 1). The products of individual phospholipases can be further metabolized in the cell (Fig. 2). For example, PA, produced by PLD, can be dephosphorylated to DAG by phosphatidate phosphatase or phosphorylated to DAG pyrophosphate by PA kinase (Munnik et al. 2000). PLC-generated DAG can be phosphorylated to PA by DAG kinase. In addition, PA can be deacylated by PLAs to free fatty acids and lysoPA (Fig. 2).

Besides the phospholipases, the other enzymes that carry out these conversions also have been identified, and many of them have been cloned from animals, yeast, or plants (Katagiri et al. 1996, Munnik et al. 1998, Topham and Prescott 1999, Marcel et al. 2000, Waggoner et al. 1999, Snedden and Blumwald 2000). Information about lipid kinases and phosphatases in lipid signalling process has increased greatly in recent years. Regulation of the levels of various lipid messengers by phosphorylation and dephosphorylation can be thought of as analogous to the well-established regulation of protein functions by protein kinases and phosphatases. Nine mammalian DAG kinases have been cloned and are grouped into five subtypes according to structural motifs (reviewed in Topham and Prescott 1999). There are two major types of lipid phosphate phosphatases (reviewed in Waggoner et al. 1999): Types I and II, which are structurally and catalytically unrelated. Type I (PAP-1) is Mg²⁺-dependent and is important in triacylglycerol synthesis, whereas type II (PAP-2) is Mg²⁺-independent and is likely involved in signal transduction. PAP-2 is a family of phosphatases that hydrolyse a variety of lipid phosphates. Thus, the members of this family are renamed lipid phosphate phosphatases (LPPs), LPP-1, LPP-2, and LPP-3 can dephosphorylate PA, lysoPA, DAGPP,
ceramide 1-phosphate, and sphingosine 1-phosphate. The type-II-like PAPs have been recently cloned in plants (Marcel et al. 2000).

The multiple metabolic pathways by which a class of lipid messengers, such as PA, DAG, lysoPA, or a free fatty acid, is generated are likely to be important in regulating the amount of messenger produced, as well as the location, timing of production, and acyl composition of the messenger (Hodgkin et al. 1998). The location of the lipid messenger production is critical, because the mobility of lipids in the cell is limited. Lipid messengers can be compartmentalized not only to different membranes, such as the plasma, endoplasmic reticulum, and nuclear membrane, but also to regions within a specific membrane (Hooper 1999). Microdomains or rafts in plasma membranes have been identified in mammalian cells. The timing of lipid messenger production is important in differentiating among potential pathways of messenger production. Mitogenic signalling in animal cells, for example, involves both PI-PLC and PC-PLD. A activation of PLC results in the initial rise of DAG level, whereas PLD activity coupled with lipid phosphate phosphatase action provides the sustained supply of DAG required for cell proliferation. Furthermore, not all PAs, DAGs, and lysoPLs are chemically identical; the acyl groups at the sn-1 and sn-2 positions are varied; thus there are numerous distinct molecular species. For example, PC and Ptdtns (4,5)P2 are distinct in acyl composition, thus there are different PLDs in plants that are interrelated with the metabolism of Ptdtns (4,5)P2. Ptdtns (4,5)P2 is required by PLDβ and PLDγ for activity and also stimulates PLDα and PLDδ (Qin et al. 1997, Pappan and Wang 1999, Fig. 2). PLDs bind Ptdtns (4,5)P2, and the binding modulates PLD's interactions with substrates (Zheng et al. 2000). On the other hand, the synthesis of Ptdtns (4,5)P2 is affected by PLD; PA, the product of PLD, activates PI-5 kinase in animal systems (Jenkins et al. 1994). PA also has been shown to bind plant PI-4 kinase although the role of PLD in plant polyphosphoinositide production is yet to be determined (Stevenson et al. 1998). Ptdtns (4,5)P2 is a minor plant lipid, and its level is regulated dynamically in the cell (reviewed in Drook et al. 1999, Stevenson et al. 2000). Thus, the Ptdtns (4,5)P2 binding and activation of PLD may regulate the levels and metabolism of phosphoinositides and thereby affect PI-PLC activity and function.

One extensively studied function of PI-PLC is the production of IP3, which acts to increase cytoplasmic Ca2+ levels (Staxen et al. 1999). Ca2+ is a required activator of various PLDs (Wang 2000) and, thus, activation of PLC may influence PLD activities by modulating the levels of cellular Ca2+. Plant PLDs contain a Ca2+/phospholipid-binding fold, called the C2 domain, which is involved in Ca2+-regulated membrane association and protein–protein interactions. The C2 domains of PLDα and PLDβ have been shown to bind Ca2+, and this binding causes conformational changes and increases the affinity of the PLDs for phospholipids (Zheng et al. 2000). The PLDα and β C2 domains display distinct binding thermodynamics, with the PLDβ C2 having a higher af-
finity for Ca$^{2+}$. In addition, the Ca$^{2+}$ requirement of PLD$\alpha$ is strongly influenced by pH and substrate lipid composition, whereas the Ca$^{2+}$ requirements of PLD$\beta$ and $\gamma$ are independent of pH (Pappan and Wang 1999). PLD$\alpha$ is active at near physiological, micromolar Ca$^{2+}$ concentrations at an acidic pH of 4.5–5.0, suggesting that PLD$\alpha$ may be activated by acidification, which occurs under some stress conditions.

PLD$\alpha$ is the most prevalent PLD and is expressed in all plants tissues examined under normal growth conditions (Fan et al. 1999, Wang 2000). This constitutive presence allows PLD$\alpha$ to be activated rapidly in cellular responses. Upon wounding, for example, the activation of PLD and the rise in PA occur before that of lysophospholipids and free fatty acids (Lee et al. 1997, Ryu and Wang 1998). The activation of PLD$\alpha$ may signal an increase in PLA activity and lead to the release of free unsaturated fatty acids for the synthesis of oxylipins, such as jasmonic acid (Fig. 2).

Indeed, suppression of PLD$\alpha$ decreased wound-induced production of not only PA, but also free polyunsaturated fatty acids and jasmonic acid in Arabidopsis (Wang et al. 2000, Zien et al. 2001). Meanwhile, the activity of PLA produces lysophospholipids, which may, in turn, attenuate PLD$\alpha$ as a feedback mechanism (Fig. 2) since lysophosphatidylethanolamine inhibits PLD$\alpha$ activity (Ryu et al. 1997). Recently, a novel Arabidopsis PLD, PLD$\delta$, has been identified, and it is activated by free unsaturated fatty acids (Wang and Wang 2001). Thus, PLD$\delta$ may be a target of PLA-derived free fatty acids, and the activation of various phospholipases may act in concert, PLD$\alpha$$\rightarrow$PLA$\rightarrow$PLD$\delta$, to regulate the production of lipid messengers as part of plant defense responses. Moreover, the discovery of the Ca$^{2+}$-independent and PX/PH-containing PLD$\zeta$s indicates further complex regulatory and functional relationships in the PLD family (Qin and Wang 2002). The distinct domain structure of PLD$\zeta$s points to the possibility that these PLD$s$ may be activated differently from the other characterized PLD$s$. The different mechanisms of regulation of PLD isoforms suggest that the isoforms carry out distinct functions in signalling pathways. Thus, networking may occur among isoforms within a phospholipase family, as well as among phospholipases in different families.

**Closing remarks**

Phospholipases are a diverse group of enzymes that are involved in a broad range of cellular functions through roles in signal transduction, membrane remodelling, and membrane degradation. Recent results on multiple PLD$s$ have provided mechanistic information on how each PLD is regulated and also insights into potential networks of various phospholipases in cell function. The networks may occur not only among different families of phospholipases, but also within the same family. One feature that distinguishes the plant PLD family from the others is that the domain structures and genomic organization of plant PLD$s$ are much more diverse than those of animals and microorganisms. To date, 12 PLD genes have been identified in Arabidopsis, but only two, PLD1 and PLD2, have been found in mammals and one, SPO14, in yeast (Qin and Wang 2002). This is in contrast to the other phospholipase families; more diversity in structures and regulatory properties has been observed for PI-PLC and PLA$\alpha$ in animals than in plants (Wang 2001). This raises an intriguing question of whether plants use PLD more than other organisms as part of the regulatory machinery in cellular functions. Studies are underway to test directly whether or how the activation and inactivation of one phospholipase affects the activation and function of other phospholipases. Understanding these interactions also requires information about the temporal and spatial activation of each of these enzymes, the molecular species of their in vivo products and substrates, and the cellular targets of their respective lipid messengers.

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**References**


