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Cellular and Physiological Functions of Phosphoinositide-Metabolizing Enzymes

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Abstract：Phosphatidylinositol 4,5-bisphosphate（$PI(4,5)P_2$）is not only a component of the plasma membrane, but it also plays various physiological functions including membrane trafficking, actin cytoskeletal reorganization, endocytosis, exocytosis, and the regulation of ion channels and transporters. $PI(4,5)P_2$ also becomes a precursor of intracellular signal transduction. The *in vivo* physiology of phosphoinositide（PI）-metabolizing enzymes has been analyzed based on the study of gene knockout mice. This review summarizes the cellular and physiological functions of PI-metabolizing enzymes.

Key words：**Phosphatidylinositol, Phospholipase C, PI-Kinase, Ca^{2+} Signaling**

Introduction

Phosphoinositides（PIs）are not abundant（less than 10 % of total cellular phospholipids），yet they function in a remarkable number of crucial cellular processes [1)–3)]. PIs undergo rapid phosphorylation/dephosphorylation at positions D3, D4, and D5 of their inositol head group by PI kinases and phosphatases（Fig. 1）. Three phosphatidylinositol monophosphates（PI(3)P, PI(4)P, and PI(5)P），three phosphatidylinositol bisphosphates（$PI(3,4)P_2$, $PI(3,5)P_2$, and $PI(4,5)P_2$），and one phosphatidylinositol trisphosphates（$PI(3,4,5)P_3$）have been identified in mammals（Fig. 1B）. $PI(4,5)P_2$ is the best characterized one（Fig. 1A）. $PI(4,5)P_2$ can be generated through two distinct phosphorylation pathways. PI(4)P 5-kinase（PIP5K）phosphorylates PI(4)P at D5 position to catalyze the synthesis of $PI(4,5)P_2$. PI(5)P, which has a very low concentration in cells, is also phosphorylated by PI(5)P 4-kinase（PIP4K）[4)]. Pulse-labeling studies and the cellular level of PI(5)P, indicate that PI(4)P phosphorylation by PIP5Ks is probably the major synthetic pathway in mammals [4)–6)]. Intact $PI(4,5)P_2$ has a role in cytoskeletal reorganization, membrane ruffling, endocytosis, regulated exocytosis, regulation of ion channels and transporters, chromatin remodeling, and recruitment of signaling molecules [1)–3), 7)–9)]. $PI(4,5)P_2$ also functions as a precursor of two independent signaling pathways. PI 3-kinases phosphorylate D3 position of $PI(4,5)P_2$ to produce $PI(3,4,5)P_3$. $PI(3,4,5)P_3$ and/or other PI derivatives are involved in multiple cellular events including cell survival, proliferation, phagocytosis, membrane ruffling, membrane trafficking, and recruitment of signaling molecules [1)–3), 7)–9)]. PI-specific phospholipase-C（PLC）hydrolyzes $PI(4,5)P_2$ producing two second messengers, inositol 1,4,5-trisphosphate（$I(1,4,5)P_3$）and diacylglycerol, which participate in intracellular Ca^{2+} mobilization and protein kinase C activation, respectively [10)–12)]. This review will summarize the recent progress in characterizing the cellular and physiological functions of PI-metabolizing enzymes.

PLC isozymes and their domain structure

There are 13 PLC isozymes that are divided into six main families, PLC-β1 to β4, PLC-γ1 and γ2, PLC-δ1, δ3 and δ4, PLC-ε, PLC-ζ, and PLC-η1 and η2（Fig. 2）[10)–12)]. All PLC isozymes contain well-conserved catalytic X and Y domains. The crystal structure of PLC-δ1 shows that

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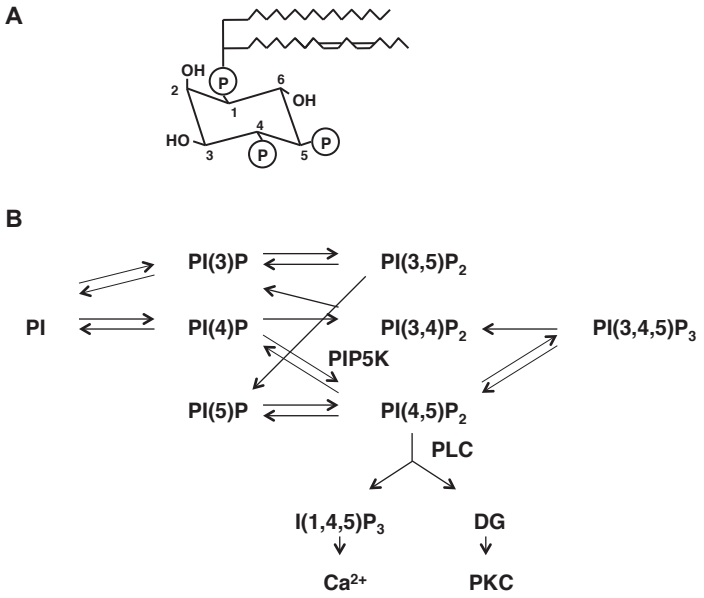

Fig. 1 Chemical structure and metabolism of the phosphoinositides. A, A schematic representation of PI(4,5)P_2. A diacylglycerol moiety is attached through a diester phosphate to the 1-hydroxyl of the inositol ring. The D4 and D5 positions of inositol ring are phosphorylated. B, Metabolic pathway of phosphoinositides. PIs are phosphorylated/dephosphorylated by PI kinases and phosphatases. PI(4,5)P_2 is mainly synthesized from PI(4)P, which is catalyzed by PIP5Ks and is hydrolyzed by PLC to produce two second messengers, I(1,4,5)P_3 and diacylglycerol.

these catalytic domains are composed of alternating α-helices and β-strands and resemble the triose phosphate isomerase (TIM) barrel [13]. Each isozyme contains their own unique domain for the regulation of activity and subcellular distribution. One of these domains is the pleckstrin homology (PH) domain. The PH domain of PLC-δ1 binds both PI(4,5)P_2 and I(1,4,5)P_3, and these interactions are important for its activity and localization in the plasma membrane [14)–16)]. The structural data from the isolated PLC-δ1 PH domain, which was co-crystallized with I(1,4,5)P_3, shows that the PH domain is composed of seven anti-parallel β-strands and one C-terminus α-helix. The positively charged amino acids on the loop between β-strands (loop1/2, loop3/4, and loop6/7) have crucial role for PI(4,5)P_2 and I(1,4,5)P_3 binding [17]. The C2 domain of PLC-δ1 contains three to four Ca^{2+} binding sites [18]. Ca^{2+} ions bind to the C2 domain and enhance the enzymatic activity of PLC-δ1 by forming a PLC-δ1/Ca^{2+}/phosphatidylserine ternary complex.

PLC-β

PLC-β has four isozymes, β1 to β4 [10)–12)]. In addition to PH, EF, X, Y, and C2 domains, they possess a unique long C-terminus region (Fig. 2). PLC-βs are activated by direct binding of heterotrimeric G proteins (Gα and Gβγ) to unique C-terminus or other regions. The *in vivo* distribution of PLC-β isozymes is tissue-dependent. PLC-β1 is widely expressed, especially in the cerebral cortex and hippocampus. Other PLC-β isozymes have a more restricted distribution. PLC-β2 is expressed in hematopoietic cells; PLC-β3 is found in the brain, liver, and parotid glands; PLC-β4 is present at the highest level in the cerebellum and retina. Knockout (KO) mice for PLC-β isozymes were developed and their phenotypes were analyzed. The PLC-β1 KO mice show epileptic seizures and related sudden death because PLC-β1 is involved in muscarinic acetylcholine receptor signaling in the cerebral cortex and hippocampus [19]. Neutrophils derived from PLC-β2 KO mice show impairment of chemoattractant-mediated elevation of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) [20]. PLC-β3 KO mice have increased sensitivity to morphine, thus suggesting that PLC-β3 is a significant negative regulator of μ opioid receptor signaling [21]. PLC-β3 KO mice show significant defects in scratching behavior induced by histamine, which suggested that PLC-β3 is required to mediate itch sensation in response to histamine [22]. The PLC-β4 KO mice exhibit ataxia because PLC-β4 is involved in the metabotropic glutamate receptor signaling in the cerebellum [19].

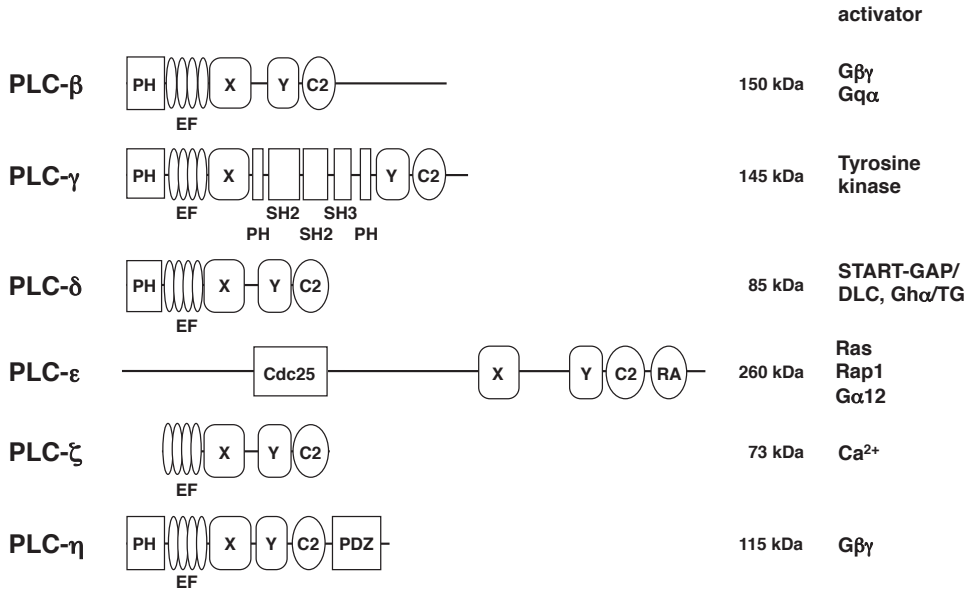

Fig. 2 A schematic representation of the mammalian PI-PLC isozymes. PH; pleckstrin homology domain which binds $PI(4,5)P_2$ and $I(1,4,5)P_3$. EF; EF-hand domain. X and Y; catalytic domain. C2; C2 domain is a Ca^{2+}-dependent phosphatidylserine binding domain. SH2 and SH3; src homology 2 and 3 domain which binds a phosphorylated tyrosine containing motif and proline rich motif, respectively. Cdc25; cdc25 domain functions as GTP/GDP exchange factor for small GTPase Rap1A. RA; RA domain, which mediates GTP-dependent interaction with small GTPase Ras or Rap. PDZ; PDZ domain is involved with protein - protein interaction. The activators of each PLC isozyme are listed on the right.

PLC-γ

PLC-γ has two isozymes, γ1 and γ2 [10)–12)]. PLC-γ possesses unique domains (a split PH domain, two SH2 domains, and one SH3 domain) between conserved catalytic X and Y domains (Fig. 2) which are implicated in the activation of PLC-γ isozymes downstream of receptor or non-receptor tyrosine kinase activity. PLC-γ1 is widely distributed in various tissues and is abundant in embryonic cortical structures, neurons, oligodendrocytes, and astrocytes. On the other hand, PLC-γ2 is expressed in the cerebellar Purkinje cells, granule cells, and the lineages of hematopoietic cells. PLC-γ1 KO mice are lethal at embryonic day 9 due to the impairment of vasculogenesis and erythropoiesis [23), 24)]. PLC-γ2 KO mice show defects in the function of B cells, platelets, mast cells, and natural killer cells [25), 26)]. Loss of PLC-γ2 signaling in human B cells causes the immunodeficiency syndrome X-linked agammaglobulinemia [27)].

PLC-δ

PLC-δ isozymes are well conserved from fungi, yeasts, plants, to mammalian cells, so they are thought to be a prototype of PLC [10)–12)]. Three isozymes, δ1, δ3, and δ4, are identified in mammalian cells. Previous studies reported that there were four isozymes of δ1 to δ4 in mammals; however, a study recently found that PLC-δ2 is the same enzyme as PLC-δ4 [28)]. The activation mechanisms of PLC-δ are poorly understood, but several candidate molecules for activating PLC-δ including START-GAP/DLC1 (p122/RhoGAP) and a subunit of a heterodimeric G protein (Ghα/transglutaminase) have been identified. In addition, it is possible that PLC-δ functions as an amplifier of Ca^{2+} signaling, since PLC-δ is activated at lower $[Ca^{2+}]_i$ in comparison to other PLCs. PLC-δ1 KO mice show a hair loss phenotype associated with abnormal hair follicle structures and epidermal hyperplasia due to an impairment of Ca^{2+} signaling and PKC activation in keratinocytes [29)]. PLC-δ3 KO mice are normal but PLC-δ1/3 double KO mice became embryonic lethal at embryonic day 11.5 to 13.5 due to a differentiation defect in placental development [30)]. PLC-δ4 KO male mice exhibit male infertility due to a failure of the zona pellucida (ZP)-induced acrosome reaction in sperm, which is a necessary step for mammalian fertilization [31)]. Single sperm imaging revealed that the ZP-induced $[Ca^{2+}]_i$ increase is not observed in PLC-δ4 KO sperm [32)].

Other PLC isozymes; PLC-ε, PLC-ζ, and PLC-η

PLC-ε has been identified as mammalian homologue of *C. elegans* PLC210 [10)–12), 33)]. PLC-ε has unique

Cdc25 homology domain which functions as a GTP/GDP exchange factor for small GTPase Rap1A and RA domain that mediates GTP-dependent interaction with small GTPase Ras or Rap [33]. Therefore, PLC-ε thought to connect between Ras-mediated and PLC-dependent signaling pathways. PLC-ε KO mice exhibit the defects in the development of the aortic and pulmonary valves [34].

PLC-ζ is a sperm specific PLC which lacks the PH domain [10)–12), 35)]. PLC-ζ is activated at the basal level of $[Ca^{2+}]_i$ ranging 10 to 100 nM and the microinjection of RNA encoding PLC-ζ into mouse eggs induces Ca^{2+} oscillation and subsequent early embryonic development [35].

The last group of the PLC isozymes is PLC-η, which was identified by homology in a database search [10)–12), 36)]. Both PLC-η1 and PLC-η2 are neuron specific isozymes. PLC-η1 is dominantly expressed in the hippocampus and cerebellar Purkinje cell layer and PLC-η2 is expressed in the hippocampus, cerebral cortex, and olfactory bulb [36]. PLC-η has comparable or more sensitivity to intracellular Ca^{2+} than PLC-δ1 for PLC activity. The Ca^{2+} signaling plays an important role in neuronal functions including regulation of axonal growth and retraction, growth-cone guidance, synapse formation, and neurotransmitter release, therefore PLC-η may function as Ca^{2+}-sensor enzymes that are activated by a small increase of $[Ca^{2+}]_i$.

PIP5K

Three PIP5K isozymes, PIP5Kα, PIP5Kβ (human and murine α and β isozymes are reversed), and PIP5Kγ, with alternative splice variants have been identified [37)–39)]. All PIP5K isozymes have a well-conserved central region which includes the kinase catalytic domain. Substrate specificity and subcellular targeting of PIP5Ks are determined by an activation loop spanning the catalytic domain [40), 41)].

$PI(4,5)P_2$ is functionally involved in cytoskeletal reorganization, membrane ruffling, endocytosis, regulated exocytosis, chromatin remodeling, and recruitment of signaling molecules [1)–3), 7)]. Spatially and temporally organized regulation of $PI(4,5)P_2$ levels by PIP5Ks as well as PLCs and PI 3-kinases could be required to maintain these various functions. In fact, an overexpression of PIP5Ks induces stress fibers [42), 43)], membrane ruffles [44], comet-like actin tails [45], and microvilli [46], accompanied by locally elevated $PI(4,5)P_2$. Moreover, recent report showed that PIP5Kγ661 isozyme, which is activated by NMDA receptor at restricted areas of postsynapses, produces $PI(4,5)P_2$ and induces AMPA receptor endocytosis during LTD [47]. $PI(4,5)P_2$ is also known to regulate ion channels and transporters including TRP channels, K_{ATP} channels, and Na^+/Ca^{2+} exchanger type 1 (NCX1) [8), 9)]. NCX1 has a $PI(4,5)P_2$-binding domain, called as XIP region, in the cytoplasmic loop. Previous electrophysiological studies showed that $PI(4,5)P_2$ exhibits two opposite effects on NCX1 activity [48]. Exogenous $PI(4,5)P_2$ ablated inactivation mechanism, resulted in enhanced activity of NCX1 in membrane patch. On the other hand, transient overexpression of either PIP5Kα or PIP4Kα increase endogenously synthesized $PI(4,5)P_2$, but decreases the NCX1 current densities in intact cells, thus suggesting that the endogenously synthesized $PI(4,5)P_2$ would promote the endocytosis of NCX1. Intriguingly, cardiac-specific PIP4Kα transgenic mice exhibited cardiac hypertrophy.

Conclusions and future issues

The PLC isozymes are redundantly expressed in numerous tissues; however, the analysis of PLC KO mice showed each PLC has their own physiological functions including neuronal activity, vasculogenesis, skin development, and fertilization. Therefore, it is necessary to determine the relationship between each of the PLC isozymes. The double or triple KO of PLC isozymes will contribute additional knowledge. Currently, there is no clinical drug that targets PLCs or PIP5Ks. The development of drugs targeting these enzymes could resolve the diseases related to PI-metabolizing disorder.

The PIs are rapidly turned over in cells. The KO mice of each PLC isozymes brought a lot of important information; however, the relationship between rapid PI-turnover and their physiological functions *in vivo* at the cellular level remains unknown. Therefore it is necessary to develop an *in vivo* PI-monitoring system. The transgenic mice with fluorescent protein fused to a PI-binding domain are therefore considered to be one of the candidates to eventually solve this problem.

References

1) Simonsen, A., Wurmser, A. E., Emr, S. D. & Stenmark, H. The role of phosphoinositides in membrane transport. Curr Opin Cell Biol 13: 485-492, 2001.
2) De Matteis, M. A. & Godi, A. PI-loting membrane traffic. Nat Cell Biol 6: 487-492, 2004.
3) Di Paolo, G. & De Camilli, P. Phosphoinositides in cell

regulation and membrane dynamics. Nature 443: 651-657, 2006.
4) Rameh, L. E., Tolias, K. F., Duckworth, B. C. & Cantley, L. C. A new pathway for synthesis of phosphatidylinositol-4,5-bisphosphate. Nature 390: 192-196, 1997.
5) Stephens, L. R., Hughes, K. T. & Irvine, R. F. Pathway of phosphatidylinositol (3,4,5)-trisphosphate synthesis in activated neutrophils. Nature 351: 33-39, 1991.
6) Whiteford, C. C., Brearley, C. A. & Ulug, E. T. Phosphatidylinositol 3,5-bisphosphate defines a novel PI 3-kinase pathway in resting mouse fibroblasts. Biochem J 323: 597-601, 1997.
7) Niggli, V. Regulation of protein activities by phosphoinositide phosphates. Annu Rev Cell Dev Biol 21: 57-79, 2005.
8) Hilgemann, D. W., Feng, S. & Nasuhoglu, C. The complex and intriguing lives of PIP_2 with ion channels and transporters. Sci STKE 2001: re19, 2001.
9) Suh, B. C. & Hille, B. Regulation of ion channels by phosphatidylinositol 4,5-bisphosphate. Curr Opin Neurobiol 15: 370-378, 2005.
10) Rebecchi, M. J. & Pentyala, S. N. Structure, function, and control of phosphoinositide-specific phospholipase C. Physiol Rev 80: 1291-1335, 2000.
11) Suh, P. G., Park, J. I., Manzoli, L., Cocco, L., Peak, J. C., Katan, M., Fukami, K., Kataoka, T., Yun, S. & Ryu, S. H. Multiple roles of phosphoinositide-specific phospholipase C isozymes. BMB Rep 41: 415-434, 2008.
12) Nakamura, Y. & Fukami, K. Roles of phospholipase C isozymes in organogenesis and embryonic development. Physiology 24: 332-341, 2009.
13) Essen, L. O., Perisic, O., Cheung, R., Katan, M. & Williams, R. L. Crystal structure of a mammalian phosphoinositide-specific phospholipase Cδ. Nature 380: 595-602, 1996.
14) Yagisawa, H., Hirata, M., Kanematsu, T., Watanabe, Y., Ozaki, S., Sakuma, K., Tanaka, H., Yabuta, N., Kamata, H. & Hirata, H. Expression and characterization of an inositol 1,4,5-trisphosphate binding domain of phosphatidylinositol-specific phospholipase C-δ1. J Biol Chem 269: 20179-20188, 1994.
15) Yagisawa, H., Sakuma, K., Paterson, H. F., Cheung, R., Allen, V., Hirata, H., Watanabe, Y., Hirata, M., Williams, R. L. & Katan, M. Replacements of single basic amino acids in the pleckstrin homology domain of phospholipase C-δ1 alter the ligand binding, phospholipase activity, and interaction with the plasma membrane. J Biol Chem 273: 417-424, 1998.
16) Fujii, M., Ohtsubo, M., Ogawa, T., Kamata, H., Hirata, H. & Yagisawa, H. Real-time visualization of PH domain-dependent translocation of phospholipase C-δ1 in renal epithelial cells (MDCK): response to hypo-osmotic stress. Biochem Biophys Res Commun 254: 284-291, 1999.
17) Ferguson, K. M., Lemmon, M. A., Schlessinger, J. & Sigler, P. B. Structure of the high affinity complex of inositol trisphosphate with a phospholipase C pleckstrin homology domain. Cell 83: 1037-1046, 1995.
18) Grobler, J. A., Essen, L. O., Williams, R. L. & Hurley, J. H. C2 domain conformational changes in phospholipase C-δ1. Nat Struct Biol 3: 788-795, 1996.
19) Kim, D., Jun, K. S., Lee, S. B., Kang, N. G., Min, D. S., Kim, Y. H., Ryu, S. H., Suh, P. G. & Shin, H. S. Phospholipase C isozymes selectively couple to specific neurotransmitter receptors. Nature 389: 290-293, 1997.
20) Jiang, H., Kuang, Y., Wu, Y., Xie, W., Simon, M. I. & Wu, D. Roles of phospholipase C β2 in chemoattractant-elicited responses. Proc Natl Acad Sci U S A 94: 7971-7975, 1997.
21) Xie, W., Samoriski, G. M., McLaughlin, J. P., Romoser, V. A., Smrcka, A., Hinkle, P. M., Bidlack, J. M., Gross, R. A., Jiang, H. & Wu, D. Genetic alteration of phospholipase C β3 expression modulates behavioral and cellular responses to μ opioids. Proc Natl Acad Sci U S A 96: 10385-10390, 1999.
22) Han, S. K., Mancino, V. & Simon, M. I. Phospholipase Cβ 3 mediates the scratching response activated by the histamine H1 receptor on C-fiber nociceptive neurons. Neuron 52: 691-703, 2006.
23) Ji, Q. S., Winnier, G. E., Niswender, K. D., Horstman, D., Wisdom, R., Magnuson, M. A. & Carpenter, G. Essential role of the tyrosine kinase substrate phospholipase C-γ1 in mammalian growth and development. Proc Natl Acad Sci U S A 94: 2999-3003, 1997.
24) Liao, H. J., Kume, T., McKay, C., Xu, M. J., Ihle, J. N. & Carpenter, G. Absence of erythrogenesis and vasculogenesis in *Plcg*1-deficient mice. J Biol Chem 277: 9335-9341, 2002.
25) Wang, D., Feng, J., Wen, R., Marine, J. C., Sangster, M. Y., Parganas, E., Hoffmeyer, A., Jackson, C. W., Cleveland, J. L., Murray, P. J. & Ihle, J. N. Phospholipase Cγ2 is essential in the functions of B

cell and several Fc receptors. Immunity 13: 25-35, 2000.

26) Hashimoto, A., Takeda, K., Inaba, M., Sekimata, M., Kaisho, T., Ikehara, S., Homma, Y., Akira, S. & Kurosaki, T. Cutting edge: essential role of phospholipase C-γ2 in B cell development and function. J Immunol 165: 1738-1742, 2000.

27) Satterthwaite, A. B., Li, Z. & Witte, O. N. Btk function in B cell development and response. Semin Immunol 10: 309-316, 1998.

28) Irino, Y., Cho, H., Nakamura, Y., Nakahara, M., Furutani, M., Suh, P. G., Takenawa, T. & Fukami, K. Phospholipase C δ -type consists of three isozymes: bovine PLCδ2 is a homologue of human/mouse PLCδ4. Biochem Biophys Res Commun 320: 537-543, 2004.

29) Nakamura, Y., Fukami, K., Yu, H., Takenaka, K., Kataoka, Y., Shirakata, Y., Nishikawa, S., Hashimoto, K., Yoshida, N. & Takenawa, T. Phospholipase Cδ1 is required for skin stem cell lineage commitment. EMBO J 22: 2981-2991, 2003.

30) Nakamura, Y., Hamada, Y., Fujiwara, T., Enomoto, H., Hiroe, T., Tanaka, S., Nose, M., Nakahara, M., Yoshida, N., Takenawa, T. & Fukami, K. Phospholipase C-δ1 and -δ3 are essential in the trophoblast for placental development. Mol Cell Biol 25: 10979-10988, 2005.

31) Fukami, K., Nakao, K., Inoue, T., Kataoka, Y., Kurokawa, M., Fissore, R. A., Nakamura, K., Katsuki, M., Mikoshiba, K., Yoshida, N. & Takenawa, T. Requirement of phospholipase Cδ4 for the zona pellucida-induced acrosome reaction. Science 292: 920-923, 2001.

32) Fukami, K., Yoshida, M., Inoue, T., Kurokawa, M., Fissore, R. A., Yoshida, N., Mikoshiba, K. & Takenawa, T. Phospholipase Cδ4 is required for Ca^{2+} mobilization essential for acrosome reaction in sperm. J Cell Biol 161: 79-88, 2003.

33) Bunney, T. D. & Katan, M. Phospholipase C epsilon: linking second messengers and small GTPases. Trends Cell Biol 16: 640-648, 2006.

34) Tadano, M., Edamatsu, H., Minamisawa, S., Yokoyama, U., Ishikawa, Y., Suzuki, N., Saito, H., Wu, D., Masago-Toda, M., Yamawaki-Kataoka, Y., Setsu, T., Terashima, T., Maeda, S., Satoh, T. & Kataoka, T. Congenital semilunar valvulogenesis defect in mice deficient in phospholipase Cε. Mol Cell Biol 25: 2191-2199, 2005.

35) Swann, K., Saunders, C. M., Rogers, N. T. & Lai, F. A. PLC-ζ (zeta) : a sperm protein that triggers Ca^{2+} oscillations and egg activation in mammals. Semin Cell Dev Biol 17: 264-273, 2006.

36) Cockcroft, S. The latest phospholipase C, PLC-η, is implicated in neuronal function. Trends Biochem Sci 31: 4-7, 2006.

37) Ishihara, H., Shibasaki, Y., Kizuki, N., Katagiri, H., Yazaki, Y., Asano, T. & Oka, Y. Cloning of cDNAs encoding two isoforms of 68-kDa type I phosphatidylinositol-4-phosphate 5-kinase. J Biol Chem 271: 23611-23614, 1996.

38) Ishihara, H., Shibasaki, Y., Kizuki, N., Wada, T., Yazaki, Y., Asano, T. & Oka, Y. Type I phosphatidylinositol-4-phosphate 5-kinases. Cloning of the third isoform and deletion/substitution analysis of members of this novel lipid kinase family. J Biol Chem 273: 8741-8748, 1998.

39) Loijens, J. C., Boronenkov, I. V., Parker, G. J. & Anderson, R. A. The phosphatidylinositol 4-phosphate 5-kinase family. Adv Enzyme Regul 36: 115-140, 1996.

40) Kunz, J., Wilson, M. P., Kisseleva, M., Hurley, J. H., Majerus, P. W. & Anderson, R. A. The activation loop of phosphatidylinositol phosphate kinases determines signaling specificity. Mol Cell 5: 1-11, 2000.

41) Kunz, J., Fuelling, A., Kolbe, L. & Anderson, R. A. Stereo-specific substrate recognition by phosphatidylinositol phosphate kinases is swapped by changing a single amino acid residue. J Biol Chem 277: 5611-5619, 2002.

42) Shibasaki, Y., Ishihara, H., Kizuki, N., Asano, T., Oka, Y. & Yazaki, Y. Massive actin polymerization induced by phosphatidylinositol-4-phosphate 5-kinase in vivo. J Biol Chem 272: 7578-7581, 1997.

43) Yamamoto, M., Hilgemann, D. H., Feng, S., Bito, H., Ishihara, H., Shibasaki, Y. & Yin, H. L. Phosphatidylinositol 4,5-bisphosphate induces actin stress-fiber formation and inhibits membrane ruffling in CV1 cells. J Cell Biol 152: 867-876, 2001.

44) Honda, A., Nogami, M., Yokozeki, T., Yamazaki, M., Nakamura, H., Watanabe, H., Kawamoto, K., Nakayama, K., Morris, A. J., Frohman, M. A. & Kanaho, Y. Phosphatidylinositol 4-phosphate 5-kinase α is a downstream effector of the small G protein ARF6 in membrane ruffle formation. Cell 99: 521-532, 1999.

45) Rozelle, A. L., Machesky, L. M., Yamamoto, M., Driessens, M. H., Insall, R. H., Roth, M. G., Luby-Phelps, K., Marriott, G., Hall, A. & Yin, H. L. Phosphatidylinositol 4,5-bisphosphate induces actin-

based movement of raft-enriched vesicles through WASP-Arp2/3. Curr Biol 10: 311-320, 2000.

46) Matsui, T., Yonemura, S., Tsukita, S. & Tsukita, S. Activation of ERM proteins in vivo by Rho involves phosphatidyl-inositol 4-phosphate 5-kinase and not ROCK kinases. Curr Biol 9: 1259-1262, 1999.

47) Unoki, T., Matsuda, S., Kakegawa, W., Van, N. T., Kohda, K., Suzuki, A., Funakoshi, Y., Hasegawa, H., Yuzaki, M. & Kanaho, Y. NMDA receptor-mediated PIP5K activation to produce PI(4,5)P_2 is essential for AMPA receptor endocytosis during LTD. Neuron 73: 135-148, 2012.

48) Yaradanakul, A., Feng, S., Shen, C., Lariccia, V., Lin, M. J., Yang, J., Kang, T. M., Dong, P., Yin, H. L., Albanesi, J. P. & Hilgemann, D. W. Dual control of cardiac Na^+-Ca^{2+} exchange by PIP_2: electrophysiological analysis of direct and indirect mechanisms. J Physiol 582: 991-1010, 2007.

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