Lakowicz, J. R., Cherek, H., & Balter, A. (1981) *J. Biochem. Biophys. Methods 5*, 131–146.
LeGrimellec, C., Giocondi, M.-C., Carriere, B., Carriere, S., & Cardinal, J. (1982) *Am. J. Physiol. 242*, F246–F253.
LeGrimellec, C., Carriere, S., Cardinal, J., & Giocondi, M.-C. (1983) *Am. J. Physiol. 245*, F227–F231.
Lentz, B. R., Moore, B. M., & Barrow, D. A. (1979) *Biophys. J. 25*, 489–494.
Lentz, B. R., Clubb, K. W., Barrow, D. A., & Meissner, G. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 2917–2921.
McElhaney, R. N., De Gier, J., & van Deenan, L. L. M. (1970) *Biochim. Biophys. Acta 219*, 245–247.
Molitoris, B. A., & Simon, F. R. (1985) *J. Membr. Biol. 83*, 207–215.
Molitoris, B. A., Alfrey, A. C., Adron Harris, R., & Simon, F. R. (1985) *Am. J. Physiol. 249*, F12–F19.
Parasassi, T., Conti, F., & Gratton, E. (1984) *Biochemistry 23*, 5660–5664.
Pates, R. D., & Marsh, D. (1987) *Biochemistry 26*, 29–39.
Poznansky, M., Tang, S., White, P. C., Milgram, J. M., & Selenen, M. (1976) *J. Gen. Physiol. 67*, 45–66.
Prendergast, F. G., Haugland, R. P., & Callahan, P. J. (1981) *Biochemistry 20*, 7333–7338.
Schroeder, F. (1983) *Eur. J. Biochem. 132*, 509–516.
Silvius, J. R., Read, B. D., & McElhaney, R. N. (1978) *Science (Washington, D.C.) 199*, 902–904.
Sklar, L. A., Hudson, B. S., & Simoni, R. D. (1977) *Biochemistry 16*, 819–828.
Sklar, L. A., Miljanich, G. P., Bursten, S. L., & Dratz, E. A. (1979a) *J. Biol. Chem. 254*, 9583–9591.
Sklar, L. A., Miljanich, G. P., & Dratz, E. A. (1979b) *Biochemistry 18*, 1707–1716.
Van Blitterswijk, W. J., van der Meer, B. W., & Hilkmann, H. (1987) *Biochemistry 26*, 1746–1756.
Verkman, A. S., & Ives, H. E. (1986) *Am. J. Physiol. 250*, F633–F643.
Wolber, P. K., & Hudson, B. S. (1981) *Biochemistry 20*, 2800–2810.
Worman, H. J., Brasitus, T. A., Dudeja, P. K., Fozzard, H. A., & Field, M. (1986) *Biochemistry 25*, 1549–1555.
Yeagle, P. L. (1985) *Biochim. Biophys. Acta 822*, 267–287.

A Fourth Type of Rabbit Protein Kinase C

Shigeo Ohno,[‡] Hiroshi Kawasaki,[‡] Yasuhiko Konno,[‡] Masaki Inagaki,[§] Hiroyoshi Hidaka,[§] and Koichi Suzuki*[,‡]

Department of Molecular Biology, The Tokyo Metropolitan Institute of Medical Science, Hon-Komagome, Bunkyo-ku, Tokyo 113, Japan, and Department of Pharmacology, Mie University School of Medicine, Edobashi, Tsu, Mie 514, Japan

Received August 28, 1987; Revised Manuscript Received October 27, 1987

ABSTRACT: Three rabbit cDNA clones coding for three types of protein kinase C (PKC α, β, and γ) have recently been identified and the structures determined [Ohno, S., Kawasaki, H., Imajoh, S., Suzuki, K., Inagaki, M., Yokokura, H., Sakoh, T., & Hidaka, H. (1987) *Nature (London) 325*, 161–166]. By use of these cloned cDNAs as hybridization probes, a fourth type (δ) of cDNA clone, which encodes a protein highly homologous to PKC α, β, and γ, was identified. PKC δ is composed of 697 amino acid residues and contains several peptide sequences determined at the protein level with the brain PKC preparation. This indicates that this molecular type (PKC δ) is, along with PKC α, β, and γ, a constituent of the brain PKC preparation. Sequence comparison among the four PKC types revealed that PKC δ is somewhat distinct from the other PKC types. PKC δ shows 99% amino acid sequence identity with rat PKC type I [Knopf, J. L., Lee, M.-H., Sultzman, L. A., Kriz, R. W., Loomis, C. R., Hewick, R. M., & Bell, R. M. (1986) *Cell (Cambridge, Mass.) 46*, 491–502], indicating relationship of these PKC types. The mRNA for PKC δ is exclusively concentrated in the brain.

Receptor-mediated hydrolysis of phospholipid is involved in a variety of agonist-specific and cell type dependent stimulus–response systems where protein kinase C (PKC) plays an essential role (Nishizuka, 1986).

We have previously described three distinct types of rabbit cDNA clones that encode closely related proteins (Ohno et al., 1987a). These proteins contain sequences identical with those determined from peptides derived from an apparently homogeneous preparation of rabbit brain PKC and were thus designated PKC α, β, and γ. The presence of a peptide that cannot be accounted for by the three PKC types suggested the existence of an additional type(s) of PKC. Thus, we screened a rabbit cDNA library in an attempt to isolate cDNA clones coding for an additional type of PKC and/or a protein related to PKC under low-stringency hybridization conditions using the cDNA fragments as probes. Here we report the isolation of cDNA clones coding for a protein (PKC δ) that shows complete amino acid sequence identity with several peptide sequences of rabbit PKC. The deduced sequence of PKC δ, in conjunction with those for PKC α, β, and γ, explains all the peptide sequences of rabbit brain PKC determined at the protein level. Comparison of amino acid sequences among PKC types from various species shows that rabbit PKC δ corresponds to rat PKC I (Knopf et al., 1986) and human and bovine PKC γ (Coussens et al, 1986).

Experimental Procedures

Screening of Rabbit Brain cDNA Libraries. Two independent rabbit brain cDNA libraries in λgt10 were constructed

[‡] The Tokyo Metropolitan Institute.
[§] Mie University.

0006-2960/88/0427-2083$01.50/0

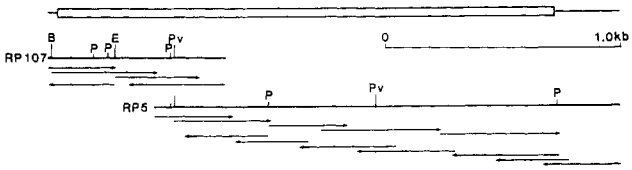

FIGURE 1: Restriction maps and sequencing strategies for cDNA clones RP5 and RP107. Arrows indicate the direction and extent of sequence determination. A white box represents the protein coding sequence. Restriction enzymes used were *Bam*HI (B), *Pst*I (P), *Eco*RI (E), and *Pvu*II (Pv).

as described (Ohno et al., 1987a,b; Huynh, 1985). The essential difference between the two is the primer used to direct the first strand synthesis. Primers used were oligo(dT) or a 22-mer oligonucleotide (Ohno et al., 1987a) for the cDNA library R or RP, respectively. cDNA fragments used as hybridization probes were β-C1 (nucleotides 18–355 and R805), γ-C1 (111–558, R812), α-C3 (1017–1954, R33), and γ-C3 (1260–1977, R812) (Ohno et al., 1987a) (Figure 6b). Screening of the cDNA libraries was done essentially as described (Maniatis et al., 1982) with the following modifications. Two sets of plaque filters were hybridized to a mixture of cDNA fragments prelabeled with ^{32}P by the multipriming procedure (Amersham) under two different hybridization conditions. One set of filters was hybridized at 65 °C in the standard solution (Maniatis et al., 1982) and washed at 65 °C in 0.1 × SSC (1 × SSC is 0.15 M NaCl/15 mM sodium citrate, pH 7.4)/0.1% sodium dodecyl sulfate (SDS) (high stringency). The other set was hybridized at 60 °C in the standard solution and washed in 6 × SSC/0.1% SDS (low stringency).

DNA Sequencing. Suitable overlapping DNA fragments from the recombinant cDNA clones were subcloned into pUC or BKS (Stratagene Cloning Systems) plasmids, and the DNA sequencing was performed by primed DNA synthesis on the denatured DNA template (Hattori et al., 1986) in the presence of dideoxy nucleotide triphosphates (Sanger et al., 1977). One-directional serial deletions were carried out by *Escherichia coli* exonuclease III and mung bean nuclease to accomplish overlapping sequencing of fragments subcloned into the BKS plasmid. No differences in the sequence were found in the overlapping region of cDNA clones RP5 and RP107. The entire protein coding region was sequenced in both strands.

Peptide Sequencing. Peptides TN40 and TN35 were obtained from a PKC preparation of rabbit brain (Inagaki et al., 1985) by digestion with a lysylendopeptidase as described (Ohno et al., 1987a). The peptides, separated by reverse-phase high-performance liquid chromatography were sequenced by a gas-phase sequencer (Applied Biosystems Model 470A).

Blot Hybridization. Blot hybridization analysis of RNA or genomic DNA was carried out as described (Ohno et al., 1987a). The probe specific to PKC δ was an 858 bp *Pvu*II fragment (486–1344) ^{32}P-labeled by a multipriming procedure. DNA probes specific to PKC α, β, and γ were as described (Ohno et al., 1987a).

RESULTS

In order to isolate cDNA clones coding for proteins related to PKC α, β, and γ, two independent rabbit brain cDNA libraries were screened under two different hybridization conditions by using cDNA fragments for rabbit brain PKC α and γ. Of 4×10^5 independent clones from the cDNA libraries R and RP, several clones gave positive signals only under hybridization conditions of low stringency. One clone, RP5, which was obtained from the RP library, was chosen for sequence analysis (Figure 1). Rescreening of the cDNA libraries using the cDNA insert of RP5 under high-stringency hybridization conditions gave only one additional signal (RP107) from the RP library. Sequence analysis of these two cDNA inserts showed that they spanned 2352 bp and contained a 2091-nucleotide open reading frame (Figure 2). This open reading frame predicts a protein of 697 amino acids (PKC δ) that is 66–68% identical with other PKC types. The deduced mRNA sequence for PKC δ is extremely rich in G/C;

```
-43                                                                                                  CCAAGACAGGCAGGATCCTGGTCCCCGCTACGTTCCTGGGGCC

  1 ATGGCGGGTCTGGGCCCCGGCGGAGGCGACTCAGAGGGGGGACCCCGACCCCTGTTTTGCAGAAAGGGGGCTCTGAGGCAGAAGGTGGTCCATGAAGTCAAGAGCCACAAGTTTACCGCCCGCTTCTTCAAGCAGCCCACCTTCTGCAGC
    M  A  G  L  G  P  G  G  G  D  S  E  G  G  P  R  P  L  F  C  R  K  G  A  L  R  Q  K  V  V  H  E  V  K  S  H  K  F  T  A  R  F  F  K  Q  P  T  F  C  S
                                                                                                                                                        50
151 CACTGCACCGACTTTATCTGGGGCATTGGGAAGCAGGGCCTGCAATGTCAAGTCTGCAGCTTTGTGGTTCACCGGCGATGCCACGAATTCGTGACCTTCGAGTGTCCAGGCGCTGGGAAGGGCCCCCAGACGGACGACCCCCGGAACAAG
    H  C  T  D  F  I  W  G  I  G  K  Q  G  L  Q  C  Q  V  C  S  F  V  V  H  R  R  C  H  E  F  V  T  F  E  C  P  G  A  G  K  G  P  Q  T  D  D  P  R  N  K
                                                                                                                                                       100
301 CACAAGTTCCGCCTGCACAGCTACAGCAGCCCCACCTTCTGCGACCACTGCGGCTCGCTCCTCTACGGGCTGGTGCACCAGGGCATGAAGTGCTCCTGCTGCGAGATGAACGTGCATCGGCGCTGTGTGCGCACGGTGCCCTCGCTGTGC
    H  K  F  R  L  H  S  Y  S  S  P  T  F  C  D  H  C  G  S  L  L  Y  G  L  V  H  Q  G  M  K  C  S  C  C  E  M  N  V  H  R  R  C  V  R  T  V  P  S  L  C
                                                                                                                                                       150
451 GGTGTAGACCACACAGAGGCGCCGCGGGCGCCTGCAGCTGGAGATCCGGGCGCCCACCTCGGATGAGATCCACGTCACCGTTGGCGAGGCCCGAAACCTCATTCCGATGGACCCCAATGGTCTGTCTGACCCCTACGTGAAGCTGAAGCTC
    G  V  D  H  T  E  R  R  G  R  L  Q  L  E  I  R  A  P  T  S  D  E  I  H  V  T  V  G  E  A  R  N  L  I  P  M  D  P  N  G  L  S  D  P  Y  V  K  L  K  L
                                                                                                                                                       200
601 ATCCCAGACCCGCGCAACCTGACCAAGCAGAAGACCCGGACCGTGAAAGCCACGCTGAACCCCGTGTGGAACGAGACTTTTGTGTTCAACCTGAAGCCGGGCGACGTGGAGCGCAGGCTCAGCGTGGAGGTGTGGGACTGGGACAGGACC
    I  P  D  P  R  N  L  T  K  Q  K  T  R  T  V  K  A  T  L  N  P  V  W  N  E  T  F  V  F  N  L  K  P  G  D  V  E  R  R  L  S  V  E  V  W  D  W  D  R  T
                                                                                                                                                       250
751 TCCCGCAACGACTTCATGGGCGCCATGTCCTTTGGCGTCTCAGAACTGCTCAAGGCGCCCGTGGACGGGTGGTACAAGTTACTGAACCAGGAGGAGGGTGAATATTACAATGTGCCAGTGGCCGATGCTGACAACTGCAGCCTCCTCCAG
    S  R  N  D  F  M  G  A  M  S  F  G  V  S  E  L  L  K  A  P  V  D  G  W  Y  K  L  L  N  Q  E  E  G  E  Y  Y  N  V  P  V  A  D  A  D  N  C  S  L  L  Q
                                                                                                                                                       300
901 AAGTTTGAGGCCTGTAACTACCCCCTGGAGTTGTATGAGCGGGTGCGGATGGGCCCCTCTTCTTCCCCCATCCCCTCCCCCTCTCCCAGTCCCACCCGACTCCAAGCGCTGCTTCTTCGGGGCCAGCCCAGGACGCCTGCACATCTCCGAC
    K  F  E  A  C  N  Y  P  L  E  L  Y  E  R  V  R  M  G  P  S  S  S  P  I  P  S  P  S  P  S  P  T  D  S  K  R  C  F  F  G  A  S  P  G  R  L  H  I  S  D
                                                                                                                                                       350
1051 TTCAGCTTCCTCATGGTCCTAGGAAAAGGCAGTTTCGGGAAAGGTGATGCTGGCCGAGCGCAGAGGCTCCGATGAGCTCTACGCCATCAAGATCCTGAAGAAGGACGTCATCGTCCAGGACGACGACGTGGACTGCACCCTGGTGGAGAAA
    F  S  F  L  M  V  L  G  K  G  S  F  G  K  V  M  L  A  E  R  R  G  S  D  E  L  Y  A  I  K  I  L  K  K  D  V  I  V  Q  D  D  D  V  D  C  T  L  V  E  K
                                                                                                                                                       400
1201 CGCGTGCTGGCGCTGGGCGGCCGAGGGCCTGGCGGCCGGCCGCACTTCCTCACCCAGCTCCACTCCACCTTCCAGACCCCGGACCGCCTGTATTTCGTGATGGAGTATGTCACCGGGGGCGACCTGATGTACCACATCCAGCAGCTGGGC
    R  V  L  A  L  G  G  R  G  P  G  G  R  P  H  F  L  T  Q  L  H  S  T  F  Q  T  P  D  R  L  Y  F  V  M  E  Y  V  T  G  G  D  L  M  Y  H  I  Q  Q  L  G
                                                                                                                                                       450
1351 AAGTTCAAGGAGCCCCACGCAGCGTTCTACGCGGCGGAGATCGCCATCGGCCTCTTCTTCCTCCATAACCAGGGCATCATCTACAGGGACCTGAAGCTGGACAACGTGATGCTGGATGCCGAGGGGCACATCAAGATCACCGACTTCGGC
    K  F  K  E  P  H  A  A  F  Y  A  A  E  I  A  I  G  L  F  F  L  H  N  Q  G  I  I  Y  R  D  L  K  L  D  N  V  M  L  D  A  E  G  H  I  K  I  T  D  F  G
                                                                                                                                                       500
1501 ATGTGTAAGGAGAACGTGTTCCCCGGCACCACCACCCGCACCTTCTGCGGGACCCCGGACTACATAGCCCCCGAGATCATCGCCTACCAGCCCTATGGGAAGTCTGTCGACTGGTGGTCCTTTGGGGTCCTGCTGTATGAGATGTTGGCG
    M  C  K  E  N  V  F  P  G  I  T  T  R  T  F  C  G  T  P  D  Y  I  A  P  E  I  I  A  Y  Q  P  Y  G  K  S  V  D  W  W  S  F  G  V  L  L  Y  E  M  L  A
                                                                                                                                                       550
1651 GGACAGCCTCCCTTCGACGGGGAGGATGAGGAGGAGCTGTTCCAGGCCATCATGGAGCAGACCGTCACCTACCCCAAGTCGCTGTCCCGGGAGGCCGTGGCCATCTGCAAGGGGTTCCTGACCAAGCACCCTGGGAAGCGCCTGGGCTCC
    G  Q  P  P  F  D  G  E  D  E  E  E  L  F  Q  A  I  M  E  Q  T  V  T  Y  P  K  S  L  S  R  E  A  V  A  I  C  K  G  F  L  T  K  H  P  G  K  R  L  G  S
                                                                                                                                                       600
1801 GGGCCCGACGGGGAGCCCACCATCCGTGCGCACGGCTTCTTCCGCTGGATCGACTGGGAGCGGCTGGAGCGGCTGGAGATCGCGCCCCCGTTCAGACCCCGCCCGTGCGGCCGCAGCGGCGAGAACTTCGACAAGTTCTTCACCCGGGCG
    G  P  D  G  E  P  T  I  R  A  H  G  F  F  R  W  I  D  W  E  R  L  E  R  L  E  I  A  P  P  F  R  P  R  P  C  G  R  S  G  E  N  F  D  K  F  F  T  R  A
                                                                                                                                                       650
1951 GCGCCGGCCGTGACGCCCCCAGACCGCCTGGTCCTGGCCAGCATCGACCAGGCGGACTTCCAGGGCTTCACCTACGTGAACCCCGACTTCGTGCACCCGGACGCCCCGCAGCCCCAGCAGCCCCGTGCCCGTGCCCGTCATGTAATGCCAC
    A  P  A  V  T  P  P  D  R  L  V  L  A  S  I  D  Q  A  D  F  Q  G  F  T  Y  V  N  P  D  F  V  H  P  D  A  R  S  P  S  S  P  V  P  V  P  V  M
                                                                                                                                                       697
2101 CTGCAGCCGCCAGGTGCGCAAGCGCCCCTCCGCCGCCACACCGCCCCCTCCCCACGCCAGACGCGGCCGCCGCGGCGCCGGCCTCCCCCCTCCCGAGCGGCCCGGGACCACCGCCGCCGCCGCCGTGCAGCCGCCAGGGCCTCGCCGCGC
2251 TCTAGGTCTTGCGGGCGTCGAGCCGCTCTCGCCCCTGCATTCAGCACTGCAC
```

FIGURE 2: Nucleotide and deduced amino acid sequences of composite cDNA for PKC δ. Nucleotides and amino acids are numbered starting at A of the presumptive initiation codon and Met-1, respectively. Amino acids that were identified by the peptide sequencing are underlined.

brain PKC	L20a FTARFF	L24 LLNQEEGEYYNVP	TN40 FPAXNYXLXLY	L20b YMLAERRGSDELYAI	TN35 ENVFPGTTT	L33 DXAFPRYIDXE
PKC α	.....	..S.....F...	.ER.KI.Q.TK	...S..K.T.....V	..IWD.V..	.....
PKC β	.....	..S.....F...	.ER.KI.Q.TK	...S..K.T.....V	..IWD.V..	.....
PKC γ	.I....	.....	.EK.KL.P.GN	....D.K.TE.....	.HMMD.V..	E.....R....
PKC δ	.....	.....	.....	.....	.....	A.G...W....

FIGURE 3: Correspondence of peptide sequences derived from the brain PKC preparation and those deduced from cDNA clones for PKC α, β, γ, and δ. The sequences of peptides TN40 and TN35 were determined as described under Experimental Procedures. Those for peptides L20a, L24, L20b, and L33 are from Ohno et al. (1987a). X indicates unidentified amino acids. Deduced amino acids not matching the peptide sequences are shown, whereas residues consistent with the peptide sequences are shown as dots.

G/C contents are 67%, 63%, and 79% for the 5′-noncoding, protein coding, and 3′-noncoding sequences, respectively. The GC content of the third letter in the protein coding sequence is unusually high (88%). mRNA having a high GC content often forms a stable secondary structure that inhibits efficient synthesis of cDNA. The reason that the content of this cDNA clone was low in cDNA libraries and that we could not isolate this type of cDNA clone from the cDNA library, R, might be explained by this unusual structural feature of the mRNA for PKC δ. In fact, mRNA for PKC δ is expressed in the brain as much as those for other PKC types such as PKC α and β (see below).

The deduced amino acid sequence of PKC δ contains five peptide sequences (comprising 51 amino acids in total) determined at the protein level with a complete match, in addition to one peptide sequence (peptide L33) with three amino acid substitutions (Figure 3). This fact indicates that PKC δ is one of the constituents of the rabbit brain PKC preparation.

Northern blot hybridization analysis of rabbit brain RNA using a cDNA fragment as a probe detected a major band of 3.4 kb that is clearly distinct from mRNA species for PKC types α, β, and γ (Figure 4a). This 3.4-kb band was detected almost exclusively in brain (Figure 4b). Southern blot hybridization analysis of rabbit genomic DNA shows a single band after digestion with *Bam*HI or *Hin*dIII, suggesting that the gene for PKC δ is a single copy (Figure 4c).

DISCUSSION

The use of cloned cDNA for PKC α, β, and γ as hybridization probes has made it possible to isolate cDNA clones coding for an additional distinct type of rabbit PKC family, PKC δ. Precise correspondence of the predicted sequences from the brain cDNA clones to those of peptides of highly purified brain PKC determined at the protein level provides a basis for the definition of PKC types. These peptide sequences (comprising 60 amino acid residues in total) appear in the sequence of at least one of the four PKC types in a complete match. On the contrary, none of the four PKC types identified thus far can explain all the peptide sequences determined at the protein level. Although peptides unique to α, β, and γ have not been identified (Figure 3), structural similarities among these PKC types, as well as the observation that their mRNAs are all expressed in the brain (Figure 4a), strongly indicate that the brain PKC preparation which gives a single band on SDS gel electrophoresis (Inagaki et al., 1985) is a mixture of PKC α, β, γ, and δ. This notion is supported by the recent demonstration that highly purified PKC from rat brain can be separated into three fractions that are immunologically distinguishable (Huang et al., 1986). These PKC preparations have enzymological properties apparently similar to those described earlier for a mixture of PKC types. The presence of at least two distinct PKC preparations from rat brain has also been demonstrated (Woodgett et al., 1987).

The amino acid sequences of the four types of PKC can be aligned as shown in Figure 5. PKC δ shows 66–68% sequence homology to PKC α, β, and γ, a lower homology than that between any two of the other three types (Figure 6). Thus, PKC δ is rather distantly related to the other three PKC types. PKC types α, β, and γ share strongly conserved sequences, C1, C2, and C3, flanked by completely divergent sequences, D1, D2, and D3, and a less divergent sequence, D4 (Ohno et al., 1987a). C3 and D4 constitute the kinase domain, and C1, C2, D1, D2, and D3 constitute the regulatory domain. These characteristics are completely conserved in PKC δ (Figure 5). PKC δ has some deletions and/or insertions in various regions as compared with other PKC types. The close relationship of PKC α, β, and γ to PKC δ at the level of the whole molecule is also seen for regional comparison as demonstrated in Figure 6. The D4 region contains the only difference between PKC α and β (Ohno et al., 1987a) and has been shown to form one exon (Kubo et al., 1987a; Ono et al., 1987; Rosenthal et al., 1987). The fact that the percentage identities of the D4 regions of the α and γ types and of the γ and δ types are comparable and higher than the homologies between other PKC types (see Figure 6) may support the idea that PKC γ

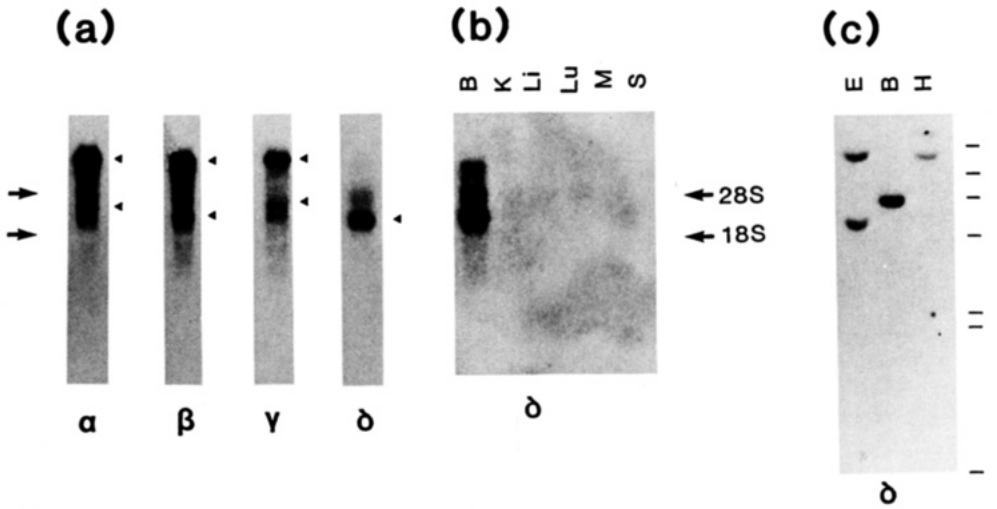

FIGURE 4: Blot hybridization analyses. (a) Northern analysis of rabbit brain RNA (4 μg) using type-specific cDNA probes (12-h exposure). Upper and lower arrows on the left show the positions of 28S and 18S ribosomal RNAs. Triangles indicate main bands. (b) Northern analysis of RNA (4 μg) from various rabbit tissues using a PKC δ cDNA probe (3-day exposure). B, K, Li, Lu, M, and S represent brain, kidney, liver, lung, muscle, and spleen, respectively. (c) Southern analysis of rabbit genomic DNA using a PKC δ cDNA probe. Genomic DNA was electrophoresed after digestion with *Eco*RI (E), *Bam*HI (B), and *Hin*dIII (H). Markers are *Hin*dIII digests of λ DNA.

FIGURE 5: Comparison of amino acid sequences among four rabbit PKC types. The four sequences are aligned and gaps are inserted to maximize homology. Boundaries of regions (C1–C3, D1–D4) are shown by vertical lines. Sequences for PKC α, β, and γ are from Ohno et al. (1987a). Horizontal arrows indicate intramolecular repeat rich in cysteine residues.

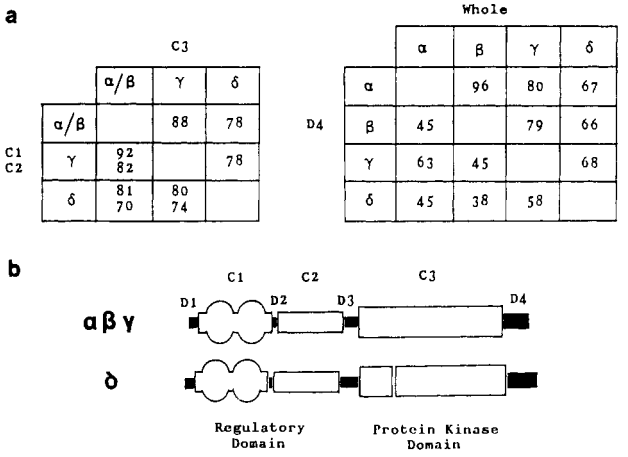

FIGURE 6: (a) Regional homologies between PKC family members. Degrees of homology are calculated on the basis of the alignment shown in Figure 5 and presented as percent identity. (b) Schematic representation of PKC molecules.

retains more of the features of the prototype PKC than the other types that have diverged more significantly. As previously noted, PKC γ is the only type whose mRNA is expressed in all cells and tissues examined, though mRNAs for α, β, and δ are expressed in a highly tissue-specific manner (Ohno et al., 1987a). This suggests that PKC γ may be involved in an essential cellular function such as cell proliferation. The notion that the γ type is the most "prototype" PKC is consistent with this suggestion.

The C1 region is the most highly conserved among PKC molecules. This region contains intramolecular repeats rich in cysteine residues. Spacings of cysteine residues are strictly conserved in the two repeats and among all PKC molecules, including PKC δ. A similar sequence is seen in the putative regulatory region of the c-raf protooncogene product (Booner et al., 1986; Ishikawa et al., 1986), DNA binding site of DNA

Table I: Four Distinct PKC Types Deduced from the cDNA Sequences

amino acid residue no.	rabbit	human	bovine	rat	proposed nomenclature[f]
673	α^a	β,[b] βII[i]	β^b	II[d] (II)[e,h]	βII
671	β^a	βI[i]		I[d] (III)[e,h]	βI
672	γ^a	$(\alpha_{16-444})^{b,g}$	α^c		α
697	δ	$(\gamma_{1-318})^{b,g}$	$(\gamma_{16-697})^{b,g}$	I[e]	γ

[a] Ohno et al. (1987a). [b] Coussens et al. (1986). [c] Parker et al. (1986). [d] Ono et al. (1986b). [e] Knopf et al. (1986). [f] Ono et al. (1987). [g] Numbers show amino acid positions of determined sequences. [h] The correspondence of these types has been documented (Brandt et al., 1987), although the sequences differ significantly from those of type II and type I described in Ono et al. (1986b). [i] Kubo et al. (1987b).

binding proteins such as steroid hormone receptors (Weinberger et al., 1985; Greene et al., 1986; Krust et al., 1986, Berg et al., 1986), and the active site of phospholipase A_2 (Maraganore et al., 1987). This raises the additional interesting question as to whether these Cys-rich sequences seen in diverged proteins have any functional similarities or not.

Independently of our group (Ohno et al., 1987a), several groups have identified cDNAs for PKC molecules from brain cDNA libraries of rat (Ono et al., 1986a,b; Makowske et al., 1986; Housey et al., 1987), bovine, and human (Parker et al., 1986; Coussens et al., 1986). All of the PKC types fall into four categories (Table I). PKC δ shows 99% amino acid identity (690/697) with rat PKC I (Knopf et al., 1986), indicating an exact correspondence of these PKC molecules. Similarly, the sequences of the corresponding PKC molecules from different species show more than 98% identity. Thus, the presence and the sequences of the four PKC types α, β, γ, and δ are strictly conserved among mammalian species. In fact, we have recently characterized human cDNA clones coding for PKCs corresponding to rabbit PKC α and β (Kubo et al., 1987b). Thus, the presence of the four distinct PKC

types α, β, γ, and δ were also shown in human in addition to in rabbit. Among these PKC molecules, rat PKC I and II (Knopf et al., 1986) and rat PKC type I and type II (Ono et al., 1986b, 1987), which correspond to rabbit PKC δ and α or β and α, respectively, have been shown to possess PKC activity after expression of the corresponding cDNA in COS cells. This also supports our notion that all four PKC types are not merely PKC-related enzymes but, in fact, PKC. We have recently demonstrated that all rabbit PKC types α, β, γ, and δ share phorbol ester binding activity by expressing the corresponding cDNAs in COS cells (Akita et al., unpublished results).

REFERENCES

Berg, J. M. (1986) *Science (Washington, D.C.) 232*, 485–487.

Bonner, T. I., Oppermann, H., Seeberg, P., Kerby, S. B., Gunnel, M. A., Young, A. C., & Rapp, U. R. (1986) *Nucleic Acids Res. 14*, 1009–1015.

Brandt, S. J., Niedel, J. E., Bell, R. M., & Young, W. S., III (1987) *Cell (Cambridge, Mass.) 49*, 57–63.

Coussens, L., Parker, P. J., Rhee, L., Yang-Feng, T. L., Chen, E., Waterfield, M. D., Franke, U., & Ullrich, A. (1986) *Science (Washington, D.C.) 233*, 859–866.

Greene, G. L., Gilna, P., Waterfield, M., Baker, A., Hort, Y., & Shine, J. (1986) *Science (Washington, D.C.) 231*, 1150–1154.

Hattori, M., & Sakaki, Y. (1986) *Anal. Biochem. 152*, 232–238.

Housey, G. M., O'Brian, C. A., Johnson, M. D., Kirschmeier, P., & Weinstein, I. B. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 1065–1069.

Huang, K.-P., Nakabayashi, H., & Huang, F. L. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 8535–8539.

Huynh, T. V., Young, R. A., & Davis, R. W. (1985) in *DNA Cloning* (Glover, D. M., Ed.) Vol. 1, pp 49–78, IRL, Oxford, U.K.

Inagaki, M., Watanabe, M., & Hidaka, H. (1985) *J. Biol. Chem. 260*, 2922–2925.

Ishikawa, F., Takaku, F., Nagao, M., & Sugimura, T. (1986) *Jpn. J. Cancer Res. 77*, 1183–1187.

Knopf, J. L., Lee, M.-H., Sultzman, L. A., Kriz, R. W., Loomis, C. R., Hewick, R. M., & Bell, R. M. (1986) *Cell (Cambridge, Mass.) 46*, 491–502.

Krust, A., Green, S., Argos, P., Kumar, V., Walter, P., Bornet, J.-M., & Chambon, P. (1986) *EMBO J. 5*, 891–897.

Kubo, K., Ohno, S., & Suzuki, K. (1987a) *Nucleic Acids Res. 15*, 7179–7180.

Kubo, K., Ohno, S., & Suzuki, K. (1987b) *FEBS Lett. 223*, 138–142.

Makowske, M., Birnbaum, M. J., Ballester, R., & Rosen, O. M. (1986) *J. Biol. Chem. 261*, 13389–13392.

Maniatis, T., Fritsch, E. F., & Sambrook, J. (1982) *Molecular Cloning*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Maraganore, J. M. (1987) *Trends Biochem. Sci. (Pers. Ed.) 12*, 176–177.

Nishizuka, Y. (1986) *Science (Washington, D.C.) 233*, 305–312.

Ohno, S., Kawasaki, H., Imajoh, S., Suzuki, K., Inagaki, M., Yokokura, H., Sakoh, T., & Hidaka, H. (1987a) *Nature (London) 325*, 161–166.

Ohno, S., Emori, Y., Sugihara, H., Imajoh, S., & Suzuki, K. (1987b) *Methods Enzymol. 139*, 363–379.

Ono, Y., Kurokawa, T., Kawahara, K., Nishimura, O., Marumoto, R., Igarashi, K., Sugino, Y., Kikkawa, U., Ogita, K., & Nishizuka, Y. (1986a) *FEBS Lett. 203*, 111–115.

Ono, Y., Kurosawa, T., Fujii, T., Kawahara, K., Igarashi, K., Kikkawa, U., Ogita, K., & Nishizuka, Y. (1986b) *FEBS Lett. 206*, 347–352.

Ono, Y., Kikkawa, U., Ogita, K., Fujii, T., Kurokawa, T., Asaoka, Y., Sekiguchi, K., Ase, K., Igarashi, K., & Nishizuka, Y. (1987) *Science (Washington, D.C.) 236*, 1116–1120.

Parker, P. A., Coussens, L., Tonny, N., Rhee, L., Young, S., Chen, E., Stabel, S., Waterfield, M. D., & Ullrich, A. (1986) *Science (Washington, D.C.) 233*, 853–859.

Rosenthal, A., Rhee, L., Yadegari, R., Paro, R., Ullrich, A., & Goeddel, D. V. (1987) *EMBO J. 6*, 433–441.

Sanger, F., Nicklen, S., & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 5463–5467.

Weinberger, C., Hollenberg, S. M., Rosenfeld, M. G., & Evans, R. M. (1985) *Nature (London) 318*, 670–672.

Woodgett, J. R., & Hunter, T. (1987) *J. Biol. Chem. 262*, 4836–4843.