PKCε Is a Unique Regulator for hsp90β Gene in Heat Shock Response*

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An early event in cellular heat shock response is the transmittance of stress signals from the cell surface into the nuclei, resulting in the induction of heat shock proteins (Hsps). Protein kinase C (PKC) has been implicated as a key player in transducing stress signals. However, mechanism(s) by which PKC regulates heat shock-induced events remains largely unknown. Here we present data that pan-PKC inhibitor G0109203X, but not classic PKC inhibitor Gö6976, specifically repressed heat shock-induced accumulation of mRNA as well as promoter activity of hsp90β, but not hsp90α, in Jurkat cells. Subcellular fractionation studies revealed that heat shock exclusively induced PKC-ε membrane translocation. Consistently, expression of a constitutively active PKC-ε(A155E) resulted in an enhanced promoter activity of hsp90β upon heat shock, whereas a dominant-negative PKC-ε(K437R) abolished this effect. In contrast, constitutively active PKC-α or dominant-negative PKC-α had no effects on heat shock induction of the gene. The effect of PKC-ε on hsp90β expression seems to be stimuli-specific, as phorbol myristate acetate-mediated hsp90β expression was PKC-ε-independent. We conclude that PKC-ε is specifically required in the signaling pathway leading to the induction of hsp90β gene in response to heat shock.

In a mammalian cell stress response, the most remarkable early events are the transmittance of stress signals from cell surface into the nucleus, resulting in the induction of a set of proteins, dominantly the heat shock protein (HSP) family. Hsps, with or without the partnerships of chaperones, may interact with various signaling molecules to form active components in signaling pathways that regulate cell growth, development, and protect the cells from extreme stress through the induction of stress-tolerance (1–3). Over- or underexpression of the molecular chaperone Hsps may result in aberrant growth control, developmental malformations, diseases, or even cell death (4–6).

As one of the most abundant cytoplasmic heat shock proteins and a member of the molecular chaperone family, Hsp90 is highly conserved during evolution and is ubiquitously expressed for cell survival in animals and plants (1). Hsp90 is capable of interacting with a wide variety of proteins both native and denatured to affect their turnover, cellular localization, and activity (7, 8). The specificity of Hsp90 may reside in its cellular “client” selection for players in transcription regulation and signal transduction pathways, such as the steroid hormone receptors, basic helix-loop-helix transcription factors, oncogenic tyrosine kinase, or cellular serine/threonine kinases, and has been defined as a specific molecular chaperone (9, 10). There are two cytoplasmic versions of the Hsp90 subfamily in mammalian cells encoded by two distinct genes, the hsp90α and hsp90β (11, 12). Although Hsp90α and Hsp90β proteins share very high homology, we have demonstrated that the regulation mechanisms of the two genes are quite different (13, 14): hsp90α is more sensitive to heat shock induction, whereas the hsp90β gene is the major cellular counterpart responding to mitogenic stimulation (15). We reported elsewhere (16) that Hsp90 is not only induced in stress response but also uniquely expressed in mitogen-activated T lymphoid cells, indicating its potential importance in cell growth. Additionally, recent studies demonstrated that in heat-shocked or other stressed cells, Hsp90 takes part in protecting cell survival and counteracts apoptosis (17–19).

PKC is a family of serine/threonine kinases that participates as a central component in signal transduction pathways responding to multiple external stimuli (2, 20). It plays a pivotal role in the regulation of many biological functions, including proliferation, differentiation and apoptosis (21). Although PKC isoforms share conserved domains, they exhibit different tissue distribution, subcellular localization, substrate specificity, and cofactor requirements (22, 23). The PKC isoforms consist of three subgroups based on their structure and cofactor requirements, the conventional isoforms (cPKC-α, -βI, -βII, and -γ) which require both Ca2+ and diacylglycerol/phorbol ester for activation; the novel isoforms (nPKC-δ, -ε, -η, and -θ) which are Ca2+-independent but diacylglycerol/phorbol ester-dependent; and the atypical isoforms (aPKC-ζ, -μ, and -τλ) which are Ca2+- and diacylglycerol/phorbol ester-independent (2, 24). These differences suggest that individual PKC isoforms may play a distinct role in regulation of cellular functions. In some cases, PKC isoforms may counteract each other in regulating cell growth, transformation, or apoptosis (21, 24). For example, PKC-ε functions as an oncogene upon overexpression, whereas PKC-δ usually promotes differentiation or apoptosis (25).

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The abbreviations used are: HSP, heat shock protein; CA, constitutively active; PKC, protein kinase C; DN, dominant negative; PMA, phorbol 12-myristate 13-acetate; RT-PCR, reverse transcriptase-PCR; iRNA, internal control RNA; Cyto, cytosolic fraction; WT, wild type; HSF, heat shock factor.

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Our early findings showed that PKC was involved in hsp90 gene expression in heat-shocked Jurkat cells (15). However, it is unclear which isoforms of the PKC family are specifically involved in the heat shock-induced responses, such as the induction of hsp90 expression. We demonstrate here that PKC-δ/H9280 plays a critical role in the induction of the hsp90α gene but not the hsp90β gene in response to heat shock in Jurkat cells. The results suggest a link between the Hsp family and the PKC family in heat shock response. It is possible that cooperation between these two families of proteins may play an important role in cell growth control in mammalian cells.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—Human Jurkat T lymphoma cells were maintained in RPMI 1640 medium (Invitrogen) supplemented with 10% fetal calf serum, 0.03% l-glutamine, 0.2% NaHCO3, 0.5% HEPES (pH 7.2), and sodium penicillin and streptomycin sulfate (100 units/ml each) in a 5% CO2 humidified atmosphere at 37°C.

For PKC inhibitor studies, Jurkat cells were pretreated with PKC inhibitor GF109203X or Go6976 at the indicated concentrations at 37°C for 2 h, followed by wash of the cells with phosphate-buffered saline. Cells in each condition were then separated into two groups and incubated at either 42°C or 37°C for 1 h.

**Reagents**—PKC inhibitors (GF109203X and Go6976) and phorbol 12-myristate 13-acetate (PMA) were purchased from Sigma. Anti-actin antibody and rabbit polyclonal antibodies that recognize PKC-δ/H9280, -ε/H9257, -θ/H9258, and -α/H9251 were obtained from Santa Cruz Biotechnology. Mouse IgG2a against PKC-δ/H9280 was obtained from BD Transduction Laboratories. Antibody against HSP90α and anti-FLAG antibody were obtained from Stressgen and Sigma, respectively.

**DNA Constructs**—A full-length cDNA clone for human PKC-δ was obtained from Dr. A. P. Fields (University of Texas Medical Branch, Galveston, TX). The human PKC-δ cDNA was subcloned into pBluescript II-SK(+) (Stratagene, La Jolla, CA), and a FLAG-epitope tag was inserted to the NH2 terminus of the PKC-δ and -α were obtained from Santa Cruz Biotechnology. Mouse IgG2a against PKC-δ was obtained from BD Transduction Laboratories. Antibody against HSP90α and anti-FLAG antibody were obtained from Stressgen and Sigma, respectively.

Our early findings showed that PKC was involved in hsp90 gene expression in heat-shocked Jurkat cells (15). However, it is unclear which isoforms of the PKC family are specifically involved in the heat shock-induced responses, such as the induction of hsp90 expression. We demonstrate here that PKC-δ plays a critical role in the induction of the hsp90β gene but not the hsp90α gene in response to heat shock in Jurkat cells. The results suggest a link between the Hsp family and the PKC family in heat shock response. It is possible that cooperation between these two families of proteins may play an important role in cell growth control in mammalian cells.

**A**

**Fig. 1. Effects of PKC inhibitor GF109203X on the expression of hsp90 genes.**

A, effects of GF109203X on the mRNA expression of hsp90 genes. Jurkat cells were pretreated with GF109203X (GF) at the indicated concentrations, followed by heat shock (HS) at 42°C for 60 min. Controls (C) were left at 37°C for 60 min. Hsp90 mRNA expression was detected by a competitive RT-PCR-based technique described under “Experimental Procedures.” White bars and black bars show mRNA levels of hsp90α genes at 37°C and 42°C, respectively. Data presented are the mean ± S.D. of normalized mRNA expression of hsp90α (left panel) and hsp90β (right panel), relative to that of cRNA. A representative electrophoretic profile of RT-PCR-amplified products is shown at the bottom of the figure. B, effects of GF109203X on the promoter activity of hsp90 genes. Jurkat cells were transfected with either hsp90α-CAT (−1039/+1531) or hsp90α-CAT (−1756/+37) along with a transfection control plasmid pM-CAT and treated as described above. The promoter activity of hsp90 genes was detected by a competitive RT-PCR. Data represented are the mean ± S.D. of normalized promoter activities of hsp90α (left panel) and hsp90β (right panel) relative to that of pM-CAT at 37°C (C, white bars) and 42°C (HS, black bars), respectively. A representative electrophoretic profile of RT-PCR-amplified products is shown at the bottom of the figure.

For PKC inhibitor studies, Jurkat cells were pretreated with PKC inhibitor GF109203X or Go6976 at the indicated concentrations at 37°C for 2 h, followed by a wash of the cells with phosphate-buffered saline. Cells in each condition were then separated into two groups and incubated at either 42°C or 37°C for 1 h.
wild-type PKC-α, PKC-ε (A25E, CA-mutant), and PKC-α (R368A, DN-mutant) were generous gifts from Dr. Jean-Guy LeHoux of the University of Sherbrooke in Quebec (27).

For quantifying the promoter activity of human hsp90 genes, the −1756/+37 fragment of the hsp90α gene and the −1039/+1531 fragment of the hsp90β gene were independently fused to the upstream of a CAT reporter gene in pBLCAT3 to form reporter plasmids of p90α-CAT and p90β-CAT, respectively (13, 14). A transfection control plasmid was constructed in which the +698/+1003-bp fragment of the CAT gene was deleted to express a mutant CAT and was designated as pm-CAT (28).

DNA Transfection—Transient transfection of Jurkat cells was carried out by electroporation (as described in Ref. 29). For promoter activity assays, cells (1.5 × 10⁶) were transfected with a mixture of 27 μg of reporter plasmid hsp90α,CAT or hsp90β,CAT and 3 μg of control plasmid pm-CAT. For assessing PKC isoform-specific effects, constructs expressing wild-type or mutants of PKC-α or PKC-ε were individually co-transfected with reporter plasmid hsp90β,CAT along with a control plasmid of pm-CAT into Jurkat cells. At 48 h after transfection, cells were separated into two groups and incubated at either 42 °C or 37 °C for 1 h. Total cellular RNA was extracted according to the method described by Chomezynski and Sacchi (30) and used for detecting mRNA expression and promoter activity of hsp90 genes.

Detection of mRNA Expression and Promoter Activity of hsp90 Genes—The mRNA levels of hsp90 genes were determined based on a competitive RT-PCR assay as described by Wang et al. (31). Briefly, an internal control RNA (ircRNA) was first transcribed in vitro from pHSL3 plasmid, which contains the same 5′ and 3′ fragments that existed in the cDNAIs of the hsp90α and hsp90β genes. An equal amount of ircRNA was then mixed with each aliquot of cellular RNA, reverse-transcribed, and amplified in the competitive RT-PCR system. The sizes of amplified fragments for hsp90α mRNA and ircRNA were 564 and 841 bp, respectively, and the amplified fragments for hsp90β mRNA and ircRNA were 337 and 625 bp, respectively.

For promoter activity assays, control plasmid pm-CAT was co-transfected into Jurkat cells with either hsp90α,CAT or hsp90β,CAT in an appropriate ratio. Total cellular RNA was then isolated for competitive RT-PCR using paired 5′ and 3′ primers corresponding to nucleotides +554/+573 and +1122/+1141 of pBLCAT3, respectively. A 588-bp fragment for wild-type CAT driven by the hsp90 promoter and a smaller fragment of 286 bp generated from control plasmid pm-CAT were co-amplified and separated by agarose gel electrophoresis.

Fluorescence intensity of each band stained with ethidium bromide was analyzed with an UltraScan XL laser densitometer (Amersham Biosciences). The ratio of the fluorescence intensity of two bands in each RT-PCR (hsp90 mRNA to ircRNA or hsp90-CAT to pm-CAT) was defined as the relative mRNA expression or the relative promoter activity of the hsp90 gene, respectively.

Subcellular Fractionation—All of the experimental procedures in subcellular fractionation were carried out at 4 °C with all of the solutions pre-chilled. The fractionation was performed as described by Ding et al. (32) with minor modifications. Briefly, Jurkat cells (2 × 10⁶) harvested were washed twice with phosphate-buffered saline and suspended in 1.5 ml of hypotonic Heps buffer (HBB: 10 mM Heps, pH 7.4, 5 mM MgCl₂, 40 mM KCl, 1 mM phenylmethylisulfonyl fluoride, 10 μg/ml aprotinin, 10 μg/ml leupeptin). After incubation on ice for 15 min, cell suspensions were homogenized by using a Dounce homogenizer for 30–50 strokes and then spun at 200 × g for 10 min. The supernatants were further centrifuged at 10,000 × g for 15 min at 4 °C. The resulting pellets were washed with HBB, suspended in 200 μl of HBB, and sonicated for 5 s, which was designated as HM for heavy membrane fraction. The resulting supernatants were further centrifuged at 150,000 × g for 90 min at 4 °C (Beckman SW-60 rotor) and were designated as the cytosolic fraction (Cyto).

Western Blot Analysis—Western blotting was performed as described previously (33) with minor modifications. Aliquots of subcellular fractions were separated by SDS-PAGE and electroblotted to nitrocellulose filters in a Trans-Blot Cell (Bio-Rad). Membranes were blocked for 1 h in a blocking solution for PKC-δ, ε, α, η, and HSP90β: 5% nonfat milk, 0.05% Tween 20 in phosphate-buffered saline, for PKC-ε, 5% nonfat milk, 0.1% Tween 20, 10 mM Tris-Cl, pH 7.5, and 100 mM NaCl and then incubated 1 h at 37 °C or overnight at 4 °C with specific antibodies in blocking solution. Protein bands were visualized with the ECL detection system (Amersham Pharmacia). The intensity of each protein band was scanned and analyzed on an UltraScan XL laser densitometer (Amersham Biosciences).

RESULTS

Novel PKCs Are Involved in Heat Shock-induced hsp90β Gene Expression—Our previous findings indicated that PKC was involved in hsp90α gene expression in heat-shocked Jurkat cells (15). To identify the specific PKC isoform(s) involved, we assessed the effects of two isoform-selective PKC inhibitors, GF109203X and Go6976, on heat shock-induced expression of hsp90α genes (34, 35). Jurkat cells were pretreated with indicated PKC inhibitors and subsequently heat shocked at 42 °C for 1 h. Controls were incubated at 37 °C for 1 h. The mRNA levels of hsp90α genes were determined by competitive RT-PCR. As shown in Fig. 1A, GF109203X, which inhibits the activity of both classical (εPKC-α and -β) and novel (nPKC-δ and -ε) PKC isoforms, specifically repressed the heat shock-induced accumulation of hsp90β mRNA in a dose-dependent manner, which was initially observed at the concentration of 0.02 μM. In contrast, GF109203X showed no effect on the induction of hsp90α mRNA by heat shock. GF109203X elicited similar effects on the promoter activity of hsp90β genes; the inhibitor repressed only the heat shock-induced promoter activity of the hsp90β gene but not the hsp90α gene (Fig. 1B). At the concentration of 2.0 μM, GF109203X abolished the heat shock-induced elevation of the hsp90β promoter activity as well as mRNA induction. The results indicated that PKC plays a specific role in mediating...
the induction of the hsp90β gene expression in heat-shocked Jurkat cells.

To further define the identity of PKC isoform(s) involved, a cPKC-specific inhibitor Gö6976 was used. As shown in Fig. 2, at various concentrations, Gö6976 exerted little inhibition on the heat shock-induced mRNA accumulation (Fig. 2, left panel) and promoter activity (Fig. 2, right panel) of the hsp90β gene. Together, the data exclude the participation of cPKC in the regulation of heat shock-induced hsp90β gene expression and suggest a role for nPKCs in this event.

**Heat Shock-induced Membrane Translocation of nPKC-ε in Jurkat Cells**—PKCs have been shown to undergo activation-dependent intracellular redistribution. In particular, a membrane translocation upon stimulation has been linked to the PKC activation. We investigated whether any nPKC isoform can be induced to translocate from the cytosol to the membrane in Jurkat cells by heat shock at 42 °C for 1 h. Cells were either maintained at 37 °C (mock controls) or heat shocked at 42 °C (experimental groups). As a positive control, cells were also stimulated with a PKC activator, PMA. Cells harvested from different treatments were subjected to subcellular fractionation followed by Western blot analysis for detecting cytosolic and membrane levels of nPKCs. Cytosol and membrane levels of each nPKC were determined by densitometric analysis and normalized to the protein loading (A, bottom panel). Results are expressed as a ratio of the level of each nPKC in the cytosol (white bars) or the membrane (black bars) to the protein loading. Similar results were obtained from at least two independent experiments.

**Fig. 3. Effects of heat shock and PMA on the cytosolic and membrane levels of novel PKC isoforms.** Jurkat cells were treated at either 37 °C or 42 °C for 1 h or stimulated with PMA (100 nm, 30 min). Subcellular fractionation was performed as described under “Experimental Procedures.” A, Western blot analysis. Equal amounts (30 μg) of proteins from each subcellular fraction were separated by SDS-PAGE, followed by Western blot analysis using PKC isoform-specific antibodies as indicated. B, quantification of cytosolic and membrane levels of nPKCs. Cytosol and membrane levels of each nPKC were determined by densitometric analysis and normalized to the protein loading (A, bottom panel). Results are expressed as a ratio of the level of each nPKC in the cytosol (white bars) or the membrane (black bars) to the protein loading. Similar results were obtained from at least two independent experiments.
PKC-ε Mediates Heat Shock-induced Expression of the hsp90β Gene—Because PKC-ε showed a distinct change in its intracellular location during heat shock, we further examined whether PKC-ε is a major player in regulating hsp90β expression and whether PKC-ε could act independently. Jurkat cells were transfected with wild type (WT), constitutively activated PKC-ε (A159) (CA-PKCε), or dominant-negative PKC-ε (K437R) (DN-PKCε); effects of PKC-ε expression on hsp90β expression were assayed by mRNA level and promoter activity in response to heat shock. Western blot analysis (Fig. 4A, bottom panel) demonstrated that transfected FLAG-tagged WT PKC-ε and its mutants were efficiently expressed in Jurkat cells. As shown in Fig. 4A, none of PKC-ε constructs notably affected the promoter activity of the hsp90β gene in the absence of heat shock (white bars). Upon heat shock, expression of CA-PKCε remarkably induced the hsp90β promoter activity (a 4-fold induction), whereas expression of DN-PKCε blocked heat shock-induced induction of the hsp90β promoter activity (Fig. 4A, lane 8 versus lanes 2, 4, and 6). Of note, expression of WT-PKCε showed no significant effect on the basal and heat shock-induced promoter activity (Fig. 4A, WT versus PCI).

To confirm the specificity of PKC-ε, we investigated the effect of PKC-α on hsp90β expression by heat shock (Fig. 4B). Consistent with PKC inhibitor studies (Fig. 2), which exclude the involvement of cPKCs in the induction of hsp90β expression upon heat shock, we found that expression of PKC-α (wild type, CA-PKCα, or DN-PKCα) had no effect on the promoter activity of the hsp90β gene in response to heat shock. Together, these data clearly suggest an exclusive role for PKC-ε in regulating the expression of the hsp90β gene by heat shock.

PKC-ε Is Not Involved in the Regulation of hsp90β Expression by PMA—Several lines of evidence indicate that PMA is capable of inducing the expression of hsp90 genes in a cell type-specific manner, which may also depend on the dosage and length of treatment adopted (38, 39). In particular, we showed previously that PMA efficiently induced hsp90β mRNA but was less effective in the induction of hsp90α mRNA in Jurkat cells (39). Because both PMA and heat shock can lead to PKC-ε translocation to the membrane in Jurkat cells (Fig. 3), we investigated whether PKC-ε also plays a role in the PMA-induced hsp90β expression. Jurkat cells were transfected with DN-PKCε (Fig. 5A, black bars) or an empty vector (Fig. 5A, white bars) and then treated with heat shock (42 °C, 1 h), PMA (100 nM, 30 min), or a combination of heat shock and PMA. The promoter activity of the hsp90β gene was assessed by competitive RT-PCR. As shown in Fig. 5A, when transfected cells were heat shocked in the absence of PMA (Fig. 5A, left panel), the heat shock-induced promoter activity of the hsp90β gene was specifically blocked by the expression of DN-PKCε (Fig. 5A, bar 4 versus bar 3). When Jurkat cells were treated with PMA alone, the promoter activity of the hsp90β gene in vector-transfected cells (bar 5) was about 2-fold over that of the untreated control (bar 1), indicating that PMA induced hsp90β gene expression. Interestingly, this PMA-induced promoter activity was not affected by the expression of DN-PKCε (bar 6 versus bar 5). Additionally, we found that when cells were treated with heat shock in addition to the PMA stimulation, there was an additive effect on the induction of hsp90β gene expression (bar 7), and DN-PKCε partially blocked this induction (bar 8 versus bar 7). These data suggest that DN-PKCε abrogated the heat shock but not PMA-induced promoter activity of the hsp90β gene. In contrast to DN-PKCε, expression of DN-PKCε showed no obvious effects on heat shock as well as PMA-mediated induction of the hsp90β promoter activity (Fig 5B, bar 4 versus bar 3 and bar 8 versus bar 7). These results further confirm our notion that PKC-ε is specifically involved in the heat shock-induced expression of the hsp90β gene. It also suggests that heat shock-induced and PMA-induced expression of the hsp90β gene are regulated by distinct mechanisms in a PKC-ε-dependent and -independent manner.

**DISCUSSION**

PKC has been implicated as a potential mediator of inflammatory stimuli-induced gene expression (22). It was shown that
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The nuclear PKC activity was stimulated by heat shock prior to the induction of hsp70 mRNA in rat liver (40, 41). Furthermore, we have shown previously that PKC inhibitor GF109203X attenuated the heat shock-induced hsp90β expression in Jurkat cells (15). These data indicate a link between PKC stimulation and heat shock response and suggest that a role for PKC in heat shock response is to modulate the expression of hsp genes.

The present study determined the mechanism by which PKC regulates the heat shock response, in particular the induction of hsp90 expression. We showed that pan-PKC inhibitor GF109203X, which inhibits both cPKCs (α and β) and nPKCs (δ and ε), preferentially affected heat shock-induced mRNA expression (Fig. 1A) and the promoter activity (Fig. 1B) of the β version of the hsp90 gene but had no effect on that of the hsp90α gene. This finding suggests that the repression of hsp90β expression is mediated by either nPKCs or cPKCs, or alternatively, by the combination of the two PKC subfamilies. To disclose which one of the two PKC subfamily members exerts the enhancing function to hsp90β, we employed a second PKC inhibitor, Gö6976, that is specific to the cPKC subfamily and found that no changes occurred on heat shock-induced mRNA expression and promoter activity of hsp90β at concentrations up to 2 μM (Fig. 2). Thus we excluded the involvement of the cPKC subfamily and suggest that members of the nPKC subfamily are responsible for mediating the induction of hsp90β expression in response to heat shock.

PKC isoforms exhibit distinct enzymological properties, differential subcellular localization, and different modes of cellular regulation. Because translocation from the cytosol to the phospholipid-rich environment such as membrane is one of the essential steps that activate the enzyme (42), we investigated the subcellular distribution of nPKC in response to heat shock and PMA treatment. Subcellular fractionation experiments indicated that Jurkat cells expressed four novel PKCs, PKC-δ, ε, η, and θ. However, only PKC-ε demonstrated a heat shock or PMA-mediated translocation from the cytosol to the membrane, evidenced by a decreased level in the cytosol and an increased level in the membrane-associated fractions, indicating that PKC-ε may be activated by heat shock in the manner similar to PMA treatment. In contradiction to our results, Holmberg et al. (40) reported that PKC-ε was incapable of translocation from the cytosol to the cellular particulate fraction upon heat shock (42 °C for 15 min) in K562 cells. It is possible that heat shock at 42 °C for 15 min (versus 42 °C for 60 min in our study) may not be sufficient to activate the enzyme (membrane translocation). Alternatively, the heat shock-induced translocation of PKC-ε is cell-type specific.

Expression of wild-type, constitutive active, or dominant-negative PKC-ε in Jurkat cells did not lead to an obvious change in basal hsp90β expression in the absence of heat shock (Fig. 4A). Upon heat shock, however, the constitutively active PKC-ε exerted a marked induction (4-fold increase) of the hsp90β gene, whereas dominant-negative PKC-ε completely blocked this effect, suggesting that PKC-ε is required only for heat shock-mediated events. It should be noted that expression of WT-PKC-ε seems to have little effect on heat shock-mediated induction of hsp90β expression, which was only slightly higher than that in the vector-transfected cells. This suggests that the endogenous PKC-ε may be sufficient for the induction of hsp90β by heat shock. In the case of expression of CA-PKC-ε, it is possible that activated PKC-ε may interact directly with Hsp90, a molecular chaperone, in a functional complex or module leading to the phosphorylation of Hsp90, which in turn stabilizes and keeps PKC-ε in a conformation amenable to full activation upon heat shock. This possibility is supported, at least in part, by our findings that, in Jurkat cells, Hsp90β translocated simultaneously with PKC-ε to the membrane upon heat shock, and after the recovery from heat shock, the membrane fractions of both hsp90β and PKC-ε were reduced.2 The specificity of PKC-ε on heat shock-induced expression of the hsp90β gene is further supported by the fact that neither PKC-δ, PKC-η, or PKC-θ demonstrated a heat shock or PMA-mediated translocation from the cytosol to the membrane.

2 J. M. Wu and Y. F. Shen, unpublished data.

FIG. 5. Effects of DN-PKC-ε and DN-PKC-α on the promoter activity of the hsp90β gene induced by heat shock or PMA. Constructs expressing DN-PKC-ε (A) or DN-PKC-α (B) and corresponding vectors were individually co-transfected with hsp90β-CAT and pT-CAT into Jurkat cells following the procedure similar to that described in Fig. 4. White bars (1, 3, 5, and 7) represent the data from vector-transfected cells; black bars (2, 4, 6, and 8) indicate the data from DN-PKC-transfected cells. Bars of 1, 2, 5, and 6 show the promoter activities in the absence of heat shock, whereas those of 3, 4, 7, and 8 indicate heat shock-induced promoter activities. PMA treatment (100 nM for 30 min) is shown in the right panels (bars 5–8). Lane 8 bar, cells were first incubated with 100 nM of PMA for 30 min and then treated at 42 °C for 1 h. Data presented are the mean ± S.D. of normalized promoter activities of the hsp90β gene from at least three independent experiments. A representative electrophoretic profile of the competitive RT-PCR-amplified products is shown in bottom panels.
CA-PKC-α nor DN-PKC-α affected the promoter activity of the hsp90β gene in response to heat shock, suggesting that heat shock-induced expression of the hsp90β gene is regulated by a PKC-ε-dependent mechanism.

Despite the fact that Hsp90α and Hsp90β are highly homologous at the protein level, we have demonstrated earlier that distinct signaling mechanisms are involved in the precise regulation of each hsp90 gene in a heat shock response. For example, we found previously that the intronic heat shock element of hsp90 preferentially binds to the heat shock factor (HSF1) and plays a critical role on heat shock induction of the gene (14). Additionally, it was reported that heat shock-induced Hsp70 expression was controlled primarily at the transcriptional level through the activation of HSFs, whereas the PMA-induced HSP70 expression resulted from a stabilization of HSP70 through the activation of HSFs, whereas the PMA-induced HSP70 expression resulted from a stabilization of HSP70 mRNA through the post-transcriptional-dependent mechanism(s) (38). These data imply that mechanism(s) that control the expression of hsp genes may also be stimuli-specific. Consistent with this notion, we found that PKC-ε is required for heat shock- but not PMA-induced induction of hsp90β expression in Jurkat cells. Because PKC has been reported to mediate the phosphorylation of HSF1 and to regulate Hsp70 expression in human cells (41), it is therefore interesting to explore whether PKC-ε-mediated heat shock response requires the participation of HSF1 in our system.

In summary, we demonstrate that PKC-ε can be activated by heat shock and is required for the induction of hsp90β gene expression in heat-shocked Jurkat cells. It is conceivable that PKC-ε and Hsp90β may participate in a functional active module to protect cells from external damage, particularly in heat shock-mediated events.

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