The overexpression of protein kinase C-δ (PKC-δ), but not PKC-ε, enables the mouse myeloid cell line 32D to differentiate into macrophages when treated with phorbol esters such as 12-O-tetradecanoylphorbol-13-acetate (TPA). To determine the domain of PKC-δ that is responsible for this isotype-specific function, cDNAs that encode reciprocal chimeras of PKC-δ and -ε (PKC-δε and PKC-εδ) were constructed by exchanging regulatory and kinase domains using polymerase chain reaction technology. Both chimeras were stably expressed in 32D cells using the pLTR expression vector and displayed protein kinase activity upon TPA treatment. TPA treatment of Leδ cells that overexpressed the PKC-εδ chimera, induced a dramatically increased cell volume, surface adherence, surface expression of Mac-1 and Mac-3, lysozyme production, and phagocytosis. These are the characteristics of the macrophage phenotype found in TPA-treated 32D cells that overexpressed PKC-δ. In contrast, little effect was seen in Leε, 32D cells that overexpressed PKC-δε, with or without TPA treatment. A PKC inhibitor directed toward the catalytic domain of PKC, GF109203X, and a selective inhibitor of PKC-δ, Rottlerin, blocked the TPA-induced differentiation of PKC-εδ-overexpressing 32D cells. These results demonstrate that the catalytic domain of PKC-δ contains the primary determinants for its activity in phorbol ester-induced macrophage differentiation.

The structure of all PKC isozymes can be functionally divided roughly in halves comprising a regulatory (N-terminal) and a catalytic (C-terminal) domain connected by a flexible hinge region that contains the primary site of protein degradation (Fig. 1). Four evolutionarily conserved domains (C1-C4) are interspersed among variable regions (V1-V5) that appear to determine the isozyme-specificity (3). The C3-V5 region has been defined as the catalytic domain, because C3 contains the ATP-binding site and C4 contains the substrate-binding site and the phosphate-transfer region. Sequences N-terminal of C3 are called the regulatory domain. C1 contains the pseudosubstrate domain and the sn-1,2-diaclyglycerol- and phorbol ester-binding region. C2 contains the Ca2+-binding domain, present in the “conventional” isozymes, PKC-α, -β and -γ, but not in the “novel” isozymes, PKC-δ, -ε, -η and -θ, or the “atypical” isozymes, PKC-ζ, -ι, -λ and -μ (2).

Marked differences have been found in the distribution of PKC isozymes in tissues and organs. PKC-α, -δ, -ε, and -ζ are nearly ubiquitously expressed, while PKC-γ, -η and -θ are more restricted to certain tissues. PKC-δ is abundant in most hematopoietic cells, but PKC-ε is expressed only in occasional B and T cell lines (4). In addition, PKC-ε is expressed at very low levels in many normal murine tissues except for the brain, which is also the richest source of PKC-γ (5, 6). These differences imply a divergence in functions. This was borne out by experiments in which we showed that PKC-δ and -ε had different effects in the murine myeloid progenitor cell line 32D: the overexpression of PKC-δ induced macrophage differentiation of 32D cells upon stimulation with TPA, but overexpression of PKC-ε had no such effect (7). What is more, the overexpression of PKC-δ and -ε has been shown to generate opposite effects on growth, morphology, and tumorigenicity in mouse, rat, and human fibroblasts (8–10). In addition, we and others showed that when individual PKC isozymes were activated in a single cell type, e.g. NIH 3T3 cells, different isozymes translocated to different subcellular locations, presumably their unique sites of action (11, 12).

In the interest of understanding the structural basis for the differences in isozyme function, we wanted to determine whether the “regulatory” or the “catalytic” region of PKC-δ and -ε contained the chief determinants for specificity of isoenzyme action. There are reports that parts of the regulatory domains of PKC-α, -δ, and -ε play a role in determining their substrate specificity (13–16). On the other hand, the catalytic domains of PKC-α, -β1, -β11, and -ζ have been implicated in their isozyme-specific functions (1, 17, 18). We wished to determine directly which of these two domains of the novel PKCs, PKC-δ and -ε, contributed to their differences in biological function. Therefore, we constructed reciprocal chimeric molecules of PKC-δ and -ε and expressed them in 32D cells to determine which

Protein kinase C (PKC)1 comprises a group of cellular Ser/Thr kinases that have been implicated in regulation of cellular differentiation and proliferation. There are at least 11 closely related PKC isozymes that are encoded by different genes (except PKC-β1 and -β11, which are the products of alternative splicing (1)). Phorbol esters, such as 12-O-tetradecanoylphorbol-13-acetate (TPA), are potent activators of most isozymes, and TPA acts at the same site on PKC as the endogenous activator, sn-1,2-diacylglycerol (2).

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1 The abbreviations used are: PKC, protein kinase C; TPA, 12-O-tetradecanoylphorbol-13-acetate; PCR, polymerase chain reaction; HAT, 0.2 mM hypoxanthine, 0.4 mM aminopterin, and 16 mM thymidine; PDBu, phorbol dibutyrate.

The Catalytic Domain of Protein Kinase C-δ in Reciprocal δ and ε Chimeras Mediates Phorbol Ester-induced Macrophage Differentiation of Mouse Promyelocytes*

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domain of PKC-δ was responsible for its ability to confer TPA-induced differentiation on this myeloid cell line.

**Experimental Procedures**

Construction of PKC-δ and -ε Chimeras—Before generating the PKC chimeras, we separately amplified the regulatory and catalytic domains of PKC-δ and -ε from their cDNAs using polymerase chain reaction (PCR) and a series of oligonucleotide primer pairs each of which includes a 21-mer that is present in the C3 region of both PKC-δ and -ε.

The location and use of these pairs are shown diagrammatically in Fig. 1A and the sequences are as follows: 5′- δ-reg, GAAATCTCCATCATG-GCACCC, and the C3-antisense primer, CTGGTGACTGCTCCGTCT-GCC; C3-sense, GGCAAGCGCTTGTCGCAAG, and 3′ δ-kin, GAATTCACTATGATGCT; 5′- ε-reg, GAATTCACTATGATGCT; and the C3-antisense primer, C3-sense, and 3′ ε-kin, GAATTCTGGGAGTTCAGCAAT.

A PCR Optimizer kit (Promega, Madison, WI) was used to find the optimal pH and MgCl2 concentrations for each reaction, and Tq polymerase (Perkin Elmer) was employed. 1 µg of the cloned mouse PKC-δ and -ε cDNAs (5, 9) were used as templates. PCR conditions were: 30 cycles of 15 s at 94°C, 30 s at 58°C, 1.5 min at 72°C.

Reciprocal chimeras of PKC-δ and -ε were generated using an overlap from the 21-base pair region in C3 that is identical in the two isoforms and oligonucleotides in each of the first round of PCR products. The PKC-εδ was generated by a second round of PCR amplification using 5′ ε-reg plus 3′ δ-kin primers and regulatory domain and δ catalytic domain templates that were generated in the first round of PCR and the same PCR conditions as above. The PKC-εδ was generated in an analogous fashion using 5′ δ-reg plus 3′ ε-kin primers and the PCR products corresponding to the regulatory and ε catalytic domains as templates. PCR products were initially cloned into a pBluescript SK+ vector, sequenced (United States Biochemical Corp.), and finally recloned into a mammalian pLTR expression vector at the EcoRI cloning site. pLTR is a mammalian expression vector based on the Harvey sarcoma virus long terminal repeat which contains the selectable marker thymidine-phosphoribosyltransferase (19). Mø, a previously reported PKC-δ overexpressor that uses the pMTH vector (7), which expresses lower levels of PKC-δ protein, was also used for comparison in Fig. 1.

Overexpression of PKCs in 32D Cells—32D cells were grown in RPMI 1640 supplemented with 10% fetal bovine serum, 10% conditioned medium from WEHI 3 cells as source of interleukin-3, 4 mL L-glutamine, and 100 units/ml penicillin and 100 µg/ml streptomycin. Cells were transfected with the expression vectors by electroporation (400 volts, 50 microfarads) and selected for 2 weeks in medium supplemented with HAT (0.2 mM hypoxanthine, 0.4 mM aminopterin, and 16 µM mycophenolic acid). This yielded the cell lines L6, Le, L6δ, and L6ε that overexpressed PKC-δ, -ε, and the PKC chimeras, respectively. The presence of the PKC proteins and the levels of their expression were determined by Western blot analysis.

Western Blot Analysis—Abs 10×106 32D cells grown with or without 10 nM TPA for 10 h were harvested in 100 µl of lysis buffer containing 0.1% Triton X-100, sonicated, and centrifuged at 100,000 g for 1 h at 4°C. The supernatants were resuspended in 100 µl of 2× SDS sample loading buffer as total kinase activity measured in the presence of TPA. Activity was calculated as the total kinase activity measured in the presence of TPA divided by the activity in the absence of TPA. Activity was also used for comparison in Fig. 1. Western blot analysis (Fig. 1b) showed that PKC-δ, -ε, and -δε were stably expressed at high levels in L6, Le, and L6δ, respectively. Early passages of L6ε (L6ε2)

**RESULTS**

Expression, Stability, and Enzyme Activity of Overexpressed PKCs in 32D Cells

The cDNAs for mouse PKC-δ and -ε and the PKC-εδ and -δε chimeras were sequenced completely, and the chimeras were found to have no introduced mutations. They were cloned into the pLTR expression vector and transfected into 32D cells. The stable transfectants were selected by growth in HAT medium plus mycophenolic acid. Western blot analysis (Fig. 1b) showed that PKC-δ, -ε, and -δε were stably expressed at high levels in L6, Le, and L6δ, respectively. Early passages of L6ε (L6ε2)
exhibited higher levels of PKC-δe than the stable line that emerged later, Lδe. Neither Lδe (Fig. 2) nor Lδe (not shown) acquired macrophage morphology when treated with TPA (see below). Lδe was unstable and could not be tested for kinase activity or other macrophage markers. Expression of PKC-δ in Lδ was unusually high, so it was compared to Mδ, a line of 32D cells bearing PKC-δ in the MTH expression vector (9). The chimeric PKC-δe was identified as a protein with the size of PKC-δ that reacted with an antibody against the C terminus of PKC-ε but not with one against the C terminus of PKC-δ (not shown). The PKC-δe protein was similar in size to PKC-ε, as expected, and was detected with both antibodies against the C terminus of PKC-δ and the N terminus of PKC-ε.

Protein kinase assays and PDBu binding assays were performed on the overexpressing cell lines and compared with wild-type 32D cells. As expected PKC kinase and PDBu binding activities were elevated in the overexpressers (Table I). Values are similar to those obtained by us (9) and others (24) for PKC overexpressers in other cell lines.

As expected, TPA (10 ng/ml) caused down-regulation of the abundant endogenous PKC-δ and the less abundant endogenous PKC-α (Fig. 2A). However, no down-regulation of the exogenous PKCs was seen in any of the four overexpressing cell lines, even after 48 h of treatment (Fig. 2B). None of the overexpressed PKCs abrogated the interleukin-3 dependence of 32D cells in the presence or absence of TPA (data not shown).

Fractionation studies showed that similar amounts of PKC-δ, -ε, and chimeric PKC-δe proteins appeared in the membrane and cytosolic fractions in the absence of TPA (Fig. 2C). In contrast, the chimeric PKC-δe was found in membrane and Triton-insoluble fractions only. Upon TPA treatment, the PKC-δe chimera and most of the overexpressed PKC-δ and -ε were found to translocate from the soluble to the membrane fractions, but the pattern of distribution of PKC-δe remained the same. The amount of each construct that was associated with the Triton-insoluble fractions remained unchanged.

| Cell lines | PKC activity | PDBu binding |
|------------|--------------|--------------|
| 32D        | 0.15 ± 0.05  | 1.9 ± 0.3    |
| Lδ         | 0.95 ± 0.20  | 23.0 ± 0.4   |
| Lε         | 0.30 ± 0.10  | 7.0 ± 0.2    |
| Lδε         | 0.29 ± 0.09  | 10.7 ± 0.4   |
| Lδe         | 0.28 ± 0.08  | 6.4 ± 0.2    |

a Values for PKC enzymatic activity represent the average ± range of two independent experiments with duplicate determinations of activity in each experiment.

b Values for PDBu binding represent the mean ± S.E. of three independent experiments with triplicate determinations of PDBu binding in each experiment.

The Effects of Chimeric PKCs on Myeloid Differentiation in 32D Cells

We detected substantial levels of PKC-δ, low levels of PKC-α, but no PKC-ε in wild-type 32D cells (Fig. 2). Activation of endogenous PKCs by TPA, however, did not cause differentiation, which may be due to the low level of expression. Overexpression of PKC-δ and -ε results in a slowing of cell growth (Table II), similar to the results reported for overexpression of PKC-δ in NIH3T3 cells (9) and COS cells (8), but macrophage differentiation required stimulation by TPA. The differentiation of 32D cells was assessed by several criteria: morphology, surface adherence, lysozyme production, phagocytosis, and macrophage-specific cell surface markers. 10 ng/ml TPA treatment for 10 h was sufficient to translocate overexpressed PKC-δ, PKC-ε, and PKC-δe (TPA-treatment had no effect on the distribution of chimeric PKC-δe, since it was absent from the cytosolic fraction), down-regulate endogenous PKC-δ and PKC-α as well as induce myeloid differentiation, whereas this concentration did not down-regulate the overexpressed PKCs.
FIG. 2. Western blots of the PKC-overexpressing lines. A, the endogenous expression of PKC-α and δ in 32D cells and their down-regulation by TPA (10 ng/ml). PKC-α was probed with anti-PKC-α catalytic domain antibody and PKC-δ with anti-PKC-δ C-terminal antibody. B, cell lysates of Le, Lδ, Lδ, and Lδ were prepared from parallel cultures of untreated cells (0 h) or cells that had been treated with 10 ng/ml TPA for the indicated times. Anti-PKC-δ N-terminal antibody was used to probe Lδ, anti-PKC-ε N-terminal antibody for Lδ, anti-PKC-ε C-terminal antibody for Lε and Lδε. C, Western blots of the fractionated lysates of PKC-overexpressing lines untreated or treated with TPA (10 ng/ml) for 10 h. PKCs were detected by the same antibodies used in B. Arrows indicate overexpressed PKC isoforms. S, soluble fractions; M, membrane fractions; I, Triton-insoluble fractions.

TABLE II
Growth rate and macrophage characteristics of wild-type 32D cells and PKC overexpressors with or without PKC activator (TPA10 = 10 ng/ml TPA treatment for 16–20 h) or inhibitors (GF15 = 1 μM GF109203X; GF30 = 10 μM GF109203X; Rott6 = 6 μM Rottlerin; Rott30 = 30 μM Rottlerin.)

| Cell lines | Doubling time | % Adherent cells | Lysozyme production (μg/107 cells) | Zymosan ingestion (particles/100 cells) |
|------------|---------------|------------------|----------------------------------|----------------------------------------|
|            | h             | No TPA + TPA10 | + TPA10 + GF15 | + TPA10 + GF30 | + TPA10 + Rott6 | + TPA10 + Rott30 | No TPA + TPA10 | No TPA + TPA10 |
| 32D        | 16            | <1               | 2.60               | 1.49               | <1               | 1.76               | <1               | 0.036              | 0.033              |
| Lδ         | 24            | <1               | 86.45             | 22.40             | <1               | 16.15             | <1               | 0.11               | 0.67               |
| Le         | 18            | <1               | 4.99              | 7.82              | <1               | 3.56              | <1               | 0.29               | 0.32               |
| Lδδ        | 20            | <1               | 90.29             | 45.76             | <1               | 46.24             | <1               | 0.19               | 0.70               |
| Lδεδ       | 20            | <1               | 7.96              | 10.04             | <1               | 4.53              | <1               | 0.16               | 0.54               |

Data from one representative experiment from three such experiments are shown.

Before addition of TPA <1% of all the overexpressers were adherent.

Each number represents the mean of three separate experiments.

Results are expressed as the number of intracellular particles per 100 cells as determined by light microscopy.

Morphology—Wild-type 32D cells displayed the phenotype of normal myeloid progenitor cells: small cells with well defined membrane structure and few if any cytoplasmic vacuoles (Fig. 3). This same morphology characterized Le cells before and after 10 h in 10 ng/ml TPA. Untreated Lδ cells were slightly larger than 32D cells and had a greater cytoplasm to nucleus ratio. Untreated Lδ and Lδε appeared even larger, with a few more vacuoles. These minor deviations from wild-type morphology in Le cells were not intensified by treatment with TPA. In contrast, Le and Lδ acquired the morphological characteristics of mature macrophages upon TPA treatment: greatly increased cell volume, increased adherence to the surface of culture vessels, numerous cytoplasmic vacuoles, and poorly defined plasma membrane. These morphological changes were even more pronounced in Lδε cells than in Lδ cells (Fig. 3).

Adherence—Lδ showed increased adherence 1 h after TPA treatment. Maximum effects were observed at 4 h. These effects were observed up to 20 h (Table II), but by 50 h after TPA addition most cells had detached. The adherence of Lδ was more persistent, starting 1 h after the addition of TPA, peaking at 8 h, and continuing for more than 70 h. In contrast, Lδε exhibited virtually no adherence in the presence of 10 ng/ml TPA. Untransfected 32D cells, as well as Le cells, transiently adhered to the surface of the tissue culture dish but without morphological changes, beginning 30 min after the addition of TPA and lasting no more than 14 h.

Lysozyme Production—Secretion of lysozyme is another characteristic of mature macrophages. Lysozyme activity was measured spectrophotometrically in the medium in which 32D cells and their PKC-overexpressing derivatives had been cultured for 16 h with or without TPA. As shown in Table II, wild-type 32D cells produced very little lysozyme with or without TPA. Low levels of lysozyme production were observed in...
Inhibition of Myeloid Differentiation by PKC Inhibitors

To confirm that the TPA-induced 32D cell differentiation into macrophages was mediated by PKC, we treated the cell lines with the PKC inhibitor GF109203X, that acts competitively at the ATP-binding site of PKC (25). The changes in morphology observed in TPA-treated Lδ, Leδ, and Lδε cells were completely blocked by the addition of 1 μM GF 109203X (Fig. 3). What is more, addition of GF109203X to uninduced overexpressers diminished the cells’ morphological differences from 32D (data not shown), suggesting that these differences in cell size were mediated by endogenous activation of a small portion of the overexpressed PKC. Importantly, 1 μM GF109203X significantly reduced the surface adherence of Lδ and Leδ (Table II), and 10 μM completely inhibited adherence (not shown).

These results were confirmed by experiments with Rottlerin, another PKC inhibitor that has been shown to have some selectivity for PKC-δ and also inhibit CaM-kinase III (26). Rottlerin showed similar effects on TPA-induced differentiation as did GF109203X (Table II): partial inhibition of differentiation was obtained at the concentration of 6 μM, and 30 μM Rottlerin was able to completely block the differentiation.

DISCUSSION

PKC δ and ε are members of the family of novel PKCs, but despite belonging to the same PKC subgroup, PKC-δ and ε have substantial differences in size and significant biochemical and biological differences. nPKCs exhibit in vitro differences in phosphorylating myelin basic protein, histones, protamine, and protamine sulfate (3, 13, 27). In vivo, activated PKC-δ and ε have distinct patterns of intracellular localization (11, 12), and overexpressed PKC-δ and ε induce distinct biological responses in a variety of cell types. Overexpression of PKC-δ in Chinese hamster ovary cells, NIH 3T3 cells and human glioma cells slowed proliferation while overexpressed PKC-ε increased growth (8, 9). Moreover, overexpression of PKC-ε but not PKC-δ in mouse or rat fibroblasts was transforming in vitro and tumorigenic in vivo (9, 10). In addition, overexpression of PKC-δ, but not PKC-ε, could reconstitute PKC-depleted rat basophil's ability to respond to antigen (28). Of particular relevance to this study, we have previously shown that PKC-δ, but not PKC-ε, could endow 32D cells with the ability to differentiate into macrophages when treated with TPA (7). The structural basis for these divergent isozyme-specific functions remains largely unknown.

To approach this issue we sought to assign the 32D-differentiation ability to either the regulatory or the catalytic half of PKC-δ. To do so, we constructed PKC-δε chimeras by swapping the regulatory and catalytic domains of PKC-δ and ε cDNAs and stably expressing them in 32D cells. Both chimeric proteins were overexpressed in 32D cells as shown by isozyme-specific antisera. The cell lines that expressed PKC chimeras exhibited increased kinase activity and showed significant PDBu binding, indicating that the chimeric proteins were functioning kinases that could be stimulated by phorbol esters.

As expected from previous experiments (7) the overexpression of PKC-δ in 32D cells induced all the signs of macrophage differentiation upon TPA treatment: slower growth rate; flat, vacuolated morphology; surface adherence; lysozyme production, phagocytic activity, and increased expression of Mac-1 and Mac-3. Similar results were obtained for PKC-εδ overexpresser but not for the reciprocal chimera, PKC-εδ. The appearance of the macrophage phenotype could be blocked by PKC-specific inhibitors: GF 109203X, a competitive inhibitor of the binding of ATP to nearly all PKCs, and Rottlerin, which shows some specificity for inhibition of the PKC-δ isofrom (26, 29). These results indicated that the PKC-δ catalytic domain contains the crucial determinants for 32D differentiation into mature macrophages. Overexpressed PKC-δε and -δε induced differentiation of TPA-treated 32D cells with different kinetics, however. The effect of PKC-εδ lasted for 70 h, whereas PKC-δ-overexpresses retrodifferentiated after 20 h. Neither PKC-δ nor -εδ were down-regulated after 48 h of TPA treatment, so the reasons for the more persistent effect of chimeric PKC-δε are...
Although neither Lδ nor Lε exhibited all the characteristics of typical macrophages after TPA treatment, some effects were seen in these 32D derivatives that overexpressed PKC-δ and PKC-ε. Like Lδ and Lεδ, overexpression of PKC-δε induced a slight macrophage-like morphology in the absence of TPA, which could be abolished using the PKC inhibitor GF109203X, although unlike Lδ and Lεδ, no further changes were seen upon TPA treatment. In contrast, the morphology of Lε, in the presence or absence of TPA, was no different from that of wild-type 32D cells. This suggests that the regulatory domain of PKC-δ may also make a contribution to the expression of macrophage differentiation markers, but it is minor compared to that of the catalytic domain.

The slight macrophage morphology noted in both untreated chimeras may be the result of two different mechanisms. That of Lεδ seems to be the result of an imperfect fit between the chimeric regulatory and catalytic domains and the concomitant incomplete inhibition of the kinase domain of one isoform by the regulatory domain of another isoform, which may also explain the relatively high phagocytic activity in Lεδ in the absence of TPA treatment. However, this explanation would not hold for Lδε, because the kinase domain of PKC-ε does not contribute to 32D differentiation. Instead, it may be that the δ regulatory domain contributes to the expression of macrophage morphology. A similar effect was seen in the lysozyme production by untreated Lδε cells, although the level of lysozyme did increase in the presence of TPA. Curiously, Lε had a higher basal level of lysozyme that did not increase in response to TPA. It is unclear at present whether this result is unrelated to the overall process of macrophage differentiation or may be the result of partial differentiation.

Our experiments with PKC chimeras have demonstrated the predominant role of the catalytic domain of PKC-δ in induction of macrophage differentiation. Several other studies have used a similar approach to dissect isotype-specific PKC functions for other biological end points. Catalytic domains were found to contain the determinants for isotype-specific function in a study using chimeric proteins of “classical” isoforms, PKC-α and -βII, in human erythroleukemia cells (18). In another study, fusion of the regulatory domain of PKC-δ to the catalytic domain of PKC-ζ gave rise to a chimera (17). Since the catalytic domain contains the substrate-binding region, it was reasonable to predict that it may be more important than the regulatory domain in determining the substrate specificity of PKC-ζ, implicating the catalytic domain in this aspect of specifying PKC isotype-specific functions. Our results are in agreement with this assumption. However, the regulatory domain may also play important roles in determining isoyme functions in other contexts. 1) PKC may regulate signaling events through direct molecular interaction with downstream effectors in addition to catalytic modification of proteins by phosphorylation. The PKC-α regulatory domain was found to activate phospholipase D in vitro through a direct protein-protein interaction that is independent of the kinase activity of PKC-α (16). 2) A
chimeric protein that contains the regulatory domain of PKC-ε fused to the catalytic domain of PKC-γ showed the substrate specificity of PKC-ε when expressed in COS1 cells (13). 3) Substrate localization signals are responsible for bringing each PKC to its appropriate intracellular compartment where different biochemical events are occurring. Three localization signals have been identified in the PKC-ε regulatory domain that appear to determine whether this isozyme associates with the plasma membrane, the cytoskeleton, or the Golgi apparatus (30). 4) A tyrosine phosphorylation site (Tyr-52) was found in the PKC-δ regulatory domain, which may be important in determining isozyme-specific functions (15, 31). In the present study, the system of untreated 32D cells expressing δ/ε chimeras may be linked to the regulatory domain as well as the catalytic domain of PKC-δ. Thus, each isozyme-specific function in each cell type must be analyzed to identify the structural element responsible for it.

In conclusion, using chimeric molecules that were constructed by fusing the regulatory domain and catalytic domain of two novel PKCs, PKC-δ and -ε, we demonstrated that the catalytic domain determined the bulk of the ability of the chimeras to enable TPA to cause macrophage differentiation in 32D cells. Further studies are needed to narrow down the regions in the catalytic domain of PKC-δ involved in regulating cell differentiation. Moreover, our studies showed that the chimeric approach remains an important tool in elucidating the structural basis for isotype-specific functions of PKCs, and reciprocal swapping of smaller regions may allow us to fine tune our structure/function analysis.

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The Catalytic Domain of Protein Kinase C-δ in Reciprocal δ and θ Chimeras Mediates Phorbol Ester-induced Macrophage Differentiation of Mouse Promyelocytes

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