Zoological Studies **34**(3): 141-148 (1995)

Zoological Studies

Review Article

Phospholipase C Signalling

Mark R. Smith[1], Ya-Lun Liu[1], Sue Goo Rhee[2] and Hsiang-Fu Kung[3,*]

[1]*Biological Carcinogenesis and Development Program, PRI/DynCorp Inc., NCI-FCRDC, Frederick, MD 21702-1201, USA*
[2]*Laboratory of Biochemistry, National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20892, USA*
[3]*Laboratory of Biochemical Physiology, BRMP, DCT, NCI-FCRDC, Frederick, MD 21702-1201, USA*

CONTENTS

ABSTRACT

Mark R. Smith, Ya-Lun Liu, Sue Goo Rhee and Hsiang-Fu Kung (1995) Phospholipase C signalling. *Zoological Studies* **34**(3): 141-148. Lipids were originally thought to play important roles only in energy storage and membrane structure. Recent experimental evidence indicate that phospholipids also play critical roles as mediators in cell activation and signal transduction. Phospholipases are the key enzymes that regulate various signalling pathways. Inositol phospholipid-specific phospholipase C (PLC) catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2), to generate inositol triphosphate (IP_3) and diacylglycerol (DAG) in response to several receptor-binding growth/differentiation factors, hormones, and neurotransmitters. The hydrolysis products serve as intracellular second messenger molecules which amplify the initial signalling events leading to cellular calcium mobilization by IP_3 and protein kinase C (PKC) activation by DAG. In this article, we address two aspects to PLC signalling: 1. characterization, purification and molecular cloning of PLC isozymes; and 2. mitogenic and catalytic activities of PLC isozymes. In addition to reviewing published data on PLC signalling, we have included new data that examine the mitogenic activity of the PLC isozymes. PLC-β and PLC-γ induce DNA synthesis after microinjection into quiescent NIH/3T3 cells, while PLC-δ does not exhibit this activity. Monoclonal antibodies to PLC-γ were shown to block serum-stimulated growth of NIH/3T3 cells and several oncogenes transformed NIH/3T3 cells (*fes, src, ras* and *mos*), yet Raf transformed cells were not inhibited by antibody injection. Thus, PLC-γ signalling is required for serum- and (*fes, src, ras* and *mos*) oncogene-induced proliferation of fibroblasts.

Key words: Phospholipase C/signal transduction.

*To whom all correspondence and reprint requests should be addressed.

INTRODUCTION

Although inositol phospholipids comprise less than 10% of the total cellular phospholipid pool, their turnover induced by agonist accounts for a significantly higher percentage of total phospholipid turnover. It is clear that the products derived from phospholipid breakdown serve as second messengers during agonist-induced receptor activation. In addition to the well-established second messengers (cAMP and cGMP), evidence is mounting for the existence of additional second messengers, e.g., inositol 1,4,5-triphosphate (IP_3) and 1,2-diacylglycerol (DAG). The production of two second messengers (IP_3 and DAG) is catalyzed by a phosphatidylinositol-specific phospholipase C (PLC) and this pathway is one of the most commonly used signal transduction mechanisms in a wide range of tissue and cells. The role of phosphoinositides in transmembrane signalling was recently reviewed by Rana and Hopkin (1990). In this article, we focus on the hydrolysis of phosphoinositide catalyzed by PLC isozymes and the effect of catalytic activity on biological responses relating to cellular proliferation.

MULTIPLE FORMS OF PLC ISOZYMES

Numerous PLC activities have been resolved chromatographically from a variety of tissues (Ryu et al. 1987a,b, Hofman et al. 1982, Manne and Kung 1987). The enzymes were most extensively purified and studied in bovine brain tissue (Ryu et al. 1987a,b). Three distinct forms of PLC were purified from bovine brain cytosol (designated PLC-I, PLC-II, and PLC-III) (Ryu et al. 1987a,b) and monoclonal antibodies to these PLC enzymes were prepared (Suh et al. 1988a). These PLC enzymes have been shown to differ in molecular weight, isoelectrical point, pH optimum, calcium dependence, and immunoreactivity, suggesting the presence of PLC isozymes. Based on SDS-PAGE, PLC-I, PLC-II, and PLC-III exhibited an apparent molecular weight of 150,000, 145,000, and 85,000, respectively. The catalytic properties of the three isozymes were studied using small unilamellar vesicles prepared from either phosphatidylinositol (PI) or phosphatidylinositol 4,5-bisphosphate (PIP_2) as substrates. Unilamellar vesicles of phosphatidylinositol (PI) and phosphatidyliniositol 4,5-bisphosphate (IP_2) containing [2-^{3}H] inositol were separately prepared as described (Hofman et al. 1982). PLC activity was measured using the PI or PIP_2 unilamellar vesicles as the substrate as described by Ryu et al. (Ryu et al. 1987a,b, Manne and Kung 1987). Hydrolysis of both PI and PIP_2 by the three enzymes was dependent on Ca^{+2}. However, at low Ca^{+2} concentrations, PIP_2 was the preferred substrate for all three enzymes. When PI was the substrate, the three enzymes exhibited similar specific activities at their optimum pH, which was 4.8 for PLC-1, 5.0 for PLC-II, and 5.5 for PLC-III, but at neutral pH, the order of specific activity was PLC-III > PLC-II > PLC-I. In contrast, the order of specific activity was PLC-1 > PLC-III > PLC-II for PIP_2 hydrolysis, which means that PIP_2 is the preferable substrate for PLC-I. The relationship between the catalytic activities of the PLC isozymes and the biological responses in cellular proliferation will be described in the following sections.

MOLECULAR CLONING OF PLC ISOZYMES

PLC-related cDNA clones corresponding to PLC-I and PLC-II were isolated by Suh et al. (1988b,c) from a rat brain expression cDNA library using specific monoclonal antibodies. Several PLC forms were also molecularly cloned in different laboratories from bovine brain cDNA libraries (Stahl et al. 1988, Katan et al. 1988). All of these PLC clones were completely sequenced. The structure of the three PLC genes is illustrated in Fig. 1. Comparisons of their deduced amino acid sequences and immunological cross-reactivity indicates that the mammalian PLCs can be divided into three types, PLC-β (or PLC-I), PLC-γ (or PLC-II), and PLC-δ (or PLC-III); each of which represents a discrete gene product. Each enzyme type contains more than one subtype which is structurally related to one of the three previously isolated PLCs (Arabic numerals are used to designate each subtype, e.g., PLC-β1, PLC-β2, PLC-γ1, PLC-γ2, etc). A total of 16 amino acid sequences have been established: 6, 5, and 5 for PLC-β, γ, and δ, respectively. The three types of PLC enzymes are dissimilar not only in molecular size but also in amino acid sequence which is consistent with the absence of immunological cross-reactivity between the three enzyme types. When the amino acid sequences of PLC-β, PLC-γ, and PLC-δ originally isolated from rat and bovine brains are compared, despite a low overall homology between the three enzyme types, a significant sequence similarity is apparent in two domains: the X domain contains about 170 amino acids and the Y domain contains about 260 amino acids. The X and Y domains are about

60% and 40% identical, respectively, between the three enzyme types. These two domains might constitute functional regions that are responsible for catalytic activities. The PLC-δ type of isozyme is readily distinguishable by the lack of a long C-terminal region after the Y domain. PLC-δ2 has been purified from bovine brain (Meldrum et al. 1991) and PLC-δ3 cDNA has been isolated from a WI-38 fibroblast library.

With regards to the nomenclature of the PLC isozymes using Greek letters, PLC-α was initially assigned to the 62-68 KDa enzyme purified from rat liver and sheep seminal vesicles and later from guinea pig uterus (Rhee et al. 1989). These 62-68 KDa enzymes appeared to be immunologically distinct from PLC-β, PLC-γ, and PLC-δ. The putative PLC-α cDNA was obtained from rat basophilic leukemic cell libraries with the use of rabbit serum raised against the 62 KDa guinea pig uterus PLC (Meldrum et al. 1991). The amino acid sequence deduced from this putative PLC-α clone showed no similarity to those of other PLC enzymes, but showed most similarity to thioredoxin and protein disulfide isomerase. No sequence corresponding to the X and Y domains could be located in the deduced sequence of PLC-α. With the expression of PLC-α cDNA, Martin et al. (1991) convincingly showed that the rat basophilic leukemia cell cDNA actually encodes an endoplasmic reticular protein that carries no PLC enzymatic activity.

Although PLC-β, -γ, and -δ are different in primary structures, they are very conserved throughout species or tissues of origin. For example, amino acid conservation is better than 95% for PLC-γ1 from rat, bovine and human brains and PLC-γ1 can be found in almost every mammalian tissue. PLC-γ2 cDNA has been isolated from libraries of human lymphocytes (Ohta et al. 1988), rat muscle (Emori et al. 1989), and HL-60 cells (Kritz et al. 1990) by low-stringency hybridization techniques. PLC-γ1 and PLC-γ2 are highly homologeous in sequence around the amino terminal 300 amino acids and in the 400-amino acid region between the X and Y domains. In the 400-amino acid insert between X and Y, PLC-γ1 and PLC-γ2 contain three regions that are related in sequence to limited portions of the *src* gene product (Stahl et al. 1988, Suh et al. 1988c). The three regions correspond to duplicates of SH2 and to SH3 (*src* homology 2 and 3), which were first recognized as highly conserved sequences in the regulatory domains of a number of non-receptor tyrosine kinases, e.g., *src, abl, yes, fgr, lyn, fyn, hcks*, and *lck* (Pawson 1988). PLC-γ isozymes can, therefore, be structurally divided into the three domains of X, Y, and SH (SH2 plus SH3). To study the function of each domain, plasmids containing mutations within these domains were constructed. A vaccinia expression vector harboring the entire coding sequence of rat liver PLC-γ1, and the construction of PLC-γ1 mutants have been described previously (Kim et al. 1991, Smith et al. 1994). Recombinant wild-type and mutant PLC-γ1 enzymes were purified through two sequential chromatographic steps, i.e., HPLC TSK DEAE-5PW column and HPLC TSK phenyl-5PW column (Smith et al. 1994), SDS-PAGE analysis indicated >95% purity for the purified enzymes. The catalytic and mitogenic activities of the wild type and mutant PLC-γs were examined (see below).

IMMUNOLOGICAL ANALYSIS OF PLC ISOZYMES

Individual murine hybridoma cell lines secreting antibodies against bovine brain PLC-β, -γ, and -δ

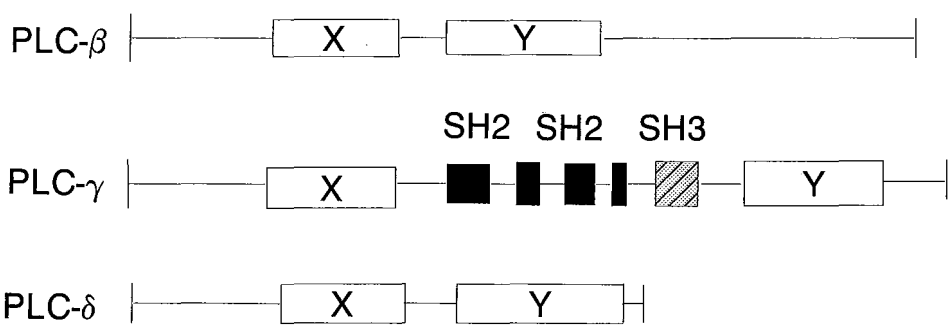

Fig. 1. Structure of three types of mammalian PLCs (β, γ, and δ types). Open boxes X and Y denote the regions of approximately 170 and 260 amino acids, respectively, of similar sequences found in the three of mammalian PLC. PLC-β, -γ, -δ contain 1,215, 1,289 and 756 amino acid residues, respectively.

were prepared and the antibodies were purified from ascites fluid. All the antibodies cross-reacted with their corresponding PLC enzymes, but not with the other two isozymes, indicating the specificity of the monoclonal antibodies (Suh et al. 1988a) and suggesting that the three enzymes contain very different antigenic determinants. Each antibody exerts different inhibitory effects on the phosphatidylinositol-hydrolyzing activity of PLC. Competitive binding studies with purified PLC enzymes and Western blot experiments with proteolytic digests revealed that distinct groups of monoclonal antibodies bind to different epitopes on the PLC isozymes. The fact that these antibodies bind to different epitopes on the same enzyme allowed us to develop a highly sensitive and specific radioimmunoassay for quantitating PLC isozymes. PLC-γ and PLC-δ were found in all tissues studied, whereas heart and lung were devoid of PLC-β. Brain is the richest source for PLC-β and PLC-γ. PLC-δ is evenly distributed in the brain, heart, lung, liver, and kidney ($\sim$20-60 ng PLC-δ/mg extract). Although significant amounts of PLC-γ are present in murine fibroblast NIH/3T3 cells, PLC-β is not detectable in the cells. Western blot analysis of the PLC isozymes are shown in Fig. 2. NIH/3T3 cells were used as a model system for our studies of PLC-induced cellular proliferation (see below).

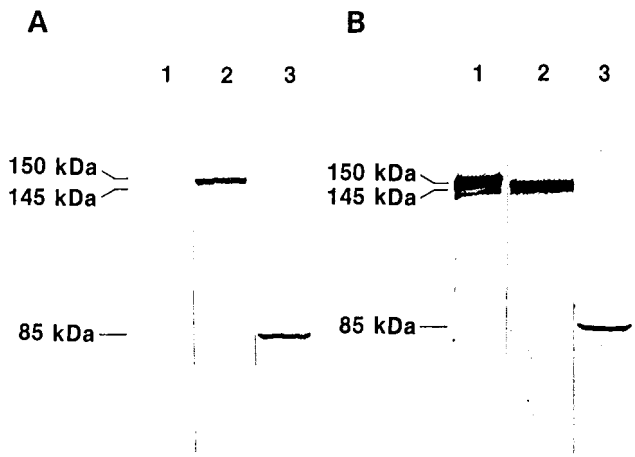

Fig. 2. Western blot analysis quantitating the expression of PLC isozymes in NIH/3T3 cells and rat brain. Lysates (20 μg) prepared from NIH/3T3 cells (A) and rat brain (B) were run out on 10% polyacrylamide gels. PLC isozymes were identified with specific anti-sera: anti-PLC-β1 (lane 1), anti-PLC-γ1 (lane 2), and anti-PLC-δ1 (lane 3). Molecular weights are indicated.

INTRACELLULAR MITOGENIC ACTIVITIES OF PLC ISOZYMES

PLC-βs and PLC-γs have been demonstrated to play important roles in cellular signalling. PLC-γs are in general activated by receptors that are protein tyrosine kinase (PTK) themselves or known to be coupled to a non-receptor PTK, whereas PLC-βs are regulated by heterotrimeric G proteins. In contrast, the regulation and mechanism of activation of PLC-δs in signal transduction is not clear. Among the PLC family, PLC-γ has been studied most extensively. PLC-γ1 contains stretches of amino acids which are homologues to regions of the c-*src* proto-oncogene products (SH2 and SH3 domains) (Stahl et al. 1988). In the case of *src*, these regions are involved in regulation of its tyrosine kinase and transforming activities. Several reports (Anderson et al. 1990, Klippel et al. 1992) have shown that the SH2 regions of PLC-γ1, GTPase activating protein (GAP), the 85 KDa subunit of phosphatidylinositol (PI)-3′ kinase and *Crk* all associate specifically with other phosphotyrosine proteins. The SH2 domains of PLC-γ1 are responsible for its association with PDGF, EGF, and FGF receptors in a ligand-dependent manner (Mohammadi et al. 1991). Elevation of PLC enzyme activity has been reported in growth factor-treated cells as well as many transformed cells (Kim et al. 1991, Nishibe et al. 1990). In our laboratory, microinjection techniques were employed to study the biological functions of PLC.

We have microinjected three PLC isozymes into quiescent NIH/3T3 cells to directly test the growth-promoting and transforming potential of these enzymes. Fig. 3A and B illustrates the induction of [^{3}H]thymidine incorporation into the nuclei of serum-starved NIH/3T3 fibroblast cells after microinjection of PLC-β and PLC-γ. In contrast, PLC-δ and BSA injection did not induce DNA synthesis (Figs. 3C, D). In all of the experiments, 100-150 cells were injected with each sample and similar results were reproduced in 8-10 separate experiments. Table 1 summarizes this data and the results are presented by fold induction of DNA synthesis. Although PLC-δ was not mitogenic, it exhibited higher specific activity than PLC-γ for PIP_2 hydrolysis at neutral pH (Ryu et al. 1987). Therefore, our results suggest that the phosphoinositide-hydrolyzing activity alone is not sufficient for the observed induction of DNA synthesis by PLC isozyme and that something else is also involved.

EFFECTS OF ANTI-PLC ANTIBODIES ON THE DNA SYNTHESIS OF TRANSFORMED NIH/3T3 CELLS

Mixtures of anti-PLC-β and anti-PLC-γ antibodies with different epitopic specifications were prepared and used to evaluate their ability to neutralize the mitogenic activity of the PLC isozymes in co-injection experiments. Anti-PLC-β and anti-PLC-γ do not cross-react with each other and the antibody mixtures were found to inhibit specifically the mitogenic activity of the respective PLC enzymes (Smith et al. 1990). NIH/3T3 cells express significant amounts of PLC-γ and PLC-δ with very little PLC-β (Fig. 2A). Since PLC-δ exhibited no mitogenic activity in quiescent NIH/3T3 cells, we have used microinjection to evaluate the biological significance of PLC-γ in growth-mediated signal transduction pathways by depleting intracellular PLC-γ with neutralizing anti-PLC-γ monoclonal antibodies during growth activation by serum factors. We have demonstrated the requirement for PLC-γ in serum-stimulated DNA synthesis of NIH/3T3 fibroblasts (Smith et al. 1990). A similar observation was seen with neutralizing anti-*ras* antibody, Y13-259 (Mulcahy et al. 1985, Kung et al. 1986).

After it was first shown that microinjection of a neutralizing monoclonal antibody to *ras* could block serum-stimulated growth of fibroblast cells (Mulcahy et al. 1985, Kung et al. 1986), several studies have used neutralizing antibodies to dissect the molecular events of membrane signal transduction (Smith et al. 1990, Mulcahy et al. 1985, Smith et al. 1986, Lazaris-Karatzas et al. 1992). Microinjection of neutralizing anti-*ras* antibody showed that the transforming signals generated by several growth factor receptor-like oncogenes

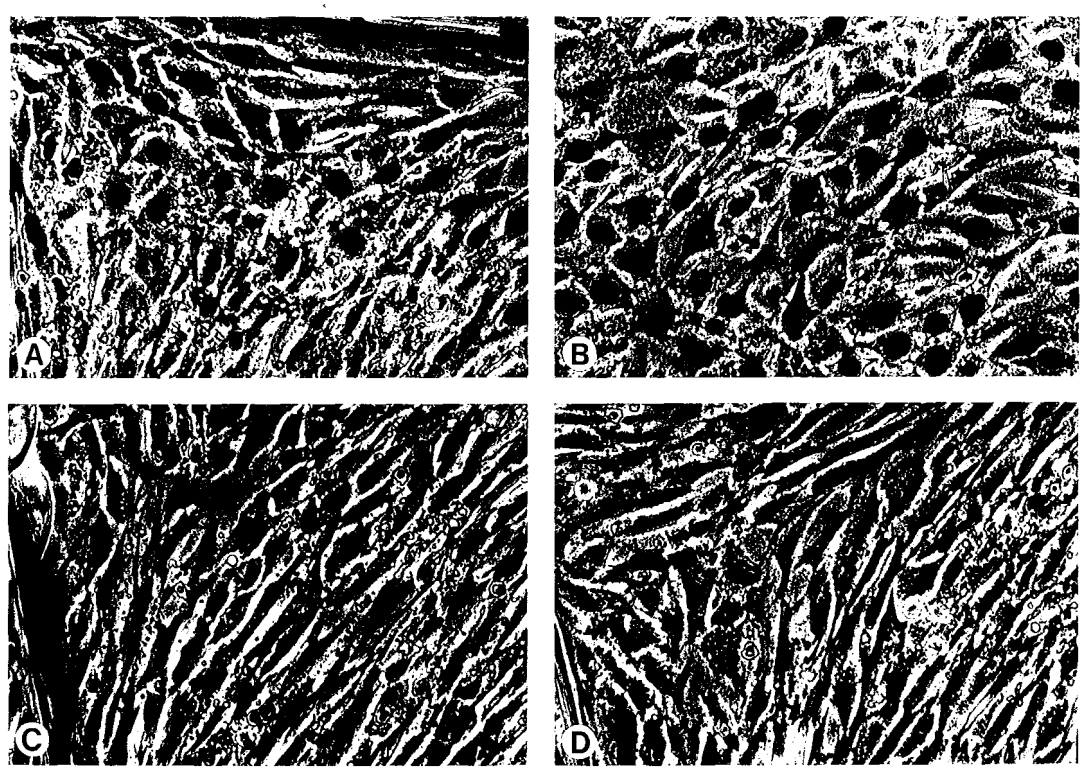

Fig. 3. Induction of DNA synthesis in quiescent NIH/3T3 cells after microinjection of PLC . Murine NIH/3T3 fibroblasts were maintained in Dulbecco's modified essential medium (DMEM; Gibco/BRL) supplemented with 10% calf serum and nonessential amino acids (Gibco/BRL). Cells were plated on etched glass coverslips in 35 mm plates at a concentration of $\sim 4\times10^4$ cells/ml. After one to two days, the contact-inhibited monolayers ($\sim$90% confluence) were made quiescent by replacing the medium with DMEM containing 0.5% (vol/vol) fetal calf serum for a period of 20-36 hr before microinjection. PLC-β, PLC-γ, and PLC-δ were purified from bovine brain as described (Ryu et al. 1987a,b). Approximately 100 cells (panels A, C, and D) and 150 cells (panel B) were injected with 250 μg/ml of A, PLC-β1; B, PLC-γ1; C, PLC-δ1; and D, BSA. Injected cultures were maintained in low serum media and pulsed with [^{3}H]thymidine (0.5 μCi/ml) between 16-20 hr after injection, washed with PBS, fixed in 2.5% glutaraldehyde, and autoradiography was performed in nuclear tracking emulsion for two days. The cultures were stained with Gemsia, counted and photographed (100×).

Table 1. Microinjection of PLC induces DNA synthesis in quiescent NIH/3T3 cells

Sample	Fold Induction of DNA Synthesis	# of Cells Injected
BSA	1.4 (0.8)	1,200
PLC-β	28.2 (4.6)	1,100
PLC-γ1	24.7 (5.3)	1,350
PLC-δ	3.5 (1.6)	1,050

NIH/3T3 cells (3×10^4) were grown to confluence on glass cover slips in DMEM supplemented with 10% calf serum. The cultures were serum starved in DMEM supplemented with 0.5% fetal calf serum for 24 hr. Cells were microinjected with approximately 0.5×10^{-11} ml of sample, incubated at 37°C for 16 hr, pulsed with [^{3}H]thymidine (0.5 μCi/ml, Amersham) for 3-5 hr, washed in PBS, and fixed in 2.5% glutaraldehyde (v/vPBS). Coverslips were mounted on glass slides and autoradiographed in nuclear tracking emulsion (Kodak) for two days. Cells were stained with Gemsia, counted and photographed (100×). The number of cells that incorporated [^{3}H]thymidine into nuclei were monitored by microscopic observation. Fold induction of DNA synthesis is defined as the ratio of injected cells that incorporated [^{3}H]thymidine into DNA divided by the ratio of uninjected cells near the injected area that incorporated [^{3}H]thymidine into DNA. The labeling efficiency is reflected by fold induction of DNA synthesis. Standard deviation from the mean from at least six different experiments is shown in parenthesis.

Table 2. Inhibition of oncogene stimulated growth after microinjection of *ras* and PLC-γ1 monoclonal antibodies into transformed NIH/3T3 cells

	Labeling Efficiency	
Cell Line	αY13-259	αPLCγ
NIH/3T3	10 (4)	21 (5)
NIH/3T3/*fes*	20 (5)	35 (6)
NIH/3T3/*src*	24 (4)	40 (7)
NIH/3T3/*ras*	12 (4)	23 (5)
NIH/3T3/*mos*	95 (8)	32 (6)
NIH/3T3/*raf*	93 (5)	96 (7)

Cell lines were derived or obtained as described previously (Mulcahy et al. 1985). Labeling efficiency is defined as the percent of microinjected cells that incorporated [^{3}H]thymidine into DNA. Monoclonal antibody Y13-259 neutralizes the biological activity of *ras* (Mulcahy et al. 1985, Kung et al. 1986) and the mixture of monoclonal antibodies raised against PLC-γ are described elsewhere (Smith et al. 1990). Antibodies were microinjected into quiescent cells (0.5% FCS media for 24 hr) and then serum-induced. The cultures were incubated at 37°C for 16 hr, pulsed with [^{3}H]thymidine (0.5 μCi/ml, Amersham) for 4-6 hr, fixed with 2.5% glutaraldehyde (v/vPBS), and autoradiography was performed in nuclear tracking emulsion exposing for 48 hr. The cells on the coverslips were stained with Gemsia, injected, and background areas were scored. Standard deviation from the mean from at least five separate experiments is shown in parenthesis.

(*fms, fes*, and *src*) were dependent on *ras* protein for activity because induction of DNA synthesis by these oncogenes was blocked by the anti-*ras* antibody (Smith et al. 1986). These injection experiments established a hierarchy within signal-transducing biochemical pathways for the oncogenes and showed that a downstream block could stop the transforming signal of an upstream oncogene. The antibodies to PLC-γ, that inhibit serum-induced DNA synthesis, also block mitogenesis when injected into *fes, src, ras*, and *mos* oncogene-transformed NIH/3T3 cells, whereas *raf*-transformed cells are not affected (Table 2). Our results suggest that PLC-γ activity is necessary for *ras*-mediated induction of DNA synthesis in NIH/3T3 cells and that *ras* acts downstream of *src* and *fes* in the signalling pathways. Although *mos* is independent of *ras* for the induction of DNA synthesis, PLC-γ is required for *mos* signalling. The molecular mechanism for this requirement remains to be determined.

In the course of our investigation of anti-PLC-γ monoclonal antibodies, we found that each antibody exerts a different inhibitory effect on the lipase activity of PLC-γ and that the inhibition of enzymatic activity does not correlate with the inhibition of DNA synthesis observed in the microinjection assay (Smith et al. 1994). It is possible that mitogenic signals other than the generation of second messengers (DAG and IP_3) may be responsible for the mitogenic activity of PLC-γ (e.g., the interaction of PLC-γ with other intracellular proteins). To determine whether the enzyme activity of PLC-γ is essential for its mitogenic activity, mutant enzymes with defective lipase activity were prepared.

BIOLOGICAL ACTIVITIES OF MUTANT PLC-γ ENZYMES

It appears that domains X and Y, but not SH, are essential for PLC enzymatic activity. Deletion in either the X or Y domain of PLC-γ1 led to a complete loss of lipase activity. To investigate the relevance of the X region of PLC-γ1 for the modulation of mitogenic activity in quiescent NIH/3T3 cells, we substituted phenylalanine for histidine at amino acid positions 335 and 380 and expressed the mutant enzymes in a vaccinia expression system. The purified mutant PLC-γ1 enzymes had markedly reduced lipase activity ($<10\%$ of wild-type PLC-γ1 activity). Although

PLC-γ1 mutant enzymes were defective in PLC enzymatic activity, they still induced intermediate levels of DNA synthesis when microinjected into quiescent NIH/3T3 cells (~50% of wild-type PLC-γ1 activity) (Smith et al. 1994). Taken together, our results suggest that the lipase-defective mutants exert their mitogenic activity through a mechanism other than the generation of second messenger molecules (DAG and IP_3). The partial induction of DNA synthesis seen after microinjection of the catalytic defective PLC-γ1 mutant was completely restored to levels seen with wild-type PLC-γ1 by co-injection of the mutant enzyme with DAG and IP_3 (Smith et al. 1994). These results suggest that the generation of second messenger molecules by PLC-γ1 and the interaction of PLC-γ1 with other signalling molecules are both responsible for the DNA synthesis inducing activity. Studies are underway to investigate the catalytic and mitogenic activities of other PLC-γ1 mutant enzymes.

THE CONCLUDING REMARKS

Inositol phospholipid-specific phospholipase C (PLC) plays a central role in cellular signal transduction pathways. It catalyzes the hydrolysis of phosphotidyl inositol 4,5-bisphosphate (PIP_2) to generate two second messenger molecules, inositol triphosphate (IP_3) and diacylglycerol (DAG), in response to several external stimuli. In addition to its catalytic activity, it exhibits mitogenic activity. Several PLC isozymes PLC-β, PLC-γ, PLC-δ were purified and molecularly cloned. The functional domains were defined. This article discusses the biological activities of these isozymes in PLC signalling.

Acknowledgements: We thank Dr. Dan L. Longo for support, Ms. Christina Schaff and Mrs. Ann E. Rogers for preparation of this manuscript. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

REFERENCES

Anderson D, CA Koch, L Grey, C Ellis, M Moran, T Pawson. 1990. Binding of SH2 domains of phospholipase C-γ1, GAP, and *src* to activated growth factor receptor. Science **250:** 979-982.

Bennett CF, JM Balcarek, A Varrichio, ST Crooke. 1988. Molecular cloning and complete amino acid sequence of form-I phosphoinositide-specific phospholipase. C. Nature **334:** 268-270.

Emori Y, Y Homma, H Sorimachi, H Kawasaki, O Naikanishi, K Suzuki, T Takerawa. 1989. A second type of rat phosphoinositide-specific phospholipase C containing a *src*-related sequence not essential for phosphoinositide-hydrolyzing activity. J. Biol. Chem. **264:** 21885-21890.

Hofman SL, PW Majerus. 1982. Identification and properties of two distinct phosphatidylinositol-specific phospholipase C enzymes from sheep seminal vesicular glands. J. Biol. Chem. **257:** 14359-14364.

Katan M, RW Kriz, N Totty, R Philip, E Meldrum, RA Aldape, JL Knopf, PJ Parker. 1988. Determination of the primary structure of PLC-154 demonstrates diversity of phosphoinositide-specific phospholipase C activities. Cell **54:** 171-177.

Kim HK, JW Kim, A Zilberstein, B Margolis, JG Kim, J Schlessinger, SG Rhee. 1991. PDGF stimulation of inositol phospholipid hydrolysis is requires PLC-γ1 phosphorylation on tyrosine residue 783 and 1254. Cell **65:** 435-441.

Klippel A, JA Escobeds, WJ Fantl, LT Williams. 1992. The C-terminal SH2 domain of p85 accounts for the high affinity and specificity of the binding of phosphatidylinositol 3-kinase to phosphorylated platelet-derived growth factor β receptor. Mol. Cell. Biol. **12:** 1451-1459.

Kritz R, LL Lin, L Suttzman, C Ellis, CH Heldin, T Pawson, J Knopf. 1990. Phospholipase C isozymes: Structural and functional similarities. Ciba Found. Symp. **10:** 112-127.

Kung HF, MR Smith, E Bekesi, V Manne, DW Stacey. 1986. Reversal of transformed phenotype by monoclonal antibodies against Ha-*ras* p21 proteins. Exp. Cell Res. **162:** 363-371.

Lazaris-Karatzas A, MR Smith, RM Frederickson, ML Jaramillo, YL Liu, HF Kung, N Sonenburg. 1992. *Ras* mediates translation initiation factor 4E-induced malignant transformation. Genes Dev. **6:** 1631-1642.

Manne V, HF Kung. 1987. Characterization of phosphatidylinositol-specific phospholipase C from human platelets. Biochem. J. **243**: 763-771.

Martin JL, NR Pumford, AC LaRosa, BM Martin, HM Gonzaga, MA Beaver, LR Pohl. 1991. A metabolic of halothane covalently binds to an endoplasmic reticulum protein that is highly homologous to phosphatidylinositol-specific phospholipase C-α but has no activity. Biochem. Biophys. Res. Commun. **178:** 679-685.

Meldrum E, RW Kriz, N Totty, PJ Parker. 1991. A second gene product of the inositol-phospholipid-specific phospholipase C delta subclass. Eur. J. Biochem. **196:**159-165.

Mohammadi M, AM Honegger, D Rotin, R Fischer, F Bellot, W Li, KA Dionne, M Jage, M Rubinstein, J Schlessinger. 1991. A tyrosine-phosphorylated caboxyl-terminal peptide of the fibroblast growth factor receptor (Flg) is a binding site for the SH2 domain of phospholipase C-γ1. Mol. Cell. Biol. **11:** 5068-5078.

Mulcahy LS, MR Smith, DW Stacey. 1985. Requirement for *ras* proto-oncogene function during serum stimulation growth of NIH/3T3 cells. Nature **313:** 241-244.

Nishibe S, MI Wahl, SMT Hernández-Sotomayor, NK Tonks, SH Rhee, G Carpenter. 1990. Increase of the catalytic activity of phospholipase C-γ1 by tyrosine phosphorylation. Science **250:** 1253-1256.

Ohta S, A Matsin, Y Nazawa, Y Kagawa. 1988. Complete cDNA encoding a putative phospholipase C from transformed human lymphocytes. FEBS Lett. **242:** 31-35.

Pawson T. 1988. Non-catalytic domains of cytoplasmic protein tyrosine kinase: Regulatory elements in signal transduction. Oncogene **3:** 491-495.

Rana RS, LE Hokin. 1990. Role of phosphoinositides in transmembrane signaling. Physiol. Rev. **10:** 115-164.

Rhee SG, PH Suh, SH Ryu, SY Lee. 1989. Studies of inositol phospholipid-specific phospholipase C. Science **244:** 546-550.

Ryu SH, KS Cho, KY Lee, PG Suh, SG Rhee. 1987a. Purification and characterization of two immunologically distinct phosphoinositide-specific phospholipase C from bovine brain. J. Biol. Chem. **262:** 12511-12518.

Ryu SH, PG Suh, KS Cho, KY Lee, SG Rhee. 1987b. Bovine brain cytosol contains three immunologically distinct forms of inositol phospholipid-specific phospholipase C. Proc. Natl. Acad. Sci. USA **84:** 6649-6653.

Smith MR, SJ DeGudicibus, DW Stacey. 1986. The requirement for c-*ras* proteins during viral oncogene transformation. Nature **320:** 540-543.

Smith MR, YL Liu, H Kim, SG Rhee, HF Kung. 1990. Inhibition of serum- and *ras*- stimulated DNA synthesis by antibodies to phospholipase C. Science **247:** 1074-1077.

Smith MR, YL Liu, NT Mattews, SG Rhee, WK Sung, HF Kung. 1994. Phospholipase C-γ1 can induce DNA synthesis by a mechanism independent of its lipase activity. Proc. Natl. Acad. Sci. USA **91:** 6554-6558.

Stahl ML, CR Ferenz, KL Kelleher, RW Kriz, JL Knopf. 1988. Sequence similarity of phospholipase C with the non-catalytic region of *src*. Nature **332:** 269-272.

Suh PG, SH Ryu, WK Choi, KY Lee, SG Rhee. 1988a. Monoclonal antibodies of three phospholipase C isozymes from bovine brain. J. Biol. Chem. **263:** 14497-14504.

Suh PG, SH Ryu, KH Moon, HW Suh, SG Rhee. 1988b. Cloning and Sequence of multiple forms of phospholipase C. Cell **54:** 161-169.

Suh PG, SH Ryu, KH Moon, HW Suh, SG Rhee. 1988c. Inositol phospholipid-specific phospholipase C: Complete cDNA and protein sequences and sequence homology to tyrosine-related oncogene products. Proc. Natl. Acad. Sci. USA **85:** 5419-5423.

Abbreviations used: PLC, inositol phopholipid-specific phospholipoase C; PIP_2, phosphatidyl inositol 4, 5-bisphosphate; IP_3, inositol triphosphate; DAG, diacylglycerol; PKC, protein kinase C; BSA, bovine serum albumin; SH, *src* homology; PTK, protein tyrosine kinase; GAP, GTPase activating protein.

磷脂酶－C之訊息傳遞

Mark R. Smith[1] Ya-Lun Liu[1] Sue Goo Rhee[2] 孔 祥 復[3]

脂類早先被認爲只在能量儲存與細胞膜構造方面扮演重要角色，最近研究顯示，磷脂亦扮演在細胞活化與訊息傳遞之介體角色。磷脂酶是調控許多訊息傳遞路徑的關鍵酶，當細胞接受（生長）分化因子、激素、及神經傳遞物刺激時，肌醇磷脂特異性之磷脂酶－C (PLC) 催化磷脂肌醇4,5-雙磷酸 (PIP_2) 水解，產生肌酸三磷酸 (IP_3) 與甘油二酯 (DAG)，此水解產物 (IP_3與DAG) 爲細胞內第二訊息傳遞者，可放大細胞初級訊息傳遞：IP_3引發細胞之鈣離子流動，DAG可活化蛋白質激酶－C。本文主要描述PLC兩方面之研究結果：㈠PLC同工酶之特性研究、純化與分子選殖，㈡PLC同工酶酵素活性及催化細胞分裂之關連性。我們除了回顧PLC訊息傳遞已發表之研究成果外，也同時敍述一些尚未發表之新結果。將PLC-β或PLC-γ注射到生長後分裂靜止之NIH/3T3細胞裡面，可引發DNA合成及細胞增殖，而PLC-δ則無此活性。PLC-γ之單株抗體可阻斷血清引發之NIH/3T3細胞增殖與致癌因子(*fes, src, ras*與*mos*)轉形之NIH/3T3細胞生長，而Raf轉形之細胞則不受抗體注射所抑制。因此，血清與*fes, src, ras*與*mos*等致癌基因引發之纖維母細胞增殖，需要透過PLC-γ之訊息傳遞。

關鍵詞：磷脂酶－C／訊息傳遞。

[1] Biological Carcinogenesis and Development Program, PRI/DynCorp Inc., NCI-FCRDC, Frederick, MD 21702-1201, USA

[2] Laboratory of Biochemistry, National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20892, USA

[3] Laboratory of Biochemical Physiology, BRMP, DCT, NCI-FCRDC, Frederick, MD 21702-1201, USA