Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 1065–1069, February 1987
Genetics

Isolation of cDNA clones encoding protein kinase C: Evidence for a protein kinase C-related gene family

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Communicated by Ronald Breslow, October 20, 1986 (received for review September 11, 1986)

ABSTRACT **We have isolated cDNA clones encoding protein kinase C by using a 53-base-pair synthetic oligonucleotide probe corresponding to a peptide that we obtained from the rat brain enzyme. We also have isolated several closely related clones using the same oligonucleotide probe. Nucleotide sequence analysis of one of the protein kinase C clones, RP41, identifies a 224-amino-acid carboxyl-terminal region with ≈40% homology to the carboxyl-terminal catalytic domains of both the cAMP-dependent and cGMP-dependent protein kinases. The levels of mRNA homologous to RP41 are very high in brain, whereas much lower levels are present in heart and liver. Nucleotide sequence analysis of a second cDNA clone, RP16, identifies a deduced amino acid sequence that shares 65% homology with the corresponding region of the protein kinase C clone RP41. The levels of mRNA corresponding to RP16 are also high in rat brain, but the transcript sizes and tissue-specific expression patterns differ from those of RP41. These and additional results provide evidence that the gene encoding protein kinase C is a member of a novel serine/threonine protein kinase multigene family.**

Protein kinase C (PKC) is a Ca^{2+}- and phospholipid-dependent protein kinase involved in mediating a wide variety of cellular responses to growth factors, hormones, neurotransmitters, and other modulators of growth control (for reviews, see refs. 1–3). When specific hormones or growth factors bind to their corresponding receptors, they induce hydrolysis of phosphatidylinositol 4,5-bisphosphate, a minor component of cellular phosphatidylinositol. This leads to the production of two hydrolysis products: inositol 1,4,5-trisphosphate and diacylglycerol (4, 5). The former compound induces the release of Ca^{2+} from intracellular storage sites in the endoplasmic reticulum (4), and the latter compound is an endogenous activator of PKC that greatly reduces the Ca^{2+} requirement of the enzyme (6). Thus, the turnover of phosphatidylinositol 4,5-bisphosphate and PKC activation play a central role in signal transduction (1–3).

PKC is also a high-affinity intracellular receptor for the phorbol ester tumor promoters (7, 8). The binding of the potent tumor promoter phorbol 12-myristate 13-acetate (PMA) to PKC activates its kinase activity both *in vivo* and *in vitro* (9–16). In intact cells exposed to PMA, PKC undergoes translocation from the cytosolic to the membrane fraction (17). The activated enzyme phosphorylates a number of diverse proteins, including the receptors for epidermal growth factor, insulin, interleukin-2, and transferrin; the Na^+,K^+-ATPase; the glucose transporter; the oncogene protein $pp60^{src}$, and many others (see ref. 3). In addition, PMA treatment induces transcription of both the c-*myc* and c-*fos* protooncogenes, presumably by a pathway initially involving the direct activation of PKC.

In view of the central importance of this enzyme in signal transduction and tumor promotion, we undertook the isolation of cDNA clones encoding this enzyme. In this paper we report the isolation of a cDNA clone encoding the carboxyl terminus of rat brain PKC. In addition, we describe a closely related yet distinct cDNA clone that appears to belong to a novel PKC-related multigene family. The availability of these clones should greatly facilitate further studies on the role of PKC in growth control, differentiation, and multistage carcinogenesis. A preliminary report of these results has appeared elsewhere (18).

MATERIALS AND METHODS

Protein Purification. Rat brain cytosolic PKC was partially purified by DEAE-Sephacel chromatography, ammonium sulfate precipitation, and AcA 34 gel filtration as described (16). The partially purified enzyme was then labeled with ^{32}P by stimulating its autophosphorylation activity in a reaction mixture containing high-specific-activity [γ-^{32}P]ATP (10 mCi/ml; 3000 Ci/mmol, New England Nuclear; 1 Ci = 37 GBq), phosphatidylserine, and PMA. After preparative polyacrylamide gel electrophoresis and autoradiography, the ^{32}P-labeled PKC was identified as a homogeneous 82-kDa band. This band was then excised from the gel, and the protein was recovered by electroelution. The purified enzyme was then reduced, carboxymethylated, dialyzed, and cleaved with endoproteinase Lys C (Pierce). Cleavage peptides were separated by reverse-phase HPLC. Several purified peptides were sequenced by the automated Edman degradation method on a gas-phase protein sequencer [Applied Biosystems (Foster City, CA) 470A]. The sequence of one of these peptides, P2, was used to design the synthesis of a corresponding oligonucleotide probe. A detailed description of the enzyme purification, proteolytic cleavage, peptide purification, amino acid analyses, and peptide sequence analyses will be published elsewhere.

Oligonucleotide Synthesis and Purification. A 53-base oligonucleotide probe (P2CODE) corresponding to peptide P2 (Fig. 1) was synthesized on an automated DNA synthesizer (Applied Biosystems 380A). The sequence of the probe (Fig. 1) was based upon mammalian codon usage frequencies as described (19), with additional third-base degeneracies as shown in Fig. 1. A 17-base primer (P2PRIM) complementary to the 3′ end of P2CODE was also synthesized (Fig. 1). P2PRIM was purified by HPLC. P2CODE was very G·C-rich and required purification on a 20 M formamide polyacrylamide gel (20).

Preparation of ^{32}P-Labeled Probe. The probe/primer mixture was prepared by adding 15 pmol of P2CODE to 150 pmol of P2PRIM in reverse transcriptase buffer (50 mM NaCl/34 mM Tris·HCl, pH 8.7 at 25°C/6 mM $MgCl_2$/5 mM dithio-

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Abbreviations: kb, kilobase(s); PKC, protein kinase C; PKA and PKG, cAMP- and cGMP-dependent protein kinases; PMA, phorbol 12-myristate 13-acetate.

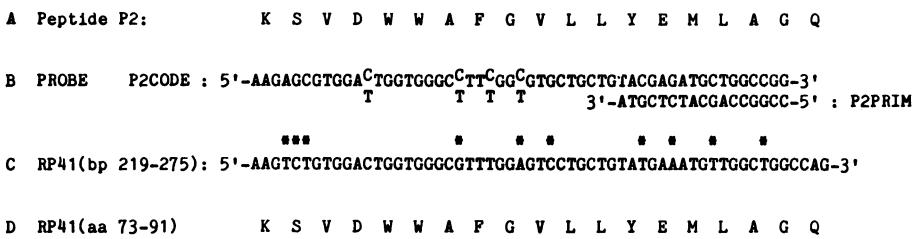

FIG. 1. Peptide and oligonucleotide sequences. (*A*) Amino acid sequence (single-letter code) of peptide P2, one of several peptides obtained from PKC and sequenced by automated Edman degradation on an Applied Biosystems 470A protein sequencer. The lysine (K) residue at the amino terminus was inferred from the cleavage method used. (*B*) P2CODE designates a 53-base coding strand probe that corresponds to peptide P2 and was synthesized based upon mammalian codon usage frequencies (19). Additional third-base degeneracies were incorporated at four positions as shown. The 17-base noncoding strand primer, P2PRIM, corresponding to the 3' terminus of P2CODE, is also shown. (*C*) Nucleotide sequence of RP41 in the probe region. Mismatches with the probe are indicated by asterisks. (*D*) Deduced amino acid sequence of RP41 (amino acids 73–91) in the probe region, showing identity at all positions with the P2 sequence shown in *A*. aa, Amino acids; bp, base pairs.

threitol). The reaction mixture was then annealed by incubating at 65°C for 5 min, followed by 55°C for 10 min, and then allowing it to cool slowly to room temperature for 30 min. The annealed probe/primer mixture was brought up to a final volume of 20 μl, containing 1 mM dATP, 1 mM dGTP, 1 mM TTP, and 125 μCi of [γ-^{32}P]dCTP (5000 Ci/mmol, New England Nuclear). Then 25 units of avian myeloblastosis virus reverse transcriptase (Life Sciences, St. Petersburg, FL) was added, and the reaction mixture was incubated at 42°C for 1 hr. Unincorporated radioactivity was removed by Sephadex G-50 chromatography as described (21). The specific activity of the resulting probe was 2×10^8 cpm/μg.

cDNA Library Screening. A rat brain λgt10 cDNA library was constructed as described (gift of J. Brosius) (22). Screening of the library, blotting, and hybridization were performed by standard methods (23).

Blotting and Hybridization. Nylon membrane (Amersham Hybond N) was substituted for nitrocellulose in all cases, including library screening. Low-stringency hybridization and wash conditions to be used for cDNA library screening with the oligonucleotide probe were first determined theoretically (19) and then tested with blot hybridizations of rat brain poly(A)$^+$ RNA (24). The low-stringency hybridization was at 37°C in 20% formamide containing 6× NaCl/Cit (1× = 0.15 M NaCl/0.015 M sodium citrate, pH 7), 5× Denhardt's solution (1× = 0.02% polyvinylpyrrolidone/0.02% Ficoll/0.02% bovine serum albumin), and 2% $NaDodSO_4$ at 37°C. Low-stringency wash conditions were 2× NaCl/Cit at 25°C for 20 min, followed by 2× NaCl/Cit containing 0.1% $NaDodSO_4$ at 37°C for an additional 20 min. High-stringency blot hybridizations were performed as described (25).

cDNA Subcloning and Nucleotide Sequencing. cDNA inserts isolated from purified phage clones were subcloned into the vectors pGEM-1 and pGEM-2 (Promega Biotec, Madison, WI). DNA sequence analyses were performed in the phage M13 vectors mp18 or mp19 by the dideoxy chain-termination method (26, 27). Sequences were determined on both strands.

Computer Analyses. Protein sequence data base searches were performed using the Protein Identification Resource data base (28)[§] on the Columbia University Cancer Center PRONUC analysis system (29) according to the algorithm of Lipman and Pearson (30). Protein sequence alignments were performed using a Protein Identification Resource implementation of the Needleman and Wunsch algorithm (31).

RESULTS

Isolation of cDNA Clones Homologous to a PKC Probe. Initial screening of 6×10^5 clones from a rat brain λgt10 cDNA library identified 41 clones that hybridized under low-stringency conditions to the ^{32}P-labeled probe prepared from the oligonucleotides designated P2CODE and P2PRIM as described in *Materials and Methods* and Fig. 1. These clones were isolated and placed into distinct groups based upon the intensity of the hybridization signal, restriction mapping, and high-stringency Southern blot analyses of rat genomic DNA. Thus far, based on the latter criteria, we have identified six distinct groups of cDNA clones. Detailed studies on two of these clones, a group I cDNA designated RP41 and a group II cDNA designated RP16, are described below.

Sequence Analysis of the cDNA Clone RP41. Restriction enzyme mapping of the RP41 clone indicated that it contained a 1.7-kilobase (kb) cDNA insert. Appropriate restriction fragments were subcloned into the phage M13 vectors mp18 and mp19, and the complete nucleotide sequence of RP41 was determined. The sequence of the 720-base-pair *Pst* I fragmentof RP41 is shown in Fig. 2*A*. This sequence displays a 224-amino acid open reading frame followed by a stop codon (TAG) and includes a region of 19 amino acids (Fig. 2*A*, amino acids 73–91; Fig. 3, region 5) that is identical with the PKC peptide P2 (Fig. 1). The latter finding, coupled with findings described below, provides strong evidence that the RP41 clone encodes the carboxyl-terminal region and catalytic domain of rat brain PKC. It is of particular interest that this sequence also exhibits homology with several domains present in almost all of the previously characterized protein kinases (33), including the conserved amino acid residues Arg-Asp-Leu, Asp-Phe-Gly, Cys-Gly-Thr, and Ala-Pro-Glu (amino acids 18–20, 37–39, 55–57, and 62–64 in Fig. 2*A*; regions 1–4 in Fig. 3). Computer searches of the Protein Identification Resource data base using the coding region of RP41 shown in Fig. 2*A* indicated that the greatest homologies in amino acid sequence (about 40% overall identity) were with the catalytic subunit of the cyclic AMP-dependent protein kinase (PKA) and the carboxyl-terminal (catalytic) domain of the cyclic GMP-dependent protein kinase (PKG). Alignments of the sequences of RP41, the catalytic subunit of PKA, and the carboxyl-terminal (catalytic) domain of PKG using the Needleman and Wunsch algorithm (31) are shown in Fig. 3. The multiple regions of homology provide strong evidence that the carboxyl-terminal region of RP41 constitutes the catalytic domain of PKC.

Region 6 (Fig. 3) of the RP41 clone will be discussed in the context of the cDNA clone RP16 (see *Discussion*). Region 7 (Fig. 3) contains the hexapeptide Asp-Thr-Ser-Asn-Phe-Asp. This sequence is also conserved in PKA and PKG. The presence of both threonine and serine residues in this sequence suggests that it may be a phosphorylation site, although the amino acid sequence differs considerably from peptide sequences that are known to be substrates for PKA or PKC (34). Thus, the biologic significance of the conservation of region 7 in PKC, PKA, and PKG merits further study. Finally, it is of some interest that an ATP binding site

[§]Protein Identification Resource (1986) Protein Sequence Database (Natl. Biomed. Res. Found., Washington, DC), Release 10.

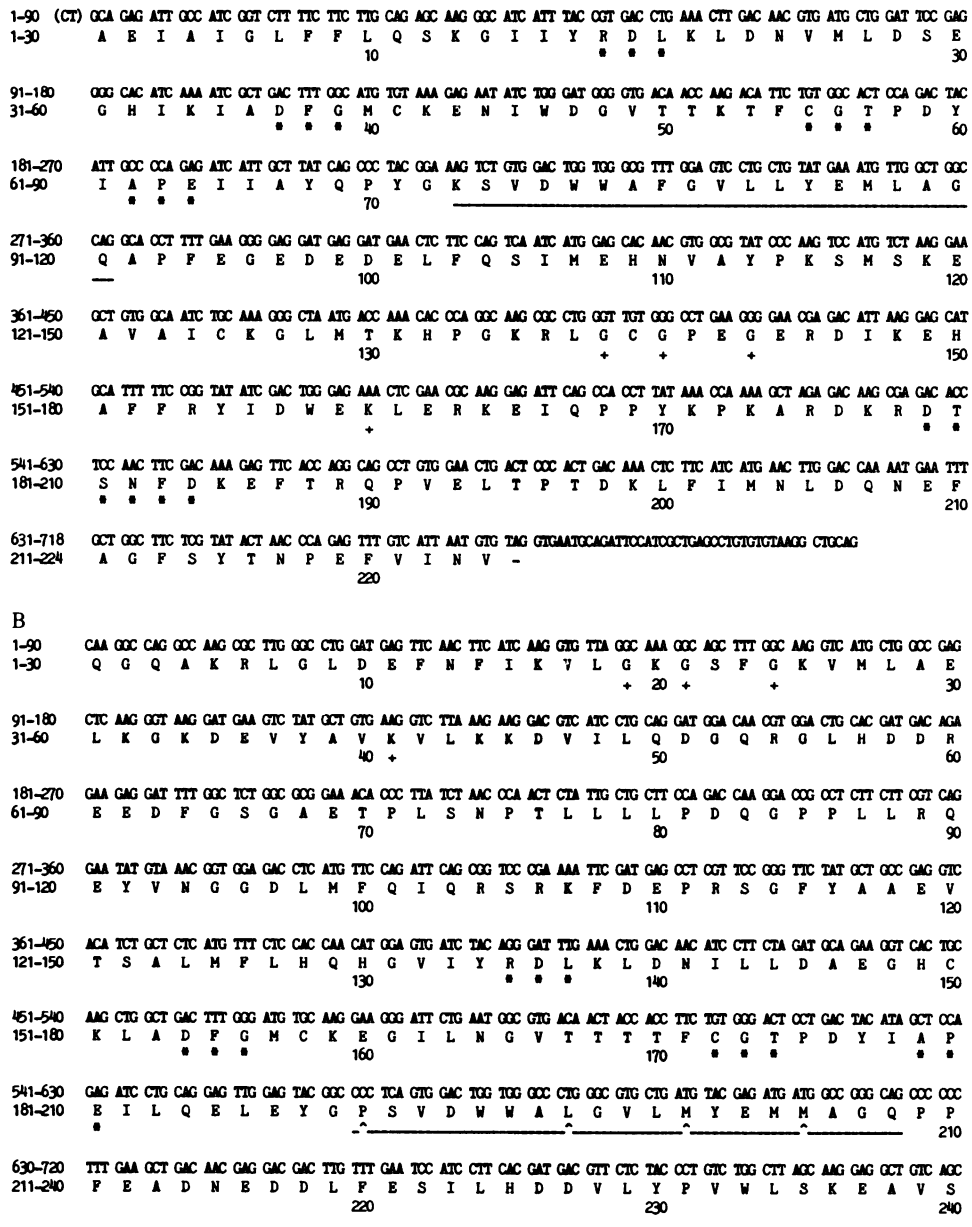

FIG. 2. Nucleotide and deduced amino acid sequences (single-letter code) of the cDNA clones RP41 and RP16. (*A*) Partial sequence of RP41. A 720-base-pair (bp) *Pst* I fragment encoding the 224-amino acid carboxyl-terminal region of rat brain PKC is displayed. Asterisks denote amino acid residues conserved among several previously described protein kinases (see ref. 32). The 19-amino acid peptide that is underlined (amino acids 73–91) corresponds exactly to PKC peptide P2 (see Fig. 1). Amino acids that appear to constitute an ATP binding site consensus sequence are denoted with a plus sign (amino acids 138, 140, 143, and 160). (*B*) Partial sequence of RP16 (a PKC-related cDNA clone) and deduced amino acid sequence of the protein. Notations are as in *A*. The 19-amino acid peptide underlined (amino acids 190–208) is identical to PKC peptide P2 at 15 of 19 positions. Circumflexes denote the four amino acids that differ between P2 and the corresponding region of RP16.

consensus sequence Gly-Xaa-Gly-Xaa-Xaa-Gly-$(Xaa)_{16}$-Lys, in which Xaa is another amino acid, occurs at amino acids 138–143 and 160 in RP41 (Fig. 2*A*) (32). The position of this sequence is very unusual when compared to other protein kinases because, in most of the other kinases (ref. 32; see also clone RP16 below), this sequence is on the amino-

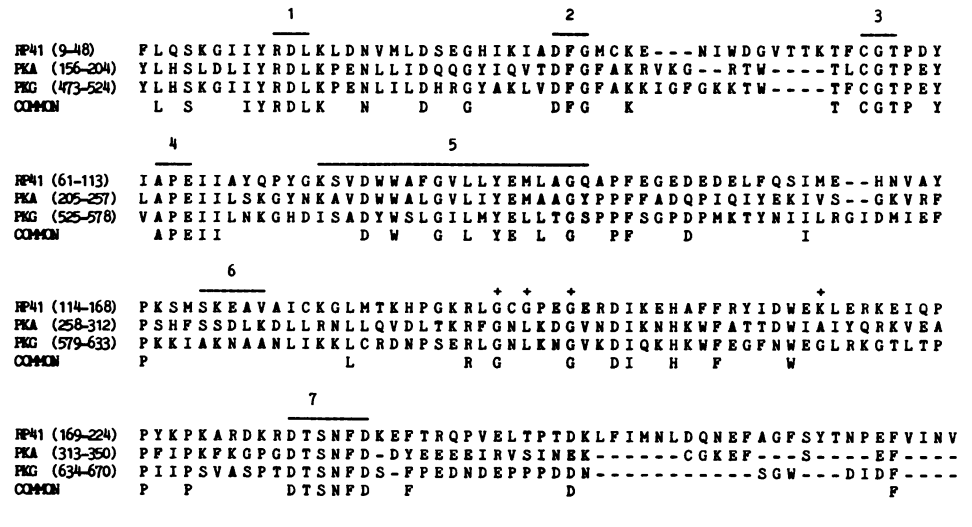

FIG. 3. Alignment of the deduced amino acid sequence (single-letter code) of RP41 with the corresponding regions of PKA and PKG. Amino acid numbering is as given in Fig. 2*A* for RP41 and as listed in the Protein Identification Resource data base for PKA and PKG. Regions 1–7 are discussed in detail in the text. An ATP binding site consensus sequence in RP41 is denoted with plus signs (see text and Fig. 2*B*). The alignment begins nine amino acids prior to the conserved Arg-Asp-Leu homology region (region 1). Region 5 corresponds to PKC peptide P2 (see Fig. 1). Region 6 is conserved only in RP41 and RP16 (see Fig. 4). Also note the complete conservation of the hexapeptide in region 7. Beyond the latter region, all three sequences diverge and then terminate.

terminal side of the Arg-Asp-Leu sequence (region 1 in Fig. 3). It will be of interest to determine if this sequence actually functions as an ATP binding site in PKC.

Sequence Analysis of the cDNA Clone RP16. Restriction enzyme mapping of the RP16 clone indicated that it contained a 2.0-kb cDNA insert. Appropriate restriction fragments were subcloned into the M13 vectors mp18 and mp19, and the complete nucleotide sequence of RP16 was determined. Fig. 2*B* shows a 720-nucleotide segment of this sequence, along with the deduced amino acid sequence, beginning 18 amino acids upstream from an ATP binding site consensus sequence Gly-Xaa-Gly-Xaa-Xaa-Gly-$(Xaa)_{16}$-Lys (amino acids 19–24 and 41 in Fig. 2*B*). As with RP41, RP16 also exhibits all of the homology domains that have been identified in other protein kinases (33), including the Arg-Asp-Leu, Asp-Phe-Gly, Cys-Gly-Thr, and Ala-Pro-Glu clusters (amino acids 135–137, 154–156, 172–174, and 179–181, respectively, in Fig. 2*B*; regions 1–4 in Fig. 4). Furthermore, in the region corresponding to the PKC peptide P2 (amino acids 190–208 in Fig. 2*B*, region 5 in Fig. 4), this clone differs at only four positions. Fig. 4 shows a portion of the amino acid sequence of RP16 aligned with the sequences of RP41, PKA, and PKG. The sequence of RP16 displayed in Fig. 4 exhibits 65% identity with the carboxyl-terminal region of RP41, whereas the homology between this region of RP41 and either PKA or PKG is only about 40%. Sequence breakpoints shown in Fig. 4 were required to obtain the optimal alignments of RP41 and RP16 with PKA and PKG. However, optimal alignments of RP41 and RP16 did not require any sequence breakpoints. Thus, the sequences of RP41 and RP16 are much more similar to each other than they are to the corresponding regions of PKA and PKG (see also *Discussion*).

Analysis of the Transcripts Related to RP41 and RP16. Having obtained a significant amount of nucleotide sequence information from the cDNA clones RP41 and RP16, it was of interest to examine the sizes and abundance of their transcripts in various tissues. Utilizing a ^{32}P-labeled probe prepared from the coding region of the PKC cDNA clone RP41, we detected by blot-hybridization analyses high levels of two distinct transcripts of about 9 and 3.5 kb in the poly(A)$^+$ RNA fraction of rat brain (Fig. 5*A*). These transcripts were also present but at low levels in the poly(A)$^+$ RNA from rat liver and heart, although only the 9-kb transcript was detectable in the latter tissue (data not shown). The relative abundance of these transcripts in these three tissues is consistent with published data on the levels of PKC enzymatic activities and the amounts of PKC determined by immunoassay present in these tissues (35, 36). Fig. 5*B* shows the results of blot-hybridization analyses when the same poly(A)$^+$ RNA samples were hybridized to a ^{32}P-labeled probe prepared from the PKC-related cDNA clone RP16. In this case a single transcript that was ≈7.5 kb in size was detected. The abundance of this transcript was also high in brain, with a moderate level in heart and a low level in liver.

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                          1                   2
                           ___                 ___
RP41 (9-48)      FLQSKGIIYRDLKLDNVMLDSEGHIKIADFGMCKE---NIWDG
RP16 (126-165)   FLHQHGVIYRDLKLDNILLDAEGHCKLADFGMCKE---GILNG
PKA (156-196)    YLHSLDLIYRDLKPENLLIDQQGYIQVTDFGFAKRVKG--RTW
PKG (473-516)    YLHSKGIIYRDLKPENLILDHRGYAKLVDFGFAKKIGFGKKTW

RP41/RP16        FL   G IYRDLKLDN  LD EGH K ADFGMCKE    I  G
RP41/PKA          L S   IYRDLK  N   D  G I   DFG  K
RP41/PKG          L SKGIIYRDLK  N  LD  G  K  DFG  K
COMMON            L     IYRDLK  N   D  G     DFG  K

                        3     4               5
                       __    __          ___________________
RP41 (49-91)     VTTKTFCGTPDYIAPEIIAYQPYGKSVDWWAFGVLLYEMLAGQ
RP16 (166-208)   VTTTTFCGTPDYIAPEILQELEYGPSVDWWALGVLMYEMMAGQ
PKA (197-235)    ----TLCGTPEYLAPEIILSKGYNKAVDWWALGVLIYEMAAGY
PKG (517-554)    ----TFCGTPEYVAPEIILNKGHDISADYWSLGILMYELLTGS

RP41/RP16        VTT TFCGTPDYIAPEI     YG SVDWWA GVL YEM AGQ
RP41/PKA             T CGTP Y APEII    Y K VDWWA GVL YEM AG
RP41/PKG             TFCGTP Y APEII       S D W  G L YE L G
COMMON               T CGTP Y APEI          D W  G L YE   G

                                             6
                                             _____
RP41 (92-123)    APFEGEDEDELFQSIME--HNVAYPKSMSKEAVA
RP16 (209-240)   PPFEADNEDDLFESILH--DDVLYPVWLSKEAVS
PKA (236-267)    PPFFADQPIQIYEKIVS--GKVRFPSHFSSDLKD
PKG (555-588)    PPFSGPDPMKTYNIILRGIDMIEFPKKIAKNAAN

RP41/RP16         PFE   ED LF SI      V YP   SKEAV
RP41/PKA          PF           I      V  P   S
RP41/PKG          PF G D       I          PK   K A
COMMON            PF           I          P
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FIG. 4. Alignment of the deduced amino acid sequences (single-letter code) of RP41 and RP16 with the corresponding regions of PKA and PKG. Each sequence was individually aligned to the others, and the resulting consensus alignment is shown. The first four lines show the alignment of these four sequences. The fifth line, marked RP41/RP16, denotes the residues common to RP41 and RP16; the sixth line, marked RP41/PKA, denotes the residues common to both RP41 and PKA, etc. The bottom line, marked COMMON, lists residues common to all four sequences. Other notations, including regions 1–6, are as described in the legend to Fig. 3. Comparisons of the fifth line, RP41/RP16, to the sixth and seventh lines, RP41/PKA and RP41/PKG, respectively, demonstrate subtle yet distinct differences among these four sequences, as discussed in the text.

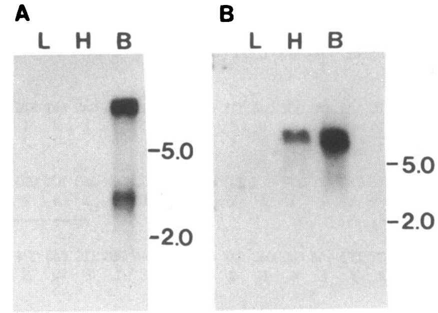

FIG. 5. Blot-hybridization analyses. Five micrograms of poly(A)$^+$ RNA from rat liver (lanes L), heart (lanes H), and brain (lanes B) were applied to each lane and electrophoresed on 1% agarose/formaldehyde gels, blotted onto nylon membranes, and hybridized with ^{32}P-labeled probes. Molecular size markers in kb are to the right of lanes B. (*A*) Results obtained with an RP41 nick-translated probe prepared from the *Pst* I coding region fragment (see Fig. 2*A*). Autoradiography was for 16 hr with intensifying screens. Although not visible in this figure, with a 72-hr exposure, 9-kb and 3.5-kb bands were also detected in the liver RNA and a 9-kb band was detected in the heart RNA. (*B*) Results obtained with an RP16 nick-translated probe prepared from the complete 2-kb *Eco*RI cDNA insert. The original film also displayed a weak 7.5-kb band in the liver RNA.

DISCUSSION

This paper reports the molecular cloning and nucleotide sequence of a cDNA clone designated RP41 that encodes the carboxyl-terminal 224 amino acids of rat brain PKC. The striking homology between this sequence and the carboxyl-terminal catalytic domains of PKA and PKG provides evidence that this region constitutes the catalytic domain of PKC as well. We assume that the amino-terminal region of PKC functions as the regulatory domain, thus mediating the effects of Ca^{2+}, phorbol esters, diacylglycerol, and phospholipids. This interpretation is consistent with the observation that limited proteolysis of PKC generates a fragment that has catalytic activity, even in the absence of Ca^{2+} and phospholipid (37). Thus, the overall structure of PKC is more analogous to PKG than to PKA, since the former contains an amino-terminal regulatory domain, whereas the latter is controlled by two different regulatory subunits encoded by separate genes. Therefore, it may be possible to eliminate the Ca^{2+}-, phospholipid-, and phorbol ester-mediated regulation of PKC by appropriate construction of a truncated cDNA that encodes only the carboxyl-terminal catalytic domain of the enzyme. The effects of such a construct on growth control when introduced into mammalian cells should be of interest.

In this paper we also report the isolation and initial characterization of a cDNA clone, RP16; the deduced amino

acid sequence of RP16 shares 65% homology with the carboxyl-terminal region of the deduced sequence of the PKC cDNA clone RP41 (Fig. 4). This clone also exhibits all of the conserved domains previously identified in other protein kinases (see *Results* and Fig. 2*B*). Furthermore, RP16 and RP41 proteins share additional common amino acid residues in the regions immediately flanking these domains, as shown in detail in Fig. 4. For example, both RP41 and RP16 proteins have the sequence Ala-*Asp-Phe-Gly*-Met-Cys-Lys-Glu in region 1, whereas PKA and PKG contain the sequences Thr-*Asp-Phe-Gly*-Phe-Ala-Lys-Arg and Val-*Asp-Phe-Gly*-Phe-Ala-Lys-Lys, respectively; a sequence Pro-Asp-Tyr-Ile located between regions 2 and 3 is present in both RP41 and RP16 proteins, and the sequence Ser-Lys-Glu-Ala-Val is present in region 6 of both RP41 and RP16 proteins, yet we have not identified this pentapeptide in any other protein sequence in the Protein Identification Resource data base. Taken together, these results suggest that RP16 is a member of a family of PKC-related genes. It is of interest that, as with expression of the PKC clone RP41, sequences homologous to RP16 are also expressed at high levels in the brain.

The existence of a PKC-related gene family may have considerable implications with respect to growth control and tumor promotion (38). It is possible, for example, that the pleiotropic effects of the phorbol ester tumor promoters (38) and of PKC (3) may reflect the activities of multiple forms of PKC or PKC-related proteins. Other laboratories have recently obtained evidence for the heterogeneity of PKC proteins as well as for the existence of multiple phorbol ester-activated protein kinases (ref. 39; S. Jaken, personal communication). During the preparation of this manuscript, three other laboratories reported the isolation of cDNA clones encoding PKC. Ono *et al.* (40) have isolated a rat brain PKC cDNA clone. The partial nucleotide sequence that they reported is identical to the sequence of RP41 that we describe in the present study. In addition, their peptide sequence data suggest the existence of more than one form of PKC. Knopf *et al.* (41) have isolated and sequenced three closely related yet distinct forms of PKC-related rat cDNA clones (PKC-I, -II, and -III) and have presented evidence for the existence of additional PKC-related genes. Their partial clone III has a deduced amino acid sequence that is virtually identical with the deduced sequence of RP41 shown in Fig. 2*A*. Parker *et al.* (42) report the isolation of a cDNA segment encoding a bovine PKC and also a homologous human clone as well as two other closely related bovine and human cDNA sequences (43). All of these sequences differ appreciably from the sequences of our clones RP41 and RP16. Thus, the results of these three studies are consistent with our evidence for the existence of a PKC-related multigene family. Furthermore, the RP16 clone differs considerably from any of the previously reported PKC-related clones and, thus, further extends the repertoire of this gene family. Additional studies with the cDNA clones that we and others have obtained should help to clarify the biologic basis of this heterogeneity and may contribute to our understanding of the molecular mechanisms involved in the pleiotropic responses of cells to tumor promoters and related agonists.

Note Added in Proof. Since the submission of this manuscript we have analyzed the 5′ terminus of a full-length cDNA clone corresponding to RP41 and have found that its sequence 5′ to the internal *Eco*RI site is identical to the 5′ terminus of PKC-II described by Knopf *et al.* (41).

We thank Janusz Wideman, Peter Kao, May Chang, and Stan Stein for advice and assistance in the peptide purifications, amino acid analyses, and gas-phase protein-sequencing studies. We also thank Jurgen Brosius for the rat brain λgt10 cDNA library and the following individuals for helpful advice and discussion: Scott Zeitlin, Paul Maddon, John Celenza, Vasso Episkopou, Robert Liskamp, Marcelo Soares, David Julius, Dan Littman and Yu-Ching Pan. The authors also express their gratitude to James Murphy for expert technical assistance. This work was supported by a grant from the National Cancer Institute (CA02656) to I.B.W.; G.M.H. is in the Medical Scientist Training Program.

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