Cross-Talk between Protein Kinase C-α (PKC-α) and -δ (PKC-δ): PKC-α Elevates the PKC-δ Protein Level, Altering Its mRNA Transcription and Degradation

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ABSTRACT: Studies utilizing the overexpression of individual isoforms indicated that both PKC-α and -δ promote a number of biological effects, including inhibition of DNA synthesis associated with rearrangements of the actin cytoskeleton in the murine B-cell lymphoma (Baf3), differentiation of the murine promyelocyte line 32D, and activation of MAP kinase in CHO fibroblasts. We postulated that these results reflect some form of cross-regulation between PKC-α and -δ rather than their functional redundancy. In this report, we show that overexpression of PKC-α in Baf3 and 32D leads to an elevation of the endogenous PKC-δ mRNA and protein levels. The elevated steady-state PKC-δ mRNA level results from a combination of increased PKC-δ transcription and mRNA stability. Upregulation of PKC-δ mRNA by PKC-α occurs even after a selective depletion of the PKC-δ protein. In addition, phorbol ester-induced elevation of PKC-δ mRNA and protein levels can be prevented by the PKC inhibitor GF109203X, an indication of the requirement for PKC kinase activity. Inhibition of new protein synthesis by cycloheximide showed that upregulation of PKC-δ mRNA, as opposed to delayed downregulation of the PKC-δ protein, is primarily responsible for the accumulation of this isoform by PKC-α. In parental Baf3 and 32D cells and PKC-α overexpressers, PKC-α and PKC-δ are uniquely involved in cross-regulation, while PKC-ϵ, PKC-η, and PKC-μ are not.

The PKC[1] family of serine/threonine kinases is involved in signaling through a number of important biochemical pathways that regulate cell cycle progression, apoptosis, differentiation, and the immune response, among others (*1*). Individual PKC isozymes have been shown to have different and sometimes opposite effects on these processes. It has been reported that PKC-α stimulates cell proliferation (*2*, *3*) and transcription of various genes that have TRE (*4*, *5*) or SRE (*6*) in their promoters. PKC-α acts as a cell cycle-promoting agent probably because of its ability to activate the MAP kinase pathway through a direct phosphorylation of c-Raf1 (*7*). In many model systems, PKC-δ has an opposite effect on cell proliferation. Activation of this isoform inhibits DNA synthesis (our unpublished data) and cell growth (*3*, *8*) and induces cell differentiation (*9*).

PKC isoforms are not highly selective for the substrates in vitro, and most substrates are phosphorylated by several, if not all PKC, isoforms (*10*, *11*). The selectivity of PKC isozymes in vivo is probably achieved by tissue-specific distribution, different requirements of cofactors, selective activation by different effectors, and differential subcellular compartmentalization (*12*−*15*). The similarities in structure and regulation of PKC family members make the assignment of specific biological roles to individual isoforms a challenging task. Overexpression of PKC isozymes is a commonly used method for the determination of the isozyme function. However, its efficacy is based on the assumption that overproduction of one member of the family, in either an active or a dominant-negative form, would not significantly affect the behavior of the others. Here we show that there is an important exception to this rule, which may have already led to misinterpretation of overexpression experiments.

In several cell systems, overexpression of PKC-α or -δ appears to exert the same biological effects. For example, overexpression of either PKC-α or -δ causes prolonged activation of MAPK and formation of polynuclear cells in hamster CHO fibroblasts (*16*), induction of myeloid differentiation of murine 32D cells (*9*), and inhibition of DNA synthesis associated with actin cytoskeleton rearrangements in CCRF CEM, a human T cell line, and Baf3, a murine pre-B cell line (our unpublished data). The detailed analysis reported here shows that there is a complex interisozyme relationship between these particular isoforms in which activated PKC-α protein upregulates the steady-state levels of PKC-δ mRNA, which causes an increase in the PKC-δ protein level.

METHODS

Cell Culture, PKC Overexpression, and Western Blot Analysis. The murine IL-3-dependent Baf3 B lymphoma and

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[1] Abbreviations: PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; dPP, 12-deoxyphorbol 13-phenylacetate.

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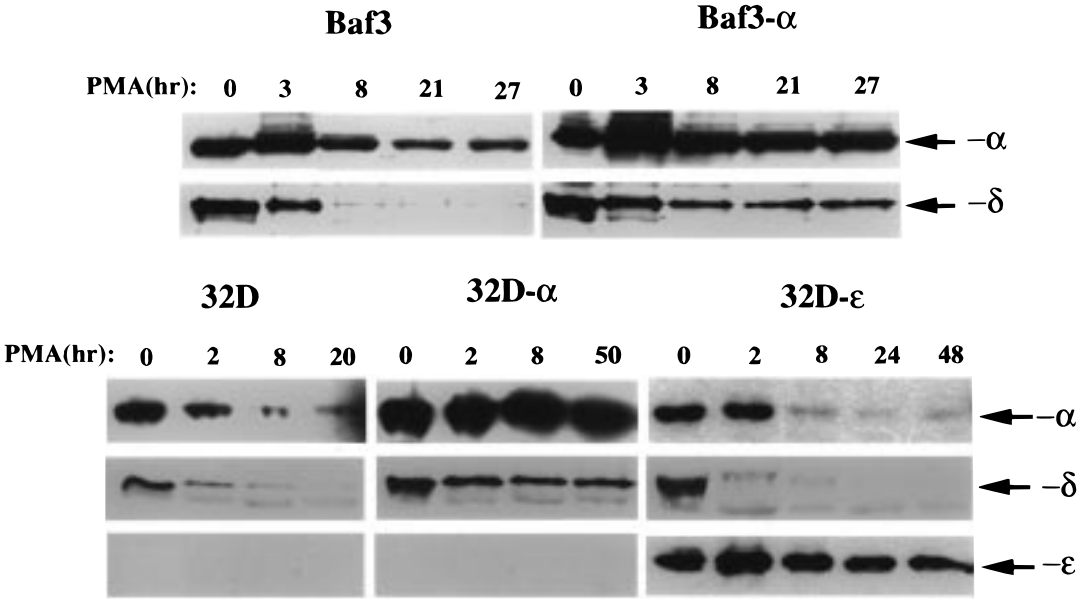

FIGURE 1: Effect of PMA on PKC in parental Baf3, 32D, and their derivatives that overexpress various PKC isoforms. The cells were treated with 100 nM PMA for the indicated periods of time. Whole cell lysates were analyzed by Western blots using anti-PKC type III (-α) (UBI), anti-PKC-δ (R&D), and anti-PKC-ϵ (BRL) antibodies. Analysis of each isoform was performed with the same exposure time for the wild type cells and PKC overexpressers. The results of one of three experiments performed with Baf3 and one of two experiments performed with 32D are presented.

32D promyelocytic cell lines were maintained in RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum and 5% WEHI-3 supernatant as a source of murine IL-3. Overexpression of PKC-α, -δ, -ϵ, and -η in the 32D cell line (e.g., 32D-α, 32D-δ, 32D-ϵ, and 32D-η) has been described earlier (*9*). The Baf3 derivative that overexpresses PKC-α (Baf3-α) was created by electroporation of Baf3 with a vector that constitutively expresses PKC-α under the control of the Moloney leukemia virus LTR promoter (*9*). The experiments were performed on two Baf3-α clones with similar levels of PKC-α expression, leading to similar results.

Western blot analysis was performed as described earlier (*17*) using polyclonal antibodies that specifically react with PKC-α, -βI, -βII, -γ, -δ, -ϵ, or -ζ (BRL), PKC-δ (R&D), PKC-δ (BRL), PKC-η, -θ (Santa Cruz), and monoclonal anti-PKC-α and -μ antibodies (Transduction Labs), anti-PKC-type III (PKC-α) antibody (UBI), and anti-PKC-λ and anti-PKC-θ (Transduction Labs).

Nuclear Run-On Assay. Analysis of mRNA transcription was performed with the nuclear run-on assay before and after a 1 h treatment of Baf3 and Baf3-α with 100 nM PMA. One hundred thousand cells per preparation were washed with cold PBS, resuspended in lysis buffer [10 mM Tris-HCl (pH 7.4), 10 mM sodium chloride, 3 mM magnesium chloride, and 0.5% (v/v) NP40], and incubated for 5 min on ice. The nuclear pellet was spun out at 500*g* for 6 min and washed in the same buffer. The repelleted nuclei were resuspended in storage buffer [50 mM Tris-HCl (pH 8.0), 40% glycerol, 5 mM magnesium chloride, and 0.1 mM EDTA] and frozen in liquid nitrogen in 100 μL portions corresponding to 1×10^7 nuclei. After being thawed on ice, nuclei were incubated for 20 min at 28 °C with 100 μL of reaction buffer [10 mM Tris-HCl (pH 8.0), 5 mM magnesium chloride, 300 mM potassium chloride, ATP and GTP (both at 0.5 mM), and 80 μCi of [α-^{32}P]UTP and [α-^{32}P]CTP (800 Ci/mmol, Amersham)]. DNase was added to a final concentration of 10 mg/μL, and the incubation was continued for 15 min at 28 °C. After addition of 200 μL of STE buffer [0.5% SDS, 100 mM Tris-HCl (pH 7.5), and 50 mM EDTA] and 20 μL of proteinase K (20 mg/mL), the samples were incubated for 1 h at 40 °C. Nuclear transcripts were separated from unincorporated nucleotides on Sephadex G-50. Five milligrams of inserts encoding murine PKC-α and -δ and GAPDH were excised from full-length cDNAs and immobilized on nitrocellulose membranes using the Hoefer slot-blot PR600 apparatus. The labeled RNA transcripts were boiled for 10 min, chilled on ice, and hybridized with DNA on the membranes for 4 days at 42 °C. Approximately 7×10^6 cpm of probe was used for each hybridization in a total volume of 1 mL of 50% formamide, 0.5% SDS, 0.05 M Hepes (pH 7), 0.75 M sodium chloride, 2 mM EDTA (pH 8), 1× Denhardt's solution, 1% poly(A), and 20% salmon sperm DNA (Sigma).

Analysis of mRNA Stability. Baf3 and Baf3-α cells were pretreated with 100 nM PMA for 1 h. After PMA was washed out, the cells were treated with 10 μg/mL actinomycin D alone or in combination with 100 nM PMA. Total mRNA was isolated at different times after actinomycin D addition and analyzed for the presence of mRNA for individual PKC isozymes expression by Northern blots.

Northern Blot Analysis. For isolation of total RNA, 5×10^7 cells were washed with cold PBS and resuspended in NP40 buffer [150 mM sodium chloride, 1.5 mM magnesium chloride, and 10 mM Tris-HCl (pH 7.4)]. After centrifugation, the pellet was resuspended in 400 μL of the same buffer containing 10 mM vanadyl ribonucleoside complex (BRL) and mixed with 50 μL of 10% NP40 and the mixture centrifuged for 15 s at full speed in a Microfuge. The supernatant was transferred to a sterile tube containing a mixture of 10 μL of 500 mM EDTA (pH 8) and 50 μL of 10% SDS. After phenol/chloroform extraction and ethanol precipitation, RNA was resuspended in TE buffer. Five milligrams of each RNA was fractionated by electrophoresis on 1% agarose gels containing 2.5% formaldehyde. The

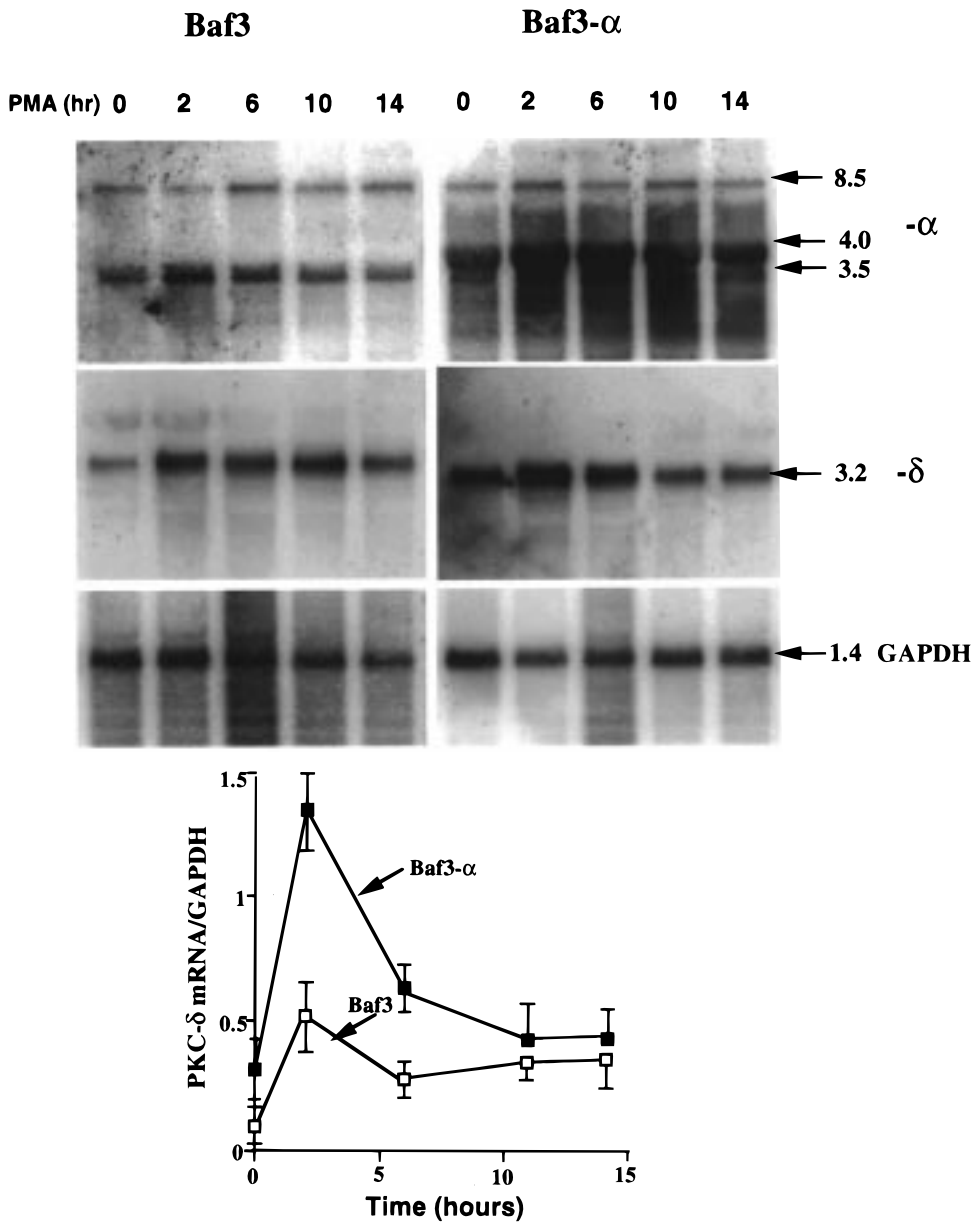

FIGURE 2: Effects of PMA and overexpression of PKC-α on the steady-state level of PKC-*δ* mRNA. Baf3 and Baf3-α cells were treated with 100 nM PMA for the indicated periods of time. Northern blots and quantitative analysis of mRNA bands, which was performed using Phospho-Image Scanning and Quant Scann Program (version 3.3) after a 48 (PKC-α or PKC-*δ*) or 16 (GAPDH) h exposure. The bands corresponding to PKC-α, PKC-*δ*, and GAPDH mRNAs and their approximate sizes (kilobases) are indicated. The endogenous PKC-α mRNAs are 3.5 and 8.5 kb; the exogenous mRNA is 4.0 kb. The endogenous PKC-*δ* mRNA is 3.2 kb. The points and error bars represent the means and standard deviations from three experiments, respectively.

RNAs were transferred to Hybond^N nylon membranes (Amersham) by capillary blotting and hybridized with radioactively labeled full-length cDNA probes specific for individual PKC isoforms (*18*).

RESULTS

Overexpression of PKC-α Raises the Level of the Endogenous PKC-δ Protein. PKC-α, -*δ*, -*μ*, -*ζ*, and -*λ* are expressed in Baf3, but no PKC-*β*, -*γ*, -*ϵ*, -*η*, or -*θ* can be detected in these cells (not shown). Of the isozymes expressed, only PKC-α and -*δ* are responsive to phorbol esters. In Baf3, cells a partial downregulation of PKC-α and an almost complete downregulation of PKC-*δ* are seen within 8 h of treatment with 100 nM PMA (Figure 1). In Baf3 that overexpresses PKC-α (Baf3-α), the ectopically overexpressed isozyme persists at a high level for at least 27 h after PMA addition. Unexpectedly, in PMA-treated Baf3-α, the level of endogenous PKC-*δ* also remains high for the same duration. The levels of PKC-*μ*, -*ζ*, and -*λ* (not shown) are not affected by PMA in Baf3 or Baf3-α. Thus, PKC-α and -*δ* are linked by some form of transregulation. As a result, PKC-*δ* persists in the phorbol ester-treated Baf3-α cells.

We also observed similar isozyme stabilization in the murine myeloid cell line 32D. PKC-α, -*δ*, -*η*, and -*ζ* are expressed in these cells (*9*). In cell lines that overexpressed PKC-α or -*ϵ* (32D-α and 32D-*ϵ*), the ectopically expressed isoforms persist in the cells for at least 2 days. In parental 32D cells, PKC-*δ* is completely downregulated by 100 nM PMA within 8 h (Figure 1). In 32D-*ϵ*, PKC-*δ* is downregu-

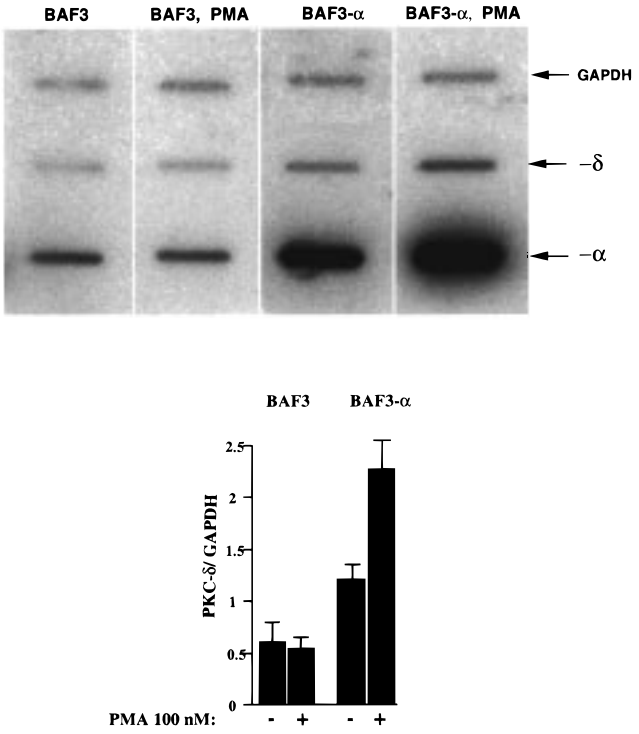

FIGURE 3: Effect of PMA treatment and PKC-α overexpression on PKC-δ mRNA transcription. The transcription of PKC-α, -δ, and GAPDH mRNA was analyzed by nuclear run-on assay before and after a 1 h treatment with 100 nM PMA. The quantitative analysis of the band intensities was performed using Phospho-Image Scanning and Quant Scann Program (version 3.3). The error bars represent standard deviation from three experiments.

lated in a manner similar to that of the parental cells. But in 32D-α, the endogenous PKC-δ persists for at least for 2 days, similar to the lifespan of overexpressed PKC-α. The level of endogenous phorbol ester-responsive PKC-η is not affected by 100 nM PMA in parental 32D or in any of the overexpressers, at least within 50 h (not shown).

From these data, we conclude that stabilization of PKC-δ by PKC-α is not unique to one cell type and that it does not involve the other PMA-responsive or nonresponsive isoforms present in these cell lines.

PKC-α Upregulates the PKC-δ mRNA Level by Elevating Its Transcription and Increasing the Stability of Its mRNA. In the absence of PMA, the endogenous 3.2 kb PKC-δ mRNA is expressed at a low steady-state level in Baf3 and Baf3-α (Figure 2). PMA induces an upregulation of the endogenous PKC-α and -δ mRNA levels in the parental cells and Baf3-α. PMA also induces activation of the pLTR promoter and consequently upregulates the level of ectopically expressed PKC-α in Baf3-α. In Baf3-α, mRNA for the endogenous PKC-δ is upregulated to a greater extent than in the parental cells (Figure 2). This indicates that, in a situation similar to the persistence of PKC-δ at the protein level (Figure 1), overexpression of PKC-α increases the steady-state level of PKC-δ mRNA.

To determine which factors contribute to PMA-induced elevation of PKC-δ mRNA levels, we examined the effects of PMA and overexpression of PKC-α on the transcription and mRNA stability of PKC-δ. Analysis of PKC-δ transcription in Baf3 and Baf3-α was performed using the

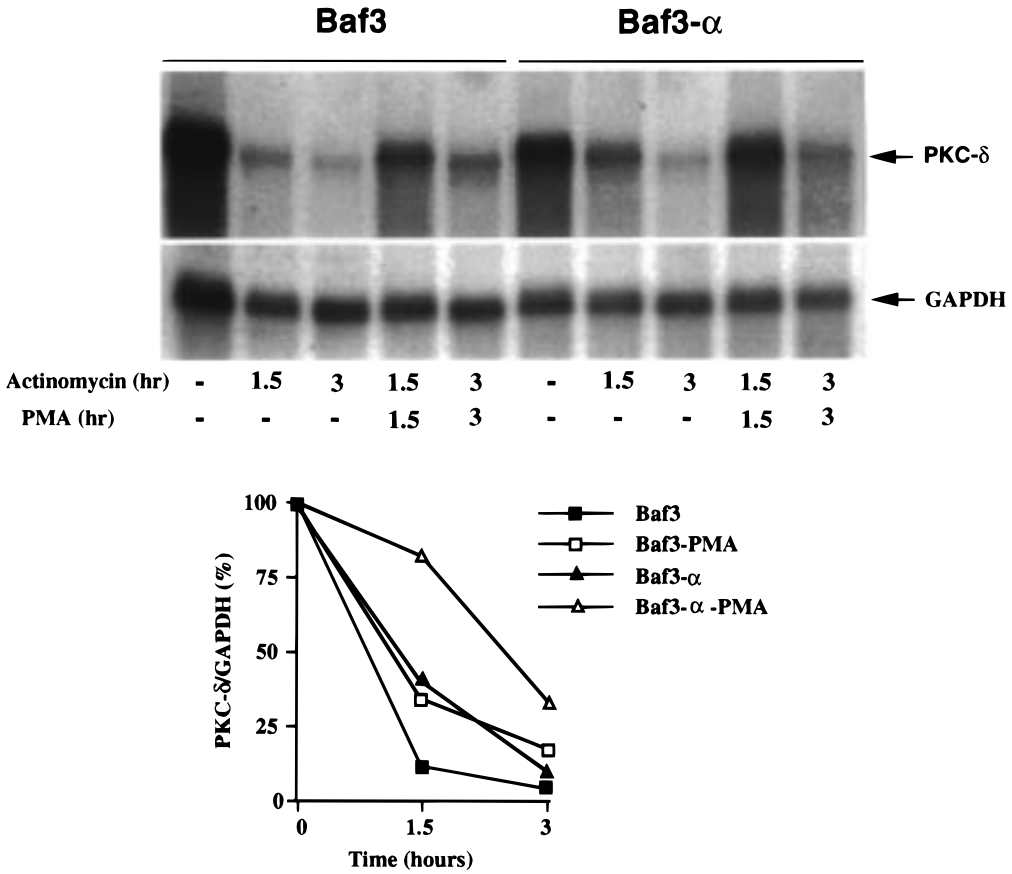

FIGURE 4: Effect of PMA on the stability of PKC-δ mRNA in Baf3 and Baf3-α. After a 1 h pretreatment of cells with 100 nM PMA, the drug was washed out. The PKC-δ mRNA degradation was followed in the presence of 10 mg/mL actinomycin D alone or with 100 nM PMA. Northern blot and quantitative analysis of PKC-δ and GAPDH mRNA expression. The result of one of three experiments is presented.

nuclear run-on assay before and after a short-term PMA treatment (Figure 3). Treatment of the parental Baf3 with PMA did not significantly affect the endogenous transcription of PKC-*δ*; however, overexpression of PKC-α elevates PKC-*δ* transcription, and treatment of Baf3-α with PMA further upregulates it (Figure 3).

To assess whether stabilization of PKC-*δ* mRNA by PKC-α also contributes to the elevated steady-state level of PKC-*δ* mRNA in PMA-treated cells, we studied the decay of PKC-*δ* mRNA in the presence of the inhibitor of mRNA synthesis, actinomycin D. We found that PMA addition to Baf3 cells stabilizes PKC-*δ* mRNA. A further stabilization of PKC-*δ* occurs in response to overexpression of PKC-α and treatment of Baf3-α with PMA (Figure 4). Thus, both elevation of PKC-*δ* mRNA transcription and an increase in its stability contribute to the upregulation of the PKC-*δ* mRNA level by activated PKC-α.

Upregulation of the PKC-δ mRNA Level Is Caused by Activation of the PKC-α Protein. Two possible mechanisms may be responsible for the upregulation of PKC-*δ* mRNA. On one hand, PKC-α may upregulate PKC-*δ* mRNA transcription. On the other hand, PKC-α may induce an accumulation of the endogenous PKC-*δ* protein through some post-translational mechanism(s), and the stabilized PKC-*δ*, in turn, could upregulate its own mRNA level. To rule out the latter mechanism, we determined whether the PKC-*δ* protein itself is needed for upregulation of its mRNA level. The PKC activator dPP (12-deoxyphorbol 13-phenylacetate), unlike PMA, has a 100 times greater affinity for PKC-*δ* than for PKC-α (*19*). As expected, treatment of Baf3 cells with dPP causes a selective activation and a rapid depletion of PKC-*δ*, but only a delayed activation and very slow depletion of PKC-α (Figure 5A). After a 3 h pretreatment with dPP, which had induced a selective depletion of PKC-*δ*, the cells were treated with PMA. Northern blot analysis of the steady-state level of PKC-*δ* mRNA (Figure 5B) showed that, even in the virtual absence of the PKC-*δ* protein, the level of PKC-*δ* mRNA increased in response to PMA. This indicates that the PKC-*δ* protein is not required for the upregulation of its own mRNA level by PKC-α.

PKC-α-Induced Upregulation of the PKC-δ mRNA Level Causes an Elevation of the PKC-δ Protein Level, a Requirement for PKC Kinase Activity. The specific PKC inhibitor GF109203X has an affinity for both "classical" and "novel" PKC isoforms, similar to that of PMA. However, GF109203X and phorbol esters interact with different PKC domains: the ATP-binding domain and the regulatory domain, respectively (*20*). As a result, the association of a phorbol ester with a PKC isoform does not prevent its inhibition by GF109203X (not shown). We also found that GF109203X does not prevent PKC translocation to the membrane and its downregulation there by proteolysis (not shown, Figure 7B).

Addition of GF109203X at a concentration that induces 50% inhibition of the phorbol ester-responsive activity (*20*) blocks PMA-induced upregulation of the PKC-*δ* mRNA level in both Baf3 and Baf3-α (Figure 6A).

To determine whether PKC activation elevates the PKC-*δ* protein level, we compared the levels of PKC-*δ* in cells treated with PMA alone or in combination with GF109203X and used this difference as a measure of the PKC-*δ* protein level elevation. Although the level of PKC-*δ* mRNA is low in the absence of PMA, the level of the PKC-*δ* protein is high, as a result of a negligible amount of PKC proteolysis. In PMA-treated cells, the level of the PKC-*δ* protein is higher than that in cells treated with a combination of PMA and GF109203X (Figure 6B). These data allow us to conclude that (i) activation of PKC-α upregulates the levels of both the PKC-*δ* mRNA and protein; (ii) cross-talk between PKC-α and PKC-*δ*, which involves both mRNA and protein alterations, takes place in wild-type Baf3 and Baf3-α cells; and (iii) in PMA-treated cells the levels of PKC-*δ* protein parallel those of PKC-*δ* mRNA, suggesting that the changes of PKC-*δ* mRNA are responsible for the changes of the PKC-*δ* protein.

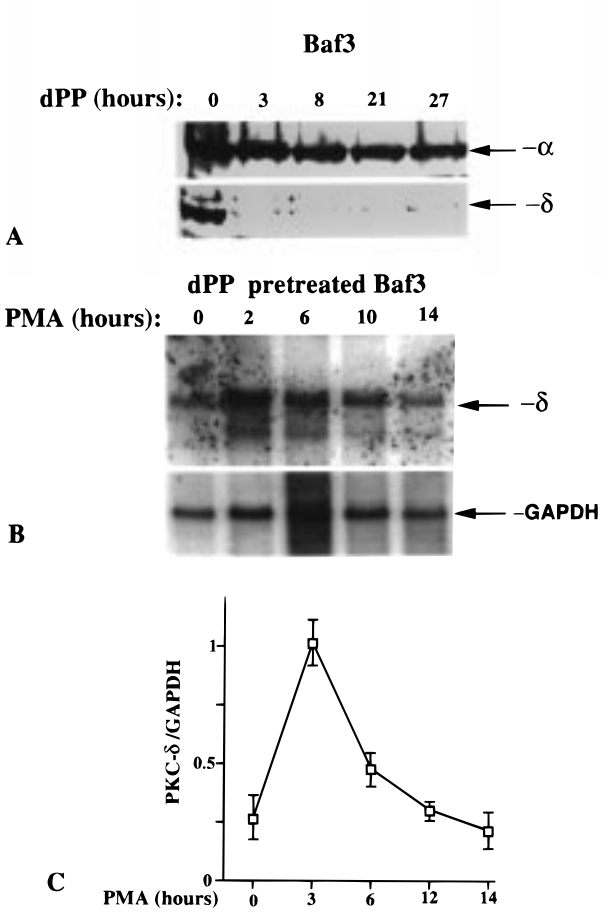

FIGURE 5: Effects of dPP alone and in combination with PMA on the expression of PKC-*δ* in Baf3. (A) Western blot analysis of PKC-α and -*δ* before and after treatment of Baf3 cells with 200 nM dPP. (B and C) A 3-h dPP pretreatment was followed by PMA addition. (B) Northern blot result of a typical experiment. (C) Quantitative analysis of PKC-*δ* mRNA expression. The error bars indicate the standard deviation from three experiments.

The steady-state level of PKC-*δ* observed by Western blot reflects two opposing phorbol ester-induced processes: (i) its elevation as a consequence of upregulation of the PKC-*δ* mRNA level and (ii) its loss as a result of PMA-induced proteolysis. We have established that the elevation of the PKC-*δ* mRNA level is involved in elevation of the PKC-*δ* protein level. However, a delayed downregulation of the PKC-*δ* protein level could be another factor that contributes to its elevation. Inhibition of newly synthesized proteins by cycloheximide permits us to investigate the phenomenon of PKC-*δ* downregulation without a contribution from PKC-*δ* mRNA synthesis. Using this approach, we showed that, compared to the cells treated with PMA alone, the addition of a combination of PMA and GF109203X delays down-

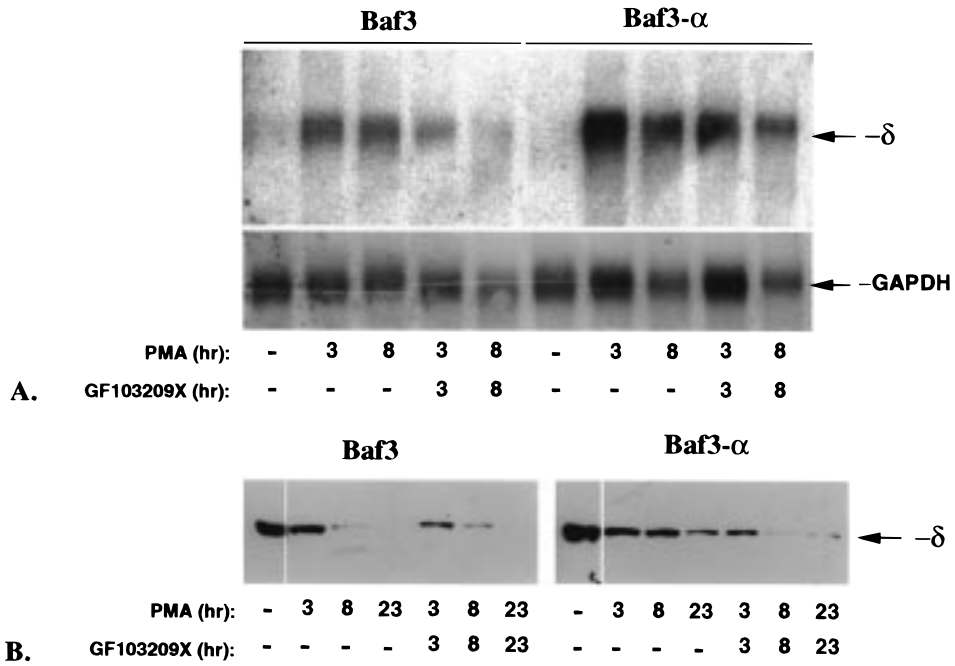

FIGURE 6: Effect of PKC inhibition on the PMA-induced upregulation of the PKC-δ mRNA and protein. Baf3 and Baf3-α cells were treated with 100 nM PMA alone or in combination with 1 mM GF109203X for the indicated periods of time. PKC-δ mRNA and protein expression were analyzed by Northern (A) and Western blots (B), respectively. The result of one of two similar experiments is presented.

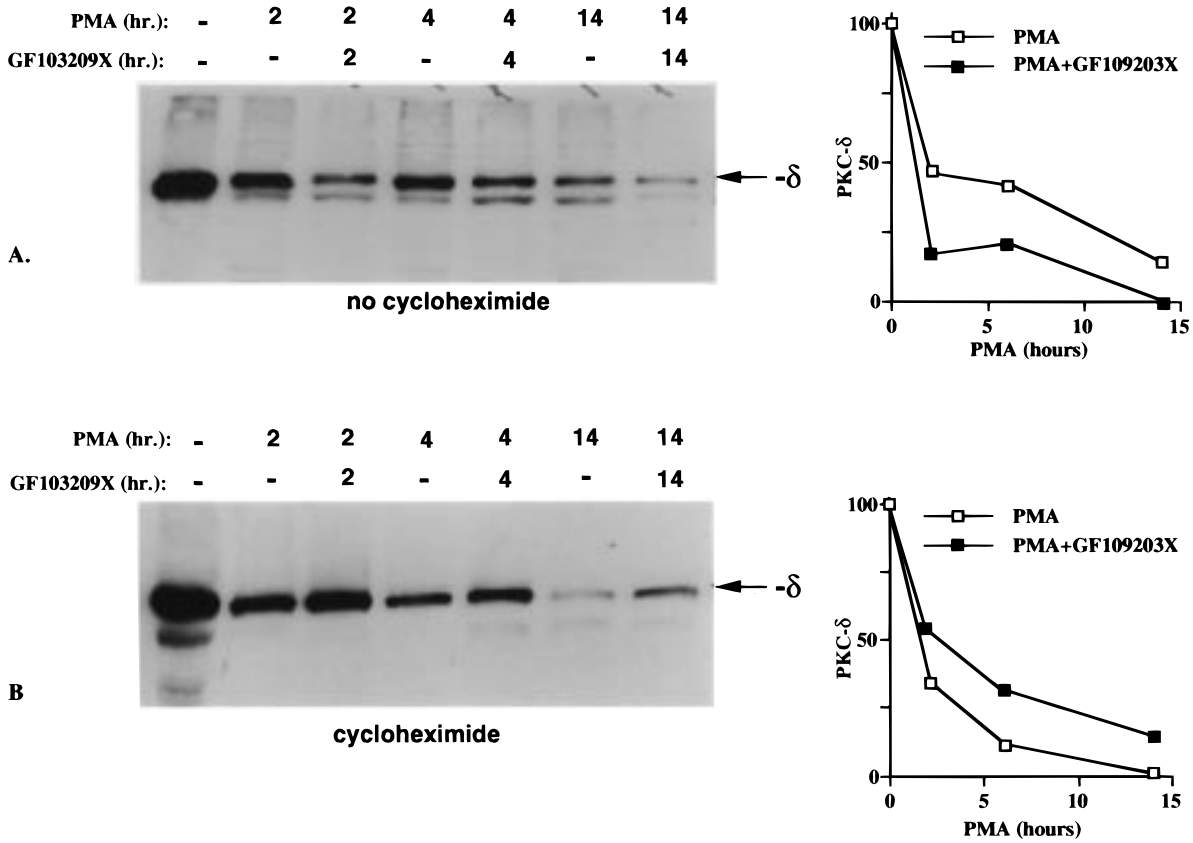

FIGURE 7: Effects of the protein synthesis inhibitor cycloheximide on the accumulation of the PKC-δ protein. Baf3 cells were treated with 100 nM PMA alone or in combination with 1 mM GF109203X in the absence (A) or in the presence (B) of 2 mg/mL cycloheximide. Western blots and quantitative analysis of PKC-δ protein expression. The result of one of two similar experiments is presented.

regulation of the PKC-δ protein (Figure 7B). Thus, we know that the addition of GF103209X to PMA-treated cells inhibits PKC-δ mRNA synthesis but delays downregulation of the PKC-δ protein. In the absence of cycloheximide, PKC inhibition by GF109203X causes a drop in the PKC-δ protein level (Figures 6B and 7A). This reflects the PKC inhibition-induced decline of the PKC-δ mRNA level rather than a delay in PKC-δ downregulation. Thus, we conclude that the change of the PKC-δ mRNA level in response to PKC activation and inhibition is the principal factor that contrib-

utes to the elevation and reduction of the PKC-δ protein level, respectively.

DISCUSSION

The principal message of this report is that PKC-α causes an accumulation of PKC-δ protein in two phorbol ester-treated murine hemopoietic cell lines, Baf3 and 32D. PKC-α-induced upregulation of the PKC-δ mRNA level, which involves both increases in transcription and mRNA stability, is the principal factor responsible for the elevation of its protein level.

Some consequences of the described phenomenon could be noted in phorbol ester-treated PKC-α overexpressers, in which endogenous PKC-δ persists as long as the ectopically overexpressed PKC-α. This may lead to misinterpretation of the PKC-α overexpression results. In the cell lines we have studied, transregulation involves only the PKC-α/PKC-δ pair of isoforms and does not affect PKC-ϵ, -η, -μ, or "atypical" -ζ and -λ isoforms. This finding may explain the data demonstrating that overexpression of either isoform of the PKC-α/PKC-δ pair, but not of PKC-η or -ϵ, endows 32D with the ability to undergo phorbol ester-induced differentiation (*9*). We have also reported that, in CCRF CEM and Baf3 lymphocytes that overexpress either PKC-α or -δ, phorbol esters produce similar rearrangements of the actin cytoskeleton associated with the inhibition of DNA synthesis (our unpublished data). However, in both cell lines, we were able to show that PKC-δ, but not PKC-α, is responsible for morphological changes of Baf3 and CCRF CEM cells. Thus, the apparent identity of functions appears to be a result of elevation of the PKC-δ isozyme level as a result of the overexpression of PKC-α.

There have been additional reports of cross-regulation between PKC isozymes. Overexpression of PKC-βII in rat fibroblasts conferred a partial resistance to PMA-induced downregulation of PKC-δ and PKC-ϵ levels (*21*). Coexpression of PKC-γ or -δ with PKC-ϵ in *Schizosaccharomyces pombe* renders the overexpressed PKC-ϵ susceptible to downregulation (*22*). Such a lack of uniformity and isoform specificity indicates that multiple mechanisms probably are involved in the apparent cross-talk between isozymes. For example, if one PKC isozyme is expressed at a high level, it may compete for proteases that participate in degradation of this and other PKC isoforms. This mechanism has been ruled out in certain systems, in which the cross-talk has been shown to require PKC activity (*22*, *23*). It was reported that autophosphorylation (*24*, *25*) or cross-phosphorylation may affect the isozymes' susceptibility to proteolysis. It appears that phosphorylation of PKC-α and -δ, which in turn is modulated by phorbol esters, may be involved in the control of their downregulation in certain systems (*26*, *27*).

It was accepted for a long period of time that PKC mRNA expression was not affected by phorbol esters (*28*−*30*). However, several reports have demonstrated that phorbol esters downregulate the expression of PKC-β in myeloid leukemia cells (*31*) and PKC-δ in A20 murine B lymphoma cells (*23*), the opposite of the effect we reported here. On the other hand, upregulation of PKC-δ levels and inhibition of PKC-α expression in response to phorbol esters were recently described in human epithelial cells (*32*), so peculiarities of the test system must determine the effect of PKC activation on expression of individual isozymes.

In Baf3 cells, PKC-α elevates the PKC-δ protein level through an upregulation of its steady-state mRNA level in a manner that requires PKC-α kinase activity. However, the relationship between the isozyme accumulation observed at the protein and mRNA levels deserves a further comment. Although the changes of PKC-δ mRNA expression may be sufficient to account for the elevation of this isoform, other factors operating on the protein level, such as phosphorylation of PKC isozymes or competition for proteases, may also be involved. We used two alternative approaches to investigate if any possible cross-talk between PKC-α and -δ at the protein level may be involved in the accumulation of the PKC-δ protein. We showed that, after depletion of PKC-δ by dPP, its mRNA level can be upregulated by the addition of PMA. This result indicates that PKC-δ is not required for upregulation its mRNA level by PKC-α.

In addition, we used the protein synthesis inhibitor cycloheximide to investigate whether another mechanism, a delayed downregulation of the PKC-δ protein level in response to activation of PKC-α, may contribute to the accumulation of the PKC-δ isoform. We found that the levels of PKC-α and -δ proteins are not affected by a 14 h cycloheximide inhibition of protein synthesis (not shown). Thus, the PKC protein is relatively long-lived. It has been also reported that calpain-μ and -m, the major proteins participating in PKC degradation, are also long-lived proteins [half-life is approximately 5 days (*33*)]. Thus, PMA-induced downregulation of the PKC-δ protein is not significantly affected by degradation of PKC isozymes or calpains. On the basis of the analysis of PMA-induced PKC-δ accumulation in the absence and presence of cycloheximide, we conclude that the upregulation of the PKC-δ mRNA level by PKC-α is the factor that is primarily responsible for the accumulation of this isoform. Thus, PKC-α upregulates the PKC-δ mRNA level, and upregulation of the PKC-δ mRNA level causes accumulation of the PKC-δ protein.

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