Journal of Biochemistry and Molecular Biology, Vol. 33, No. 2, March 2000, pp. 97-102

JOURNAL OF
Biochemistry and Molecular Biology

Identification and Characterization of the Interaction between Heat-Shock Protein 90 and Phospholipase C-γ1

Su-Jeong Kim, Myung Jong Kim, Yong Kim, Fu Chun Si, Sung Ho Ryu and Pann-Ghill Suh*
Department of Life Science, and School of Environmental Engineering,
Pohang University of Science and Technology, Pohang 790-784, Republic of Korea

Received 7 October 1999, Accepted 9 November 1999

Phosphoinositide-specific phospholipase C-γ1 (PLC-γ1) is a pivotal mediator in the signal transduction cascades induced by many growth factors. Using a yeast two-hybrid system, heat-shock protein 90 (Hsp90) was identified as a PLC-γ1-binding protein. A co-immunoprecipitation experiment, using anti-PLC-γ1 antibody, demonstrated an *in vivo* interaction between Hsp90 and PLC-γ1 in the NIH-3T3 cells. The interaction in NIH-3T3 was unaffected by the PDGF treatment, inducing phosphorylation and activation of PLC- γ1. Direct interaction between Hsp90 and PLC-γ1 was confirmed by *in vitro* binding experiments using purified Hsp90 and PLC-γ1. Furthermore, Hsp90 increased the PIP_2-hydrolyzing activity of PLC-γ1 up to 2-fold at 0.1μM *in vitro*. Taken together, we show for the first time, the interaction of PLC-γ1 with Hsp90, both *in vivo* and *in vitro*. We suggest that Hsp90 may play a role in PLC-γ1-mediated signal transduction.

Keywords: Hsp90, *In vitro binding* experiment, PLC-γ1, Protein-Protein Interaction, Yeast two-hybrid system.

Introduction

Phosphoinositide-specific phospholipase C (PLC)[1] hydrolyzes phosphatidylinositol 4,5-bisphosphate in order to generate inositol trisphosphate (IP_3) and diacylglycerol (DAG) in response to many growth hormones. Multiple PLC isozymes were purified from various tissues, and multiple mammalian PLC cDNAs were cloned and sequenced. On the basis of the amino acid sequence homology, and immunological cross-reactivity, these mammalian PLCs were divided into three types: PLC-β, -δ, -γ (Rhee and Bae, 1997; Noh *et al.*, 1995).

PLC-γ1 subtypes were reported to play an important role in several signaling pathways leading to cellular events, such as cell growth and differentiation (Rhee and Bae, 1997; Noh *et al.*, 1995). Recently, it was reported that the mitogenic activity of PLC-γ1 does not exclusively result from the enzymatic activity of the lipase, and that another activity (inherent to the PLC-γ1) can induce mitogenesis (Smith *et al.*, 1989; Smith *et al.*, 1990; Smith *et al.*, 1994; Huang *et al.*, 1995). Besides enzymatic catalytic domains (X, Y domain), the PLC-γ1 molecule contains the c-Src homology (SH) domain 2, 3, which are known to be binding motifs for protein-protein interactions in intracellular signaling pathway (Koch *et al.*, 1991). SH2 and SH3 domains are conserved among cytoplasmic signaling molecules, including PLC-γ1, ras-GTPase activating protein, phosphatidylinositol 3'-kinase, and Src and Src-family tyrosine kinase (Koch *et al.*, 1991; Pawson, 1995). Previously it was reported that PLC-γ1 binds to phosphorylated tyrosine residues of cell surface receptors, such as EGF receptor, PDGF receptor and non-receptor type tyrosine kinase family via SH2 domains. It also binds to cytoskeletal proteins, such as dynamin, integrin via SH3 domain (Kamat and Carpenter, 1997; Rhee and Bae, 1997). These interactions are important in cellular events, such as DNA synthesis, cytokinesis, change of morphology, motility and adhesion that is induced by mitogenic signal (Pawson, 1995).

In order to identify proteins that bind to SH2, 3 domains of PLC-γ1 were used in the yeast two-hybrid system. Here, we report that Hsp90 is a novel PLC-γ1-binding protein.

Experimental procedures

Materials All yeast strains, plasmid and human B-lymphocyte MATCHMAKER cDNA library for two-hybrid library screening were purchased from Clontech (Palo Alto, USA). The mammalian cell culture media and PDGF were purchased from GIBCO-BRL (Grand Island, USA). Mouse monoclonal anti-PLC-γ1 antibodies (F7, E8-4) were prepared, as previously described (Suh *et al.*, 1988). Mouse anti-human Hsp90 antibody was purchased from Transduction Laboratories (Lexington, USA). A horseradish peroxidase that conjugated secondary antibody to

*To whom correspondence should be addressed.
Tel: 82-562-279-2293; Fax: 82-562-279-2199
E-mail: pgs@postech.ac.kr.

mouse IgG and enhanced chemiluminescence (ECL) reagent was purchased from Amersham Corp (Arlington Heights, USA). Protein A-Sepharose beads were obtained from Pharmacia Biotech Inc. (Uppsala, Sweden). Phosphatidyl-inositol 4,5-bisphosphate, phosphatidyl-ethanolamine and phosphatidyl [^{3}H]inositol 4,5-bisphostate were purchased from Amersham Corp (Arlington Heights, USA).

Yeast two hybrid screening The region (amino acid 465-952) between X and Y domain of rat PLC-γ1 was fused to the GAL4 DNA binding domain in vector pAS2 (Harper *et al.*, 1993) to produce pAS2-PLC-γ1-SH223, which was used as the 'bait' in the screening. Yeast genetic techniques and media composition were as described (Harper *et al.*, 1993). The histidine selection system (Durfee *et al.*, 1993), which utilizes two distinct GAL4--dependent reporter genes, HIS3 (a histidine biosynthetic enzyme) and lacZ (β-galactosidase), was used. Histidine selective medium contained 1 mM 3-aminotriazole (Sigma, St. Louis, USA). The pACT human B-lymphocyte library was screened in strain HF7c (MAT *ura3-52 his3-200 ade2-191 lys2-801 trp1-901 leu2-3112 gal4-542 gal80-538* Lys2::GAL1$_{UAS}$ GAL$_{TATA}$-HIS3 URA3:: GAL4$_{17mer(x3)}$-CyC1$_{TATA}$-*lacZ*). Approximately 1.2×10^6 double transformants of a human B-lymphocyte cDNA library were screened. To eliminate false positives, the isolated pACT/clones were also re-transformed alone; or co-transformed with pAS2-control vector, pAS2-PLC-γ1SH223, pAS2-PLC-β4-N (rat phospholipase C-β4, a.a 1-120), pAS2-PLC-δ1-N (rat phospholipase C-δ1, a.a 1-110), or pAS2-RhoA (full open reading frame of RhoA) in yeast, and clones that showed positive LacZ activity were eliminated as false positives. Finally, twenty-eight distinct clones, which show the reporter gene activity (His$^+$ and LacZ$^+$ activities) dependent on their interaction with the SH2-SH2-SH3 domain of PLC-γ1, was finally isolated. Plasmid DNAs in positive yeast clones were subjected to DNA sequencing.

Cell culture and extraction NIH-3T3 cells were grown in Dulbecco modified Eagle medium supplemented with 10% bovine calf serum. After serum starvation for 15 hrs, NIH-3T3 cells were stimulated for the indicated time with 50 ng/ml PDGF. After washing with cold Ca^{2+}, Mg^{2+}-free phosphate-buffered saline (dPBS) cells were lysed for 30 min at 4°C in 1 ml TBST buffer (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, supplemented with protease inhibitors, 10 μg/ml aprotinin, 1 μg/ml leupeptin, 1 μg/ml pepstatin A). Nuclear and cell debris were removed by centrifugation at 15,000g for 15 min at 4°C.

Co-Immunoprecipitation After the pre-clearing of cell lysate for 1 hr at 4°C with protein A Sepharose, anti-PLC-γ1 antibody (E8-4) was added in the lysate and incubated for 1 hr at 4°C. Then protein A-Sepharose, coupled with rabbit anti-mouse IgG-antibody, was added into cell lysate and incubated for 1 hr at 4°C. The resin was washed five times with TBST buffer, and immune-complexes were eluted by boiling in a SDS-PAGE sample buffer (Kim et al., 1999). The protein samples were separated in SDS-PAGE (8%), electrophoretically blotted onto nitrocellulose paper (Joo and Kim, 1998; Shin et al., 1998), and immunostained with antibody against PLC-γ1 (F7) and Hsp90.

Purification of Hsp90 Hsp90 was purified to homogeneity from bovine brain, as described previously (Itoh et al., 1990), with slight modifications, as judged by immunoblotting pattern using mouse monoclonal anti-Hsp90 antibody. Bovine brain was homogenized with 3 volume of 10 mM Tris buffer (pH 7.5) containing 1 mM phenylmethylsulfonyl-fluoride (PMSF) and centrifuged at 18,000×g for 20 min at 4°C. The supernatant was fractionated with ammonium sulfate (Sigma, St. Louis, USA) at a concentration of 30-50%. After overnight dialysis, the sample was separated with QA-52 column (Whatman, Lexington, USA) using a 0.3-0.6M NaCl gradient in Tris buffer (pH 7.5) containing 15 mM β-mercaptoethanol. Hsp90 was eluted between 0.4-0.5 M NaCl. Collected fractions was dialyzed overnight in 0.01 M potassium phosphate buffer (pH 7.0) and then separated on hydroxyapatite column (Bio Rad, Richmond, USA) with 0.01-0.5 M potassium phosphate gradient. Hsp90 was eluted between 0.2-0.3 M potassium phosphate. CBB stain on SDS-PAGE showed a single band at molecular size 90 kDa (data not shown).

In vitro binding PLC-γ1 was purified from HeLa cells transfected with recombinant vaccinia virus containing corresponding cDNA. Purified PLC-γ1 was mixed with purified Hsp90 in a TBST buffer and incubated for 10 min at 36°C. Anti-PLC-γ1 antibody was added in the mixture and incubated for 1 h at 4°C. After adding rabbit anti-mouse Ig antibody-coupled protein A sepharose suspension, it was incubated for 1 hr at 4°C. The resin was washed five times with TBST buffer, and immune-complexes were eluted by boiling in a SDS-PAGE sample buffer. Resulting protein samples were separated in SDS-PAGE and analyzed with immuno-blotting.

PLC enzyme activity assay In vitro PLC activity was measured, as previously described (Hepler et al., 1993). Briefly, substrate was prepared as sonicated vesicles of 75 μM [^{3}H]PIP_2 (9,000-10,000 cpm/assay), and 750 μM phosphatidyl-ethanolamine in 50 mM HEPES buffer (pH 7.0) containing 2 mM EGTA. $CaCl_2$ was added to the assay mixture to give the 0.2 μM free Ca^{2+}, which were calculated as described (Fabiato, 1988). Reactions were performed for 10 min at 36°C in 50μl final volume and terminated by addition of 1 ml of chloroform/methanol/HCl (50 : 50 : 0.3) and 0.45 ml of 1 N HCl. The mixtures were mixed and centrifuged for 10 min at 2000 rpm. The aqueous phase containing released [^{3}H]IP_3 was collected and radioactivity was measured by liquid scintillation spectroscopy. The effect of Hsp90 was demonstrated by adding the indicated amount of Hsp90 to PLC-γ1 assay mixture.

Results

Identification of Hsp90 as a PLC-γ1-binding protein using yeast two-hybrid system To identify the PLC-γ1-binding proteins, we used a yeast two-hybrid system using the SH2-SH2-SH3 domain of PLC-γ1. The pACT human B-lymphocyte library was screened, and twenty-eight distinct clones, which show the reporter gene activity (His+ and LacZ+ activities) dependent on their interaction with the SH2-SH2-SH3 domain of PLC-γ1, was finally isolated. Among

Table 1. Specific interaction between PLC-γ1 and Hsp90.

Transforming Plasmid	Colony Color (LacZ activity)	Histidine Prototrophy
pACT-Hsp90 + pAS2-γ1SH223	Blue	+
pACT-Hsp90 + pAS2-PLC-β4-N	White	-
pACT-Hsp90 + pAS2-PLC-δ1-N	White	-
pACT-Hsp90 + RhoA	White	-

To test whether reporter gene activity is dependent on the interaction between Hsp90 and SH2-SH2-SH3 domain of PLC-γ1, the isolated pACT-Hsp90 (human Heat shock protein 90α, a.a. 207-737) was co-transformed either with pAS2-PLC-γ1SH223, pAS2-PLC-β4-N (rat phospholipase C-β4 (a.a 1-130), pAS2-PLC-δ1-N (rat phospholipase C-δ1 (a.a 1-110), or pAS2-RhoA (full open reading frame of RhoA) in HF7c cells, and checked LacZ+ activity (colony color) and His+ activity (Histidine prototrophy). + and - denote positive and negative reporter gene activities, respectively.

twenty-eight positive clones, two of them were Hsp90α. These two clones contained identical cDNA insert (a.a 208 737) of Hsp90α. They showed specific interaction with the SH2-SH2-SH3 domain of PLC-γ1 in the yeast two-hybrid system (Table. 1).

Hsp90 associates with PLC-γ1 in NIH-3T3 cell To demonstrate *in vivo* interaction between Hsp90 and PLC-γ1, a co-immunoprecipitation experiment was performed. Since PLC-γ1 was reported phosphorylated and activated in NIH-3T3 by PDGF treatment (Rhee and Bae, 1997; Kamat and Carpenter, 1997), the effect of PDGF on this interaction was examined. Serum-starved NIH-3T3 cells were stimulated with PDGF for various time periods and lysed. These cell lysates were immunoprecipitated with either monoclonal anti PLC-γ1 antibody, or control monoclonal antibody (monoclonal anti-PLC-β4 antibody). As shown in Fig. 1A, Hsp90 was specifically co-precipitated with PLC-γ1. Although PDGF treatment induced tyrosyl phosphorylation of PLC-γ1 (Fig. 1B), the interaction between Hsp90 and PLC-γ1 was unchanged (Fig. 1A). Therefore, these data demonstrate the physical interaction between Hsp90 and PLC-γ1 *in vivo*.

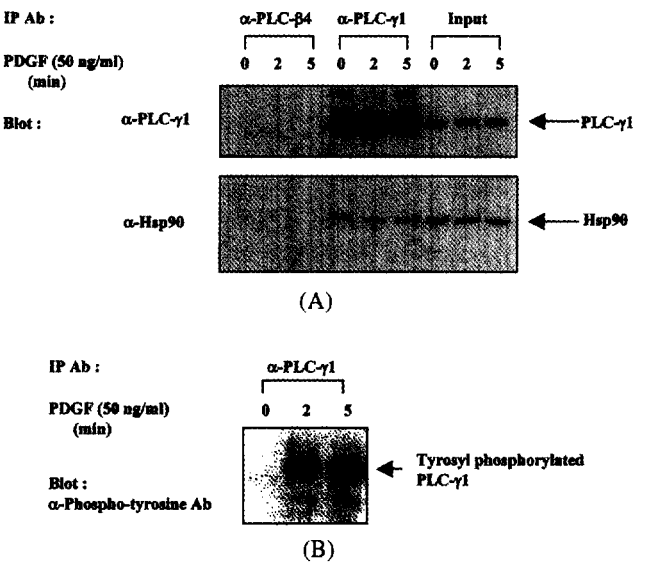

Fig. 1. Interaction between PLC-γ1 and Hsp90 in NIH-3T3 cell. A: Confluent NIH-3T3 cells grown in 100mm dish were serum-starved for 15 hrs and stimulated with 50 ng/ml PDGF for the indicated time. After cell lysis, PLC-γ1 was immunoprecipitated with either anti-PLC-γ1 antibody, or control monoclonal anti-PLC-β4 antibody. Resulting immune-complexes were subjected to SDS-PAGE (8%) and immunoblotting with anti-PLC-γ1, Hsp90 antibody. Input lanes were 2% of total cell lysate used in immunoprecipitatation. B: PDGF-induced tyrosine phosphorylation of PLC-γ1 in NIH3T3 cell. The same blot in Figure 1A was detached with a detaching solution (62.5 mM Tris-CI pH 6.8 100 mM β-mercaptoethanol, 2% SDS) and re-probed with anti-phospho-tyrosine antibody.

Hsp90 forms complex with PLC-γ1 in vitro To further confirm the apparent direct association of Hsp90 with PLC-

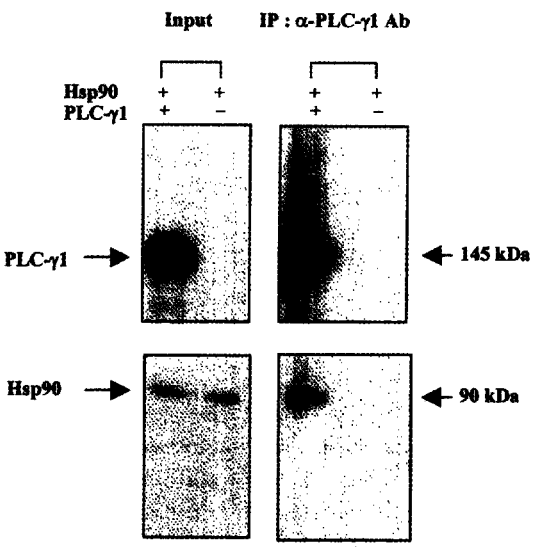

Fig. 2. Interaction between purified PLC-γ1 and Hsp90 *in vitro*. Purified Hsp90 was incubated with, or without, pure PLC-γ1 in TBST buffer (20mM Tris-CI, pH 7.5, 150mM NaCI, 1% Triton X-100, 1 mM EDTA, protease inhibitors) for 10 min at 37°C. After immunoprecipitation with anti-PLC-γ1 antibody, co-precipitated Hsp90 was detected by immunoblotting using anti-Hsp90 antibody.

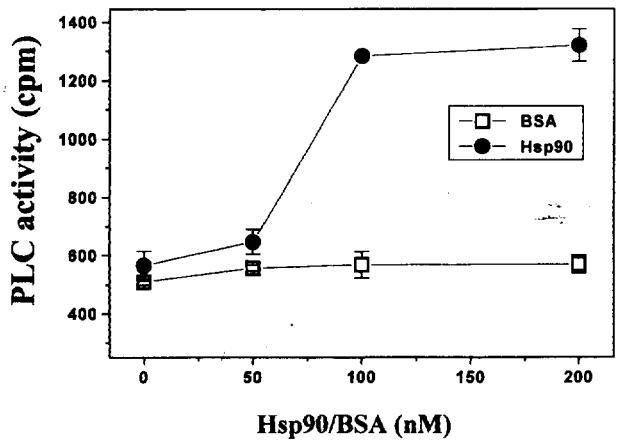

Fig. 3. Effect of Hsp90 on PLC-γ1 activity. By adding the indicated amount of Hsp90 to pure PLC-γ1, enzymatic activity of PLC-γ1 was examined (see Experimental Procedures). Sonicated vesicle of 75 μM [^{3}H]PIP_2 and 750 nM PE was used as substrate. Data are the average +/- standard deviation of the three experiments.

γ1, *in vitro* binding experiments were performed by using pure Hsp90 and PLC-γ1 (Fig. 2). Purified Hsp90 and PLC-γ1 were mixed, and then the mixture was precipitated with monoclonal anti-PLC-γ1 antibody. The immunoblotting showed that Hsp90 was co-precipitated with PLC-γ1 (Fig. 2). When PLC-γ1 was not included in the mixture, Hsp90 was not precipitated with anti-PLC-γ1 antibody. Thus, these data demonstrate that precipitation of Hsp90 by PLC-γ1 antibody is mediated by the direct binding of Hsp90 with PLC-γ1.

The effect of Hsp90 on PLC-γ1 activity To check the effect of Hsp90 on PLC-γ1, we investigated the effect on enzymatic activity of PLC-γ1 by Hsp90. As shown in Fig. 3, Hsp90 increased the PLC-γ1 activity up to 2-fold at 100 nM. Whereas, BSA, used as a control, showed no effect on the enzymatic activity of PLC-γ1. This data suggests that Hsp90 can play a role as an activator of PLC-γ1.

Discussion

We demonstrated the direct association of PLC-γ1 with Hsp90, and the potential for PLC-γ1 activity by Hsp90. Hsp90 is an essential protein that is required for cell viability (Borkovich *et al.*, 1989) and thought to function as a molecular chaperone (Hendrick and Hartl, 1993). Biochemical analysis demonstrated that Hsp90 forms heteromeric complexes with a 50-kDa protein (p50) and other proteins, such as steroid hormone receptors (Joab *et al.*, 1984), dioxin receptor (Perdew, 1988), actin (Miyata and Yahara, 1991), Src tyrosine kinase (Brugge, 1986), eIF-2a kinase (Rose *et al.*, 1987), casein kinaseII (Miyata and Yahara, 1992) and c-raf (Stancato *et al.*, 1993). The meanings of these associations were not clearly elucidated. Previous studies, however, suggest a broad spectrum of the functional roles for the binding of Hsp90 to proteins. Hsp90 is complexed with a glucocorticoid receptor, and it was found that the binding to Hsp90 is required for receptor trafficking to the nucleus and hormone stimulated gene expression (Joab *et al.*, 1984). In the case of c-raf, the binding with Hsp90 is required for protein stability and translocation to membrane. In addition, Hsp90 binding causes stimulation of casein kinase II (Miyata and Yahara, 1992) and eIF-α kinase (Rose *et al.*, 1987). Together, these data suggested that several signaling proteins could be regulated by interaction with Hsp90.

Our study demonstrates that PLC-γ1 is a novel protein associated with Hsp90 and that the activity of PLC-γ1 can be up regulated by direct association with Hsp90. PLC-γ1was reported to exist mainly in cytosolic fractions of mammalian tissues and cultured cells and to translocate from cytosol to membrane in response to various extra cellular signaling molecules (Yang *et al.*, 1994). The existence of modulators of PLC-γ1 activation and translocation was suggested (Kim *et al.*, 1990; Disatnik *et al.*, 1994). It is possible that Hsp90 is cytosolic regulator of PLC-γ1 and involved in PLC-γ1 translocation from cytosol to membrane.

Hsp90 was reported to be up regulated by several cytokines and mitogen (Colotta *et al.*, 1990), and super-induced in human B cell transformation by Epstein-Barr virus in susceptible (immuno-deficient) hosts. Transformed cells rapidly develop into fatal lymphoma (Cheung and Dosch, 1993). The blockade of Hsp-induction prevented transformation. PLC-γ1 and was overexpressed in human colorectal cancer (Noh *et al.*, 1994), human breast carcinomas (Arteaga *et al.*, 1991), human skins in hyper-proliferate conditions (Nanney *et al.*, 992). The microinjection of PLC-γ1 into NIH-3T3 cells induced DNA-synthesis, which was blocked by co-injection of antibodies to PLC-γ1 (Smith *et al.*, 1989; Smith *et al.*, 1990; Smith *et al.*, 1994; Huang *et al.*, 1995). Altogether, the relation between cell growth control and the expression of Hsp90 and PLC-γ1 can be suggested.

A number of stresses, other than hypothermia, induce the rapid over-expression of Hsp90. These include exposure to heavy metals, thiol-reactive chemicals, oxy-radicals, vitamins, and certain pathogenic viruses (Polla *et al.*, 1987). Recently, it was reported that the EGF receptor is clustered and activated in response to stress, such as osmotic-shock, radiation (Rosette and Karin, 1996), which is a similar mode found in a mitogen signal involving PLC-γ1. Therefore, it is possible that PLC-γ1 is involved in the stress signal pathway. Indeed, the involvement of PLC-γ1 in JNK/SAPK-activation was reported recently (Assefa *et al.*, 1999).

Previously, heat-shock was known to activate PLC activity in cells (Calderwood and Stevenson, 1993; Yang *et al.*, 1995), and PLC-γ1 isozyme studied in this report also was known to be activated by heat shock (Liu and Carpenter, 1992). These reports suggest that there may be a functional relation between heat shock proteins and PLC-γ1. Although there is no direct evidence that Hsp90 can activate PLC-γ1 in cells in response to heat shock, our findings that Hsp90 directly associates with

PLC-γ1, and that Hsp90 activates PLC-γ1 *in vitro,* raise the possibility that Hsp90 may be responsible for the PLC-γ1 activation in response to heat shock.

In conclusion, we demonstrated that Hsp90 is a novel binding protein of PLC-γ1, and that Hsp90 can activate PLC-γ1 activity. Therefore, we suggest the possibility that Hsp90 is involved in the regulation of PLC-γ1.

Acknowledgments This work was supported in part by the Korea Science and Engineering Foundation (KOSEF) through the Center for Cell Signaling Research at Ewha Womans University, a KOSEF grant (98-0403-08-01-5), and by the Brain Science & Engineering Research Program (98-J04-02-01-A-03) of the Ministry of Science and Technology in Korea. Also, the authors wish to acknoweldge the financial support of the Korea Reserch Foundation made in the program year of (1996).

References

Arteaga, C. L., Johnson, M. D., Todderud, G., Coffey, R. J., Carpenter, G., and Page,. L. (1991) Elevated content of the tyrosine kinase substrate phospholipase C-gamma 1 in primary human breast carcinomas. *Proc. Natl. Acad. Sci. USA.* **88**, 10435-10439.

Assefa, Z., Valius, M., Vantus, T., Agostinis, P., Merlevede, W. and Vandenheede, J.R. (1999) JNK/SAPK activation by platelet-derived growth factor in A431 cells requires both the phospholipase C-gamma and the phosphatidylinositol 3-kinase signaling pathways of the receptor. *Biochem. Biophys. Res. Commun.* **261**, 641-645.

Borkovich, K. A., Farrelly, F. W., Finkelstein, D. B., Taulien, J. and Lindquist, S. (1989) hsp82 is an essential protein that is required in higher concentrations for growth of cells at higher temperatures. *Mol. Cell. Biol.* **9**, 3919-3930.

Brugge, J. S. (1986) Interaction of the Rous sarcoma virus protein pp60src with the cellular proteins pp50 and pp90. *Curr. Top. Microbiol. Immunol.* **123**, 1-22.

Calderwood, S. K. and Stevenson, M. A. (1993) Inducer of the heat shock response stimulate phospholipsae C and phospholipase A2 activity in mammalian cells. *J. Cell. Physiol.* **155**, 248-256.

Cheung, R. K. and Dosch, H. M. (1993) The growth transformation of human B cells involves superinduction of hsp70 and hsp90. *Virology.* **193**, 700-708.

Colotta, F., Polentarutti, N., Staffico, M., Fincato, G. and Mantovani, A. (1990) Heat shock induces the transcriptional activation of c-fos protooncogene. *Biochem. Biophys. Res. Commun.* **168**, 1013-1019.

Disatnik, M. H., Hernandez-Sotomayor, S. M., Jones, G., Carpenter, G. and Mochly-Rosen, D. (1994) Phospholipase C-gamma 1 binding to intracellular receptors for activated protein kinase C. *Proc. Natl. Acad. Sci. U S A.* **91**, 559-563.

Durfee, T., Becherer, K., Chen, P. L., Yeh, S. H., Yang, Y., Kilburn, A. E., Lee, W. H. and Elledge, S. J. (1993) The retinoblastoma protein associates with the protein phosphatase type 1 catalytic subunit. *Genes Dev.* **7**, 555-569.

Fabiato, A. (1988) Computer programs for calculating total from specified free or free from specified total ionic concentrations in aqueous solutions containing multiple metals and ligands. *Methods. Enzymol.* **157**, 378-417.

Harper, J. W., Adami, G. R., Wei, N., Keyomarsi, K. and Elledge, S. J. (1993) The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin- dependent kinases. *Cell* **75**, 805-816.

Hendrick, J. P. and Hartl, F. U. (1993) Molecular chaperone functions of heat-shock proteins. *Annu. Rev. Biochem.* **62**, 349-384.

Hepler, J. R., Kozasa, T., Smrcka, A. V., Simon, M. I., Rhee, S. G., Sternweis, P. C. and Gilman, A. G. (1993) Purification from Sf9 cells and characterization of recombinant Gq alpha and G11 alpha. Activation of purified phospholipase C isozymes by G alpha subunits. *J. Biol. Chem.* **268**, 14367-14375.

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-gamma 1. *FEBS. Lett.* **358**, 287-292.

Itoh, H., Toyoshima, I., Mizunuma, H., Kobayashi, R. and Tashima, Y. (1990) Three-step purification method and characterization of the bovine brain 90-kDa heat shock protein. *Arch. Biochem. Biophys.* **282**, 290-296.

Joab, I., Radanyi, C., Renoir, M., Buchou, T., Catelli, M. G., Binart, N., Mester, J. and Baulieu, E. E. (1984) Common non-hormone binding component in non-transformed chick oviduct receptors of four steroid hormones. *Nature* **308**, 850-853.

Joo, H. S. and Kim, S. S. (1998) Purification and characterization of the catalytic a-acetolactate synthase from Serratia marcescens. *J. Bolchem. Mol. Biol.* **31**, 37-43.

Kamat, A., and Carpenter, G. (1997) Phospholipase C-gamma1: regulation of enzyme function and role in growth factor-dependent signal transduction. *Cytokine. Growth. Factor. Rev.* **8**, 109-117.

Kim, M. J., Hwang, J. I., Chang, J. S., Ryu, S. H. and Suh, P. G. (1999) The SH3 domain of Phospholipase C-γ1 associates with Shc. *J. Biochem. Mol. Biol.* **32**, 119-126.

Kim, U. H., Kim, H. S. and Rhee, S. G. (1990) Epidermal growth factor and platelet-derived growth factor promote translocation of phospholipase C-gamma from cytosol to membrane. *FEBS. Lett.* **270**, 33-36.

Koch, C. A., Anderson, D., Moran, M. F., Ellis, C. and Pawson, T. (1991) SH2 and SH3 domains: elements that control interactions of cytoplasmic signaling proteins. *Science* **252**, 668-674.

Liu, S. M. and Carpenter, G. (1992) Activity of the epidermal-growth-factor receptor and phospholipase C-gamma1 in heat-stressed fibroblasts and A-431 cells. *Biochem. J.* **286**, 541-547.

Miyata, Y. and Yahara, I. (1992) The 90-kDa heat shock protein, HSP90, binds and protects casein kinase II from self-aggregation and enhances its kinase activity. *J. Biol. Chem.* **267**, 7042-7047.

Miyata, Y. and Yahara, I. (1991) Cytoplasmic 8 S glucocorticoid receptor binds to actin filaments through the 90-kDa heat shock protein moiety. *J. Biol. Chem.* **266**, 8779-8783.

Nanney, L. B., Gates, R. E., Todderud, G., King, L. E., Jr., and Carpenter, G. (1992) Altered distribution of phospholipase C-

gamma1 in benign hyperproliferative epidermal diseases. *Cell. Growth. Diff.* **3**, 233-239.

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-gamma 1 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**, 99-114.

Pawson, T. (1995) Protein modules and signaling networks. *Nature* **373**, 573-580.

Perdew, G. H. (1988) Association of the Ah receptor with the 90-kDa heat shock protein. *J. Biol. Chem.* **263**, 13802-13805.

Polla, B. S., Healy, A. M., Wojno, W. C. and Krane, S. M. (1987) Hormone 1 alpha, 25-dihydroxyvitamin D3 modulates heat shock response in monocytes. *Am. J. Physiol.* **252**, C640-649.

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

Rose, D. W., Wettenhall, R. E., Kudlicki, W., Kramer, G. and Hardesty, B. (1987) The 90-kilodalton peptide of the heme-regulated eIF-2 alpha kinase has sequence similarity with the 90-kilodalton heat shock protein. *Biochemistry.* **26**, 6583-6587.

Rosette, C. and Karin, M. (1996) Ultraviolet light and osmotic stress: activation of the JNK cascade through multiple growth factor and cytokine receptors. *Science* **274**, 1194-1197.

Shin, S. Y., Park, J. H., Jang, S. Y., Lee, M. K., and Hahm, K. S. (1998) Characterization and epitope mapping of KI-41, a murine monoclonal antibody specific for the gp41 envelope protein of the human immunodeficiency virus-1. *J. Biol. Mol. Biol.* **31**, 58-63.

Smith, M. R., Lin, 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., Matthews, N. T., Rhee, S. G., Sung, W. K., and Kung, H. F. (1994) Phospholipase C-gamma 1 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.

Stancato, L. F., Chow, Y. H., Hutchison, K. A., Perdew, G. H., Jove, R. and Pratt, W. B. (1993) Raf exists in a native heterocomplex with hsp90 and p50 that can be reconstituted in a cell-free system. *J. Biol. Chem.* **268**, 21711-21716.

Suh. P. G., Ryu, S. H., Choi, K. Y., and Rhee, S. G. (1988) Monoclonal antibodies to three phospholipase C isozymes from bovine brain. *J. Biol. Chem.* **263**. 14497-14504.

Yang, J. M., Chin, K. V. and Hait, W. N. (1995) Involvement of phospholipase C in heat-shock-induced phosphorylation of P-glycoprotein in multidrug resistant human breast cancer cells. *Biochem. Biophys. Res. Commun.* **210**, 21-30.

Yang, L. J., Rhee, S. G. and Williamson, J. R. (1994) Epidermal growth factor-induced activation and translocation of phospholipase C-gamma 1 to the cytoskeleton in rat hepatocytes. *J. Biol. Chem.* **269**, 7156-7162.