EXPERIMENTAL and MOLECULAR MEDICINE, Vol. 32, No. 3, 101-109, September 2000

The mechanism of phospholipase C-γ1 regulation

Myung Jong Kim[1], Euikyung Kim[1], Sung Ho Ryu[1] and Pann-Ghill Suh[1,2]

[1] Department of Life Science, Division of Molecular and Life Science Pohang University of Science and Technology, Pohang 790-784, Kyungbuk, Korea

[2] Corresponding author: Tel, +82-54-279-2293; Fax, +82-54-279-2199; E-mail, pgs@postech.edu

Accepted 22 September 2000

Abbreviations: PLC, phospholipase C; EGF, epidermal growth factor; SH, Src-homology; IP_3, inositol trisphosphate; DAG, diacylglycerol

Overview

Phospholipase C (PLC)[1] hydrolyzes phosphatidylinositol 4,5-bisphosphate to generate the second messengers, inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 induces a transient increase in intracellular free Ca^{2+}, while DAG directly activates protein kinase C. Upon stimulation of cells with growth factors, PLC-γ1 is activated upon their association with and phosphorylation by receptor and non-receptor tyrosine kinases. In this review, we will focus on the activation mechanism and regulatory function of PLC-γ1.

Keywords: phospholipase C, protein kinase C

Introduction

Phosphoinositide-specific phospholipase C (PLC) plays a pivotal role in transmembrane signaling. In response to various extracellular stimuli, such as hormones, growth factors, and neurotransmitters, PLC hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP_2) producing two second messengers, diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3). IP_3 induces a transient increase in intracellular free Ca^{2+}, while DAG is a direct activator of protein kinase C (PKC) (Nishizuka, 1986; Berridge and Irvine, 1989). These processes have been implicated in many cellular physiological functions, such as secretion, cell proliferation, cell growth and differentiation (Rana and Hokin, 1990). In spite of low overall homology in the predicted amino acid sequences of the multiple PLC-isozymes, there are significant sequence similarities in two domains which are designated as the X- and Y-domain (Noh *et al*., 1995; Rhee and Bae, 1997; Sekiyama *et al*., 1999). So far, ten mammalian PLC-isozymes have been characterized at the cDNA level; they can be subdivided into three types (β, γ, δ) on the basis of relative locations of the X- and Y-domains in the primary structure (Noh *et al*., 1995; Rhee and Bae, 1997). The β type includes four PLCs (PLC-β1 through PLC-β4), the γ type includes two PLCs (PLC-γ1 and PLC-γ2), and the δ type includes four enzymes (PLC-δ1 through PLC-δ4) (Suh *et al*., 1988; Noh *et al*., 1995; Rhee and Bae, 1997) (Figure 1).

The existence of multiple forms of PLC enzymes suggests that each isozyme may differ in some respect, in tissue distribution, intracellular localization, regulatory mechanisms, and other downstream functions. Emerging evidence suggests that an additional level of diversity may exist beyond the above subgroup classification, because individual genes may yield multiple PLC products due to alternative splicing of the mRNA (Bahk *et al*., 1994; Kim *et al*., 1998). The diversity among the PLC enzymes point to selective coupling of individual PLCs to different receptors and signaling pathways. However, very little is known about the identity of the specific signaling pathways for each isozymes or how each isozymes function *in vivo*. Although PLC enzymes have been purified and extensively characterized biochemically, their function *in vivo* remains to be clarified.

The catalytic activities of the PLC-β type isozymes *in vivo* are mediated by α- and βγ subunits of heterotrimeric G-proteins (Smrcka *et al*., 1991; Taylor *et al*., 1991; Wu *et al*., 1992). PLC-β type are activated by G-protein-coupled receptors such as thromboxane A2, bradykinin, angiotensin, histamine, acetylcholine, etc. (Shenker *et al*., 1991; Noh *et al*., 1995; Rhee and Bae, 1997). The regulatory mechanism of the PLC-δ isozymes, however, is not clear at all as yet. PLC-β types appear to have a unique tissue distribution. A large amount of PLC-β1 has been detected in the brain (Bahk *et al*., 1994), while PLC-β3 was found in a variety of tissues and cell lines (Jhon *et al*., 1993). PLC-β4 is expressed primarily in the retina and cerebellum (Kim *et al*., 1998). The expression of PLC-β2, however, has been largely confined to the hematopoietic lineage with minor immuno-reactivity observed in other tissues (Park *et al*., 1992). Numerous efforts are being made to elucidate the activation mechanism of PLC-β isozymes in detail, by identifyng PLC-β-binding proteins. We have recently identified that the second PDZ domain of NHERF2 selectively interacts with the C-terminal end of PLC-β3, and enhances PLC-β3-activation (Hwang *et al*., 2000). In addition, CortBP1, cortactin-binding protein, specifically bind to the PLC-β1 through PDZ-domain (Hwang *et al*., unpublished data).

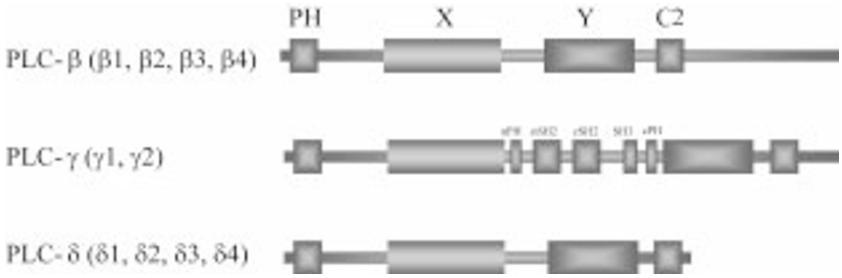

Figure 1. Linear representation of three types of PLC isozymes. The linear arrangement of SH2, SH3, PH, C2, and catalytic domain (X and Y) are shown.

The two γ-type PLCs, PLC-γ1 and -γ2, but no β- and δ-type isozymes, are activated by phosphorylation through growth factor receptor tyrosine kinases or non-receptor tyrosine kinases. While PLC-γ1 shows a ubiquitous expression pattern, PLC-γ2 is mainly expressed in B-cells.

Domain structure of PLC-γ1

The protein structure of PLC-γ1 is shown in Figure 2. All phosphoinositide-specific PLC isozymes contain the catalytic domains designated as X and Y (Figure 2). These two domains are believed to form the catalytic core through association. Mutation studies have revealed that several residues are particularly essential for the catalytic activity (Horstman *et al*., 1996; Horstman *et al*., 1999). Among them, His 356 and His 335 have been reported to be important for *in vitro* PLC-γ1 catalytic activity (Smith *et al*., 1994). PLC-γ1 harbors two SH2 domains and one SH3 domain between the X and Y domains (Figure 2). These domains are known to facilitate the PLC-γ1 association with other proteins. The SH2 domains recognize phosphotyrosine sequences in other proteins (Gergel *et al*., 1994; Pawson 1994; Sillman and Monroe, 1995; Valius *et al*., 1995; Yablonski *et al*., 1998; Poulin *et al*., 2000), while the SH3 domain mediates interactions with proteins containing proline-rich sequences (PXXP motif) (Pawson and Gish, 1992; Pawson and Nash, 2000). Cbl, SOS1, and dynamin are considered to bind to the SH3 domain of PLC-γ1 (Seedorf *et al*., 1994; Graham *et al*., 1998; Kim *et al*., 2000). Additionally, the regions of PLC-γ1 containing the SH domains are thought to be involved in the negative regulation of the catalytic activity of the enzyme. It has been suggested that the SH domain-containing region of PLC-γ1 may function as an intra-molecular "cap" or "lid" covering the active site in the absence of activating events. Tyrosine phosphorylation and/or protein binding to the SH domains may induce conformational change of PLC-γ1, which open the "lid" and make its substrate accessible to the catalytic active site (Capenter and Ji, 1999). Mutation in C-terminal SH2 domain in PLC-γ1 has resulted in considerable increase of basal PLC-γ1 activity in cells (Bae *et al*., unpublished data). In addition to the SH domains, PLC-γ1 also contains a C2 domain and two putative PH domains, one of which is split. These domains are shared with beta and delta members of the PLC family and are likely to serve some general mech-

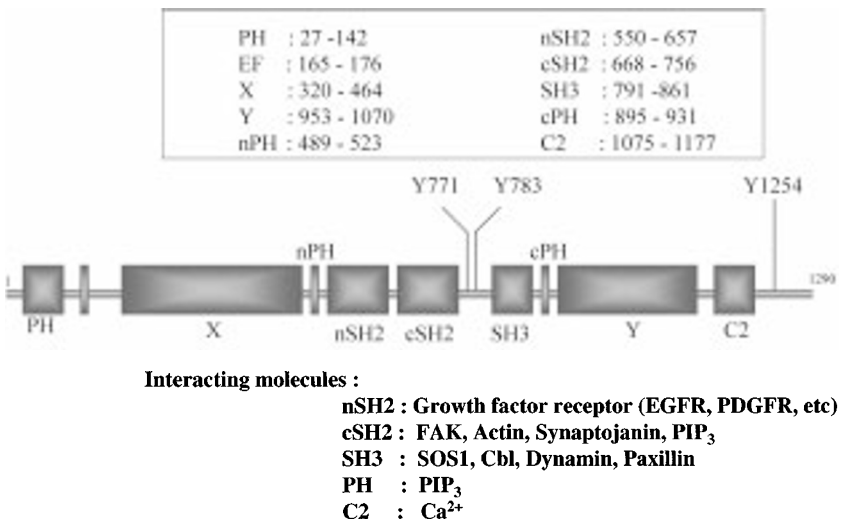

Figure 2. Domain structure and tyrosine phosphorylation sites of PLC-γ1. Domains (PH, EF, X, Y, nPH, nSH2, cSH2, SH3, cPH, and C2) of PLC-γ1 are shown in box in middle panel, and tyrosine phosphorylation sites in PLC-γ1 are also shown. Region in rat PLC-γ1 corresponding to each domain was described in the upper box. Interacting molecules to each domain are listed in bottom panel.

anism of the catalysis of PIP_2. The C2 domain of PLC-γ1 is known to mediate interaction with Ca^{2+}/phospholipids, while the PH domains in PLC-γ1 are thought to recognize polyphosphoinositides (PIP_2 and/or PIP_3) (Figure 2).

PLC-γ1 in cellular proliferation

Unlike other PLC-isozymes (PLC-β and PLC-δ isozyme), PLC-γ1 is involved in cellular proliferation. The expression level of PLC-γ1 is increased in human colorectal cancer, human breast carcinomas, familial adenomatous polyposis, and human skins under hyperproliferative conditions (Arteaga *et al*., 1991; Nanney *et al*., 1992; Park *et al*., 1994; Noh *et al*., 1994). In addition, the transcriptional activity of PLC-γ1 is up-regulated in cancer tissues. The critical regions responsible for the PLC-γ1 gene expression in the PLC-γ1 promoter have been mapped in our laboratory. These regions are named GES1, GES2, and GES3. Distinct transcription factors seem to bind to this transcriptional element in PLC-γ1 promoter. The binding of the transcription factor to these regions was elevated in colorectal carcinoma and breast cancer and so was the level of PLC-γ1, implying that they may play important roles in tumor genesis (Lee *et al*., 1995). The rise of PLC-γ1 level in cancer tissues was accompanied with increase of the PIP_2-hydrolyzing activities. Using specific antibodies, the expression levels of all three distinct PLC isozymes (PLC-β1, -γ1, and -δ1) were investigated in adenoma and carcinoma tissues from familial adenomatous polypis patients. Even though there were no apparent increases in levels of PLC-β1 and PLC-δ1, a significant increase in the PLC-γ1 level was detected in adenoma and carcinoma as compared with nomal mucosal tissue. Furthermore, the level of PLC-γ1 increased as the normal mucosa progresses from adenoma to carcinoma sequentially. Familial adenomatous polypis. The comparable levels of PLC-γ1 activity was also found in adenoma and carcinoma (Noh *et al*., 1994; Park *et al*., 1995). Increase of PIP_2-hydrolyzing-PLC-γ1 activity, downstream signaling molecules, is generally believed to be associated with rise in PKC-activity and intracellular Ca^{2+}-concentration (Berridge *et al*., 1984; Nishizuka, 1992). The activation of PKC is known to promote raf-1 activation (Morrison *et al*., 1997). Therefore, it can be expected that the PLC-γ1 is likely involved in ERK-signaling pathway (Figure 3). Additionally, phosphatidylcholine-hydrolyzing phospholipase D activity is also increased by the potentiation of PLC-γ1 activity (Lee *et al*., 1994). However, which isoforms of PKC are regulated by the activated PLC-γ1 or *vice versa* remains to be clarified. Up to date, ten isozymes of PKC were reported (Newton, 1997). Interesting question regarding specific relation between PLC-γ1 and each PKC-isozyme still remains to be clarified.

Some functional role(s) of PLC-γ1 in cellular proliferation-signaling was obtained in the studies of Smith *et*

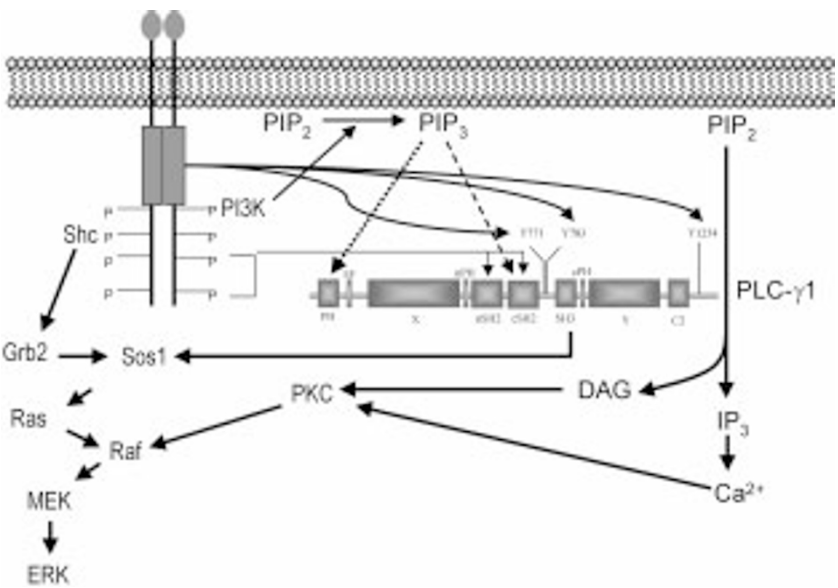

Figure 3. Schematic display of the activation process and down-stream signaling of PLC-γ1. Upon growth factor stimulation, growth factor receptors are multimerized and activated. Activated growth factor receptor autophosphorylate multiple tyrosine residues in cytoplasmic tail. SH2 domain-containing signaling molecules such as Grb2, Shc, and PLC-γ1 are recruited to specific phospho-tyrosine residues of the growth factor receptor. Growth factor receptor trigger PI-3K activation, Shc/Grb2/SOS1/Ras signaling, and tyrosine phosphorylation (Y771, Y783, Y1254)/activation of PLC-γ1. Two SH2 domains (nSH2, and cSH2 domain) in PLC-γ1 are involved in the interaction with phospho tyrosine residues of growth factor receptor. Activated PI-3K by growth factor- stimulation converts PIP_2 to PIP_3 to activate PLC-γ1. PH domain and cSH2 domain are known to be responsible for the interaction with PIP_3. Activated PLC-γ1 by PIP_3 or/and tyrosine phosphorylation hydrolyzes PIP_2 to generate IP_3 and DAG. DAG in turn activates PKC and IP_3 increases intracellular calcium concentration. Activated PKC relay a signal to raf-1. The SH3 domain of PLC-γ1 plays a role in the ras activation through the direct interaction with SOS1.

al. Micro-injection of purified PLC-γ1 into quiescent NIH 3T3 cells induced DNA synthesis and the injection of antibodies against PLC-γ1 into the same cells blocked the serum induced DNA synthesis (Smith *et al*., 1989; Smith *et al*., 1990). Recent studies have shown that a PLC-γ1 mutant lacking the lipase activity still induced DNA synthesis, implying that regions other than the catalytic domain may be responsible for the mitogenic effect (Smith *et al*., 1994; Huang *et al*., 1995). Among multiple PLC-isozymes, only PLC-γ isozyme contains the Src-homology domains (SH domain) (two SH2 domains and one SH3 domain) that are also found in a number of proteins involved in the regulation of cell proliferation and differentiation (Pawson and Nash, 2000). The SH2 domains of PLC-γ1 are known to mediate the association between PLC-γ1 and phosphorylated tyrosines in the activated receptor tyrosine kinase or the src tyrosine kinase. The SH3 domain of PLC-γ1 is known to be responsible for the mitogenic effect of PLC-γ1. Namely, micro-injection of the GST-fused SH3 domain of PLC-γ1 into G0 growth arrested NIH 3T3 cells induced DNA synthesis (Huang *et al*., 1995; Smith *et al*., 1996). This mitogenic activity of PLC-γ1 or possible effector protein that could bind to the SH3 domain of PLC-γ1 has been the intense subject for study in many laboratories. We observed that SOS1, one of the activators of p21ras, can specifically interact with the SH3 domain of PLC-γ1 and that PLC-γ1 can mediate p21ras-activation by direct interaction with SOS1 in a lipase activity-independent manner (Kim *et al*., 2000) (Figure 3). Furthermore, 3Y1 cells, a rat embryonic fibroblast cell line, over-expressing either wild type PLC-γ1 or the SH2-SH2-SH3 domain of PLC-γ1 showed a comparatively elevated p21Ras activity (Kim *et al*., 2000) and transplantion of these cells into nude mouse resulted in the tumor formation (Chang *et al*., 1997). Thus, raising a possible involvement of the p21Ras signaling pathway in the PLC-γ1 SH3-induced mitogenesis of cells. Recently, we have elucidated that ectopic overexpression of PLC-γ1 can up-regulate the protein levels of PCNA, CDC2, CDK2, Cyclin D1, which play roles in the G1/S-transition. The level of Cip1/Waf-1, which inhibits G1/S transition, was dramatically diminished in the PLC-γ1-overexpressed cells compared with vector control cells. These observations suggest that PLC-γ1 can promote cell cycle progression by regulating the levels of cell cycle regulators (Lee *et al*., unpubli-

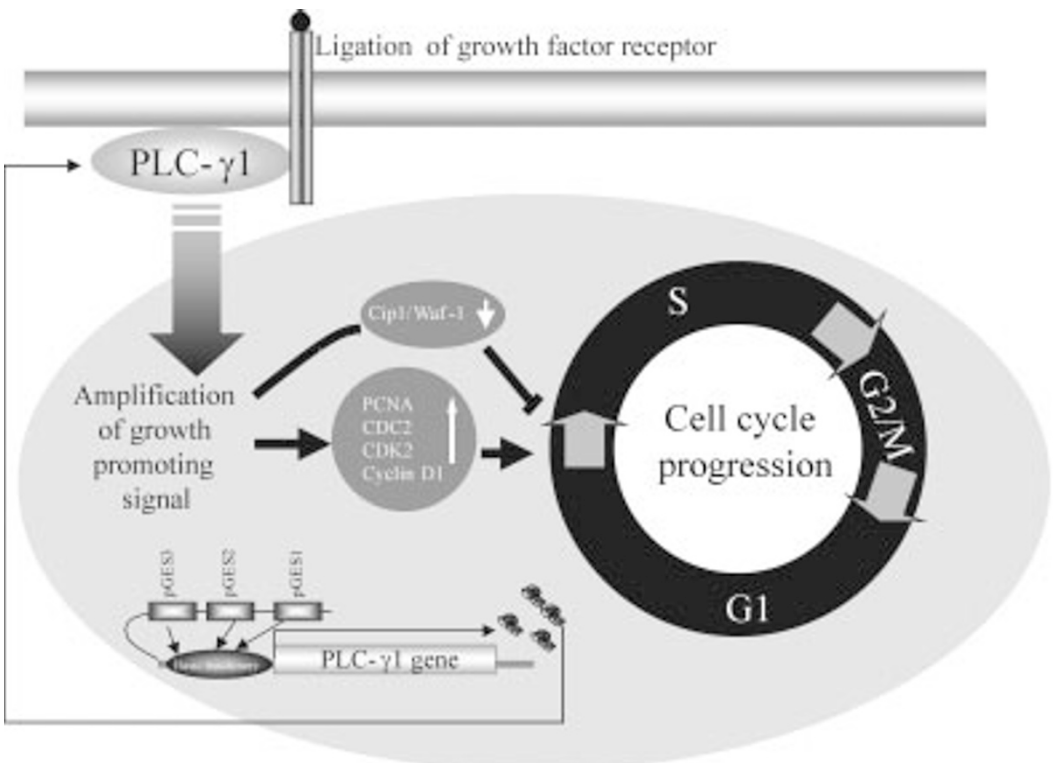

Figure 4. Transcriptional regulation of PLC-γ1 and effect on cell cycle regulator of PLC-γ1 overexpression. The transcription of PLC-γ1 is mainly up-regulated by the transcription factors (pGES1, pGES2, and pGES3) binding to *cis*-elements (GES1, GES2, and GES3) in PLC-γ1 promoter. In colorectal cancer and breast cancer tissues, the binding avctivities of pGES1, pGES2, and pGES3 to GES1, GES2, and GES3-*cis*-elements are increased to overexpress PLC-γ1 in these cancer tissues. Expressed PLC-γ1 amplifies growth-promoting signals inside cells. Growth promoting signals are transferred to nucleus, and protein levels of cell cycle regulators are modulated. In PLC-γ1-overexpressed cell line, levels of PCNA, CDK2, CDC2, and cyclin D1 are upregulated. Whereas the level of Cip1/Waf-1 are down-regulated. Resulting changes of cell cycle regulators lead to cell cycle progression.

shed data) (Figure 4).

Activation of PLC-γ1

Growth factor receptor ligation induces PLC-γ1 tyrosine phosphorylation, IP_3 formation, and Ca^{2+} mobilization. Three tyrosine residues in PLC-γ1 have been identified as the sites of receptor tyrosine kinase phosphorylation. Tyr 783 is known to be essential for IP_3 formation, phosphorylation of Tyr 771 is known to be dispensable, and phosphorylation of Tyr 1254 is known to be necessary to achieve maximal IP_3 generation (Kim *et al*., 1991) (see Figure 2). However, until now, a protein tyrosine phosphatase acting on tyrosyl phosphorylated PLC-γ1 has not been identified. Discovery of an *in vivo* protein tyrosine phosphatase for PLC-γ1 would widely extend our knowledge of the regulatory mechanism of PLC-γ1. Recently we have identified that PTP-1B, a cytosolic protein tyrosine phosphatase, can dephosphorylate PLC-γ1 *in vitro* and *vvivo* (Kim *et al*., unpublished data). Moreover, the larger proportion of PIP_2 in cells is localized in caveolin-enriched micro-domains (caveoli) (Pike and Casey, 1996). Caveoli are known to be signal initiation sites where many intracellular signaling molecules, such as EGF receptor, G-α subunit, ras, etc. are concentrated (Smart *et al*., 1999). Since the substrate of PLC-γ1 is PIP_2, there should be some mechanism by which activated PLC-γ1 could be recruited into the caveoli. However, it remains unknown how PLC-γ1 is targeted to this micro-environment.

The EGF receptor is known to phosphorylate and activate PLC-γ1 *in vivo* (Nishibe *et al*., 1990*)*. Upon EGF-stimulation, the EGF receptor undergoes autophosphorylation, and the resulting phosphotyrosine-containing sequences in the activated EGF receptor are now recognized by the SH2 domains of PLC-γ1 (Meisenhelder *et al*., 1989). No single autophosphorylation site mediates this association with the SH2 domains of PLC-γ1, but rather there are several sites involved (Chattopadhyay *et al*., 1999). This association process between the phosphotyrosine-containing sequences and the SH2 domains of PLC-γ1 can also be applied to the case of platelet-derived growth factor (PDGF) (Larose *et al*., 1993; Valius *et al*., 1993), fibroblast growth factor (FGF) (Peters *et al*., 1992), and nerve growth factor (NGF) receptors (Obermeier *et al*., 1993; Middlemas *et al*., 1994). In contrast to the EGF receptor-signaling, there is one specific autophosphorylated tyrosine that specifies an association with the PLC-γ1 SH2 domains for platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and nerve growth factor (NGF) receptors (for example, the Tyr1021 of the β-type PDGF receptor) (Valius *et al*., 1993). Both SH2 domains of PLC-γ1 have been shown to be required for growth factor-induced receptor association and Ca^{2+} mobilization and also for association with the EGF receptor (Chattopadyay *et al*., 1999; Poulin *et al*., 2000). The N-terminal SH2 domain of PLC-γ1 is known to play a major role in the association with the activated growth factor receptor, while the C-terminal SH2 domain is considered to play a minor role (Chattopadyay *et al*., 1999; Poulin *et al*., 2000). However, recently, it has been reported that the C-terminal SH2 domain of PLC-γ1 is involved in interactions with synaptojanin (Ahn *et al*., 1998), the actin-cytoskeleton (Pei *et al*., 1996), PIP_3 (Bae *et al*., 1998; Rameh *et al*., 1998) and FAK (Zhang *et al*., 1999). These observations suggest that the two SH2 domains of PLC-γ1 are dissimilar and may mediate the recognition of specific phosphorylation sites.

Importantly, growth factor-stimulation not only induces receptor association but also translocation of PLC-γ1 from the cytosol to the membrane (Todderud *et al*., 1990). Until now, the driving force behind this translocation remains unknown, but it is suspected that the membrane-targeting is linked to growth factor receptors. The amount of PLC-γ1 that translocates to the membrane (particulate fraction) is not related to the amount of growth factor receptor available. Thus it is possible that there are another anchoring protein(s) which can specifically recognize phosphorylated PLC-γ1 and target it to other membrane. This hypothesis is supported by a recent report that showed the presence of phosphorylated PLC-γ1 not only in the plasma membrane but also in the actin cytoskeleton/peri-nuclear membrane (Yu *et al*., 1998). Another possible membrane targeting element is the N-terminal PH domain of PLC-γ1. One study has suggested such a role for the N-terminal PH domain of PLC-γ1 based on inhibitor competition experiments. In this study, they used a GST (or GFP) protein fused to the N-terminal PH domain of PLC-γ1. The PLC-γ1/PH domain fusion protein migrated to the plasma membrane when the cells were stimulated by growth factor, and the translocation was mediated via interaction with PIP_3 (Falasca *et al*., 1998). Importantly, this over-expressed PH-domain fusion protein also reduced the formation of IP_3 in EGF-treated cells by about 60%, suggesting that it interfered with the membrane localization of endogenous PLC-γ1.

Other activation mechanisms

A variety of receptors activate PI 3-kinase, which phosphorylates the D3 position of PIP_2 to produce phosphatidylinositol-3,4,5-trisphosphate (PIP_3) (Rameh and Cantley, 1999). The influence of PIP_3 on the PLC-γ1 activity has been looked at more closely. In these reports, the investigators suggested that PIP_3 recognized the C-terminal SH2 domain of PLC-γ1 *in vitro* (Bae *et al*., 1998; Rameh *et al*., 1998). Moreover, they demonstrated that inhibition of the PI-3K activity using a specific inhibitor could attenuate PLC-γ1 activation (Bae *et al*., 1998; Rameh *et al*., 1998). This is contrary to the report

that the amino-terminal PH domain of PLC-γ1 is responsible for interaction with PIP_3 (Falasca *et al*., 1998). To date, it still remains controversial which domain is involved in the interaction with PIP_3 *in vivo*.

In addition, PLC-γ1 could be activated directly by several lipid-derived second messengers in the absence of tyrosine phosphorylation. Phosphatidic acid could thus activate purified PLC-γ1 by acting as an allosteric modifier *in vitro*. PLC-γ1 can also be stimulated by arachidonic acid (AA) in the presence of the microtubule-associated protein tau (in neuronal cells) (Hwang *et al*., 1996) or tau-like protein (AHNAK in non-neuronal cells) (Sekiya *et al*., 1999). The effect of tau and AA was specific on PLC-γ1, and it was strongly inhibited by phosphatidylcholine (PC). However, it is still not clear whether PA or AA is a real activator of PLC-γ1 *in vivo*.

Activation of PLC-γ1 in T cells

In T cells, TCR-ligation activates PLC-γ1 isozymes. The role of Ca^{2+} mobilization by PLC-γ1 in this process is to activate phosphatase calcineurin, which in turn dephosphorylates and activates NF-AT (Rao *et al*., 1997). The activation of PLC-γ1 in T cells is mediated by non-receptor tyrosine kinases. During T cell activation, members of three different cytosolic tyrosine kinase families are involved in PLC-γ1 tyrosine phosphorylation. Src family members are indirectly required, as these kinases serve to phosphorylate specific tyrosine residues within immunoreceptor tyrosine-based activation motifs (ITAMs) present on the cytoplasmic domains of T cell receptor polypeptide chains (Van Leeuwen and Samelson, 1999). Phosphorylated ITAM motifs then serve as docking sites for members of the ZAP-70/Syk tyrosine kinase family, which contain SH2 domains. When ZAP-70/Syk is associated with the receptor ITAMs, they become tyrosine phosphorylated at specific sites and activated. These kinases are then able to form a complex with the SH2 domains of PLC-γ1 and are therefore more likely candidates for kinases that are directly responsible for the phosphorylation of PLC-γ1 in T cells.

Another different feature of the PLC-γ1 activation in T cells is the requirement for specific adaptor proteins, such as LAT and SLP-76. LAT is a 38-kD integral membrane protein that becomes highly tyrosine phosphorylated following T cell activation. The kinase that phosphorylates LAT is thought to be ZAP-70, and, once phosphorylated, LAT associates with multiple signaling proteins that contain SH2 domains, including PLC-γ1 (Clement *et al*., 1999; Kennedy, *et al*., 1999; Van Leeuwen and Samelson, 1999). Also, the association with LAT is necessary for PLC-γ1 tyrosine phosphorylation and activation (Kennedy, *et al*., 1999). It has been suggested that the two SH2 domains of PLC-γ1 are used simultaneously in the interaction with ZAP-70 and LAT to increase both tyrosine phosphorylation and membrane localization. LAT is palmitoylated within its transmembrane domain and this modification is required for its tyrosine phosphorylation activity, association with PLC-γ1, and activation of PLC-γ1 in T cells (Kennedy, *et al*., 1999). However, it remains to be determined whether PLC-γ1 is activated within these micro-domains.

Perspectives

Here, recent advances in the regulation of PLC-γ1 were categorically summarized. A number of experimental evidences are demonstrating that PLC-γ1 is a pivotal element in growth factor signaling and T-cell receptor signaling. But there remain a number of unsolved questions regarding to the regulation of PLC-γ1 and *in vivo* function of PLC-γ1. For example, the mechanism of how PLC-γ1 is targeted to particulate fraction/detergent-resistant membrane raft and the detailed mechanism by which PLC-γ1 promotes cell cycle-progression. However, these problems in the PLC-γ1 study could readily be clarified by the identification and characterization of specific binding proteins of PLC-γ1. The development of experimental techniques such as yeast two-hybrid system and mass spectrometry could accelerate the discovery of novel regulation mechanism and tumorigenic mechanism of PLC-γ1.

Acknowledgement

*This work was supported by the POSTECH Research Fund, and by the Frontier 21 Fund. We appreciate reviewers for critical reading of the manuscript.

References

Ahn, S. J., Han, S. J., Mo, H. J., Chung, J. K., Hong, S. H., Park, T. K. and Kim, C. G. (1998) Interaction of phospholipase C-γ1 via its COOH-terminal SRC homology 2 domain with synaptojanin. *Biochem. Biophys. Res. Commun.* 244: 62-67

Arteaga, C. L., Johnson, M. D., Todderud, G., Coffey, R. J., Carpenter, G. and Page, D. L. (1991) Elevated content of the tyrosine kinase substrate phospholipase C-γ1 in primary human breast carcinomas. *Proc. Natl. Acad. Sci. USA* 88: 10435-10439

Bae, S. S., Ryu, S. H. and Suh, P. G. Mutation in C-terminal SH2 of PLC-gamma1 results in increase of basal PLC-γ1 activity. Unpublished data

Bae, Y. S., Cantley, L. G., Chen, C. S., Kim, S. R., Kwon, K. S. and Rhee, S. G. (1998) Activation of phospholipase C-γ by phosphatidylinositol 3,4,5-trisphosphate. *J. Biol. Chem.* 273: 4465-4469

Bahk, Y. Y., Lee, Y. H., Lee, T. G., Seo, J., Ryu, S. H. and Suh, P. G. (1994) Two forms of phospholipase C-β1 generated by alternative splicing. *J. Biol. Chem.* 269: 8240-8245

Berridge, M. J. and Irvine, R. F. (1989) Inositol phosphates and cell signalling. *Nature* 341: 197-205

Carpenter, G. and Ji, Q. (1999) Phospholipase C-γ as a signal-transducing element. *Exp. Cell. Res.* 253: 15-24

Chang, J. S., Noh, D. Y., Park, I. A., Kim, M. J., Song, H., Ryu, S. H. and Suh, P. G. (1997) Overexpression of phospholipase C-γ1 in rat 3Y1 fibroblast cells leads to malignant transformation. *Cancer Res.* 57: 5465-5468

Chattopadhyay, A., Vecchi, M., Ji, Q., Mernaugh, R. and Carpenter, G. (1999) The role of individual SH2 domains in mediating association of phospholipase C-γ1 with the activated EGF receptor. *J. Biol. Chem.* 274: 26091-26097

Clements, J. L., Boerth, N. J., Lee, J. R. and Koretzky, G. A. (1999) Integration of T cell receptor-dependent signaling pathways by adapter proteins. *Annu. Rev. Immunol.* 17: 89-108

Falasca, M., Logan, S. K., Lehto, V. P., Baccante, G., Lemmon, M. A. and Schlessinger, J. (1998) Activation of phospholipase C-γ by PI 3-kinase-induced PH domain- mediated membrane targeting. *EMBO. J.* 17: 414-422

Gergel, J. R., McNamara, D. J., Dobrusin, E. M., Zhu, G., Saltiel, A. R. and Miller, W. T. (1994) Identification of amino acids in the N-terminal SH2 domain of phospholipase C-γ1 important in the interaction with epidermal growth factor receptor. *Biochemistry* 33: 14671-14678

Graham, L. J., Stoica, B. A., Shapiro, M., DeBell, K. E., Rellahan, B., Laborda, J. and Bonvini, E. (1998) Sequences surrounding the Src-homology 3 domain of phospholipase C-γ1 increase the domain's association with Cbl. *Biochem. Biophys. Res. Commun.* 249: 537-541

Horstman, D. A., DeStefano, K. and Carpenter, G. (1996) Enhanced phospholipase C-γ1 activity produced by association of independently expressed X and Y domain polypeptides. *Proc. Natl. Acad. Sci. USA* 93: 7518-7521

Horstman, D. A., Chattopadhyay, A. and Carpenter, G. (1999) The influence of deletion mutations on phospholipase C-γ1 activity. *Arch. Biochem. Biophys.* 361: 149-155

Huang, P. S., Davis, L., Huber, H., Goodhart, P. J., Wegrzyn, R. E., Oliff, A. and Heimbrook, D. C. (1995) An SH3 domain is required for the mitogenic activity of microinjected phospholipase C-γ1. *FEBS Lett.* 358: 287-292

Hwang, S. C., Jhon, D. Y., Bae, Y. S., Kim, J. H. and Rhee, S. G. (1996) Activation of phospholipase C-γ by the concerted action of tau proteins and arachidonic acid. *J. Biol. Chem.* 271: 18342-18349

Hwang, J. I., Heo, K., Shin, K. J., Kim, E., Yun, C., Ryu, S. H., Shin, H. S. and Suh, P. G. (2000) Regulation of phospholipase C-β3 activity by Na+/H+ exchanger regulatory factor 2. *J. Biol. Chem.* 275: 16632-16637

Hwang, J. I., Ryu, S. H. and Suh, P. G. CortBP1 binds to Phospholipase C-β1. unpublished data

Jhon, D. Y., Lee, H. H., Park, D., Lee, C. W., Lee, K. H., Yoo, O. J. and Rhee, S. G. (1993) Cloning, sequencing, purification, and Gq-dependent activation of phospholipase C-β3. *J. Biol. Chem.* 268: 6654-6661

Kennedy, J. S., Raab, M. and Rudd, C. E. (1999) Signaling scaffolds in immune cells. *Cell. Calcium* 26: 227-235

Kim, H. K., Kim, J. W., Zilberstein, A., Margolis, B., Kim, J. G., Schlessinger, J. and Rhee, S. G. (1991) PDGF stimulation of inositol phospholipid hydrolysis requires PLC-γ1 phosphorylation on tyrosine residues 783 and 1254. *Cell* 65: 435-441

Kim, M. J., Min, D. S., Ryu, S. H. and Suh, P. G. (1998) A cytosolic, Gaq- and bg-insensitive splice variant of phospholipase C-β4. *J. Biol. Chem.* 273: 3618-3624

Kim, M. J., Chang, J. S., Park, S. K., Hwang, J. I., Ryu, S. H. and Suh, P. G. (2000) Direct interaction of SOS1 Ras exchange protein with the SH3 domain of phospholipase C-γ1. *Biochemistry* 39: 8674-8682

Kim, M. J. Ryu, S. H. and Suh, P. G. PTP1-B dephosphorylates phospholipase C-γ1 *in vivo*. Unpublished data

Larose, L., Gish, G., Shoelson, S. and Pawson, T. (1993) Identification of residues in the beta platelet-derived growth factor receptor that confer specificity for binding to phospholipase C-γ1. *Oncogene* 8: 2493-2499

Lee, Y. H., Kim, H. S., Pai, J. K., Ryu, S. H. and Suh, P. G. (1994) Activation of phospholipase D induced by platelet-derived growth factor is dependent upon the level of phospholipase C-γ1. *J. Biol. Chem.* 269: 26842-26847

Lee, S. J., Lee, S. D., Park, J. G., Kim, C. M., Ryu, S. H. and Suh, P. G. (1995) Overexpression of phospholipase C-γ1 in colorectal carcinomas is associated with overexpression of factors that bind its promoter. *J. Biol. Chem.* 270: 16378-16384

Lee, Y. H., Ryu, S. H. and Suh, P. G. Overexpression of phospholipase C-γ1 alters levels of cell cycle regulator. Unpublished data

Meisenhelder, J., Suh, P. G., Rhee, S. G. and Hunter, T. (1989) Phospholipase C-γ is a substrate for the PDGF and EGF receptor protein-tyrosine kinases *in vivo* and *in vitro*. *Cell* 57: 1109-1122

Middlemas, D. S., Meisenhelder, J. and Hunter, T. (1994) Identification of TrkB autophosphorylation sites and evidence that phospholipase C-γ1 is a substrate of the TrkB receptor. *J. Biol. Chem.* 269: 5458-5466

Morrison, D. K. and Cutler, R. E. (1997) The complexity of Raf-1 regulation. *Curr. Opin. Cell. Biol.* 9: 174-179

Nanney, L. B., Gates, R. E., Todderud, G., King, L. E., Jr. and Carpenter, G. (1992) Altered distribution of phospholipase C-γ1 in benign hyperproliferative epidermal diseases. *Cell Growth Differ.* 3: 233-239

Newton, A. C. (1997) Regulation of protein kinase C. *Curr. Opin. Cell. Biol.* 9: 161-167

Nishibe, S., Wahl, M. I., Wedegaertner, P. B., Kim, J. W., Rhee, S. G., Carpenter, G. and Kim, J. J. (1990) Selectivity of

phospholipase C phosphorylation by the epidermal growth factor receptor, the insulin receptor, and their cytoplasmic domains *Proc. Natl. Acad. Sci USA* 87: 424-428

Nishizuka, Y. (1986) Studies and perspectives of protein kinase C. *Science* 233: 305-312

Noh, D. Y., Lee, Y. H., Kim, S. S., Kim, Y. I., Ryu, S. H., Suh, P. G. and Park, J. G. (1994) Elevated content of phospholipase C-γ1 in colorectal cancer tissues. *Cancer* 73: 36-41

Noh, D. Y., Shin, S. H. and Rhee, S. G. (1995) Phosphoinositide-specific phospholipase C and mitogenic signaling. *Biochim. Biophys. Acta.* 1242: 99-113

Obermeier, A., Halfter, H., Wiesmuller, K. H., Jung, G., Schlessinger, J. and Ullrich, A. (1993) Tyrosine 785 is a major determinant of Trk--substrate interaction. *EMBO J* 12: 933-941

Park, D., Jhon, D. Y., Kriz, R., Knopf, J. and Rhee, S. G. (1992) Cloning, sequencing, expression, and Gq-independent activation of phospholipase C-β2. *J. Biol. Chem.* 267: 16048-16055

Park, J. G., Lee, Y. H., Kim, S. S., Park, K. J., Noh, D. Y., Ryu, S. H. and Suh, P. G. (1994) Overexpression of phospholipase C-γ1 in familial adenomatous polyposis. *Cancer Res.* 54: 2240-2244

Pawson, T. (1994) SH2 and SH3 domains in signal transduction. *Adv. Cancer Res.* 64: 87-110

Pawson, T. and Gish, G. D. (1992) SH2 and SH3 domains: from structure to function. *Cell* 71: 359-362

Pawson, T. and Nash, P. (2000) Protein-protein interactions define specificity in signal transduction. *Genes Dev.* 14: 1027-47

Pei, Z., Yang, L. and Williamson, J. R. (1996) Phospholipase C-γ1 binds to actin-cytoskeleton via its C-terminal SH2 domain *in vitro*. *Biochem. Biophys. Res. Commun.* 228: 802-806

Peters, K. G., Marie, J., Wilson, E., Ives, H. E., Escobedo, J., Del Rosario, M., Mirda, D. and Williams, L. T. (1992) Point mutation of an FGF receptor abolishes phosphatidylinositol turnover and Ca^{2+} flux but not mitogenesis. *Nature* 358: 678-681

Pike, L. J. and Casey, L. (1996) Localization and turnover of phosphatidylinositol 4,5-bisphosphate in caveolin-enriched membrane domains. *J. Biol. Chem.* 271: 26453-26456

Poulin, B., Sekiya, F. and Rhee, S. G. (2000) Differential roles of the Src homology 2 domains of phospholipase C-γ1 (PLC-γ1) in platelet-derived growth factor-induced activation of PLC-γ1 in intact cells. *J. Biol. Chem.* 275: 6411-6416

Rameh, L. E. and Cantley, L. C. (1999) The role of phosphoinositide 3-kinase lipid products in cell function. *J. Biol. Chem.* 274: 8347-8350

Rameh, L. E., Rhee, S. G., Spokes, K., Kazlauskas, A., Cantley, L. C. and Cantley, L. G. (1998) Phosphoinositide 3-kinase regulates phospholipase C-γ mediated calcium signaling. *J. Biol. Chem.* 273: 23750-23757

Rana, R. S. and Hokin, L. E. (1990) Role of phosphoinositides in transmembrane signaling. *Physiol. Rev.* 70: 115-64

Rao, A., Luo, C. and Hogan, P. G. (1997) Transcription factors of the NFAT family: regulation and function. *Annu. Rev. Immunol.* 15: 707-747

Rhee, S. G. and Bae, Y. S. (1997) Regulation of phosphoinositide-specific phospholipase C isozymes. *J. Biol. Chem.* 272: 15045-15048

Seedorf, K., Kostka, G., Lammers, R., Bashkin, P., Daly, R., Burgess, W. H., van der Bliek, A. M., Schlessinger, J. and Ullrich, A. (1994) Dynamin binds to SH3 domains of phospholipase C-γ and GRB-2. *J. Biol. Chem.* 269: 16009-16014

Sekiya, F., Bae, Y. S., Jhon, D. Y., Hwang, S. C. and Rhee, S. G. (1999) AHNAK, a protein that binds and activates phospholipase C-γ1 in the presence of arachidonic acid. *J. Biol. Chem.* 274: 13900-13907

Shenker, A., Goldsmith, P., Unson, C. G. and Spiegel, A. M. (1991) The G protein coupled to the thromboxane A2 receptor in human platelets is a member of the novel Gq family. *J. Biol. Chem.* 266: 9309-9313

Sillman, A. L. and Monroe, J. G. (1995) Association of p72syk with the src homology-2 (SH2) domains of PLC-γ1 in B lymphocytes. *J. Biol. Chem.* 270: 11806-11811

Smart, E. J., Graf, G. A., McNiven, M. A., Sessa, W. C., Engelman, J. A., Scherer, P. E., Okamoto, T. and Lisanti, M. P. (1999) Caveolins, liquid-ordered domains, and signal transduction. *Mol. Cell. Biol.* 19: 7289-7304

Smith, M. R., Liu, Y. L., Kim, H., Rhee, S. G. and Kung, H. F. (1990). Inhibition of serum- and ras-stimulated DNA synthesis by antibodies to phospholipase C. *Science* 247: 1074-1077

Smith, M. R., Liu, Y. L., Kim, S. R., Bae, Y. S., Kim, C. G., Kwon, K. S., Rhee, S. G. and Kung, H. F. (1996) PLC-γ1Src homology domain induces mitogenesis in quiescent NIH 3T3 fibroblasts. *Biochem. Biophys. Res. Commun.* 222: 186-193

Smith, M. R., Liu, Y. L., Matthews, N. T., Rhee, S. G., Sung, W. K. and Kung, H. F. (1994) Phospholipase C-γ1 can induce DNA synthesis by a mechanism independent of its lipase activity. *Proc. Natl. Acad. Sci. USA* 91: 6554-6558

Smith, M. R., Ryu, S. H., Suh, P. G., Rhee, S. G. and Kung, H. F. (1989) S-phase induction and transformation of quiescent NIH 3T3 cells by microinjection of phospholipase C. *Proc. Natl. Acad. Sci. USA* 86: 3659-3663

Smrcka, A. V., Hepler, J. R., Brown, K. O. and Sternweis, P. C. (1991) Regulation of polyphosphoinositide-specific phospholipase C activity by purified Gq. *Science* 251: 804-807

Suh, P. G., Ryu, S. H., Moon, K. H., Suh, H. W. and Rhee, S. G. (1988) Cloning and sequence of multiple forms of phospholipase C. *Cell* 54: 161-169

Taylor, S. J., Chae, H. Z., Rhee, S. G. and Exton, J. H. (1991) Activation of the beta 1 isozyme of phospholipase C by alpha subunits of the Gq class of G proteins. *Nature* 350: 516-518

Todderud, G., Wahl, M. I., Rhee, S. G. and Carpenter, G. (1990) Stimulation of phospholipase C-γ1 membrane association by epidermal growth factor. *Science* 249: 296-298

Valius, M., Bazenet, C. and Kazlauskas, A. (1993) Tyrosines 1021 and 1009 are phosphorylation sites in the carboxy terminus of the platelet-derived growth factor receptor beta subunit and are required for binding of phospholipase C-γ and a 64-kilodalton protein, respectively. *Mol. Cell. Biol.* 13: 133-143

Valius, M., Secrist, J. P. and Kazlauskas, A. (1995) The GTPase-activating protein of Ras suppresses platelet-derived growth factor beta receptor signaling by silencing phospholipase C-γ1. *Mol. Cell. Biol.* 15: 3058-3071

van Leeuwen, J. E. and Samelson, L. E. (1999) T cell antigen-receptor signal transduction. *Curr. Opin. Immunol.* 11: 242-248

Wu, D. Q., Lee, C. H., Rhee, S. G. and Simon, M. I. (1992) Activation of phospholipase C by the alpha subunits of the Gq and G11 proteins in transfected Cos-7 cells. *J. Biol. Chem.* 267: 1811-1817

Yablonski, D., Kuhne, M. R., Kadlecek, T. and Weiss, A. (1998) Uncoupling of nonreceptor tyrosine kinases from PLC-γ1 in an SLP-76- deficient T cell. *Science* 281: 413-416

Yu, H., Fukami, K., Itoh, T. and Takenawa, T. (1998) Phosphorylation of phospholipase C-γ1 on tyrosine residue 783 by platelet-derived growth factor regulates reorganization of the cytoskeleton. *Exp. Cell. Res.* 243: 113-122

Zhang, X., Chattopadhyay, A., Ji, Q. S., Owen, J. D., Ruest, P. J., Carpenter, G. and Hanks, S. K. (1999) Focal adhesion kinase promotes phospholipase C-γ1 activity. *Proc. Natl. Acad. Sci. USA* 96: 9021-9026