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Structure, Regulation, and Function of Phospholipase C Isozymes

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Phospholipase C (PLC) is a key enzyme in phosphatidyl inositol turnover and generates two second messengers, inositol 1,4,5-bisphosphate (IP_3) and diacylglycerol (DAG), from phosphatidyl inositol 4,5-bisphosphate [PI(4,5)P_2] in response to activation of receptors by hormones, neurotransmitters, growth factors, and other molecules. IP_3 induces calcium mobilization, and DAG induces activation of protein kinase C. PI(4,5)P_2 is a substrate for PLC, and PI 3-kinase. In addition, PI(4,5)P_2 directly regulates a variety of cell functions, including cytoskeletal reorganization, exocytosis, and channel activity; therefore, strict regulation of PI(4,5)P_2 levels by PLC or other converting enzymes is necessary for homeostasis.

Eleven PLC isozymes have been identified in mammals, and they are divided into four classes, β(1-4)-, γ(1,2)-, δ(1-4)-, and ε(1)-type, on the basis of structure and regulatory activation mechanism (*1, 2*). Each isozyme is composed of subtype-specific domains and conserved domains. Structural analyses of the domains (*3*) have revealed the detailed mechanisms of protein-protein and protein-lipid interaction needed for anchoring the enzymes to the plasma membrane. The regulatory mechanisms of β-type and γ-type PLCs have been analyzed extensively. Association of heterotrimeric G proteins of the Gq family stimulates activity of β-type PLC, and γ-type isozymes are regulated primarily by receptor and cytosolic tyrosine kinases. In contrast, δ-type PLC isozymes are thought to be regulated by calcium. ε-type PLC was recently identified as an effector of Ras protein and is regulated by Ras in a GTP-dependent manner.

Molecular and genome-based technologies have provides new insights onto the roles of PLC in cell signaling and development. This review focuses on recent advances and physiological functions of PLC isozymes, highlighting a novel type of PLC, PLCε, and the functional analysis of each enzyme by gene-disruption techniques. In addition, the mechanisms by which the isozymes act in concert and independently will be discussed.

Structure and regulation of PLC isozymes

All PLC isozymes contain catalytic X and Y domains as well as various regulatory domains. Several domains, including the C2 domain, EF-hand domain, and pleckstrin homology (PH) domain, are conserved among the PLCs. Subtype-specific domains contribute to the specific regulatory mechanisms. These domains include the src homology (SH) domain in PLCγ and the Ras-associating (RA) domain and Ras-GTPase exchange factor (RasGEF)–like domain in PLCε (Fig. 1). PLC is a soluble protein that is localized mainly in the cytosol, and it is translocated to the plasma membrane, where it functions to hydrolyze PI(4,5)P_2, in response to receptor activation. Thus, targeting of PLC to the plasma membrane is a critical event for transducing signals.

PLCβ is composed of the N-terminal PH domain, EF-hand domain, catalytic X and Y domains, and C2 domain. In addition to these domains, β-type PLC isozymes have C-terminal extensions of approximately 400 amino acids. The PH domain of PLCδ1, which comprises approximately 100 residues, binds tightly to PI(4,5)P_2 and IP_3 (see below); however, that of β-type PLC isozymes binds neither to PI(4,5)P_2 nor to IP_3. Instead, it binds specifically to PI(3)P, although the concentration of PI(3)P in mammalian cells is very low, even when PI3-kinase is activated (*4*). The PH domain of PLCβ2 and PLCβ3 also binds the heterotrimeric G protein subunit, Gβγ (*5*). In contrast, GTP-bound Gαq binds the C2 domain of PLCβ1 and PLCβ2 (*6*). Deletion analysis showed that C-terminal basic residues in cluster regions are responsible for this binding (*7, 8*). Comparison of the ligand-binding affinities of different PLCβ isozymes revealed that PLCβ2 and PLCβ3 are more sensitive to the βγ subunit than are PLCβ1 and PLCβ4. The affinities of Gαq for PLCβ1 and PLCβ3 are higher than that for PLCβ2. These results suggest that each isozyme is regulated differently by both subunits of Gq. Interestingly, however, PLCβ2 isozymes were not activated by various stimuli in platelets from Gαq–deficient mice, showing that Gαq is essential and cannot be replaced by Gβγ (*9*). All PLC-β isozymes contain a C-terminal PDZ-binding motif, (Ser/Thr)-X-(Val/Leu)-COOH, and PLCβ3 binds specifically to Na^+/K^+ exchanger regulatory factors (NHERFs), which have two PDZ domains (*10*). These findings indicate that PLCβ might be targeted to the plasma membrane through interactions with specific partners. The *Caenorhabditis elegans* genome project revealed that genes encoding transcription factors and G-protein–coupled receptors are the most numerous ones. Through a database search, we identified several types of PLCs in *C. elegans*: two PLCβ types, and one each of PLCγ, PLCδ, and PLCε type. Therefore, PLCβ-mediated signaling may be a common pathway in *C. elegans*, as well as mice and human.

PLCγ isozymes are characterized by two SH2 domains and one SH3 domain that are located between the X and Y domains. When growth factors such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF), and nerve growth factor (NGF) engage their respective recep-

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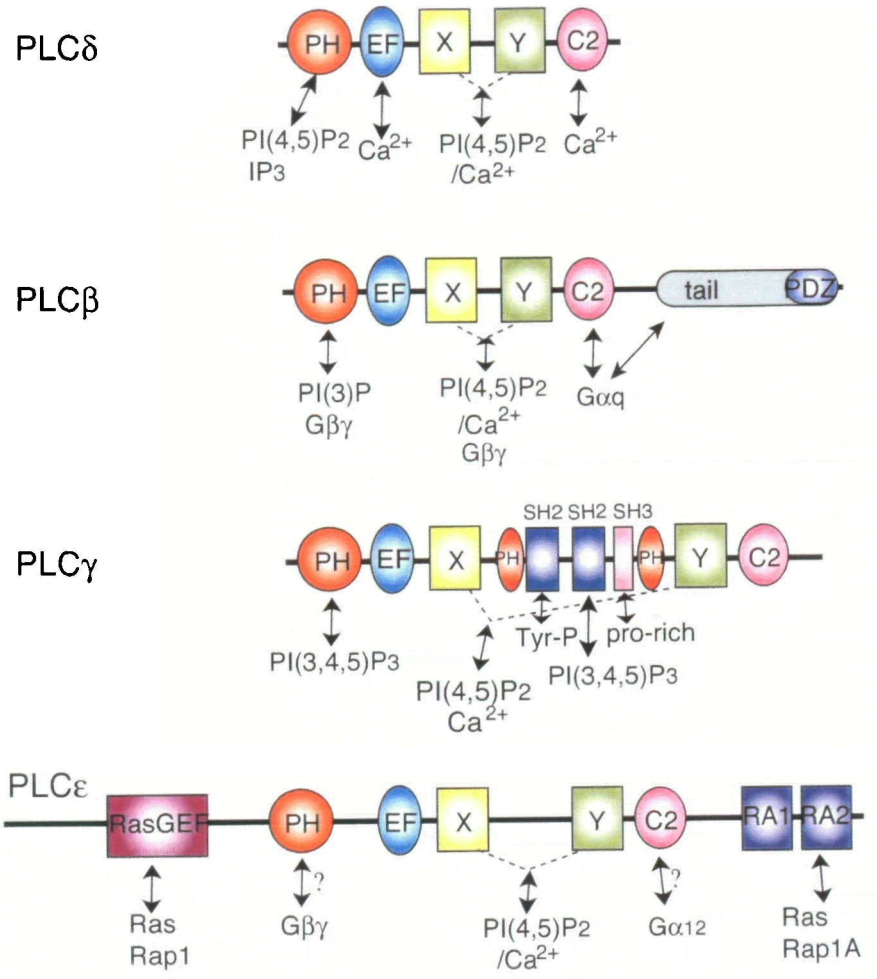

Fig. 1. **Domain structure of each type PLC.** Catalytic and regulatory domains and their interacting molecules are shown. PH, pleckstrin homology domain; EF, EF-hand domain,; X and Y domain, PLC catalytic domain; C2,C2 domain; PDZ, PDZ-binding motif; SH, src homology domain; RasGEF, Ras GTPase exchange factor–like domain; RA, Ras associating domain; Tyr-P, phosphotyrosine residues; pro-rich, proline-rich sequence.

tors, which are intrinsic tyrosine kinase, increases in cellular calcium and activation of multiple protein cascades are observed (*11, 12*). The SH2 domains of PLCγ1 are required to target PLCγ1 to tyrosine-autophosphorylated receptors in this process. PLCγ1 is then tyrosine-phosphorylated and activated. This phosphorylation is necessary but not sufficient for full activation. A recent study indicated that PDGF-induced generation of $PI(3,4,5)P_3$, a product of PI3-kinase, contributes to translocation and activation of PLCγ (*13, 14*). Membrane anchoring is mediated by an interaction between $PI(3,4,5)P_3$ and the PH domain or C-terminal SH2 domain of PLCγ1. The PH domain of PLCγ has a high affinity for $PI(3,4,5)P_3$, which appears to be responsible for this membrane targeting. In contrast, $PI(3,4,5)P_3$ is dephosphorylated by PTEN or other polyphosphoinositide-specific phosphatases soon after receptor stimulation. So PLCγ1 activity is strictly regulated by generation and elimination of partners that interact. The SH3 domain of PLCγ1 has been shown to bind to SOS1 *via* a proline-rich domain and to stimulate the guanine nucleotide exchange activity.

Comparison of DNA sequences suggests an evolutionary relationship in which PLCδ appeared in primitive eukaryotes (Fig. 2). Yeasts, slime molds, and plants contain only PLCδ-related PLCs. The PLCβ and γ subtypes arose later. PLCδ is archetypal and contains only the N-terminal PH domain, EF-hand domain, X-domain, Y-domain, and C-terminal C2 domain. Recent molecular analyses revealed that PH domain of PLCδ1 associates tightly with $PI(4,5)P_2$ and IP3, and the binding ability of the former is correlated with PLCδ1 activity (*15, 16*). Three-dimensional crystal structure analysis supported the following interaction mechanism (*17, 18*). The 4-phosphoryl groups of IP_3 interact with the Lys32 and Lys57 residues, and the 5-phosphoryl groups interact with the Lys30, Arg40, and Lys57 residues. These basic amino acids are needed to form the inositol phosphate–binding pocket; however, they are not well conserved in PLCβ and γ subtypes, suggesting that the PH domain is specific for different lipids and the Gβγ subunit. Lemmon *et al.* proposed the "tether and fix" theory, by which the binding to plasma membrane and activation of PLCδ1 was described (*19*). First, PLCδ1 is tethered to the plasma membrane through interactions of the PH domain and $PI(4,5)P_2$. This is an inactive form, which does not catalyze a high rate of hydrolysis. Further interactions with the membrane mediated by the C2 domain and catalytic domains expose the active site. This fixed (active) form hydrolyzes $PI(4,5)P_2$ efficiently. PLCδ1 is then released from the membrane depending on production of IP_3.

Although all PLC isozymes require calcium for activity, the PLCδ type is the most sensitive to calcium, suggesting that it may be regulated by calcium (*20*). There are several

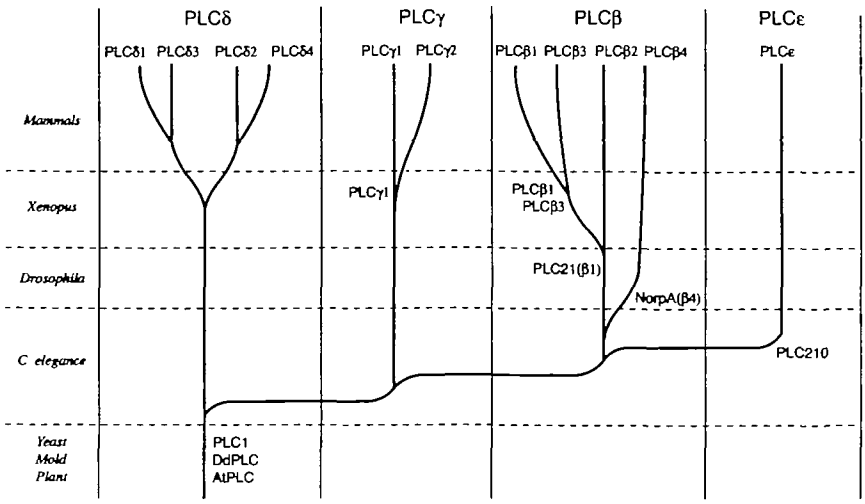

Fig. 2. **Evolutionary appearance of PLC.**

calcium-binding sites in PLCδ type. Structual analysis of the X and Y domains of PLCδ1 in the presence of calcium and IP_3 revealed that the Glu341, Asp343, Glu390, and Asn312 residues of PLCδ1 are involved in calcium-binding (*17*). The C2 domain of PLCδ1 is predicted to contain three calcium-binding sites. Recently Lomasney *et al.* reported that calcium binding to the C2 domain of PLCδ increases PLC activity by producing a ternary complex of protein-phosphatidyl serine-calcium, which facilitates enzyme access to $PI(4,5)P_2$ vesicles (*21*). In addition, calcium binding to the EF-hand domain of PLCδ1 is required for association of the PH domain with $PI(4,5)P_2$ (*22*). This calcium dependency appears to be related to the hypothesis that PLCδ type is activated secondarily to the increase in intracellular calcium induced by PLCβ or PLCγ type activation (*23*). However, its evolutionary appearance shows that PLCδ has roles even when no other PLC type exists. PLCδ type may be a relatively primitive form that can be activated by concentrations of calcium that are too low to activate PLCβ and γ. Finally, it has been reported that the α subunit of a novel class of G protein, tissue transglutaminase TGII (Gαh), activates PLCδ1 directly *in vitro* (*24*). Gh has transglutaminase activity as well as the ability to bind and hydrolyze GTP. Recent evidence suggests that PLCδ1 associated with Gh is inactive and becomes active when released from the complex by GTP in response to receptor activation (*25*). Furthermore, PLCδ1 acts as a guanine nucleotide exchange factor for TGII in a coupling system involving TGII and α1B-adrenoreceptor (*26*), suggesting that PLCδ1 plays an important role in α_{1B}-adrenoreceptor function.

PLCε, as Ras regulator/effector

Recently a novel PLCε was identified as an effector of Ras protein. Ras is a monomeric GTP-binding protein that is involved in a variety of cell functions including cell growth, differentiation, and oncogenesis. The *ras* gene is mutated in approximately 15% of all human tumors; therefore, an understanding of signaling pathways mediated by Ras is important. In 1996, Ponting and Benjamin (*27*) proposed the existence of a motif of approximately 100 amino acids, known as the RA region, that is conserved in Ras effectors RalGDS and AF-6. They also identified a variety of other proteins containing this domain. At that time a PLC-like protein was proposed to have this PA domain. Kataoka's group, using a yeast two-hybrid system, first identified this novel PLC, PLC210, in a *C. elegans* cDNA library (*28*). The mammalian homologue, PLCε, was then isolated (*29–31*). Sequence analysis revealed that PLCε contains two RA domains (RA1 and RA2) in the C-terminus and a Ras-GEF–like domain at the N-terminus as well as the catalytic domains, PH domain, EF hand, and C2 domain. Therefore, PLCε is predicted to bind activated Ras. In fact, the interaction between Ras or Ras-related protein Rap1 and RA of PLCε was confirmed to occur in a GTP-dependent manner (*29, 30*). Furthermore, a complete correlation was found between the mutational effect on Ras-binding to the RA2 domain and the resulting ability of Ras to stimulate PLCε activity. This finding suggests that the binding of activated Ras to the RA2 domain stimulates PLCε activity. Evolutionary comparison of all known PLC isozymes indicated that PLCε is most similar to the PLCβ type, which is regulated by the G protein Gα and Gβγ subunits. PLCε has a long C-terminal extension similar to that of PLCβ, which is needed for interaction with Gα. Therefore, Lopez *et al.* examined the effect of Gα on PLCε activity (*31*). Among the various Gα subunits, $G\alpha_{12}$ stimulated PLC activity most effectively. In addition to Gα, Gβγ was recently reported to activate PLCε by Wing *et al.* (*32*). Because the PH domains of PLCβ2 and PLCβ3 interact with Gβγ, they investigated whether Gβγ modulated the activity of PLCε. When PLCε and $G\beta_1\gamma_2$ were co-transfected into COS-7 cells, PLC activity increased remarkably. This increase was observed even when a RA-domain deletion mutant of PLCε was used, indicating that the effect of Gβγ on PLCε activity is not mediated by Ras. Furthermore, ectopically expressed PLCε is translocated to the plasma membrane in response to epidermal growth factor stimulation (*29*), suggesting the importance of interactions mediated by RA, C-terminal, and PH domains for activation of PLCε.

Because PLCε contains a Ras-GEF–like domain, it is interesting to examine if PLCε works as Ras-GEF and activates Ras. Jin *et al.* used an *in vitro* assay to study the specificity of PLCε-RasGEF activity and found that PLCε has an activity toward Rap1, but not to Ras, Rap2A or Rho (*33*). Lopez *et al.* (*31*) reported that transfection of cells with

PLCε or the PLCε-GEF domain alone stimulates MAP kinase, a downstream target of Ras; activation of Ras, however, resulted in decreased PLC activity. This finding differs from those of Kelly *et al.* and Song *et al.*, who reported that Ras activates PLCε activity. This may because of differences in assay systems or types of cells used for expression of PLCε. Thus PLCε acts as both a positive regulator and positive/negative effector of Ras.

Schmidt *et al.* clarified a novel pathway from adenyl cyclase-coupled receptors to calcium mobilization mediated by cAMP, Rap GTPase, and PLCε. The cAMP- and phosphatidylinositol-mediated pathways are common intracellular signaling systems for cell surface receptors (*34*). Extracellular signals are integrated differentially. In certain signaling systems, cAMP inhibits PLC activity through PKA-dependent phosphorylation of PLCs. In contrast, cAMP-mediated signaling stimulates PLC activity in some cases, where both signaling systems function coordinately. The mechanism by which this occurs has not been clarified. A transient increase in calcium was observed when HEK-293 cells expressing β_2-adrenergic receptor (β_2-AR) were treated with adrenalin or the adenyl cyclase activator, forkolin, indicating that this increase was mediated by cAMP. Furthermore, co-expression of an inactive Rap2B mutant reduced PLC activity, whereas co-expression of PLCε with β_2-AR enhanced the calcium level in cells. These results indicate that PLCε functions as a novel cross-talk signaling pathway that may be involved in a specific function. Therefore, PLCε may be a target for new medicines that limit the function of adrenalin.

Mammalian tissue distribution and involvement in diseases

The distributions of PLC isozymes often reflect their physiological functions, which have been confirmed in studies of knockout mice. The distribution of different isozymes as determined by Northern blot, Western blot, and histochemical analyses are summarized in Table I.

The expressions of PLCβ isozymes have been well characterized. PLCβ1 is the most widely expressed, especially in brain. PLCβ1 is expressed at high level in the cerebral cortex and hippocampus, whereas PLCβ4 levels are the highest in the cerebellum and retina and are almost negligible in the cerebral cortex and hippocampus (*35*). The *Drosophila* PLCβ4 homologue, NorpA, was identified as an eye-specific gene, and PLCβ4 was first isolated from cerebellum and retina (*36*). Expression of PLCβ2 is restricted to hematopoietic cells (*37*). PLCβ3 is distributed in brain, liver, and parotid gland, but its expression is low throughout the brain.

PLCγ1 is expressed ubiquitously, especially in brain and lung (*38*). In adult rat brain, expression of PLCγ1 protein is highest in neurons, followed by oligodendrocytes and astrocytes (*39*). Significantly high level of expression is observed in embryonic cortical structure. It is worth noting that expression of PLCγ1 is enhanced and phosphorylation of PLCγ1 on tyrosine residues is detected in primary human breast or colorectal carcinoma cells (*40*). On the other hand, PLCγ2 is abundant in hematopoietic cells (*38*), a finding that supports the theory that PLCγ2 is essential for B cell development (*41*).

PLCδ1 is the most abundant and widely expressed PLCδ isozyme. PLCδ1 is distributed abundantly in brain, heart, lung, skeletal muscle, and testis (*42*). In rat brain, it is concentrated in astroglial cells and to a lesser extent in neurons. A remarkable aberrant accumulation of PLCδ1 is observed in brain from Alzheimer-disease patients (*43*), but its role in the brain remains unclear. PLCδ2 was isolated from bovine brain library, and although its localization in other tissues has not been analyzed, it is thought to be involved in cancers including intestinal metaplasia and adenocarcinoma (*44*). PLCδ3 is detected abundantly in brain, skeletal muscle, and heart (*45*), whereas expression of PLCδ4 is restricted to testis, brain, and skeletal muscle

TABLE I. **Mammalian PLC isozymes.** Species: (H), human; (B), bovine; (R), rat; (M), mouse.

Isozyme	Residues (species)	Locus: human mouse	Regulation mechanism	Distribution	Function from KO mice
PLCβ1	1,216(H,B,R) 1,173(M) 1,115(H)	20p12 2	Gαq (*6*) PI(*3*)P (*4*)	brain (cerebrum hippocampus) adrenal gland lung parotld gland (*35,38*)	induce epilepsy (*35*) (inhibitory neuron)
PLCβ2	1,181(H) 1,183(R)	15q15 2	Gβγ (*5*),Gαq (*6*)	hematopoietic cells (neutrophill, platelet) (*37*)	chemoattactant-induced superoxide production (*37*)
PLCβ3	1,234(H,M) 1,223(H)	11q13 19	Gβγ (*5*),Gαq, PDZ-motif (*10*)	brain, liver, parotid gland, platelet (*20*)	μ-opioid–mediated response (*50*)
PLCβ4	1,022(H,B) 1,175(R,M)	20p12 2	Gαq	brain (cerebellum, retina) (*35*)	visual response (*48*), ataxia (*35*), long-term depression (*49*)
PLCγ1	1,290(H,B,R)	20q12-13.1 2	Tyrosine kinase (*11*), PI(3,4,5)P_3 (*13,14*), Tau	lung, thymus, brain (neuron) (*38,39*)	embryonic lethality (*51*)
PLCγ2	1,265(H,r)	16q24.1 8	Tyrosine kinase (*11*), PI(3,4,5)P_3	spleen, thymus, lung (*38*)	B cell development (*41*)
PLCδ1	756(H,R,M)	3p22→p21.3 8	Calcium (*17,20,22*), Gαh (*24,25*) PI(4,5)P_2 (*15,16,19*)	brain, heart, lung, testis, skeletal muscle, spleen (*38,42*)	—
PLCδ2	764(B)	2p 12q	—	brain	—
PLCδ3	737(H)	17q21 11	Calcium, cAMP (*45*), PI(4,5)P_2, phosphatidic acid	skeletal muscle, heart, brain (*45*)	—
PLCδ4	771(R)	2q35 1	Calcium, PI(4,5)P_2	testis, brain, skeletal muscle (*46,47*)	acrosome reaction in sperm (*52*)
PLCε	2,303(H) 2,281(R)	10 1	Ras (*29,30*), Gβγ (*32*), Gα12 (*31*)	lung, liver, heart, skeletal muscle (*30,31*)	—

(*46, 47*).

The mRNA encoding PLCε is most abundant in the heart, followed by the lung and kidney, but it is not found in brain. This remarkable expression in the heart, along with correlation of adrenalin function, suggests that PLCε has important physiolosical functions in this organ.

Functional analysis with knockout mice

Null mutations of PLCβ1, 2, 3, and 4 have been generated, and analyses of the resulting phenotypes support their roles in specific signaling and distinct biological functions. PLCβ4 gene–deficient mice were first reported in 1996 (*48*). As mentioned above, PLCβ4 is localized in retina and shares homology with *NorpA*, which mediates the phototransduction cascade in *Drosophila* photoreceptors, and it was therefore expected to be involved in mammalian visual processes. Jiang *et al.* reported that PLCβ4$^{-/-}$ mice have defective visual responses, whereas they retain their auditory capabilities. In addition, a reduction in the maximal amplitude of the rod a- and b-wave components of electroretinograms was observed in PLCβ4$^{-/-}$ mice. However, there was no obvious change in rod morphology, suggesting that PLCβ4 plays a role in distal rod-mediated signaling in the retina.

Kim *et al.* generated PLCβ1- and PLCβ4-deficient mice, and reported that PLCβ1$^{-/-}$ mice develop epilepsy and that PLCβ4$^{-/-}$ mice have ataxia (*35*). PLCβ4$^{-/-}$ mice showed a waddling gait, with the rear body swinging left-right. The ataxia was not due to bone deformation or muscle weakness. Histological analysis of PLCβ4$^{-/-}$ mice revealed that the cerebellar development was retarded, with an aberrant pattern of folia and incompletely migrated external granule cells at P15. They further clarified that PLCβ4 is involved in metabotropic glutamate receptor–mediated signal transduction in the cerebellum. Yoshioka's group reported that PLCβ4 is involved in induction of long-term depression through glutamate receptor 1 in the cerebellum (*49*).

In contrast to the above findings in PLCβ4$^{-/-}$ mice, most PLCβ1$^{-/-}$ mice die suddenly from epileptic-like seizures starting the third week after birth. The seizures were of a generalized type characterized as tonic-clonic or by tonic extension of the entire body. Behavior data obtained after administration of pentamethylenetetrazole, which blocks the inhibitory neuronal pathway, or kainic acid, which activates the excitatory neuronal pathway, suggested that PLCβ1 is essential for normal function of the neuronal inhibitory pathway. In addition, carbachol-induced phosphoinositide hydrolysis is markedly attenuated in PLCβ1$^{-/-}$ hippocampus, indicating that PLCβ1 is required for muscarinic acetylcholine receptor signaling.

PLCβ3 is reported to be involved in μ-opioid–mediated responses. Xie *et al.* reported that mice lacking PLCβ3 exhibit a 10-fold decrease in morphine sensitivity in producing antinociception (*50*). They also showed that the μ-opioid–induced increase in intracellular calcium does not occur in PLCβ3$^{-/-}$ cells, whereas the bradykinin- or bombesin-induced calcium increase, which is mediated by the Gαq pathway, is not affected. Because PLCβ2 and PLCβ3 are potently activated by Gβγ *in vitro*, these data indicate that PLCβ3 participates in a pathway involved in inhibition of opioid responses mediated by Gβγ.

Li *et al.* carried out further analyses of the *in vivo* functions of PLCβ2 and PLCβ3 (*37*). They found that PLCβ2 contributes mainly to chemoattractant-mediated production of superoxide and regulation of protein kinases but not to chemotaxis. They also indicated that large amounts of antigen-specific antibodies were produced when PLCβ3$^{-/-}$ mice or PLCβ2/3$^{-/-}$ mice were immunized with T cell–independent antigen, suggesting that PLCβ2 and PLCβ3 play defined roles in immune function.

With respect to PLCγ, Ji *et al.* generated PLCγ-deficient mice (*51*). Homologous disruption of the PLCγ1 gene resulted in lethality at approximately embryonic day 9. The gross morphology of PLCγ1$^{-/-}$ embryos at day E8.5 is normal, but their growth and development at day E9.5 is markedly retarded in compared with wild-type embryos. Fibroblasts derived from these embryos were used to show that growth factor–induced DNA synthesis, cell growth, and cell migration were unaffected. However, the PLCγ1$^{-/-}$ embryo fibroblasts did not mobilize in response to EGF. PLCγ1 is widely expressed in embryos, especially in the dorsal aorta and limbs. However, the relation of its distribution to embryonic lethality is unclear.

PLCγ2-deficient mice have a number of defects in signaling through immunogloblin superfamily receptors. PLCγ2$^{-/-}$ mice have decreased numbers of mature B cells because of blocked pro-B cell differentiation (*41*). IgM receptor–induced calcium increase and proliferation of B cell mitogens are also absent. The phenotype is similar to those of Btk- and Blnk-deficient mice, suggesting that PLCγ2 is downstream of Btk/Blnk signaling. In addition, collagen-induced platelet aggregation is defective, indicating that PLCγ2 plays a critical role in signaling through a receptor requiring the FcRγ chain.

Recently, we reported that PLCδ4 gene–disrupted male mice either produced a few smaller litters or are sterile (*52*). *In vitro* fertilization studies showed that insemination with PLCδ4$^{-/-}$ sperm resulted in significantly fewer eggs becoming activated and that the calcium transients associated with fertilization are absent or delayed. These results suggest that PLCδ4 in sperm plays an essential role in an early step of fertilization. Histochemical analysis of testes revealed that PLCδ4 is concentrated in the anterior acrosomal region of sperm. Furthermore, PLCδ4$^{-/-}$ sperm were unable to initiate the acrosome reaction, an exocytotic event required for fertilization that is induced by interaction with the egg coat, the zona pellucida. These data demonstrate that PLCδ4 functions in the zona pellucida–induced acrosome reaction during mammalian fertilization.

Conclusion

The subtype-specific regulations of PLC isozymes by tyrosine kinase, heterotrimeric G proteins, and calcium have been extensively analyzed; however, recent advances have provided additional information regarding specific regulators. For activation, agonist-induced anchoring of PLCs to the plasma membrane is the first step. This is followed by conformational changes. These associations are mediated by protein–protein or protein–lipid interactions through relatively large molecular domains and small molecules such as lipids and phosphotyrosine-containing peptides.

The most remarkable recent finding is the identification of a novel mammalian PLC, PLCε. Although it is not yet clear if PLCε is a regulator or effector of Ras, PLC and PI3-kinase appear to have similar regulators, G protein and

tyrosine kinase, and effector, Ras. In addition, these enzymes have a common substrate, $PI(4,5)P_2$. The mechanism by which these enzymes act cooperatively in signal transduction pathways needs to be elucidated.

Gene-targeting technologies have contributed significantly to our understanding of physiological functions of PLC isozymes. Each PLC isozyme apparently plays a decisive but specific role in a particular function, even when different isozymes show a similar distribution. The isozymes act in concert, each contributing to a specific aspect of the cellular response. This combination of individual but coordinated functioning may be why so many PLC isozymes exist in mammalian cells. Further analysis of targeted mice should provide additional data to aid in clarifying the function of PLC isozymes.

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