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Phospholipase C-γ1 Is Required for Survival in Heat Stress: Involvement of Protein Kinase C-Dependent Bcl-2 Phosphorylation[1]

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The consequences of heat-induced phospholipase C-γ1 (PLC-γ1) phosphorylation are not known. We investigated the role of PLC-γ1 activation and its downstream targets during the cellular response to heat stress using mouse embryonic fibroblasts genetically deficient in PLC-γ1 (*Plcg1* null MEF) and its wild type (wt MEF) as models. Treatment of wt MEF with heat resulted in temperature- and heating duration-dependent tyrosine phosphorylation of PLC-γ1. HSP70 synthesis and the activation of extracellular signal-regulated kinases 1/2 (ERK1/2) and c-Jun N-terminal protein kinase (JNK) increased equally following heat treatment in both cell lines. However, heat-induced protein kinase C (PKC) activation was dramatically reduced in *Plcg1* null MEF compared with wt MEF. Importantly, the mitochondrial localization of PKCα, PKC-dependent phosphorylation of Bcl-2, and cell viability in *Plcg1* null MEF following heat treatment, were significantly decreased compared with the wild type. Furthermore, pretreatment with bryostatin-1, a PKC activator, enhanced Bcl-2 phosphorylation and cellular resistance to heat-induced apoptosis in *Plcg1* null MEF. Taken together, these results suggest that PLC-γ1 activation enhances cell survival through the PKC-dependent phosphorylation of Bcl-2 during the cellular response to heat stress.

Key words: Bcl-2, heat stress, phospholipase C-γ1, phosphorylation, protein kinase C, survival.

Exposure of cells to elevated temperatures leads to the expression of heat shock responses, in which the induction of a cohort of heat shock proteins (HSPs) is accompanied by the expression of heat resistance and cell survival (*1, 2*). If the stress is too severe, a signal that leads to programmed cell death (apoptosis) is activated, thereby providing a finely tuned balance between survival and death (*3*).

The signaling pathways utilized by hyperthermia to modify cell behavior are not known. However, protein kinases such as phosphatidylinositol 3-kinase (PI 3-K) (*4*) and mytogen-activated protein kinase (MAPK), including extracellular signal-regulated kinases (ERKs) (*5*), c-Jun N-terminal protein kinase (JNK) (*6*), and p38 kinase (*7*), are reported to be activated and involved in the cellular response to heat. On the other hand, protein kinase C (PKC) has been shown to play a role in the modulation of heat-induced HSPs and the acquisition of thermotolerance (*8–10*). Also, PKC may serve as an activator of Bcl-2 by phosphorylating it at Ser^{70}, and eventually play an anti-apoptotic role under some stress applications (*11–13*).

Phospholipase C-γ1 (PLC-γ1) is activated by the phosphorylation of tyrosine residues upon occupation of cell surface receptors by growth factors, and plays an important role in controlling cellular proliferation and differentiation (*14, 15*). The activated PLC-γ1 catalyzes the hydrolysis of phosphatidyl 4,5-bisphosphate to generate second messengers, diacylglycerol and IP_3. The former functions to activate PKC; the latter stimulates the release of Ca^{2+} from internal stores.

Several laboratories have demonstrated that PLC-γ1 undergoes tyrosine phosphorylation in response to heat (*16, 17*) or oxidant exposure (*18*). Recently it was shown that oxidative stress-induced PLC-γ1 activation enhances cell survival (*19*). However, the physiologic consequences of PLC-γ1 activation in heat stress responses remain unclear. Furthermore, the downstream targets of PLC-γ1 activation in the stress response are not known. We hypothesized that PLC-γ1 might also be important for transducing survival signals resulting from heat exposure. In this study, we confirmed this possibility and further implicated the PKC-dependent phosphorylaton of Bcl-2 acting as a downstream effector of PLC-γ1 activation in heat stress using normal mouse embryo fibroblasts (wt MEF) and MEF rendered

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Abbreviations: Cyt, cytosol; DAG, diacylglycerol; DMEM, Dulbecco's modified Eagle's medium; ECL, enhanced chemiluminescence; ERK1/2, extracellular signal-regulated kinases 1/2; HSP, heat shock protein; IP_3, inositol-1,4,5-phosphate; JNK, c-Jun N-terminal protein kinase; MAPK, mytogen-activated protein kinase; PI 3-kinase, phosphatidylinositol 3-kinase; PKC, protein kinase C; *plcg1* null MEF and wt MEF, mouse embryonic fibroblasts genetically deficient in PLC-γ1 and its wild type; PLC-γ1, phospholipase C-γ1; PVDF, polyvinylidene difluoride.

deficient for PLC-γ1 by targeted disruption of both *plc-γ1* alleles (*Plcg1* null).

MATERIALS AND METHODS

Cell Culture and Treatment—Immortalized normal mouse embryo fibroblasts (wt MEF) and PLC-γ1–deficient MEF (*Plcg1* null) produced by gene targeting techniques were obtained as described previously (*20, 21*). Both cells lines were cultured in DMEM medium (GIBCO/BRL) supplemented with 10% (v/v) fetal bovine serum (GIBCO/BRL) and a 1% (v/v) antibiotics mixture in a humidified 95% air, 5% CO_2 atmosphere. Cell heating was done by incubating cells in a temperature-regulated circulating water bath.

Cell Survival and DNA Fragmentation Assay—Cells were heated at the indicated temperature for various durations as described in the figure legends and then cultured for 24 h at 37°C in complete media. Cells were harvested and stained with trypan blue, and the number of viable cells was counted in triplicate using a hemocytometer. For DNA fragmentation analysis, stimulated cells were lysed in 0.5 ml of lysis buffer (10 mM Tris-HCl, pH 7.5, 10 mM EDTA, 200 mM NaCl, 0.4% Triton X-100, and 0.1 mg/ml proteinase K) for 20 min at room temperature, followed by a 30-min incubation with 0.1 mg/ml RNase A at 50°C. DNA fragmentation was analyzed using a 1.5% agarose gel in the presence of 0.5 μg/ml ethidium bromide.

Immunoprecipitation and Immunoblot Analysis—Cells were washed in ice-cold phosphate-buffered saline, then harvested in 1 ml of lysis buffer (20 mM Hepes, pH 7.4, 2 mM EGTA, 50 mM β-glycerophosphate, 1 mM Na_3VO_4, 5 mM NaF, 1% Triton X-100, 10% glycerol, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 5 μg/ml leupeptin, 5 μg/ml aprotinin). Equal amounts of protein were incubated with 4 μg of monoclonal anti–PLC-γ1 antibody (Santa Cruz) and 30 μl of a 50% slurry of protein A–Sepharose (Sigma) overnight at 4°C. Immune complexes were washed four times with the same lysis buffer and resuspended in 2× sample buffer. For immunoblot analysis, whole-cell lysate or immunoprecipitates were analyzed on SDS–polyacrylamide gels and transferred electrophoretically to polyvinylidene difluoride (PVDF) membranes. The membranes were blocked with 10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.1% Tween 20, containing 5% milk, and then probed with different antibodies (Santa Cruz). Proteins were detected using enhanced ECL reagents (Santa Cruz).

PKC Kinase Assay—PKC assay were performed using the PKC assay system manufactured by Promega. PKC activity was assayed by measuring the incorporation of ^{32}P from [γ-^{32}P]ATP (New England Nuclear) into biotinylated peptide substrate (Neurogranin, AAKIQAS*FRGHMARKK) according to manufacturer's recommendations.

P44/42 MAP Kinase (ERK1/2) Assay—ERK1/2 was performed using nonradioactive kits manufactured by Cell Signaling Technology according to the manufacturer's recommendations. Briefly, activated ERK was precipitated from cell lysates using anti–phospho-ERK1/2 (Thr^{202}/Tyr^{204}) antibody, and precipitates were incubated with a specific substrate, Elk-1, and ATP. The reaction was terminated by adding boiling gel loading buffer. ERK activity was detected by immunoblotting the products of the kinase reaction with anti-phospho-Elk (Ser^{383}) antibody.

Phosphorylation of Bcl-2—Cells were labeled with [^{32}P]-orthophosphoric acid (New England Nuclear) and Bcl-2 proteins were immunoprecipitated using Bcl-2 antibody (Santa Cruz). Immunoprecipitates were analyzed on SDS-polyacrylamide gels and transferred to a PVDF membrane, then exposed to Koda X-Omat film for 16 h at –80°C. The same blot was then used for immunoblotting analysis with the anti–Bcl-2 antibody.

Cell Fractionation and PKC Immunolocalization Studies—Stimulated cells were swelled in ice-cold hypotonic Hepes buffer (10 mM Hepes at pH 7.4, 5 mM $MgCl_2$, 40 mM KCl, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 10 μg/ml leupeptin) for 30 min, aspirated repeatedly through a 25-gauge needle (25 strokes), and centrifuged at 200 ×*g* to separate the nuclei. The resulting supernatant was centrifuged at 10,000 ×*g* to pellet the heavy membrane (HM) fraction containing mitochondria. The resulting HM supernatant was centrifuged at 150,000 ×*g* to pellet the plasma membranes (light membranes), and the supernatant represented the cytosol. The nuclear membranes were isolated by centrifuging the nuclei through a 2 M sucrose cushion at 150,000 ×*g*. Subcellular fractions were subjected to denaturing electrophoresis in a 12% acrylamide, 0.1% SDS gel and transferred to a PVDF membrane for PKCα-specific Western blotting as described above.

RESULTS

Heat Shock Stimulates Tyrosine Phosphorylation of PLC-γ1—To examine the effect of heat treatment on PLC-γ1 phosphorylation, wt MEF were subjected to heat shock at different temperatures (37–51°C) for 20 min or for different time periods (5–60 min) at 45°C. Then the tyrosine phosphorylation of PLC-γ1 was examined by precipitating PLC-γ1 with a PLC-γ1 antibody, followed by Western blotting analysis with a phosphotyrosine-specific antibody. The

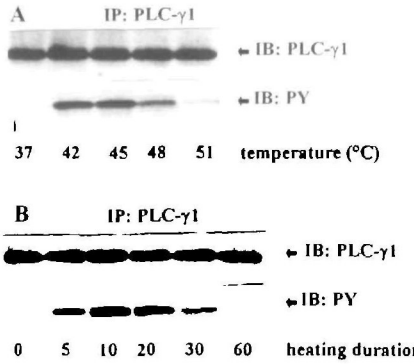

Fig. 1. **Temperature- and heating duration-dependent changes in PLC-γ1 tyrosine phosphorylation by heat treatment in wt MEF.** A: Temperature response of PLC-γ1 phosphorylation. PLC-γ1 was immunoprecipitated from cell lysates prepared from wt MEF treated at various temperatures (37–51°C) for 20 min using an anti–PLC-γ1 antibody. One half of the immunoprecipitate was immunoblotted with the same antibody and the other half with an anti-phosphotyrosine antibody. B: Heating duration course of PLC-γ1 phosphorylation. Cell lysates were prepared after treatment with at 45°C for the indicated periods of time (5–60 min). Phosphorylation of PLC-γ1 was determined as described above. Data are from three independent experiments. Typical blots are presented for A and B. IP, immunoprecipitation; IB, immunoblot; PY, anti-phosphotyrosine antibody.

results demonstrated that heat treatment results in PLC-γ1 tyrosine phosphorylation in a temperature- and heating duration-dependent manner (Fig. 1, A and B). Maximal levels of PLC-γ1 phosphorylation were recorded at 42–45°C after 10–20 min. Severe or long term heat stress (45°C, 60 min or 51°C, 20 min), however, failed to induce PLC-γ1 phosphorylation in MEF (Fig. 1, A and B).

PLC-γ1 Phosphorylation Enhances Cellular Resistance to Heat Stress–Induced Apoptosis—To examine the relationship between PLC-γ1 activation and cell survival in the heat stress response, wt MEF and *Plcg1* null MEF were subjected to heat shock at 45°C for 20 min, and then the tyrosine posphorylation of PLC-γ1 was examined. Cell viability and DNA fragmentation were determined after culture for another 24 h at 37°C in complete media as described in "MATERIAL AND METHODS." The results showed that about 20% of wt MEF in which PLC-γ1 phosphorylation was stimulated died after heat shock, whereas more than 70% of *Plcg1* null MEF, or wt MEF pretreated with 5 μM U73122 (Calbiochem), a phospholipase C–specific inhibitor, in which no PLC-γ1 phosphorylation was detected died under similar conditions (Fig. 2, A and B). Furthermore, DNA ladder formation was detected in MEF after heat treatment, as shown in Fig. 2C. A deficiency of PLC-γ1 phosphorylation in *Plcg1* null MEF or wt MEF pretreated with U73122 produced a significantly enhanced apoptotic response based on DNA fragmentation. These results suggest that PLC-γ1 phosphorylation plays a role in the protection of fibroblasts from heat stress-induced apoptosis.

Heat-Induced PKC, but Not JNK or ERK1/2, Activation Is PLC-γ1–Dependent—It has been demonstrated that heat shock stimulates JNK (*7*), ERK1/2 (*6*), and PKC (*11*) in many cell types. To determine whether PKC acts downstream of PLC-γ1 in heat stress and rule out the possibility that the differential sensitivity of wt MEF and *Plcg1* null MEF to heat might be attributable to effects of PLC-γ1 on the ERK1/2 and JNK signaling pathway, *Plcg1* null MEF and wt MEF were incubated at 45°C for different times (5–60 min), then PKC, ERK1/2 activity and JNK phosphorylation were determined as described in "MATERIALS AND METHODS." Exposure of wt MEF to heat shock increased PKC, ERK1/2 activity, and JNK phosphorylation, and the increases were heating duration–dependent (Fig. 3). The increase in PKC activity was observed at 5 min and reached a maximum at 20 min. But heating cells at 45°C for 40 min caused a considerable decrease in PKC activity. In contrast, the heat-induced activation of JNK, ERK1/2 reached a maximum at 45°C for 10 min and began to decrease after 20 min. In *Plcg1* null MEF, however, the activation of PKC was dramatically reduced ($p < 0.01$) at all durations, but there was no effect on ERK1/2 and JNK activation compared that of wt MEF (Fig. 3). These data show that PKC, but not JNK or ERK1/2, is activated by heat treatment in a PLC-γ1–dependent manner.

PLC-γ1 Is Not Involved in Heat-Induced HSP70 Synthesis—PKC has been shown to regulate the heat induction of HSP70 (*8, 9*). To determine the role of PLC-γ1 in the heat-

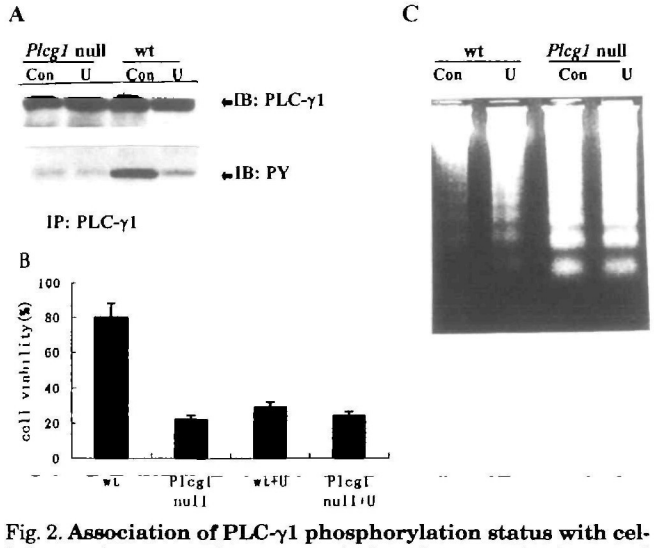

Fig. 2. **Association of PLC-γ1 phosphorylation status with cellular resistance to heat stress-induced apoptosis.** A: wt and *Plcg1* null MEF were pretreated with or without 5 μM U73122, a phospholipase C–specific inhibitor, and then subjected to heat shock at 45°C for 20 min, Tyrosine phosphorylation of PLC-γ1 was detected as described previously. B: wt and *Plcg1* null MEF were treated as in A. After heat shock, the cells were cultured for another 24 h at 37°C in complete media and cell viability was determined by trypan blue exclusion assay. C: Cells were treated as in B and the cell lysates were subjected to DNA fragmentation analysis as described under "MATERIALS AND METHODS." IP, immunoprecipitation; IB, immunoblot; PY, anti-phosphotyrosine antibody; Con, control; U, U73122.

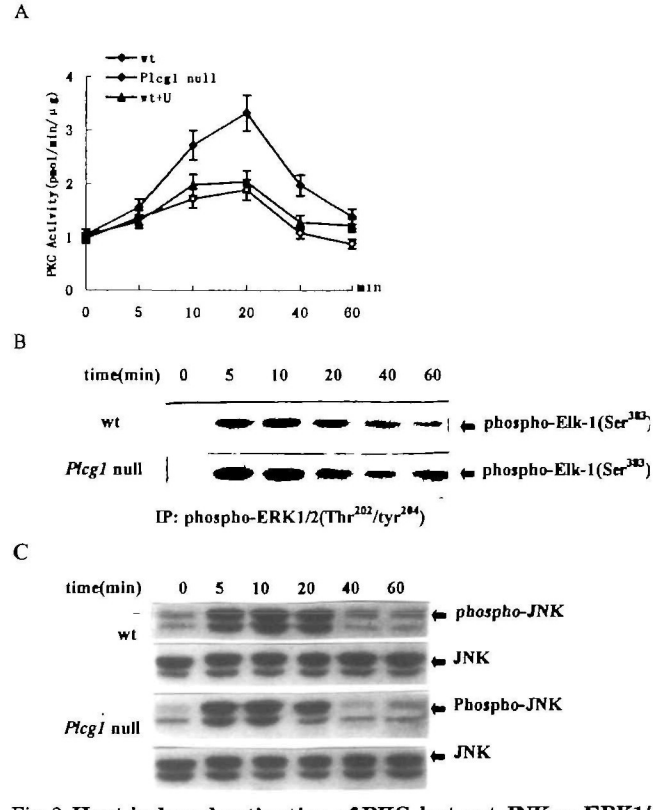

Fig. 3. **Heat-induced activation of PKC, but not JNK or ERK1/2, is PLC-γ1–dependent.** *Plcg1* null MEF and wt MEF were incubated at 45°C for different times (5–60 min). Then cell extracts were subjected to PKC (A), ERK1/2 (B) kinase assay according to the manufacturer's recommendations as described in "MATERIAL AND METHODS." Also, JNK phosphorylation was detected by Western blotting using anti-JNK and phospho-specific JNK antibodies (C).

induced synthesis of HSP70, *Plcg1* null MEF and wt MEF cells were treated at 45°C for 20 min, and then cultured for another 6 h at 37°C in complete media. The level of HSP70 expression was detected by Western blotting with anti-HSP70 antibody. The results showed that heat shock induces significant HSP70 synthesis in both wt MEF and *Plcg1* null MEF cells (Fig. 4). Pretreatment with 5 μM U73122 had no effect on heat-induced HSP70 synthesis. However, pretreatment with 1 μM Staurosporine (Calbiochem), a PKC inhibitor, abolished heat induced HSP70 synthesis in both cell lines (Fig. 4). This suggests that PLC-γ1 is not required for heat-induced HSP70 synthesis.

PLC-γ1 Plays a Role in Heat-Induced Bcl-2 Phosphorylation—Bcl-2 plays an important role in cell survival in some stress responses, and phosphorylation of Bcl-2 at Ser^{70} may be required for its full and potent anti-apoptotic function (*11–13*). To determine whether heat induces Bcl-2 phosphorylation and whether PLC-γ1 is involved in this process, *Plcg1* null MEF and wt MEF cells were labeled with [^{32}P]-orthophosphoric acid and then treated at 45°C for the indicated times. The cell lysates were then examined to determine Bcl-2 phosphorylation. Phosphorylation of Bcl-2 began to increase after heat treatment at 45°C for 10 min in wt MEF, and maximal levels of Bcl-2 phosphorylation were recorded at 20–30 min. Long term heat stress (more than 60 min), however, failed to induce Bcl-2 phosphorylation (Fig. 5). In *Plcg1* null MEF, this heat-induced phosphorylation was dramatically decreased compared with that of wt MEF cells (Fig. 5). These results suggest that PLC-γ1 plays a role in the phosphorylation of Bcl-2 in heat shock response.

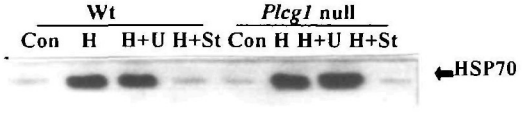

Fig. 4. **Effect of PLC-γ1 on heat-induced HSP70 synthesis.** wt and *Plcg1* null MEF pretreated with or without 5 μM U73122 or 1 μM Staurosporine, a PKC inhibitor, were treated at 45°C for 20 min, and then cultured for another 6 h at 37°C in complete media. The levels of HSP70 expression were detected by Western blotting with anti-HSP70 antibody. Con, control; H, heat; U, U73122; St, Staurosporine.

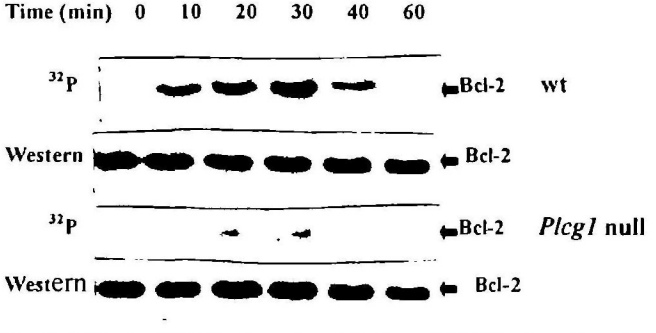

Fig. 5. **Effect of PLC-γ1 on heat-induced Bcl-2 phosphorylation.** wt and *Plcg1* null MEF were labeled with [^{32}P]orthophosphoric acid, then incubated at 45°C for the indicated times. Bcl-2 protein was analyzed by autoradiography and immunoblotting as described in "MATERIALS AND METH-ODS."

Effects of PLC-γ1 on Bcl-2 Phosphorylation and Cell Survival in Heat Stress Are PKC-Dependent—Several reports support a role for PKC as a functional Bcl-2 kinase that can suppress cell apoptosis (*11, 13*). To determine whether the role of PLC-γ1 on Bcl-2 phosphorylation and cell survival in heat stress is due to PKC activation, wt MEF and *Plcg1* null MEF pretreated with or without 1 μM staurosporine or 150 nM Bryostatin-1 (Calbiochem), a PKC activator, were subjected to heat shock at 45°C for 20 min. The PKC activity, phosphorylation of Bcl-2, and cell viability were then measured as described previously. Consistent with previous results, the activity of PKC, levels of Bcl-2 phosphorylation, and cell viability after heat treatment in *Plcg1* null MEF were significantly lower than in wt MEF cells. Pretreatment with 150 nM Bryostatin-1 produced a dramatic increase in PKC activation, Bcl-2 phosphorylation,

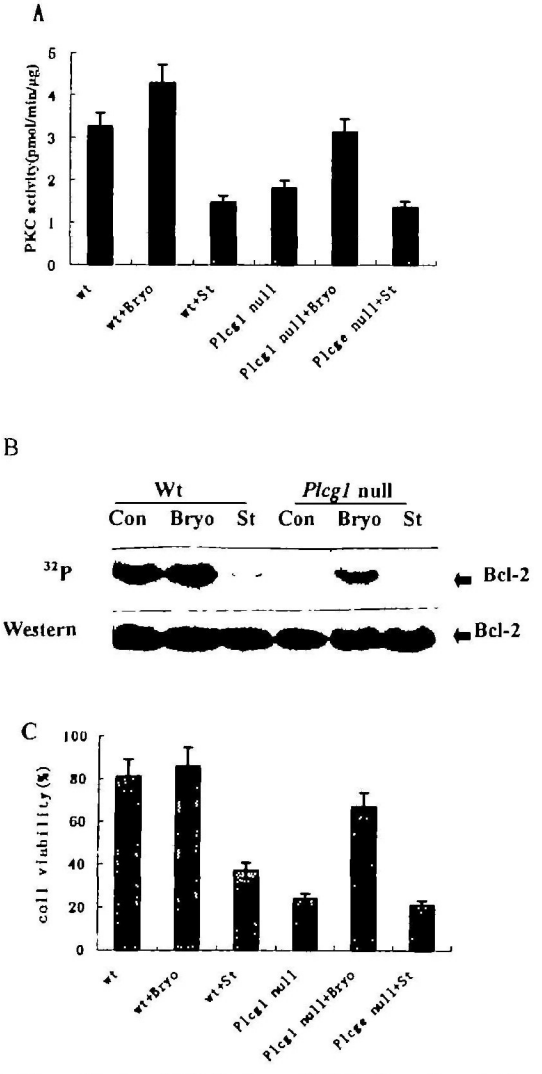

Fig. 6. **The effects of PLC-γ1 on Bcl-2 phosphorylation and cell survival in heat stress are PKC-dependent.** wt and *Plcg1* null MEF pretreated with or without 1 μM Staurosporine or 150 nM Bryostatin-1, a PKC activator were subjected to heat shock at 45°C for 20 min, PKC activity (A), Bcl-2 phosphorylation (B) and cell viability (C) were determined as previously described. Byro, Bryostatin-1; St, Staurosporine.

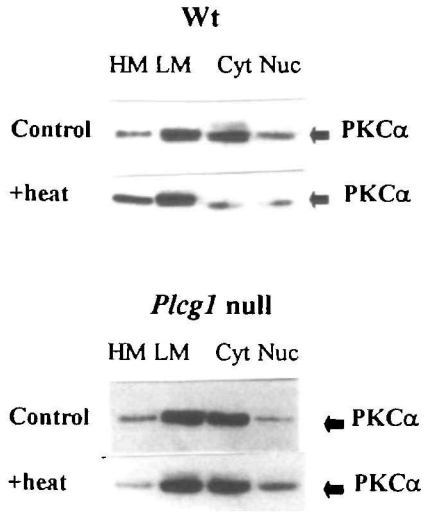

Fig. 7. **Effect of PLC-γ1 on heat-induced mitochondrial localization of PKCα.** *Plcg1* null and wt MEF were subjected to heat shock at 45°C for 20 min and then subcellular fractionation studies were performed as described in "MATERIALS AND METHODS" on the isolated HM, light membrane (LM), cytosol (Cyt), and nuclear membrane (Nuc) fractions. Western blot analyses using 40 μg of protein from each subcellular fraction were performed.

and cell viability in *Plcg1* null MEF (Fig. 6, A, B, and C). Furthermore, pretreatment with 1 μM Staurosporine abolished heat-induced PKC activation and significantly decreased Bcl-2 phosphorylation and cell viability in wt MEF (Fig. 6, A, B, and C). These results suggest that PKC-dependent Bcl-2 phosphorylation is required for PLC-γ1–mediated survival signals in the heat stress response.

PLC-γ1 Is Required for the Heat-Induced Mitochondrial Localization of PKCα—Bcl-2 is localized in the endoplasmic reticulum and nuclear membranes. It has been demonstrated that the mitochondrial localization of PKCα is a response to Bcl-2 phosphorylation under the stress of chemotherapy, and that PKCα can phosphorylate Bcl-2 directly *in vitro* (*13*). We further investigated whether heat treatment also induces PKCα localization and the role of PLC-γ1 in this process. wt MEF and *Plcg1* null MEF were subjected to heat shock at 45°C for 20 min, and then subcellular fractionation studies were carried out to determine whether PKCα and Bcl-2 colocalized and, if so, whether a potential defect might occur in *Plcg1* null MEF cells. The results indicate that PKCα is expressed in the HM fraction containing the mitochondria in wt MEF after heat treatment (Fig. 7). In contrast, little PKCα is localized to the HM fraction in *Plcg1* null MEF, suggesting a possible mechanism to account, at least in part, for the decreased Bcl-2 phosphorylation observed above (Fig. 7). This suggests that PLC-γ1 may be involved in the mitochondrial localization of PKCα in heat stress.

DISCUSSION

In this study, we have demonstrated that PLC-γ1 is tyrosine-phosphorylated in a temperature- and heating duration–dependent manner in response to heat treatment, and that this activation enhances cellular resistance to heat stress–induced apoptosis using *Plcg1* null and wt MEF as a model system. We further provide evidence that the PKC-dependent phosphorylation of Bcl-2, but not ERK1/2, JNK or HSP70, acts as a downstream target of PLC-γ1 signaling in this process. Taken together, the current study suggests that PLC-γ1 plays an important regulatory role in the cellular response to heat stress through the PKC-dependent phosphorylation of Bcl-2.

PLC-γ1 is known to play an important role in the regulation of cell proliferation through its interations with both receptor and non-receptor tyrosine kinases (*14, 15*). However, despite the existence of significant overlap between the proliferative and stress signaling pathways, the role for PLC-γ1 in mediating cellular responses to stress has not been appreciated (*19*). Recently, a survival role of PLC-γ1 in oxidative stress was identified by an approach that utilized MEF derived from embryos in which *Plcg1* expression was eliminated by targeted gene disruption (*16*). Few reports suggest a role of PLC-γ1 in heat stress response, and only demonstrate that PLC-γ1 decreases after whole body hyperthermia (*22*), undergoes tyrosine phosphorylation (*16*), and is involved in the phosphorylation of P-glycoprotein following heat treatment (*17*). The consequences of PLC-γ1 phosphorylation and its role in cell survival following heat treatment remain unclear. Our analysis of *Plcg1* null MEF, which has no capacity for PLC-γ1 functions, supports the view that this signaling molecule is required for survival following heat stress.

We further attempted to detect the downstream targets of PLC-γ1 signaling in heat stress. Heat stress has been reported to activate some major signaling processes, such as p44/42 MAPK (ERK1/2) (*5*), JNK (*6*), PKC (*8–10*), which influence cell survival through the modulation of HSPs synthesis or other pathways during heat shock response. Several reports have demonstrated that ERK1/2 (*23*), JNK (*24*), and PKC (*11–13*) also serve as activators of Bcl-2 in some stresses, and eventually play an anti-apoptotic role. PLC regulates PKC activity by controlling the levels of intracellular diacylglycerol and Ca^{2+} in some cellular responses (*25*). Our results show that all these kinases are activated following heat stress, but only the heat-induced PKC activation is PLC-γ1–dependent. Thus, PKC, but not the ERK1/2 and JNK signaling pathways, appears to be involved in the influence of PLC-γ1 on cell survival.

Inducible HSP70 has been suggested to have multiple roles in cytoprotection against apoptosis (*26*). Our results suggest that heat-induced HSP70 synthesis is PKC-dependent and PLC-γ1–independent. The result raises the question of why the increase in PKC activity in wt MEF does not cause more HSP70 synthesis than in *Plcg1* null MEF following heat treatment. One possibility is that the PLC-γ1–dependent PKC isoenzymes during heat stress response are not the ones regulating HSP70 synthesis. It remains to be determined which PKC isoenzyme(s) is involved in the regulation of HSP70 synthesis in heat shock response.

Recent studies have shown that the phosphorylation of Bcl-2 is required for the antiapoptotic function of Bcl-2, and that this phosphorylation may be mediated by both PKC-dependent and independent (*i.e.* ERK1/2 or JNK) signaling pathways (*11–13, 23, 24*). Our results show that Bcl-2 is phosphorylated in a PLC-γ1– and PKC-dependent manner during heat stress, and this phosphorylation is required for the antiapoptotic function. Pretreatment with Bryostatin-1, a PKC activator, resulted in a dramatic increase in PKC

activation, Bcl-2 phosphorylation, and cell viability in *Plcg1* null MEF. Furthermore, we find that the mitochondrial localization of PKCα, a process response to Bcl-2 phosphorylation in the stress of chemotherapy (*13*), is also PLC-γ1– and PKC-dependent. While these findings strongly support a role for PKC-dependent Bcl-2 phosphorylation as a downstream target of PLC-γ1 activation that can enhance cell resistance to heat stress, they do not exclude the possibility that another Bcl-2 kinase (*i.e.* ERK1/2 or JNK) may also play a role in this process.

In conclusion, this study demonstrates that PLC-γ1 activation enhances the survival of MEF in response to heat shock through the PKC-dependent phosphorylation of Bcl-2. Further characterization of the mechanisms leading to the activation of PLC-γ1 and the localization of PKCα will provide important information for clarifying the potential anti-apoptotic role of heat shock-induced PLC-γ1 activation.

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