J. Biochem. **131**, 301–306 (2002)

Regulation and Possible Role of Mammalian Phospholipase D in Cellular Functions

Yoshiko Banno[1]

Department of Biochemistry, Gifu University School of Medicine, Gifu 500-8705

Received November 30, 2001; accepted December 26, 2001

Key words: apoptosis, cell growth, MAP kinase, phosphatidic acid, phospholipase D.

Phospholipase D (PLD), an enzyme widely distributed in bacteria, fungi, plants, and animals, catalyzes the hydrolysis of phosphatidylcholine (PC) and other phospholipids to generate phosphatidic acid (PA). PLD activity can lead to the generation of phosphatidylalcohol in the presence of a primary alcohol. This reaction, referred to as transphosphatidylation, is not only a hallmark of PLD activity but has also been used to block the production of PA in functional studies. PLD activity in mammalian cells is transiently increased following the occupation of many cell surface receptors, including those of the heterotrimeric G-protein and tyrosine kinase families (*1–4*). The generated PA and the further metabolites, 1,2-diacylglycerol (DG) and lysoPA, are important biologically-active products that are able to recruit or modulate specific target proteins. This review will focus on recent developments in regulation of the mammalian PLD isozymes involved in cell stimulation and on the functional roles of PLD signaling.

The phospholipase D gene superfamily

Eukaryotic PC-hydrolysing PLD was first cloned from a plant, then from yeast, and finally from mammalians. Wang and his colleagues first isolated a castor-bean endosperm cDNA (PLD-α) that encoded a 92-kDa protein exhibiting both hydrolytic and transphosphatidylating Ca^{2+}-dependent PLD activity with PC as substrate. Plant PLDs have been cloned in three forms, PLDα, PLDβ, and PLDγ (*3*). A yeast sporulation gene, called SPO14/PLD1 and having sequence homology to plant PLD, has been isolated from *Saccharomyces cerevisiae* and identified as a gene exhibing Ca^{2+}-independent PLD activity. On the basis of the plant and yeast genes, a human PLD cDNA having 1,074 amino acids and a molecular mass of 124 kDa was cloned and termed PLD1a (*5*). A shorter splice variant of hPLD1a (hPLD1b), which lacks a 38-amino-acid region and has similar regulatory properties, has been identified (*6*). Another PLD (PLD2), a 106-kDa protein with 933 amino acids and 51% amino acid sequence identity with hPLD1a, has been cloned from a mouse embryonic library (*7*). Another fungal PLD gene has been cloned from *Candida albicans* (*8*). In addition, several putative PLD genes, from the nematode *Caenorhabditis elegans* and *Schizosaccharomyces pombe*, and three bacterial PLDs from *Streptomyces antibioticus*, *Streptoverticillium cinnamoneum*, and *Yersinia pestis*, respectively, comprise the PLD family tree (Fig. 1A), which was created using the CLUSTAL W software based on BLAST analyses of the individual sequences (*3*). These PLD genes all belong to an extended gene superfamily that also includes phosphatidyltransferases, bacterial phospholipid synthases, endonucleases, and pox envelope proteins (*1–3*). Mammalian-like PLDs have also been found to be present in the protozoa *Leishmania donovani* and *Tetrahymena pyriformis*.

PLD superfamily members all share a conserved (HXKX4DX6GG/S) (HKD) motif that is involved in catalysis and that confers a similar mechanism of action (*1–3*). The catalytic core of all eukaryotic PLDs is comprised of domains I–IV. These four domains are also found in the bacterial PLDs. Mutagenesis and structural studies have indicated that the HKD motifs are required for catalytic activity and that they may dimerize to form an active center. In addition to domains I–IV, other domains (PH, PX) are conserved in the yeast, human, and nematode sequences, but are absent from the plant and bacterial PLDs (Fig. 1B). Plant PLDs exhibit an N-terminal C2 domain (*3*). Analysis of the crystal structure of an endonuclease PLD superfamily member revealed that the HKD motif acts as a dimer and nucleophile that forms a covalent phosphohistidine intermediate (*3*).

Regulation of mammalian PLD1 and PLD2 in receptor signaling

The recombinant mammalian PLD1a and 1b are activated by ADP-ribosylation factor ARF, Rho protein family (Rho, CDC42, and Rac), RalA, and conventional protein kinase C (PKCα, β) *in vitro* assay (*1–4*). On the other hand, recombinant PLD2 exhibits high basal activity and is only modestly regulated by ARF. Both PLD1 and PLD2 require PIP_2 for activity. PIP_2 binds to a highly conserved region containing basic and hydrophobic amino acids (amino acids 691–712 for PLD1 and amino acids 554–575 for PLD2), but the PH domain is not necessary for PIP_2 interactions (*9*). The domain in PLD1 with which Rho interacts is localized within the C-terminal 362 amino acids. PKC interacts with a domain in the N-terminus (amino acids 1–325, which contain the PX and PH domains) of PLD1 (*2*). The site of ARF interaction has not yet been identified.

In intact cells, agonist-induced PLD activity is regulated by various protein kinases, including PKC, protein tyrosine kinase, and the MAP kinase family, in addition to ARF and Rho family proteins (*1–4*). Phorbol ester activates PLD in many cell lines, and PLD activity induced by various agonists is abolished by PKC inhibitors, implying that PKC is a major factor in the regulation of PLD *in vivo* (*1*). Some studies have shown that PKC can directly activate PLD1 in an ATP-independent manner. A PLD1 mutant unrespon-

[1] For correspondence: E-mail: banno@cc.gifu-u.ac.jp

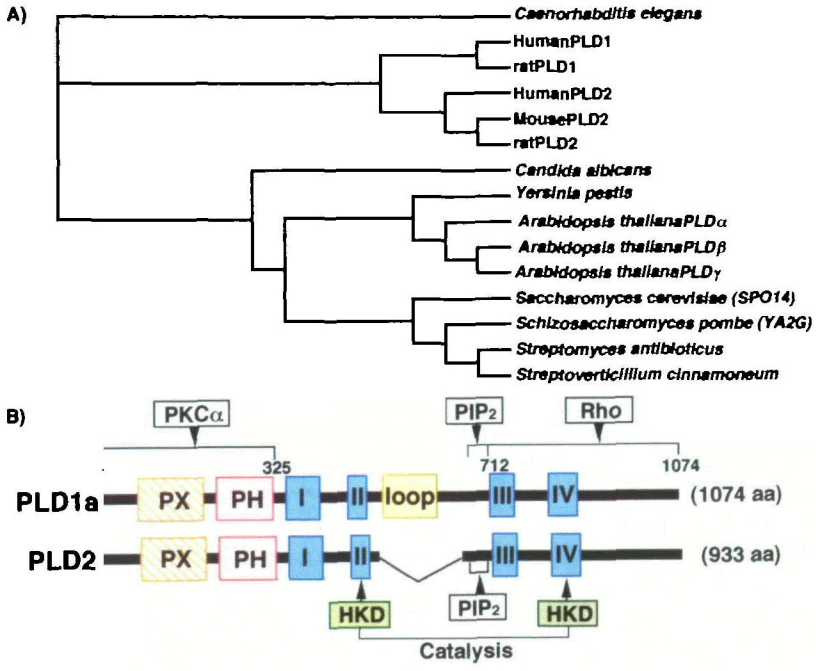

Fig. 1. **Phylogenetic tree of the PLD family and domain structure of mammalian PLD1 and PLD2.** (A) The multiple alignment and phylogenetic tree were generated by using the CLUSTALW software with PLD sequences obtained from the DNA data bank of Japan. (B) Regions of conserved sequences of PLD1a and PLD2 are shown. PX, phox homology domain; PH, pleckstrin homology domain; motifs I, II, III and IV, regions of sequence conserved among all PLD isozymes. Motifs HKD are found in II and IV. Regions of PLD1a identified as interacting with protein kinase C and Rho, and PIP_2 binding regions of PLD1a and PLD2 are indicated.

sive to PKC cannot be activated by G-protein coupled receptor stimulation when expressed in HEK 293 cells, suggesting that direct interaction with PKC is important for PLD1 activation (*10*). On the other hand, another report has suggested that a phosphorylation-dependent mechanism is important in the cells. PMA-induced phosphorylation sites of endogenous PLD1 have been identified at serine 2, threonine 147, and serine 561 in rat fibroblast 3Y1 cells, and mutation of these sites significantly decreased PMA-induced PLD1 activity (*11*). Many receptors also stimulate phosphatidylinositol-specific phospholipase C (PI-PLC) activity, leading to an increase in cellular Ca^{2+} as well as DG, which activates PKC, suggesting that PI-PLC activation is upstream of PLD activation (*1, 12, 13*). PKNα and PKNβ, serine/threonine kinases binding to RhoA, directly interact with PLD1 at the residues 228–598 for PKNα and the residues 1–228/229–598 for PKNβ when transfected in COS-7 cells (*14*). Significant stimulation of PLD1 activity by PKNα was observed in the presence of arachidonic acid.

Although recombinant PLD2 exhibits high basal activity, PLD2 expressed in the cells and in the membrane exhibits low activity. Therefore, it is suggested that the PLD2 activity may be inhibited *in vivo* by inhibitory proteins. α- and β-synucleins, clathrin assembly protein AP180, fodrin, and synaptojanin have been identified as cytosolic inhibitory proteins (*2*). The regulatory mechanism of PLD2 is less understood. PLD2 expressed in HEK 293 cells and in Sf9 cells can be regulated by PMA, indicating a positive regulation of PLD2 by PKC. Co-expression of PLD2 and PKCα results in increase in PLD2 activity (*12, 15*). ARF also increases PLD2 activity when co-expressed in HEK 293 cells (*12*). PLD2 is found to be associated with cytoskeletal proteins such as gelsolin, α-actinin and β-actin, and these proteins inhibit PLD2 activity (*16, 17*). The inhibition by a-actinin could be reversed by ARF1 (*16*). These data suggest that ARF can also regulate PLD2 activity under certain conditions in the cells.

The possible involvement of protein tyrosine kinase (PTK) and other serine/threonin kinases in the PLD activation has been suggested in response to various stimuli. H_2O_2 stimulates PLD2 activity in PC12 cells and endothelial cells, and the pretreatment with the PTK inhibitors abolished the PLD activation, suggesting that PLD2 is regulated by PTK (*18, 19*). PLD2, but not PLD1, forms a physical complex with the EGF receptor, and its Tyr 11 becomes phosphorylated in response to EGF stimulation (*20*). However, the tyrosine phosphorylation of PLD2 has no effect on the enzyme activity.

Recent evidence suggests the involvement of a MAP kinase (ERK and p38 MAP kinase) pathway in the stimulation of PLD activity in some cells. We suggested that the H_2O_2-induced PLD2 activation in PC12 cells was mediated through a signaling cascade of Src-type PTK, a calcium-dependent proline-rich tyrosine kinase-2 Pyk2, ERK, and p38 MAP kinase (*18, 21*). A similar pathway has been proposed in glucose-induced PLD activation in muscle and adipose tissues, where PLD functions downstream of the PYK2/ERK pathway and upstream of PKCζ/λ (*22*). Furthermore, norepinephrine-induced PLD1 activity is mediated *via* the ras/ERK pathway by a phosphorylation-dependent mechanism in aortic smooth muscle cells (*23*). Both PLD1 and PLD2 directly associate with p38 MAPK and are phosphorylated in response to diperoxovanadate stimulation in endothelial cells (*24*). However, phosphorylation by ERK or p38 MAPK failed to affect either PLD isoform activity *in vitro*.

Biological functions of PLD

PLD activation causes changes in the physiological properties of cellular membranes by reducing PC to increase PA, which remains in the membrane and interacts with various proteins located in the membrane or cytosol. Many enzyme activities have been reported to undergo changes

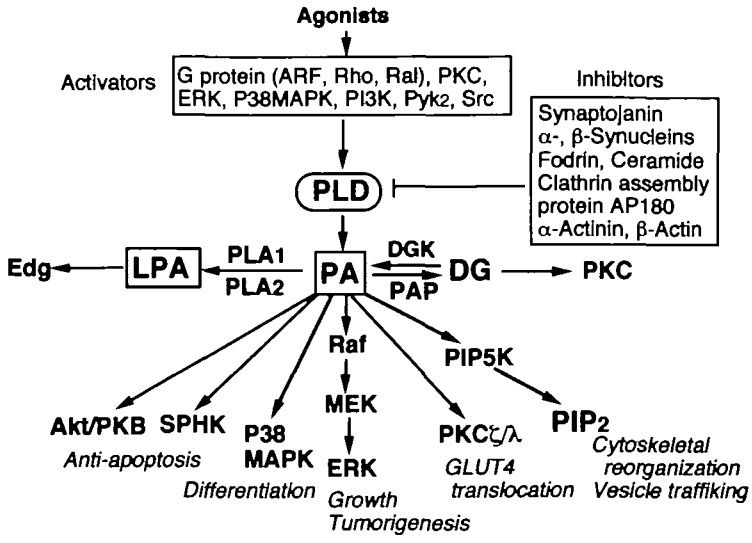

Fig. 2. **Regulation and functions of PLD activity.** PI3K, phosphatidylinositol 3-kinase; PKC, protein kinase C; ARF, ADP-ribosylation factor; LPA, lysophosphatidic acid; $PLA_{1,2}$, phospholipase $A_{1,2}$; PLD, phospholipase D; PA, phosphatidic acid; DGK, diacylglycerol kinase; PAP, PA phosphohydrolase; PIP5K, phosphatidylinositol 4-phosphate 5-kinase; MAPK, mitogen-activated protein kinase; MEK, MAP kinase kinase; ERK, extracellular signal–regulated kinase; SPHK, sphingosine kinase; Edg, endothelial differentiation gene.

induced by PA *in vitro*. Some proteins, including coatomer, ARF, and Raf-1, have been identified as specific PA-binding proteins from brain cytosol (*25*). Therefore, it is hoped that elucidation of the roles of PLD in cellular regulation will allow us to identify cellular targets affected by its reaction product, PA (Fig. 2).

To understand the function of PLD activity, it is important to determine its location within cells. Numerous studies in which epitope-tagged forms of PLDs were overexpressed in several different cell types have suggested that both PLD1 and PLD2 can be found both at the plasma membrane and in intracellular compartments including the endoplasmic reticulum, Golgi, lysosomes, endosomes, and secretory granules (*3, 4*). It is possible that transient overexpression of PLDs leads to mislocalization of the enzyme. Recent study using antibodies of endogenous enzymes have comfirmed that PLD1 is tightly associated with the Golgi complex, and also located in cell nuclei (*26*). PLD1 is translocated to the plasma membrane (containing caveolae) when stimulated with PMA (*11, 27*). Other recent studies have demonstrated that endogenous PLD2 is located in caveolar membrane and in the perinuclear region containing the Golgi (*3, 27*). We have found that PLD2 is present in isolated nuclei from hepatoma cells. However, it has not been clearly demonstrated that different PLD isoforms are involved in different physiological processes.

Vesicle trafficking and cytoskeletal reorganization. Several lines of evidence implicate ARF-dependent PLD1 in agonist-dependent cellular secretion and vesicle trafficking (*4*). Interestingly, a recent study with inactive PLD1- and PLD2-transfected epithelial cells has demonstrated that PLD1 and PLD2 regulate protein transit between the trans-Golgi network and the apical plasma membrane at different steps: in the resting cells, constitutive secretion of protein appears mainly regulated by PLD2; but the increase in secretion triggered by PMA stimulation is PLD1-dependent (*27*).

A possible role of PLD1 and PLD2 has been demonstrated in GLUT-4 translocation. PLD1 is associated with GLUT-4 containing intracellular membrane and acts to promote the mechanism of GLUT-4 translocation by insulin (*28*). Insulin-mediated activation of PLD1 is induced through the ARF pathway in adipocytes, which is consistent with the hypothesis that stimulation of PI 3-kinase by insulin may cause enhancement of ARNO, which in turn activates the ARF/PLD. On the other hand, PLD has also been implicated in glucose-induced increases in GLUT4 translocations in adipocytes and muscle cells (*22*). In these tissues, GLUT4 translocation appears to require activation of PKCζ/λ, which is activated by PA. The glucose-induced activation of PKCζ/λ is independent of the PI3 kinase pathway, but is mediated by a mechanism requiring PYK2, ERK, and PLD (most likely PLD2).

PLD may participate in regulating agonist-induced reorganization of the cytoskeleton, such as membrane ruffling and stress fiber formation. One possible function of PLD that would explain its role in cytoskeletal reorganization is related to the ability of PA to regulate PI(4)P 5-kinase, which is required for synthesis of PIP_2. In HeLa cells, PI(4)P 5 kinase, ARF6, and PLD2 are colocalized in the ruffles upon stimulation with EGF, and PI(4)P 5 kinase is activated synergistically with ARF6 and PLD2 product PA to increase local production of PIP_2 in the plasma membrane (*29*). RhoA-dependent-PLD1 has been found to play a role in stimulation of actin stress fiber formation. Actin stress fiber formation in response to LPA is inhibited by expression of the catalytically inactive form of PLD1 but not PLD2 (*30*).

Growth and differentiation. PLD is activated in mammalian cells in response to a variety of mitogenic stimuli involving EGF, PDGF, insulin, Src, and LPA, and primary alcohol, which inhibits PA generation by PLD, suppresses cell proliferation, suggesting that PLD activity plays a role in cell proliferation. This notion has been supported by evidence that PLD2 is involved in insulin-induced ERK activation by inducing membrane translocation of Raf-1, which is directly bound to PA (*25, 31*). Thus, PLD2 may be involved in mitogenic signaling *via* cross-talk with the ras/ERK signaling cascade.

Numerous studies have suggested that PLD might be involved in tumorigenesis. Enhanced expressions and increases of activity of two PLD isoforms have been found in cancer cells and tissues (*32–34*). We have observed that ARF-activated PLD activity is transiently increased in

regenerating liver nuclei, and that PLD2 level is markedly elevated in nuclei of Hepatoma cells and kidney tumors (*34, 35*). Other evidence has shown that the PLD2 levels of caveolae are markedly elevated in oncogenic stimulated cells and multidrug-resistant cancer cells (*36*). More direct evidence has shown that overexpression of PLD1 and PLD2 in mouse fibroblasts induces colony formation in soft agar, and both transformants induce undifferentiated sarcoma when transplanted into nude mice (*37*). These studies suggest that overexpression of the two PLD isozymes may play an important role in neoplastic transformation.

Our studies have implicated PLD in the regulation of cell differentiation in several cell types. mRNA expressions of PLD1a and PLD1b, and Rho- or ARF-dependent PLD1 activity were increased during differentiation of dbcAMP-induced HL60 cells and C6 glioma cells, and of NGF-induced PC12 cells (*38*). Elevation of PLD1a expression and the activity is also observed in differentiation of epidermal keratinocytes in response to 1,25-dihydroxyvitamin D3 (*39*). On the other hand, ARF-dependent PLD activity and PLD1 protein level are concomitantly decreased during differentiation of F9 cells into pariental endoderm (*40*). These data suggest a role for PLD1 and PLD2 in regulation of differentiation in diverse cell types. Although the mechanism(s) of regulation by PLD of cell differentiation remains unclear, one possible explanation may lie in recent results implicating PLD1 and PLD2 activities in the P38 MAP kinase pathway, which plays a role in cell differentiation as an upstream or downstream regulator (*21, 24, 41*).

Apoptosis and cell survival. Accumulating evidence suggests that sphingomyelin metabolites such as ceramide and sphingosine 1-phosphate (S1P) are involved in regulation of apoptosis and survival, respectively. In several cell types, cell-permeable ceramides decreased PLD activity (*42*). *In vitro* studies indicate that ceramide-mediated decreases of the two PLD isozyme activities are due to direct effects of ceramides on PLD per se or on its activating factors. The PLD product PA is able to inhibit ceramide-induced apoptotic events, caspase activation, and PARP proteolysis via inhibition of protein phosphatase 1 (*43*). These findings suggest that PLD activities may be involved in apoptosis, and other evidence supports this hypothesis. In hematopoietic cells (Jurkat T cells, A20 B cells, and HL60 cells), apoptosis-inducing drugs such as actinomycin D, anti-Fas antibody, TNF-α, and anti-cancer drugs induce up-regulation of PLD activity together with apoptotic changes (*42*). The PLD activity in Jurkat cells is oleate-dependent, which may be PLD2, since oleate can activate PLD2 but inhibit PLD1. On the other hand, PLD activity in A20 cells and HL60 cells is regulated by PC-PLC/PKC. These PLD activations are considered to be an anti-apoptotic event. In this context, it has been demonstrated that PC12 cells overexpressing wild-type PLD2, but not the inactive mutant, show suppressive effects on H_2O_2- or hypoxia-induced cell death and caspase-3 activation in PC12 cells (*44, 45*). Accumulating evidence in support of this hypothesis includes the finding that sphingosine-1-phosphate (S1P), an important mediator in mitogenic and survival signaling, stimulates PLD activity through Edg-1, Edg-3, or Edg-5 in various cells (*13, 46, 47*). Further, sphingosine kinase, an S1P-producing and pro-apoptotic enzyme, is stimulated by the PLD product PA (*48*). Recently, we have presented evidence that PLD may participate in Edg-3 receptor–mediated activation of the Akt/PKB pathway, which plays a crucial role in anti-apoptotic or pro-survival signaling (*46*). These studies were based on the finding that 1-butanol, but not 2-butanol, prevented sphingosine kinase and PI3K/Akt activation in response to agonist stimulation. However, the exact mechanisms by which PLD activates these enzymes remain to be defined.

Concluding remarks

Cloning of PLD genes has provided further insight into the regulation and catalytic mechanisms of the PLD. Recent evidence suggests that PLD participates in various signal-transduction cascades, and that there is cross-talk between PLD pathways and complex signal-tranduction networks, such as the Ras/MAPK pathway, Arf/PI4P-5K, sphingomyelin metabolites, and PI3K/Akt. This cross-talk, characteristic of important cellular signal-transductions, implies that PLD also plays important roles in regulation of glucose transport, the actin cytoskeleton reorganization and membrane ruffling, secretion, cell growth and apoptosis/cell survival. In seeking deeper insight into these PLD biological functions, it will be important to fully understand the downstream events that occur in consequence of PLD activation, and to identify target proteins that are directly or indirectly regulated by PA in the cells.

REFERENCES

1. Exton, J.H. (1998) Phospholipase D. *Biochem. Biochim. Acta* **1436**, 105–115
2. Frohman, M.A. and Morris, A.J. (1999) Phospholipase D structure and regulation. *Chem. Phys. Lipids* **98**, 127–140
3. Liscovitch, M., Czarny, M., Fiucci, G., and Tang, X. (2000) Phospholipase D: molecular and cell biology of a novel gene family. *Biochem. J.* **345**, 401–415
4. Cockcroft, S. (2001) Signalling roles of mammalian phospholipases D1 and D2. *Cell. Mol. Life Sci.* **58**, 1674–1687
5. Hammond, S.M., Altshuller, Y.M., Sung, T.C., Rudge, S.A., Rose, K., Engebrecht, J., Morris, A.J., and Frohman, M.A. (1995) Human ADP-ribosylation factor-activated phosphatidylcholine-specific phospholipase D defines a new and highly conserved gene family. *J. Biol. Chem.* **270**, 29640–29643
6. Hammond, S.M., Jenco, J.M., Nakashima, S., Cadwallader, K., Gu, Q., Cook, S., Nozawa, Y., Prestwich, G.D., Frohman, M.A., and Morris, A.J. (1997) Characterization of two alternatively spliced forms of phospholipase D1: activation of the purified enzymes by phosphatidylinositol 4,5-bisphosphate, ADP-ribosylation factor, and Rho family monomeric GTP-binding proteins and protein kinase C-α. *J. Biol. Chem.* **271**, 3860–3868
7. Colley, W.C., Sung, T.C., Roll, R., Jenco, J., Hammond, S.M., Altshuller, Y., Bar-Sagi, D., Morris, A.J., and Frohman, M.A. (1997) Phospholipase D2, a distinct phospholipase D isoform with novel regulatory properties that provokes cytoskeletal reorganization. *Curr. Biol.* **7**, 191–201
8. Kanoh, H., Nakashima, S., Zhao, Y., Sugiyama, Y., Kitajima, Y., and Nozawa, Y. (1998) Molecular cloning of a gene encoding phospholipase D from the pathogenic and dimorphic fungus, *Candida albicans*. *Biochim. Biophys. Acta* **1398**, 359–364
9. Sciorra, V.A., Rudge, S.A., Prestwich, G.D., Frohman, M.A., Engebrecht, J., and Morris, A.J. (1999) Identification of a phosphoinositide binding motif that mediates activation of mammalian and yeast phospholipase D isozymes. *EMBO J.* **20**, 5911–5921
10. Zhang, Y., Altshuller, Y.M., Hammond, S.M., Morris, A.J., and Frohman, M.A. (1999) Loss of receptor regulation by a phospholipase D1 mutant unresponsive to protein kinase C. *EMBO J.* **18**, 6339–6348
11. Kim, Y., Han, J.M., Han, B.R., Lee, K.A., Kim, J.H., Lee, B.D.,

Jang, I.H., Suh, P.G., and Ryu, S.H. (2000) Phospholipase D1 is phosphorylated and activated by protein kinase C in caveolin-enriched microdomains within the plasma membrane. *J. Biol. Chem.* **275**, 13621–13627
12. Slaaby, R., Du, G., Altshuller, Y.M., Frohman, M.A., and Seedorf, K. (2000) Insulin-induced phospholipase D1 and phospholipase D2 activity in human embryonic kidney-293 cells mediated by the phospholipase Cγ and protein kinase a signaling cascade. *Biochem. J.* **351**, 613–619
13. Banno, Y., Fujita, H., Ono, Y., Nakashima, S., Ito, Y., Kuzumaki, N., and Nozawa, Y. (1999) Differential phospholipase D activation by bradykinin and sphingosine 1-phospahte in NIH 3T3 fibroblasts overexpressing gelsolin. *J. Biol. Chem.* **274**, 27385–27391
14. Oishi, K., Takahashi, M., Mukai, H., Banno, Y., Nakashima, S., Kanaho, Y., Nozawa, Y., and Ono, Y. (2001) PKN regulates phospholipase D1 through direct interaction. *J. Biol. Chem.* **276**, 18096–18101
15. Siddiqi, A.R., Srajer, G.E., and Leslie, C.C. (2000) Regulation of human PLD1 and PLD2 by calcium and protein kinase C. *Biochim. Biophys. Acta* **1497**, 103–114
16. Park, J.B., Kim, J.H., Kim, Y., Ha, S.H., Kim, J.H., Yoo, J.S., Du, G., Frohman, M.A., Suh, P.G., and Ryu, S.H. (2000) Cardiac phospholipase D2 localizes to sarcolemmal membranes and is inhibited by α-actinin in an ADP-ribosylation factor-reversible manner. *J. Biol. Chem.* **275**, 21295–21301
17. Lee, S., Park, J.B., Kim, J.H., Kim, Y., Kim, J.H., Shin, K.J., Lee, J.S., Ha, S.H., Suh, P.G., and Ryu, S.H. (2001) Actin directly interacts with phospholipase D, inhibiting its activity. *J. Biol. Chem.* **276**, 28252–28260
18. Ito,Y., Nakashima, S., and Nozawa, Y. (1997) Hydrogen peroxide-induced phospholipase D activation in rat pheochromocytoma PC12 cells: possible involvement of Ca^{2+}-dependent protein tyrosine kinase. *J. Neurochem.* **69**, 729–736
19. Natarajan, V., Vepa, S., Verma, R., and Scribner, W.M. (1996) Role of protein tyrosine phosphorylation in H_2O_2-induced activation of endothelial cell phospholipase D. *Am. J. Physiol.* **271**, L400–L408
20. Slaaby, R., Jensen, H.S., Hansen, M., Frohman, M.A., and Seedorf, K. (1998) PLD2 complexes with the EGF receptor and undergoes tyrosine phosphorylation at a single site upon agonist stimulation. *J. Biol. Chem.* **273**, 33722–33727
21. Banno, Y., Wang, S., Ito, Y., Izumi, T., Nakashima, S., Shimizu, T., and Nozawa, Y. (2001) Involvement of ERK and p38 MAP kinase in oxidative stress-induced phospholipase D activation in PC12 cells. *Neuroreport* **12**, 2271–2275
22. Bandyopadhay, G., Sajan, M.P., Kanoh, Y., Standaert, M.L., Quon, M.J., Reed, B.C., Dikic, I., and Farese, R.V. (2001) Glucose activates protein kinase C-ζ/λ through prolin-rich tyrosine kinase–2, extracellular signal-regulated kinase, and phospholipase D. *J. Biol. Chem.* **276**, 35537–35545
23. Muthalif, M.M., Parmentier, J.H., Benter, I.f., Karzoun, N., Ahmed, A., Khandekar, Z., Adl, M.Z., Bourgoin, S., and Malik, K.U. (2000) Ras/mitogen-activated protein kinase mediates norepinephrine-induced phospholipase D activation in rabbit aortic smooth muscle cells by a phosphorylation-dependent mechanism. *J. Pharm. Exper. Ther.* **293**, 268–274
24. Natarajan, V., Scribmer, W.M., Morris, A.J., Roy, S., Vepa, S., Yang, J., Wadgaonkar, R., Reddy, S.P.M., Garcia, J.G.N., and Prinandi, N.L. (2001) Role of p38 MAP kinase in diperoxovanadate-induced phospholipase D activation in endothelial cells. *Am. J. Physiol. Lung Cell Mol. Physiol.* **281**, L435–L449
25. Manifava, M., Thuring, J.W.J.F., Lim, Z.Y., Packman, L., Holmes, A.B., and Ktistakis, N.T. (2001) Differential binding of traffic-related proteins to phosphatidic acid- or phosphatidylinositol(4,5)-bisphosphate-coupled affinity reagents. *J. Biol. Chem.* **276**, 8987–8994
26. Freyberg, Z., Sweeney, D., Siddhanta, A., Bourgoin, S., Frohman, M., and Shielda, D. (2001) Intracellular localization of phospholipase D1 in mammalian cells. *Mol. Biol. Cell* **12**, 943–955
27. Denmat-Ouisse, L.A., Phebidias, C., Honkavaara, P., Robin, P., Geny, B., Min, D.S., Bourgoin, S., Frohman, M.A., and Raymond, M.N. (2001) Regulation of constitutive protein transit by phospholipase D in HT29-cl19A cells. *J. Biol. Chem.* **276**, 48840–48846
28. Emoto, M., Klarlund, J.K., Waters, S.B., Hu, V., Buxton, J.M., Chawia, A., and Czech, M.P. (2000) A Role for phospholipase D in GLUT4 glucose transporter translocation. *J. Biol. Chem.* **275**, 7144–7151
29. Honda, A., Nogami, M., Yokozeki, T., Yamazaki, M., Nakamura, H., Watanabe, H., Kawamoto, K., Nakayama, K., Morris, A.J., Frohman, M.A., and Kanaho, Y. (1999) Phosphatidylinositol 4-phosphate 5-kinase α is a downstream effector of the small G protein ARF6 in membrane ruffle formation. *Cell* **99**, 521–532
30. Kam, Y. and Exton, J.H. (2001) Phospholipase D activity is required for actin stress fiber formation in fibroblasts. *Mol. Cell. Biol.* **21**, 4055–4066
31. Rizzo, M.A., Shome, K., Vasudevan, C., Stolz, D.B., Sung, T.C., Frohman, M.A., Watkins, S.C., and Romero, G. (1999) Phospholipase D and its product, phosphatidic acid, mediate agonist-dependent Raf-1 translocation to the plasma membrane and the activation of the mitogen-activated protein kinase pathway. *J. Biol. Chem.* **274**, 1131–1139
32. Yoshida, M., Okamura, S., Kodaki, T., Mori, M., and Yamashita, S. (1998) Enhanced levels of oleate-dependent and Arf-dependent phospholipase D isoforms in experimental colon cancer. *Oncol. Res.* **10**, 399–406
33. Noh, D.Y., Ahn, S.J., Lee, R.A., Park, I.A., Kim, J.H., Suh, P.G., Ryu, S.H., Lee, K.H., and Han, J.S. (2000) Overexpression of phospholipase D1 in human breast cancer tissues. *Cancer Lett.* **161**, 207–214
34. Zhao, Y., Ehara, H., Akao, Y., shamoto, M., Nakagawa, Y., Banno, Y., Deguchi, T., Ohishi, N. Yagi, K., and Nozawa, Y. (2000) Increased activity and intranudeate expression of phospholipase D2 in human renal cancer. *Biochim. Biophys. Res. Commun.* **278**, 140–143
35. Banno, Y., Tamiya-Koizumi, K., Oshima, H., Morikawa, A., Yoshida, S., and Nozawa, Y. (1997) Nuclear ADP-riosylation factor (ARF)- and oleate-dependent phospholipase D (PLD) in rat liver cells. Increases of ARF-dependent PLD activity in regenerating liver cells. *J. Biol. Chem.* **272**, 5208–5213
36. Fiucci, G., Czarny, M., Lavie, Y., Zhao, D., Berse, B., Blusztajn, K., and Liscovitch, M. (2000) Changes in phospholipase D isoform activity and expression in multidrug-resistant human cancer cells. *Int. J. Cancer* **85**, 882–888
37. Min, D.S., Kwon, T.K., Park, W.s., Chang, T.S., Park, S.K., Ahn, B.H., Ryoo, Z.Y., Lee, Y.H., Lee, S.S., Rhie, D.J., Yoon, S.H., Hahn, S.J., Kim, M.S., and Jo, Y.H. (2001) Neoplastic transformation and tumorigenesis associated with overeexpression of phospholipase D isozymes in cultured murine fibroblasts. *Carcinogenesis* **22**, 1641–1651
38. Nakashima, S. and Nozawa, Y. (1999) Possible role of phospholipase D in cellular differentiation and apoptosis. *Chem. Phys. Lipids* **98**, 153–164
39. Griner, R.D., Qin, F., Jung, E., Sue-Ling, C.K., Crawford, K.B., Mann-Blakeney, R., Bollag, R.J., and Bollag, W.B. (1999) 1,25-Dihydroxyvitamin D3 induces phospholipase D-1 expression in primary mouse epidermal keratinocytes. *J. Biol. Chem.* **274**, 4663–4670
40. Min, D.S., Shin, K.S., Kim, E.G., Kim, S.R., Yoon, S.H., Kim, M.S., and Jo, Y.H. (1999) Down-regulation of phospholipase D during differentiation of mous F9 teratocarcinal cells. *FEBS Lett.* **454**, 197–200
41. Bechous, S. and Daniel, L.W. (2001) Phospholipase D is required in the signaling pathway leading to p38 MAPK activation in neutrophil-like HL60 cells, stimulated by N-formyl-methionyl-leucyl-phenylalanine. *J. Biol. Chem.* **276**, 31752–31759
42. Nozawa, Y. (2002) Role of phospholipase D in apoptosis and pro-survival signaling. *Biochim. Biophys. Acta*, in press
43. Kishikawa, K., Chalfant, C.E., Perry, D.K., Bielawska, A., and Hannun, Y. (1999) Phosphatidic acid is a potent and selective inhibitor of protein phosphatase 1 and an inhibitor of ceramide-

mediated responses. *J. Biol. Chem.* **274**, 21335–21341
44. Lee, S.D., Lee, B.D., Han, J.M., Kim, J.H., Kim, Y., Suh, P.G., and Ryu, S.H. (2000) Phospholipase D2 activity suppresses hydrogen peroxide-induced apoptosis in PC12 cells. *J. Neurochem.* **75**, 1053–1059
45. Yamakawa, H., Banno, Y., Nakashima, S., Sawada, M., Yamada, J., Yoshimura, S., Nishimura, Y., Nozawa, Y., and Sakai, N. (2000) Increased phospholipase D2 activity during hypoxia-induced death of PC12 cells: its possible anti-apoptotic role. *Neuroreport* **11**, 3647–3650
46. Banno, Y., Takuwa, Y., Akao, Y., Okamoto, H., Osawa, Y., Naganawa, T., Nakashima, S., Suh, P.G., and Nozawa, Y. (2001) Involvement of phospholipase D in sphingosine 1-phosphate-induced activation of phosphatidylinositol 3-kinase and Akt in Chinese hamster ovary cells overexpressing EDG3. *J. Biol. Chem.* **276**, 35622–35628
47. Orlanti, S., Porceli, A.M., Hrelia, S., Brocklyn, J.R.V., Spiegel, S., and Rugolo, M. (2000) Sphingosine-1-phosphate activates phospholipase D in human airway epithelial cells via a G protein-coupled receptor. *Arch. Biochem. Biophys.* **375**, 69–77
48. Melendez, A., Floto, R.A., Gillooly, D.J., Harnett, M.M., and Allen, J.M. (1998) FcγRI coupling to phospholipase D initiates sphingosine kinase-mediated calcium mobilization and vesicular trafficking. *J. Biol. Chem.* **273**, 9393–9402