Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 1406–1410, February 1995
Biochemistry

Protein kinase Cε is localized to the Golgi via its zinc-finger domain and modulates Golgi function

CSABA LEHEL*, ZOLTAN OLAH*, GABOR JAKAB†, AND WAYNE B. ANDERSON*‡

*Laboratory of Cellular Oncology, National Cancer Institute, and †Clinical Neuroscience Branch and Laboratory of Experimental Neuropathology, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892

Communicated by Tibor Farkas, Institute of Biochemistry, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary, October 14, 1994

ABSTRACT **Protein kinase C (PKC) is a multigene family of serine/threonine kinases that are central to many signal transduction pathways. Among the PKC isozymes, only PKCε has been reported to exhibit full oncogenic potential. PKCε also displays unique substrate specificity and intracellular localization. To examine the interrelationship between the biological effects and domain structure of PKCε, NIH 3T3 cells were stably transfected to overexpress different epitope-tagged fragments of PKCε. The overexpressed proteins each contain the ε-tag peptide at the C terminus to allow ready detection with an antibody specific for the tag. The holo-PKCε was found to localize with the Golgi network and other compartments, whereas the zinc-finger domain localized exclusively at the Golgi. Golgi-specific glycosaminoglycan sulfation was strongly inhibited in cells overexpressing either holo-PKCε or its zinc-finger domain, while the secretion of sulfated glycosaminoglycans into the medium was impaired in cells expressing the PKCε zinc-finger domain. Thus, these results suggest that PKCε may be involved in specifically regulating Golgi-related processes. Further, the results indicate that PKCε domains other than the kinase domain may also have biological activity and that the zinc-finger domain may function as a subcellular localization signal.**

Protein kinase C (PKC) consists of a family of more than 10 closely related phospholipid-dependent protein phosphotransferase isozymes (1, 2). The various PKC isozymes show considerable diversity in their domain structure, regulatory properties, and biological effects (1, 2). Although overexpression of most of the PKC isozymes has some effect on the morphology and growth characteristics of cells, only PKCε has been reported to exhibit full oncogenic potential (3, 4). PKCε also has been implicated in regulating other biological processes, such as antiviral resistance (5), neuropeptide signal transduction (6), and transporter regulation (7). PKCε has unique substrate specificity (8), and a portion of PKCε can always be detected in a membrane-associated state (3, 4, 7).

NIH 3T3 cells have been reported to contain PKCε (2, 9). To study the interrelationship of the function, subcellular localization, and domain organization of PKCε, we used NIH 3T3 cell lines overexpressing holo-PKCε and various truncated derivatives of PKCε. For purposes of uniform detection, the C-terminal 12 amino acids of PKCε were added to the C termini of all constructs as an antibody epitope tag. The zinc-finger domain of PKCε was found to contain all the information necessary for exclusive localization to the Golgi. Further, sulfate uptake and Golgi-specific sulfation of glycosaminoglycan (GAG) chains were inhibited in cell lines overexpressing either PKCε or its zinc-finger domain, indicating that PKCε is involved in modulating Golgi function.

MATERIALS AND METHODS

Materials. Dulbecco's modified Eagle's medium (DMEM), G418, and the antibody recognizing the C-terminal 12 amino acids of PKCε were from GIBCO/BRL. [^{3}H]Phorbol 12,13-dibutyrate ([^{3}H]PDBu, 20 Ci/mmol; 1 Ci = 37 GBq) and Na$_2$[^{35}S]SO$_4$ were obtained from DuPont/NEN. Brefeldin A (BFA) was obtained from Epicentre Technologies (Madison, WI) and 4-methylumbelliferyl β-D-xyloside (xyloside) was from Sigma.

Generation of the PKCε Fragment Overexpressor Lines. The development of the ε epitope-tagging system is described in detail by Olah *et al.* (10). Briefly, pεMTH contains a Zn^{2+}-inducible metallothionein promoter, an ATG translational start codon, *Xho* I and *Mlu* I restriction enzyme sites, and the sequence coding for the ε-tag. In-frame cloning of a PCR-generated cDNA fragment into the *Xho* I and *Mlu* I sites results in the addition of a start codon at the N terminus and the ε-tag at the C terminus. With the use of Vent polymerase (New England Biolabs), and the mouse cDNA coding for PKCε [a generous gift from H. Mischak (GSF Institut for Clinical Molecular Biology, Munich)] to serve as template, PCR fragments were generated for fragment ε2 (primer 1, CGATCTCGAGGGATCATCGGGCGAAGCC; primer 2, CTTGGCAATTCCGCGCGCGTCCAC), fragment ε3 (primer 1, CAGGGTCGACCAGGTCAATGGCCACAAG; primer 2, same as primer 2 for ε2), and holo-ε (primer 1, CCGCGTCGACCATGGTAGTGTTCAATGG; primer 2, ATTCGCGCGCTCAGGGCATCAGGTCTTCAC). The cycle parameters for 15 cycles were 60°C for 45 sec, 72°C for 45 sec (for ε2 and ε3) or 130 sec (for holo-ε), and 95°C for 1 min. The fragments were cloned into the pεMTH vector and the resulting constructs were transfected into NIH 3T3 cells by the calcium phosphate precipitation method. G418-resistant colonies then were selected (G418 at 800 μg/ml), and the stably transfected cell lines were maintained in DMEM supplemented with 10% (vol/vol) fetal bovine serum and G418 (200 μg/ml). The PKCδ overexpressor cell line was a gift from H. Mischak and was generated as described (3). Cells were used at low (passages 7–15) passage number for the experiments described.

Western Blot Analysis. Confluent Zn^{2+}-induced cells were lysed, the proteins were separated by SDS/PAGE, and the recombinant proteins were detected by immunoblot analysis with a polyclonal antibody raised against the C-terminal 12 amino acids of PKCε. The ECL (Amersham) protocol was used to visualize the immunoreactive bands.

***In Vivo* [^{3}H]PDBu Binding.** *In vivo* [^{3}H]PDBu binding was performed as described (7). Briefly, confluent cells in 24-

---

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: PKC, protein kinase C; GAG, glycosaminoglycan; PDBu, phorbol 12,13-dibutyrate; BFA, brefeldin A; PMA, phorbol 12-myristate 13-acetate.

‡To whom reprint requests should be addressed at: Laboratory of Cellular Oncology, National Cancer Institute, National Institutes of Health, Building 37, Room 1E14, Bethesda, MD 20892.

well plates were serum-deprived in the presence or absence of 20 μM zinc acetate for 16 h and then incubated with 2 nM [^{3}H]PDBu for 5 min at 37°C, followed by three washes in ice-cold PBS. The amount of [^{3}H]PDBu bound was determined by liquid scintillation counting of aliquots of cell lysates.

Immunocytochemical Localization of the Overexpressed Proteins. The cells were fixed with paraformaldehyde and permeabilized with Triton X-100, and the recombinant proteins were detected with a polyclonal antibody raised against the ε-tag peptide as described (10). For peroxidase staining, the peroxidase–antiperoxidase complex was visualized with a Vector VIP substrate kit. For double-labeling experiments, the fragment ε3 overexpressor cells were treated with BFA (2 μg/ml) for 1 h and then fixed and permeabilized. The BFA-induced disruption of Golgi was monitored with BODIPY-conjugated wheat germ agglutinin (Molecular Probes). Cyanine (Cy3)-conjugated anti-rabbit goat IgG (Jackson ImmunoResearch) was used as the secondary antibody to detect fragment ε3.

GAG Release. As a measure of Golgi function, GAG sulfation and subsequent GAG release were determined by a modification of the GAG release assay described by Miller and Moore (11), with further adaptation to a 96-well format. Briefly, the cells were grown to confluence in 24-well plates and then serum-deprived for 16 h in DMEM supplemented with 25 μM zinc acetate to upregulate the expression of the recombinant proteins. The serum-deprived zinc-induced cells were preincubated with either 1 mM xyloside or 0.2% dimethyl sulfoxide (as a solvent control) in buffer A [20 mM Hepes·NaOH, pH 7.2/110 mM NaCl/5.4 mM KCl/0.9 mM Na_2HPO_4/10 mM $MgCl_2$/2 mM $CaCl_2$/glucose (1 g/liter)] for 30 min. The cells then were labeled with $Na_2[^{35}S]SO_4$ (5 μCi per well in 200 μl of buffer A) for 15 min, rapidly washed with 200 μl of DMEM/4 mM unlabeled Na_2SO_4, and further incubated in DMEM for 10 min to allow secretion. The medium was completely and carefully transferred to a 96-well plate, and the cells were lysed in buffer B (10 mM Hepes·NaOH, pH 7.5/1.5 mM $MgCl_2$/10 mM KCl/0.1% Triton X-100) at 200 μl per well for 10 min at 4°C. The cell lysates were also transferred to a 96-well plate, proteinase K was added (5 μl per well from a stock solution at 20 mg/ml), and the samples were incubated at 50°C for 1 h. Aliquots (100 μl per well) of the medium and cell lysates were transferred into wells of a 96-well nitrocellulose filtration plate (Millipore). Then, chondroitin sulfate as a carrier (5 μl from a stock at 10 mg/ml) and cetylpyridinium chloride [30 μl from a 10% (wt/vol) solution] were added to each well, and the GAG chains were precipitated at 37°C for 1 h. The precipitate was collected on the filters and washed three times with 1% cetylpyridinium chloride/40 mM Na_2SO_4. The amount of ^{35}S-labeled GAG chains in the precipitate was determined by liquid scintillation counting.

Sulfate Uptake. Sulfate uptake into intact cells was determined by incubating cells in sulfate-free buffer A for 30 min and then incubating with $Na_2[^{35}S]SO_4$ (5 μCi per well in 200 μl of buffer A) for 15 min. The labeled cells were rapidly washed three times with DMEM/4 mM Na_2SO_4 (400 μl per well) and then lysed in 200 μl of buffer B. ^{35}S incorporation was determined by liquid scintillation counting of 100-μl aliquots.

RESULTS AND DISCUSSION

To study the interrelationship between the biological function and domain organization of PKCε, NIH 3T3 cells were stably transfected to overexpress different fragments of PKCε. Fragment ε3 contains only the zinc-finger domain, and fragment ε2 has a short N-terminal addition that includes the pseudosubstrate sequence (Fig. 1*A*). To facilitate uniform detection of the overexpressed fragments, the C-terminal 12-amino acid sequence of PKCε was added to the C termini of the PKCε fragments as an antibody epitope tag by cloning the corresponding cDNA fragments into the Zn-inducible vector pεMTH. The overexpression of the various recombinant proteins was analyzed on immunoblots with an antibody raised against the ε epitope peptide (Fig. 1*B*).

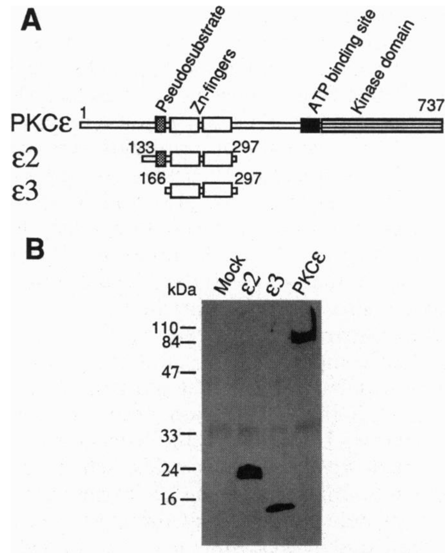

FIG. 1. Overexpression of PKCε constructs in NIH 3T3 cells. (*A*) Domain organization of PKCε and its truncated derivatives. The numbers denote the N- and C-terminal amino acids of the various constructs. (*B*) Immunoblot analysis of the cell lines transfected with the vector pεMTH (mock) or with the various constructs.

The phorbol ester binding capacity of the overexpressor lines was measured *in vivo* by using the water-soluble nonhydrolyzable diacylglycerol analog [^{3}H]PDBu. This assay exploits the finding that PKC or its phorbol-receptor zinc-finger region (12) binds phorbol esters with high affinity only in a membrane environment (13), thus allowing the determination of membrane-associated pools of PKC *in vivo* (7, 14). Fig. 2 demonstrates that inducible overproduction of each of the PKCε constructs resulted in similar increases in [^{3}H]PDBu binding, suggesting that each construct exhibits at least partial association with membranes.

To further characterize their membrane association, we compared the localization of the recombinant proteins by immunocytochemistry (Fig. 3 *a–d*). The holoenzyme was found to localize to the perinuclear region resembling Golgi,

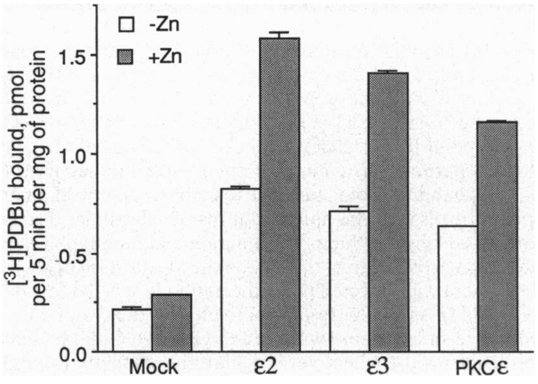

FIG. 2. *In vivo* [^{3}H]PDBu binding by the cells expressing the various PKCε constructs. Confluent cells were serum-deprived in the presence or absence of 20 μM zinc acetate for 16 h and then incubated with 2 nM [^{3}H]PDBu for 5 min at 37°C. Bound [^{3}H]PDBu was measured in total cell lysates. Results are representative of three similar experiments. Error bars indicate the range of duplicate assays.

was diffusely distributed in the cytoplasm, and also was detected at the plasma membrane. The ε2 protein was localized at the plasma membrane and at the perinuclear region. Interestingly, the ε3 zinc-finger construct was exclusively localized to the perinuclear region. To verify that the perinuclear staining found with all recombinant proteins represents Golgi localization, ε3 overexpressor cells were treated with BFA, a drug known to disintegrate Golgi (15), and fluorescence double labeling was performed. Fig. 3 *e–j* demonstrates that the perinuclear staining of ε3 was disrupted by BFA treatment, providing evidence that ε3 was indeed localized to the Golgi. The ε3 fragment also was highly enriched in the Golgi fraction prepared from ε3 overexpressor cells by the method of Balch *et al.* (16), when compared to the total cell extract (data not shown). These results indicate that the zinc-finger region of PKCε contains all the information necessary for exclusive binding to the Golgi. Interestingly, the N-terminal extension of the ε3 construct with 33 amino acids, which includes the pseudosubstrate motif, yielding the ε2 fragment, restored the ability of this protein to localize at the plasma membrane in addition to the Golgi. It is possible that "anchorage" proteins, reported to exist for at least several protein kinases (17), may define the intracellular localization of ε2 and ε3. The Golgi localization of the PKCε zinc-finger region appears to be isozyme specific, since the similarly epitope-tagged PKCα and PKCδ zinc-finger domains were found predominantly in the cytoplasm, with no detectable localization to the Golgi (unpublished observation).

Several reports have appeared (18–20) that suggest that PKC may be involved in regulating Golgi-related processes. Based on the differential downregulation of PKC isozymes by phorbol 12-myristate 13-acetate (PMA), De Matteis *et al.* (19) suggested that the PKCβ isozyme was responsible for modulating Golgi function in rat basophilic leukemia cells. In addition to rat basophilic leukemia cells, NIH 3T3 cells have been shown to exhibit elevated levels of GAG release upon short-term activation of PKC by PMA (19). NIH 3T3 cells, however, do not contain the PKCβ isozyme (2, 9). Thus, this PMA effect, at least in this cell line, must be attributable to another PKC isoform. To determine whether Golgi-associated PKCε might regulate Golgi function in NIH 3T3 cells, we measured the effects of the overproduced PKCε constructs on Golgi-specific sulfation and xyloside-induced secretion of protein-free ^{35}S-labeled GAG chains (11). Overexpression of fragment ε3 resulted in marked inhibition of GAG secretion, even though overexpression of PKCε holoenzyme had no appreciable effect (Fig. 4*A*). These results suggest that the ε3 zinc-finger construct may localize to specific PKCε binding domains at the Golgi to inhibit (interfere with) the normal function(s) of endogenous holo-PKCε to regulate secretion from the Golgi. That PKCε may act as a modulator of Golgi function is in accordance with the recent finding that PKCε expression is the rate-limiting factor for prolactin secretion in rat pituitary GH$_4$C$_1$ cells (20).

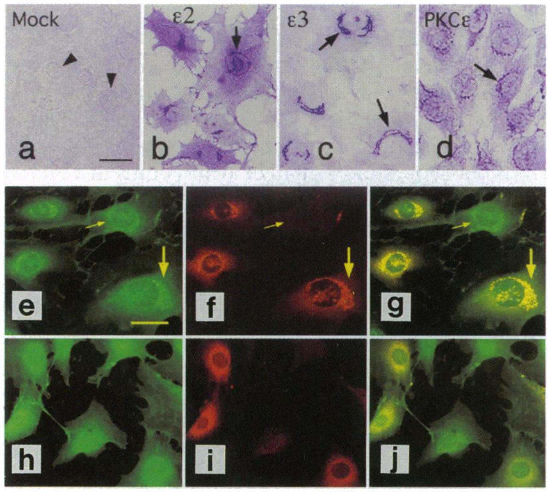

FIG. 3. Intracellular localization of holo-PKCε and its truncation mutants, ε2 and ε3. Immunocytochemical analysis of NIH 3T3 cell lines transfected with the pεMTH vector lacking an insert (*a*) or overexpressing fragment ε2 (*b*), fragment ε3 (*c*), or holo-PKCε (*d*). Arrowheads point to the nuclei of mock-transfected cells (*a*). Arrows point to the perinuclear accumulation of immunoreactivity (*b–d*). Lamellae resembling Golgi network can be recognized in the ε3 overexpressor cells (*c*). The noted patterns of subcellular distribution were confirmed by subcellular fractionation and immunoblot analysis (data not shown). (*e–j*) Effect of BFA on the localization of ε3 protein. The ε3 fragment was detected using the antibody specific for the ε-tag peptide (*f* and *i*), and a fluorescent derivative of the Golgi-decorating lectin wheat germ agglutinin was used to visualize Golgi (*e* and *h*). In untreated cultures the wheat germ agglutinin reactivity (*e*) and anti-ε-tag immunoreactivity (*f*) were colocalized at the Golgi (large arrows) and could be superimposed (*g*). Treatment with BFA resulted in dissociation of the staining patterns for both wheat germ agglutinin and ε3 reactivities (*h–j*). The lack of overexpression of the ε3 protein noted with a few cells (small arrows) is in accordance with our observation of the gradual loss of the overexpressor cell population during routine cell passage. (Bars = 20 μm.)

Previous studies have established that the Golgi network is the exclusive site for sulfation reactions (21–23). In contrast to the differential effects noted on GAG secretion, the level of total (cellular and secreted) GAG sulfation was markedly inhibited in cells overproducing either fragment ε3 or holo-PKCε (Fig. 4*B*). This inhibition was not due to decreased xyloside uptake since GAG sulfation was inhibited to a similar extent in these cells even without xyloside pretreatment (data not shown).

Rather, GAG sulfation may be influenced by sulfate uptake. As shown in Fig. 4*C*, the overexpression of PKCε resulted in a 65% decrease in sulfate uptake into the cell, while in cells expressing the ε3 fragment sulfate uptake was decreased by 40%. Xyloside pretreatment of the cells to initiate GAG chain synthesis and thus provide additional substrate for sulfation enhanced the level of sulfate uptake (Fig. 4*C*). This xyloside-induced increase in sulfate uptake was also decreased in cells overexpressing PKCε and fragment ε3 by 53 and 45%, respectively. In recent studies, we found that overexpression of either of the PKC isotypes ε and δ (both of which are endogenously expressed in NIH 3T3 cells) resulted in stimulation of sodium-dependent phosphate uptake (7). However, the inhibition of sulfate uptake and GAG sulfation by PKCε appears to be more specific since overexpression of PKCδ did not result in inhibition of these activities (Fig. 4 *B* and *C*). In addition, short-term treatment of these overexpressor cell lines with PMA did not further modify sulfate uptake, GAG sulfation, or GAG secretion. Further, overexpression of fragment ε3 or PKCε did not significantly alter the growth properties of these cells even though Golgi function was decreased (data not shown).

Since the ε3 fragment localizes exclusively to the Golgi, it is not apparent how this fragment might act to decrease sulfate uptake. Overexpression of fragment ε2, which exhibits significant localization to the plasma membrane, had little effect on sulfate uptake and GAG sulfation (Fig. 4 *B* and *C*). Thus, the exclusive Golgi localization of the ε3 protein makes it likely that the zinc-finger domain fragment may directly affect Golgi function (GAG secretion) and in turn may influence sulfate uptake and GAG sulfation through a feedback type of mechanism. Possible targets for this regulation could involve the sulfate transporter itself, the proteins required to translocate 3′-phosphoadenosine 5′-phosphosulfate to the Golgi (24), or the Golgi-localized transmembrane sulfotransferases (25).

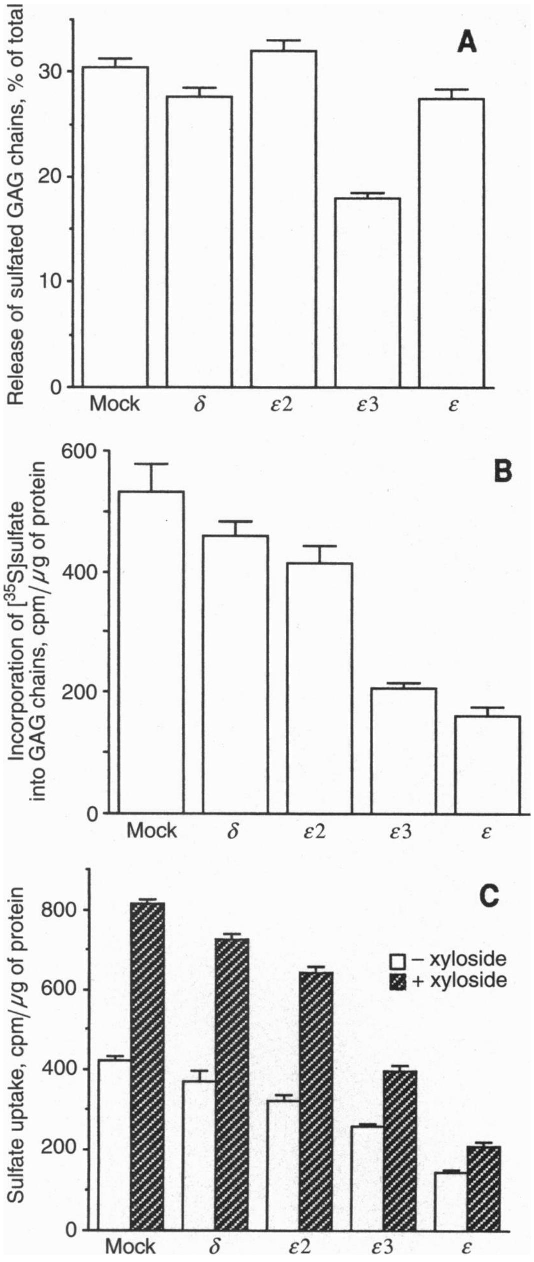

FIG. 4. Modulation of Golgi functions by the various PKCε constructs. Overexpressor cells grown to confluence were serum-deprived for 16 h in DMEM and supplemented with 25 μM zinc acetate to upregulate the expression of the recombinant proteins. (*A* and *B*) Secretion and total synthesis of ^{35}S-labeled GAG chains. Cells were pretreated with 1 mM xyloside for 30 min and labeled with [^{35}S]SO$_4$ for 15 min. Secretion was allowed to proceed for 10 min; the [^{35}S]GAG chains then were precipitated from the medium and from cell lysates and quantitated by liquid scintillation counting. (*A*) Secretion of [^{35}S]GAG chains, as determined by the ratio of [^{35}S]GAG chains in the medium to the total amount of [^{35}S]GAG chains. (*B*) Total synthesis of ^{35}S-labeled GAG chains. The [^{35}S]GAG chains present in the medium and cell lysates were quantitated and added together. (*C*) Effect of xyloside pretreatment on sulfate uptake. The cells were pretreated with either 1 mM xyloside or 0.2% dimethyl sulfoxide (as a control) for 30 min, and sulfate uptake was performed. After three washes with DMEM/4 mM Na$_2$SO$_4$, the cells were lysed and radioactivity was quantitated by liquid scintillation counting. Results are representative of three similar experiments. Error bars indicate the range of triplicate assays.

While the different PKC isozymes have been reported to localize to various subcellular compartments, none previously have been reported to associate with the Golgi (26–31). Thus, the observed association of PKCε with the Golgi may relate to some of the unique properties attributed to this isozyme, including oncogenic effects (3, 4). The observation that the zinc-finger domain of PKCε localizes exclusively to the Golgi and exhibits biological activity also is of importance. This provides supportive evidence for the suggestion that this region also may have effector function (32). Further, it has been reported that the regulatory domain of PKC may be liberated during PKC downregulation (33). In light of these findings, the possible biological roles of *in vivo*-generated fragments of this domain require careful assessment.

We thank Drs. P. M. Blumberg, Z. Szallasi, I. J. Kopin, H. D. Webster, and S. H. Yuspa for critical review of the manuscript and Dr. H. Mischak for providing the mouse PKCε cDNA and the cell line overexpressing the mouse PKCδ protein.

1. Nishizuka, Y. (1992) *Science* **258,** 607–614.
2. Hug, H. & Sarre, T. F. (1993) *Biochem. J.* **291,** 329–343.
3. Mischak, H., Goodnight, J., Kolch, W., Baron, G. M., Schaechtle, C., Kazanietz, M. G., Blumberg, P. M., Pierce, J. H. & Mushinski, J. F. (1993) *J. Biol. Chem.* **268,** 6090–6096.
4. Cacace, A. M., Guadagno, S. N., Krauss, R. S., Fabbro, D. & Weinstein, I. B. (1993) *Oncogene* **8,** 2095–2104.
5. Pfeffer, L. M., Eisenkraft, B. L., Reich, N. C., Improta, T., Baxter, G., Daniel-Issakani, S. & Strulovici, B. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 7988–7992.
6. Mangoure, D. & Dawson, G. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 2915–2919.
7. Lehel, C., Olah, Z., Mischak, H., Mushinski, J. F. & Anderson, W. B. (1994) *J. Biol. Chem.* **269,** 4761–4766.
8. Schaap, D., Parker, P. J., Bristol, A., Kriz, R. & Knopf, J. (1989) *FEBS Lett.* **243,** 351–357.
9. Szallasi, Z., Smith, C. B., Pettit, G. R. & Blumberg, P. M. (1994) *J. Biol. Chem.* **269,** 2118–2124.
10. Olah, Z., Lehel, C., Jakab, G. & Anderson, W. B. (1994) *Anal. Biochem.* **221,** 94–102.
11. Miller, S. G. & Moore, H.-P. H. (1991) *J. Cell Biol.* **112,** 39–54.
12. Kazanietz, M. G., Krausz, K. W. & Blumberg, P. M. (1992) *J. Biol. Chem.* **267,** 20878–20886.
13. Leach, K. L., James, M. L. & Blumberg, P. M. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 4208–4212.
14. Persons, D. A., Wilkinson, W. O., Bell, R. M. & Finn, O. J. (1988) *Cell* **52,** 447–458.
15. Klausner, R. D., Donaldson, J. G. & Lippincott-Schwartz, J. (1992) *J. Cell Biol.* **116,** 1071–1080.
16. Balch, W. E., Dunphy, W. G., Braell, W. & Rothman, J. E. (1984) *Cell* **39,** 405–416.
17. Hubbard, M. J. & Cohen, P. (1993) *Trends Biochem. Sci.* **18,** 172–177.
18. Ozawa, K., Szallasi, Z., Kazanietz, M. G., Blumberg, P. M., Mischak, H., Mushinski, J. F. & Beaven, M. A. (1993) *J. Biol. Chem.* **268,** 1749–1756.
19. De Matteis, M. A., Santini, G., Kahn, R. A., Tullio, G. D. & Luini, A. (1993) *Nature (London)* **364,** 818–821.
20. Akita, Y., Ohno, S., Yajima, Y., Konno, Y., Saido, T. C., Mizuno, K., Chida, K., Osada, S., Kuroki, T., Kawashima, S. & Suzuki, K. (1994) *J. Biol. Chem.* **269,** 4653–4660.
21. Young, R. W. (1973) *J. Cell Biol.* **57,** 175–189.
22. Vertel, B. M., Walters, L. M., Flay, N., Kearns, A. E. & Schwartz, N. B. (1993) *J. Biol. Chem.* **268,** 11105–11112.
23. Bennet, G. & Wild, G. (1991) *J. Electron Microsc. Tech.* **17,** 132–149.
24. Milla, M. E. & Hirschberg, C. B. (1988) *Proc. Natl. Acad. Sci. USA* **86,** 1786–1790.
25. Niehrs, C., Stinchcombe, J. C. & Huttner, W. B. (1992) *Eur. J. Cell Biol.* **58,** 35–43.
26. Kraft, A. & Anderson, W. B. (1983) *Nature (London)* **301,** 621–623.
27. Jaken, S., Leach, K. & Klauck, T. (1989) *J. Cell Biol.* **109,** 697–704.
28. James, G. & Olson, E. (1992) *J. Cell Biol.* **116,** 863–874.
29. Divecha, N., Banfic, H. & Irvine, R. F. (1993) *Cell* **74,** 405–407.

30. Thomas, T. P., Talwar, H. S. & Anderson, W. B. (1988) *Cancer Res.* **48,** 1910–1919.
31. Spudich, A., Meyer, T. & Stryer, L. (1992) *Cell Motil. Cytoskeleton* **22,** 250–256.
32. Murray, A. W., Fournier, A. & Hardy, S. J. (1987) *Trends Biochem. Sci.* **12,** 53–54.
33. Ohno, S., Konno, Y., Akita, Y., Yano, A. & Suzuki, K. (1990) *J. Biol. Chem.* **265,** 6296–6300.