



ELSEVIER

Biochimica et Biophysica Acta 1450 (1999) 99–106

BIOCHIMICA ET BIOPHYSICA ACTA

BBA

Short sequence-paper

PKC ν , a new member of the protein kinase C family, composes a fourth subfamily with PKC μ ¹

Akiko Hayashi ^a, Naohiko Seki ^{a,b}, Atsushi Hattori ^{a,c}, Sumie Kozuma ^a,
Toshiyuki Saito ^{a,*}

^a Genome Research Group, National Institute of Radiological Sciences, Anagawa 4-9-1, Inage-ku, Chiba 263-8555, Japan

^b Laboratory of Gene Function II, Kazusa DNA Research Institute, 1532-3 Yana, Kisarazu, Chiba 292-0812, Japan

^c Biotechnology and Medical Engineering Field, Aisin Cosmos R & D Co., Ltd., 5-2-11 Sotokanda, Chiyoda-ku, Tokyo 101-0021, Japan

Received 23 February 1999; accepted 9 March 1999

Abstract

Members of the protein kinase C (PKC) family of serine/threonine kinases are thought to play critical roles in the regulation of cellular differentiation and proliferation in many cell types. An additional member of the PKC family was identified through human expressed sequence tag (EST) database search and its full length cDNA was isolated. Sequence analysis revealed that the predicted translation product was composed of 890 amino acid residues and that the protein has 77.3% similarity to human PKC μ (PKC μ) and 77.4% similarity to mouse PKD (the mouse homolog of PKC μ). We designated the new member as protein kinase C ν (PKC ν). The PKC ν messenger RNA was ubiquitously expressed in various tissues when analyzed by Northern blots and reverse transcriptase-coupled polymerase chain reaction (PCR) analyses. The chromosomal location of the gene was determined between markers WI-9798 and D2S177 on chromosome 2p21 region by PCR-based methods with both a human/rodent monochromosomal hybrid cell panel and a radiation hybrid mapping panel. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Protein kinase C; Chromosome mapping; 2p21; PKC μ ; PKD; Cysteine-rich motif; PH domain

Protein kinase C (PKC) enzymes define a family of serine/threonine kinases which should contribute to the regulation of cellular differentiation and proliferation in various types of cells [1]. They are typically activated by the second messenger diacylglycerol and participate in cellular responses to various agonists like hormones, neurotransmitters, and growth factors [1–4].

Molecular cloning of various PKC isoforms has established that PKC is a multigene family [5]. To date, ten members have been identified, which can be grouped in three classes according to their primary structure and in vitro activation requirements [1,3]. The first group, the conventional PKCs (PKC α , β 1, β 2, and γ), require Ca²⁺ to be activated in the presence of phosphatidylserine. The second group, the novel PKCs (PKC δ , ϵ , η , θ), are Ca²⁺-independent. The third group, the atypical PKCs (PKC ζ and λ) are Ca²⁺-independent and are not stimulated by diacylglycerol or the tumor promoter 12-*O*-tetradecanoyl phorbol-13-ester. All PKC isozymes share a conserved catalytic kinase domain in the carboxy

* Corresponding author. Fax: +81-43-251-9818;
E-mail: t_saito@nirs.go.jp

¹ The nucleotide sequence data reported in this paper have been deposited in the DDBJ, EMBL and GenBank databases under the accession number AB015982.

terminal region and an amino terminal regulatory site (C_1). Common features of the C_1 domain are a conserved pseudosubstrate site and two adjacent amino terminal cysteine cluster that are responsible for phorbol ester binding [1,3].

An additional lone isoform, PKC μ , which is also referred to as PKD in mouse, has been described [6,7]. Lack of the typical pseudosubstrate site as well as the presence of two unique amino-terminal hydrophobic domains together with its unusually large molecular size are characteristic features of this PKC isozyme. Furthermore, the presence of a pleckstrin homology (PH) domain in the regulator region [8] is an additional characteristic feature. From the functional analysis of the PKD, diacylglycerol, phorbol ester, and L- α -phosphatidyl-D-myoinositol-4,5-bisphosphate were found to promote PKC μ /PKD kinase activity [8,9].

Database search against the public EST division (GenBank release, April 1997) using the tBLASTN program to compare with the conserved serine/threonine kinase domain reported several ESTs showing the highest similarity to PKC μ /PKD as follows: GenBank/EMBL accession numbers AA379132, AA483305, N28764 and N46176. Utilizing the consensus sequence of these ESTs, the initial fragment of the cDNA was amplified from an 8-week human fetus cDNA library using primers, 5'-GAAAACA-TAGAAAGACTGGGAGGGATGTGG-3' (corresponds to nt 2333 to 2362) and 5'-AGACATA-CAACTCAGCACTTAGACCAGCAG-3' (corresponds to nt 4017 to 3988). To obtain a full-length cDNA of the new PKC member, 5'- and 3'-rapid amplification of cDNA end (RACE) were performed. For 5'-RACE, nested primers, 5'-ATGCAGATT-CCTCAAAGCAACAAGTATCTG-3' (corresponds to nt 2628 to 2599) and 5'-TGACTTTCTTG-TTTTGTGGGAATCTCATC-3' (corresponds to nt 2411 to 2382) were used with Marathon cDNA amplification kit (Clontech, USA). The second 5'-RACE experiments were performed using nested primers, 5'-ATTATGAGAGCTGTCCCCATTGT-TCTCACC-3' (corresponds to nt 2076 to 2047) and 5'-TGCTGCTCTTCTCTTTGTGTGCTTGA-TGG-3' (corresponds to nt 1801 to 1772), and the third 5'-RACE experiments were performed using nested primers, 5'-TCACCACAGTAATCACAGA-AAGTAGGAGCT-3' (corresponds to nt 1073 to

1044) and 5'-TAGAGAGTATGTGGACGAATC-TGGAAGT-3' (corresponds to nt 1028 to 1001). To mask possible artificial mutation occurring in the PCR process, multiple 5'-RACE products were cloned and sequenced by the dideoxy chain-termination method with a 377 DNA sequencer (Applied Biosystems, USA) according to the supplier's instructions. The resultant consensus sequence was employed as the correct cDNA sequence.

The determined nucleotide sequence and deduced amino acid sequence are shown in Fig. 1. The cDNA of 4017 bp contains an open reading frame of 2670 bp with a 5'-untranslated region of 555 bp, and a 3'-noncoding region of 792 bp. The open reading frame encodes a putative protein of 890 amino acid residues having a molecular mass of 100.5 kDa (Fig. 1). The nucleotide sequence data reported here will appear in the DDBJ, EMBL, and GenBank nucleotide sequence databases under accession number AB015982.

A homology search of the cDNA clone revealed that it best matched with human PKC μ (accession number X75756, 69.4% identity and 77.3% similarity) and mouse PKD (accession number Z34524, 69.1% identity and 77.4% similarity). Thus, the isolated cDNA represents a new member of PKC family, which composes a fourth PKC subgroup with PKC μ /PKD. This novel PKC and its gene symbol were designated as PKC nu (PKC ν) and PRKCN, respectively, according to the proposal by the Human Gene Nomenclature Committee. Comparison of the predicted amino acid sequence of PKC ν with PKC μ and PKD sequences revealed that they compose a fourth PKC subgroup (Fig. 2). By hydropathy analysis, PKC μ appeared to harbor an NH₂-terminal hydrophobic sequence (Pro-35 to Ile-55), but PKC ν contained no putative transmembranous portion. The cysteine-rich motif, His-X₁₂-Cys-X₂-Cys-X₁₀₋₁₄-Cys-X₂-Cys-X₄-His-X₂-Cys-X₇-Cys, which complexes with the heavy metal ions zinc and cadmium and is responsible for phorbol ester binding [2], is fully conserved among the three enzymes (Fig. 1). The catalytic domain contains all conserved amino acid motifs considered to be functionally critical for the enzymatic activity of protein kinases [10]. And the residues of similar structure in serine/threonine protein kinase catalytic domain are conserved in PKC ν except Asn-689 (aa 576–832). The PKC ν con-

aaagttcatccccccagaatgaaaaatgaggacatttgagaaggtgatttaaggtgtggac	60
atttgagaaggtgtcctatcaaattagtaaaccaaggaagactgaatagattaatc	120
caaacacttactgttttttaaacgaagaggatttcaccttgacagaaaaaactttta	180
ttcaatatgtatttcctgaaattaaagagacaagtacagactgaaaggaaaatagattcg	240
taaaataagctacgtcaactctatcctgctgaggatagctcagtgatgttaaactctttac	300
aaatccctggtgtgtcttctacagacaagactgctttttgatgggactgatattaagaga	360
aataggacctttggggcattcaactccttgataaaaacttaaagtatcggcatgagtggc	420
ttaacagaggaataaagaagttttcaactaaatccaaaagtgcggtcattttctttact	480
gctgttatttttaaaaacctcttcataaccattgaaaaagaatcgacaactattttaaaag	540
attaaagaaagcagATGTCTGCAATAATCCCTCCATCAGCCAGAAGTCTGTATTA	600
M S A N N S P P S A Q K S V L	15
CCCACAGCTATTCCTGTGTGCTTCCAGCTGCTTCTCCGTGTTCAAGTCTTAAGACGGGA	660
P T A I P A V L P A A S P C S S P K T G	35
CTCTCTGCCGACTCTCTAATGGAAGCTTCAGTGCACCATCACTCACCAACTCCAGAGGC	720
L S A R L S N G S F S A P S L T N S R G	55
TCAGTGCATACAGTTTCACTTACTGCAAATGGCCTCACACGGGAGAGTGTACCATT	780
S V H T V S F L L Q I G L T R E S V T I	75
GAAGCCCAGGAAGTGTCTTTATCTGCTGTCAAGGATCTTGTGTGCTCCATAGTTTATCAA	840
E A Q E L S L S A V K D L V C S I V Y Q	95
AAGTTTCCAGAGTGTGGATTCTTTGGCATGTATGACAAAATTCTTCTTTTCGCCATGAC	900
K F P E C G F F G M Y D K I L L F R H D	115
ATGAACTCAGAAAACATTTTGCAGCTGATTACCTCAGCAGATGAAATACATGAAGGAGAC	960
M N S E N I L Q L I T S A D E I H E G D	135
CTAGTGAAGTGGTTCTTTCAGCTTTAGCCACAGTAGAAGACTTCCAGATTTCGTCCACAT	1020
L V E V V L S A L A T V E D F Q I R P H	155
ACTCTCTATGTACATTCTTACAAAGCTCCTACTTTCTGTGATTACTGTGGTGAGATGCTG	1080
T L Y V H S Y K A P T F C D Y C G E M L	175
TGGGATTGGTACGTCAAGGACTGAAATGTGAAGGCTGTGGATTAATTAACATAAACRA	1140
W G L V R Q G L K C E G C G L N Y H K R	195
TGTGCTTCAAGATTCCAATAACTGTAGTGGAGTAAGAAAAGAGACGTCTGTCAAATGTA	1200
C A F K I P N N C S G V R K R R L S N V	215
TCCTTACCAGGACCCGGCTCTCAGTTCGAAGACCCCTACAGCCTGAATATGTAGCCCTT	1260
S L P G P G L S V P R P L Q P E Y V A L	235
CCCAGTGAAGAGTACATGTCCACCAGGAACCAAGTAAGAGAATTCCTTCTTGGAGTGGT	1320
P S E E S H V H Q E P S K R I P S W S G	255
CGCCAATCTGGATGGAAAAGATGTAATGTGCAGAGTAAAAGTTCCACACACATTTGCT	1380
R P I W M E K M V M C R V K V P H T F A	275
GTTCACTCTTACCCGTCACGATATGTCACTACTGCAAGCGGTTACTGAAAGGCCTC	1440
V H S Y T R P T I C O Y C K R L L K G L	295
TTTCGCCAAGGAATGCAGTGTAAAGATTGCAAATTCAACTGCCATAAACGCTGTGCATCA	1500
F R Q G M Q C K D C K F N C H K R C A S	315
AAAGTACCAAGAGACTGCCTTGGAGAGGTTACTTTCAATGGAGAACCCTCCAGTCTGGGA	1560
K V P R D C L G E V T F N G E P S S L G	335
ACAGATACAGATATACCAATGGATATTGACAATAATGACATAAATAGTGATAGTAGTCGG	1620
T D T D I P M D I D N N D I N S D S S R	355
GGTTTGGATGACACAGAAGGCCATCACCCCAAGATAAGATGTTCTTCTTGGATCCA	1680
G L D D T E E P S P P E D K M F F L D P	375
TCTGATCTCGATGTGGAAAGAGATGAAGAAGCCGTTAAAACAATCAGTCCATCAACAAGC	1740
S D L D V E R D E E A V K T I S P S T S	395
AATAATATTCGCTAATGAGGGTTGTACAATCCATCAAGCACACAAAGAGGAAGAGCAGC	1800
N N I P L M R V V Q S I K H T K R K S S	415

Fig. 1. Nucleotide and deduced amino acid sequences of PKCv. The amino acid sequence is shown below the nucleotide sequence. Asterisks indicate in-frame stop codons. The cysteine-rich domains are underlined and the PH domain is double-underlined.

ACAATGGTGAAGGAAGGGTGGATGGTCCATTACACCAGCAGGGATAACCTGAGAAAGAGG	1860
<u>T M V K E G W M V H Y T S R D N L R K R</u>	435
CATTATTGGAGACTTGACAGCAAATGTCTAACATTATTTTCAGAATGAATCTGGATCAAAG	1920
<u>H Y W R L D S K C L T L F Q N E S G S K</u>	455
TATTATAAGGAAATTCACACTTTCAGAAATTCCTCCGCATATCTTCACCACGAGATTTTACA	1980
<u>Y Y K E I P L S E I L R I S S P R D F T</u>	475
AACATTTTACAAGGCAGCAATCCACACTGTTTTGAAATCATTACTGATACTATGGTATAC	2040
<u>N I S Q G S N P H C F E I I T D T M V Y</u>	495
TTCGTTGGTGAGAACAATGGGGACAGCTCTCATAATCCTGTTCTTGTCTGCCACTGGAGTT	2100
<u>F V G E N N G D S S H N P V L A A T G V</u>	515
GGACTTGATGTAGCACAGAGCTGGGAAAAGCAATTCGCCAAGCCCTCATGCCTGTTACT	2160
<u>G L D V A Q S W E K A I R Q A L M P V T</u>	535
CCTCAAGCAAGTGTGGCACTTCTCCAGGGCAAGGAAAAGATCACAAAAGATTTGTCTACA	2220
<u>P Q A S V C T S</u> P G Q G K D H K D L S T	555
AGTATCTCTGTATCTAATTTGTCAGATTCAGGAGAATGTGGATATCAGTACTGTTTACCAG	2280
S I S V S N C Q I Q E N V D I S T V Y Q	575
ATCTTTSCAGATGAGGTGCTTGGTTCAGGCCAGTTTGGCATCGTTTATGGAGGAAAACAT	2340
I F A D E V L G S G Q F G I V Y G G K H	595
AGAAAGACTGGGAGGGATGTGGCTATTAAGTAATTGATAAGATGAGATTCGCCACAAAA	2400
R K T G R D V A I K V I D K M R F P T K	615
CAAGAAAGTCAACTCCGTAATGAAGTGGCTATTTTACAGAATTTGCACCATCCTGGGATT	2460
Q E S Q L R N E V A I L Q N L H H P G I	635
GTAACCTGGAATGTATGTTGAAACCCAGAACGAGTCTTTGTAGTAATGGAAAAGCTG	2520
V N L E C M F E T P E R V F V V M E K L	655
CATGGAGATATGTTGAAATGATTCATCCAGTGAGAAAAGTCGGCTTCCAGAACGAATT	2580
H G D M L E M I L S S E K S R L P E R I	675
ACTAAATTCATGGTCACACAGATACTTGTGGCTTTGAGGAATCTGCATTTTAAGAATATT	2640
T K F M V T Q I L V A L R N L H F K N I	695
GTGCACTGTGATTTAAAGCCAGAAAATGTGCTGCTTGCATCAGCAGAGCCATTTCCCTCAG	2700
V H C D L K P E E N V L L A S A E P F P Q	715
GTGAAGCTGTGTGACTTTGGATTTGCACGCATCATTGGTGAAAAGTCATTCAGGAGATCT	2760
V K L C D F G F A R I I G E K S F R R S	735
GTGGTAGGAACTCCAGCATACTTAGCCCTGAAGTTCTCCGGAGCAAAGGTTACAACCGT	2820
V V G T P A Y L A P E V L R S K G Y N R	755
TCCCTAGATATGTGGTCAGTGGGAGTTATCATCTATGTGAGCCTCAGTGGCACATTTCCCT	2880
S L D M W S V G V I I Y V S L S G T F P	775
TTTAATGAGGATGAAGATATAAATGACCAAATCCAAAATGCTGCATTTATGTACCCACCA	2940
F N E D E D I N D Q I Q N A A F M Y P P	795
AATCCATGGAGAGAAATTTCTGGTGAAGCAATTGATCTGATAAACAATCTGCTTCAAGTG	3000
N P W R E I S G E A I D L I N N L L Q V	815
AAGATGAGAAAACGTTACAGTGTGACAAATCTTTAGTCATCCCTGGCTACAGGACTAT	3060
K M R K R Y S V D K S L S H P W L Q D Y	835
CAGACTTGGCTTGACCTTAGAATTTGAAACTCGCATTTGGAGAACGTTACATTACACAT	3120
Q T W L D L R E F E T R I G E R Y I T H	855
GAAAGTGATGATGCTCGCTGGGAAATACATGCATACACATAACCTTGTATACCCAAAG	3180
E S D D A R W E I H A Y T H N L V Y P K	875
CACTTCATTATGGCTCCTAATCCAGATGATATGGAAGAAGATCCTTAAAtcactgagctaa	3240
H F I M A P N P D D M E E D P *	890
cctaataaggaaggatttcattttatggactgatattttgctgtgtaacttgttcttcg	3300
tagattgtcatctgcagtctgcaaagatatgaagaatatgataacgaataagtacac	3360

Fig. 1 (continued).

```

cagtactgtagttcataatgagtaggtacaggcgggaaactgaataataagaagtcataa 3420
tggaaatcaaggtgaagcctttttataaaacttttttagcctaagcaataactggttttgtat 3480
tttttcttaatccttcactttaataacaataggctcaacttaatttgctctccattttctct 3540
ttatataatataatataataaaaaataaataatagtttggttggttggttttttaa 3600
ggaaaaacaagtcagctagcatccagttactatagcttggtctaaattataacaagact 3660
tacaagattgattactcgacaggccttgatattaagagataactgtgaggttaccattatg 3720
tgatgttactataaggacttttaacattggtttaacaaacatagaggcattgaagggtt 3780
tttcttagatgcctagaaaaagcacactgggctgttttacctttcttttttaggtcaatc 3840
aagactccaaaatagtgattcctaaccctttttggagttgctctgctactctgaatatgtt 3900
ctatacagcataaggattgtcaccttctgtgtgttgcaacagcttctaagataattaggg 3960
acaaatgatgttacaaaaggaagagtagctgctggtctaagtgtgagttgtatgtct 4017

```

Fig. 1 (continued).

tains a PH domain (aa 417–542) inserted between the cysteine-rich domain and catalytic domain presented in carboxyl terminus. The PH domain was initially identified as a homologous region of approximately 120 amino acids, that is duplicated in pleckstrin, the major substrate of protein kinase C in platelets [11,12]. It was subsequently found that this domain is present in large variety of proteins involved in cellular signaling or cytoskeletal functions [13,14]. It has been suggested that PH domains mediate intermolecular and/or intramolecular interactions, but their function and binding partners remain unclear. Interestingly, PDGF stimulates PKD through the activation of PLC γ and other PKC [15] and the PKD PH domain plays a negative role in the regulation of enzyme activity [16]. Conservation of PH domain between PKC ν and PKC μ /PKD as well as the overall homology suggests that they may share a common interacting factor(s) and be regulated in a similar fashion.

Tissue expression of PCK ν was investigated by Northern blot analysis. A single 6.2-kb transcript was detected in varying degrees in all adults tissues examined (Fig. 3). We also performed reverse transcription-coupled PCR (RT-PCR) in 12 human tissues with the primer set, 5'-GAAAACATAGAAA-GACTGGGAGGGATGTGG-3' (corresponds to nt 2333 to 2362) and 5'-ATGCAGATTCCTCAAAG-CAACAAGTATCTG-3' (corresponds to nt 2628 to 2599). The RT-PCR were carried out as described previously [17–20]. Templates of the human tissue poly(A)⁺ RNAs were purchased from Clontech. A single 300 bp amplified product was detected in all tissues at the same level (data not shown). Considering its ubiquitous expression in a wide variety of

human tissues, PKC ν seems to be involved in a basic housekeeping function in cells.

PCR-based chromosome mapping was carried out basically following our previous papers [17–20]. The human–rodent monochromosomal somatic cell hybrid panel (Mapping panel #2) and the radiation hybrid panel (Genebridge 4) were purchased from the National Institute of General Medicine Service, Coriell Cell Repositories and Research Genetics, USA, respectively. Primers used for PCR amplification were 5'-AAGTGATGATGCTGCCTGGGA-AATACATGC-3' (corresponds to nt 3122 to 3152) and 5'-AGACATACAACTCAGCACTTAGACCA-GCAG-3' (corresponds to nt 4017 to 3988). An apparent single amplified product with an expected size of 888 bp was amplified only in the lane of the hybrid containing human chromosome 2 (data not shown). The same signal was also detected from control human genomic DNA and cDNA. We determined the further subchromosomal location of the gene by the radiation hybrid mapping method. PCR analysis of the radiation hybrid panel was performed with the same primers used in the above assay. The radiation hybrid data were statistically processed and analyzed by the RHMAPPER software package (<http://carbon.wi.mit.edu:8000/cgi-bin/contig/rhmapper.pl>). The data vector for PKC ν gene was 1000010000 0111100010 1101001011 0000100000 0200211000 0000010112 0000101011 1100110001 1101010100 001 and the consequent report indicated the gene was mapped between markers WI-9798 and D2S177, both of which have been cytogenetically mapped to 2p21 region (Fig. 4). The position of the gene is 5.76cR proximal from WI-9798 (lod > 3.0). The position is 22.39 cR distal from the

PKCmu	1	-----MSAPPVLRPPSPLLPVAAAAAALVPGSGPGPAPFLAPVAAPVGGI
PKD	1	-----MSVPPILRPPSPLLPAAAAAALVPGS.GPAPFPAPGAAPAGGI
PKCnu	1	MSANNSPPSAOKSVLPTAIPAVLPAASPCSSPKTGLSARLSNGSFSAPSITNSRGSVHTV
PKCmu	50	SFHLOIGLSREPVLLQDSSGDYSLAHVREMACSIVDOKFPECGFYGYDKILLFRHDP
PKD	48	SFHLOIGLSREPVLLQDSSGDYSLAHVREMACSIVDOKFPECGFYGYDKILLFRHDP
PKCnu	61	SFHLOIGLIRESVTTI...EAQETSLSAVKDILVCSIVVOKFPECGFYGYDKILLFRHDMN
PKCmu	110	SENILQLVKAASDIOEGDLIEVVLSRSATFEDFQIRPHALFVHSYRAPAFCDHCGEMLWG
PKD	108	SDNILQLVKIASDIOEGDLIEVVLSASATFEDFQIRPHALFVHSYRAPAFCDHCGEMLWG
PKCnu	118	SENILQLITSADEIHEGDLIEVVLSALATVEDFQIRPHALFVHSYRAPAFCDHCGEMLWG
PKCmu	170	LVRQGLKCEGCGLNHYHKRCAPKIPNNCSGVRRRRLSNVSLTGVSTIRTSAAELSTSA
PKD	168	LVRQGLKCEGCGLNHYHKRCAPKIPNNCSGVRRRRLSNVSLTGVSTIRTSAAELSTSA
PKCnu	178	LVRQGLKCEGCGLNHYHKRCAPKIPNNCSGVRRRRLSNVSLPQPG.....LSVP..
PKCmu	230	PLL.....OKSPSESFIGREKRSNSQSYIGRPIHLDKILMSKVVPHTFVIVHSYTRP
PKD	228	PLLSPVSPGFEOKSPSESFIGREKRSNSQSYIGRPIQLDKILMSKVVPHTFVIVHSYTRP
PKCnu	226	...RPLQPEYVALPSEESHVHQEPKSRIPSWSGRPWMEKVMVCKVVPHTFAVHSYTRP
PKCmu	282	TVCQYCKKLLKGLFRQGLQCKDCRFNCHKRCAPKVPNNCLGEVTINGDILLSPGAESDVVM
PKD	288	TVCQYCKKLLKGLFRQGLQCKDCRFNCHKRCAPKVPNNCLGEVTINGDILLSPGAESDVVM
PKCnu	283	TTCQYCKRLLKGLFRQGLQCKDCRFNCHKRCASKVERDCLGEVTFNGEPSSITGTDITPM
PKCmu	342	EEGSDDDNSERNISGLMDDMEAMVODAEAMAECONDSEMODPDDEEDANRTISPSTS
PKD	348	EEGSDDDNSERNISGLMDDMEAMVODTEAMAECONDSEMODPDADQEDSNRTISPSTS
PKCnu	343	DIDNNDINSISSRGLDDTEEPSPPEDKMFFLDPSDLVBERD.....EAVKTIISPSTS
PKCmu	402	NNIPLMRVVQSVKHTKRKSSSTMKEGWMVHYTSKDTLRKRHYWRDLSKICITLFQNDTGSR
PKD	408	NNIPLMRVVQSVKHTKRKSSSTMKEGWMVHYTSKDTLRKRHYWRDLSKICITLFQNDTGSR
PKCnu	396	NNIPLMRVVQSVKHTKRKSSSTMKEGWMVHYTSKDTLRKRHYWRDLSKICITLFQNDTGSR
PKCmu	462	YYKEIPLSEIISLEPVKTSALIPNGANPHCFEITTANVVYVGENVVPSSPSPNNSVIT
PKD	468	YYKEIPLSEIISLEPAKPSALTPVGGATPHCFEITTANVVYVGENVVPSSPSPNNSVIT
PKCnu	456	YYKEIPLSEIIRISSPRDFTNISQGSNPHCFEITTDTMVYVGGE...NNGDSSHPVIAA
PKCmu	522	SGVGAADVARMWEIAIQHALMPVIPKGS...SVGSGSNLHHDISVSVSNCOIQENVDIS
PKD	528	SGVGAADVARMWEIAIQHALMPVIPKGS...SVGSGSNLHHDISVSVSNCOIQENVDIS
PKCnu	513	TGVGLDVAQSWEKAIRQALMPVTPQASVCTSPGQGD.HKDTSTISVSNCOIQENVDIS
PKCmu	579	TVYQIFPDEVLGSGQFGIVYGGKHKRKTGRDVAIKIIDKLRFPPTKQESQLRNEVAILQNLH
PKD	585	TVYQIFPDEVLGSGQFGIVYGGKHKRKTGRDVAIKIIDKLRFPPTKQESQLRNEVAILQNLH
PKCnu	572	TVYQIFADEVLGSGQFGIVYGGKHKRKTGRDVAIKVIDKLRFPPTKQESQLRNEVAILQNLH
PKCmu	639	HPGVNLECMFETPERVFVMEKLGDMLEMILSSEKGRLEPHITKFLITQILVALRHLH
PKD	645	HPGVNLECMFETPERVFVMEKLGDMLEMILSSEKGRLEPHITKFLITQILVALRHLH
PKCnu	632	HPGVNLECMFETPERVFVMEKLGDMLEMILSSEKGRLEPHITKFLITQILVALRHLH
PKCmu	699	FKNIVHCDLKPENVLLASADPPQVKLCDFGFARIIGEKSFRRSVVGTTPAYLAPEVLRNK
PKD	705	FKNIVHCDLKPENVLLASADPPQVKLCDFGFARIIGEKSFRRSVVGTTPAYLAPEVLRNK
PKCnu	692	FKNIVHCDLKPENVLLASADPPQVKLCDFGFARIIGEKSFRRSVVGTTPAYLAPEVLRNK
PKCmu	759	GYNRSLDMWSVGVIIYVSLSGTFPFNEDEDIHDQIQNAAFMYPPNPWKEISHEAIDLINN
PKD	765	GYNRSLDMWSVGVIIYVSLSGTFPFNEDEDIHDQIQNAAFMYPPNPWKEISHEAIDLINN
PKCnu	752	GYNRSLDMWSVGVIIYVSLSGTFPFNEDEDIHDQIQNAAFMYPPNPWKEISHEAIDLINN
PKCmu	819	LLQVKMRKRYSDKTLSHPWLDYQOTWLDLRELECRIGERYITHESDDLRWEKYAGEORL
PKD	825	LLQVKMRKRYSDKTLSHPWLDYQOTWLDLRELECRIGERYITHESDDLRWEKYAGEORL
PKCnu	812	LLQVKMRKRYSDKTLSHPWLDYQOTWLDLRELECRIGERYITHESDDLRWEKYAGEORL
PKCmu	879	QYPHHLINPSASHSDTPEETEEMKALGERVSIL*
PKD	885	QYPAHLISLSASHSDSPEAEREMKALSERVSIL*
PKCnu	872	VYPKHFI...MAPNPDMDPEP*-----

Fig. 2. Amino acid sequence alignment of the PKCv with PKCμ (accession number X75756) and PKD (accession number Z34524) proteins. The amino acid sequences of PKCv, PKCμ, and PKD were analyzed and aligned by GCG software. The predicted amino acids are represented in one-letter designation. The most identical amino acids at each position are black-boxed and similar ones are shadowed.

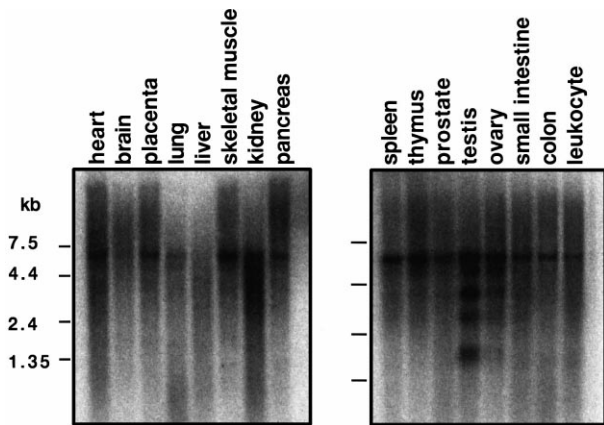


Fig. 3. Northern blot analysis of PKCv. Northern blot filters containing adult human poly(A)⁺ RNAs (2 µg/lane) were purchased from Clontech Laboratories (Palo Alto, CA), and hybridization and washing were performed following the manufacturer's instructions. A cDNA fragment corresponding to 3122–3988 was labeled with [α -³²P]dCTP and used as a hybridization probe. Size markers (left) are in kilobases.

upstream intermediary, PKCε [21], the location of which has been determined by RH mapping method also in our hands (Seki et al., unpublished data). Recently, genomic amplification was observed on human chromosome 2p21 region in thyroid cancer by

use of the comparative genome hybridization (CGH) method [22]. The sequence analysis of a BAC clone from the amplified genomic region revealed that the BAC contained a fragment of PKCε [22], suggesting that PKCε may play a role in thyroid cancer development. Fig. 4 shows a schematic representation of chromosomal positions of the PKCv and PKCε genes relative to other genetic markers on the framework of radiation hybrid mapping. Our precise chromosomal positioning data of such genes would contribute toward on-going positional candidate approaches for the disease genes linked to this genomic locus.

References

- [1] Y. Nishizuka, Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C, *Science* 258 (1992) 607–614.
- [2] R.M. Bell, D.J. Burns, Lipid activation of protein kinase C, *J. Biol. Chem.* 266 (1991) 4661–4664.
- [3] H. Hug, T.F. Sarre, Protein kinase C isoenzymes: divergence in signal transduction?, *Biochem. J.* 291 (1993) 329–343.
- [4] L.V. Dekker, P.J. Parker, Protein kinase C – a question of specificity, *Trends Biochem. Sci.* 19 (1994) 73–77.

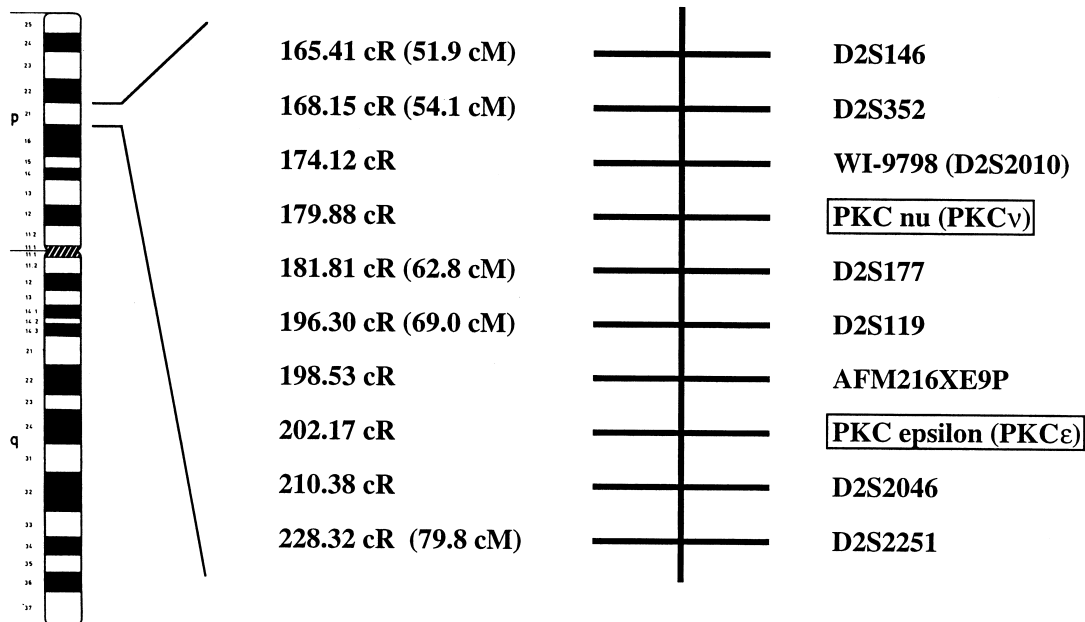


Fig. 4. Chromosomal placement of human PKCv and PKCε genes at a relative distance to framework markers on the WICGR (Whitehead Institute/MIT Center of Genome Research) radiation hybrid map of the human genome. The approximate corresponding cytogenetic location of the gene on 2p21 region. Distances are in centirays (cR) and centimorgan (cM) from top of chromosome 2 linkage group.

- [5] J.L. Knopf, M.H. Lee, L.A. Sultzman, R.W. Kriz, C.R. Loomis, R.M. Hewick, R.M. Bell, Cloning and expression of multiple protein kinase C cDNAs, *Cell* 46 (1986) 491–502.
- [6] F.J. Johannes, J. Prestle, S. Eis, P. Oberhagemann, K. Pfizenmaier, PKC μ is a novel, atypical member of the protein kinase C family, *J. Biol. Chem.* 269 (1994) 6140–6148.
- [7] A.M. Valverde, J. Sinnott-Smith, J. Van Lint, E. Rozengurt, Molecular cloning and characterization of protein kinase D: A target for diacylglycerol and phorbol esters with a distinctive catalytic domain, *Proc. Natl. Acad. Sci. USA* 91 (1994) 8572–8576.
- [8] S. Dieterich, T. Herget, G. Link, H. Bottinger, K. Pfizenmaier, F.J. Johannes, In vitro activation and substrates of recombinant, baculovirus expressed human protein kinase C μ , *FEBS Lett.* 381 (1996) 183–187.
- [9] J.V. Van Lint, J. Sinnott-Smith, E. Rozengurt, Expression and characterization of PKD, a phorbol ester and diacylglycerol-stimulated serine protein kinase, *J. Biol. Chem.* 270 (1995) 1455–1461.
- [10] S.K. Hanks, A.M. Quinn, T. Hunter, The protein kinase family: conserved features and deduced phylogeny of the catalytic domains, *Science* 241 (1988) 42–52.
- [11] R.J. Haslam, H.B. Koide, B.A. Hemmings, Pleckstrin domain homology, *Nature* 363 (1993) 309–310.
- [12] B.J. Mayer, R. Ren, K.L. Clark, D. Baltimore, A putative modular domain present in diverse signaling proteins, *Cell* 73 (1993) 629–630.
- [13] T.J. Gibson, M. Hyvonen, A. Musacchio, M. Saraste, E. Birney, PH domain: the first anniversary, *Trends. Biochem. Sci.* 19 (1994) 349–353.
- [14] M.A. Lemmon, K.M. Ferguson, J. Schlessinger, PH domains: diverse sequences with a common fold recruit signaling molecules to the cell surface, *Cell* 85 (1996) 621–624.
- [15] J. Van Lint, Y. Ni, M. Valius, W. Merlevede, J.R. Vandenhede, Platelet-derived growth factor stimulates protein kinase D through the activation of phospholipase C γ and protein kinase C, *J. Biol. Chem.* 273 (1998) 7038–7043.
- [16] T. Iglesias, E. Rozengurt, Protein kinase D activation by mutations within its pleckstrin homology domain, *J. Biol. Chem.* 273 (1998) 410–416.
- [17] T. Saito, N. Seki, H. Ishii, M. Ohira, A. Hayashi, S. Kozuma, T. Hori, Complementary DNA cloning and chromosomal mapping of a novel phosphatidylinositol kinase gene, *DNA Res.* 4 (1997) 301–305.
- [18] T. Saito, N. Seki, M. Ohira, A. Hayashi, S. Kozuma, A. Hattori, T. Hori, Assignment of the ZIP kinase gene to human chromosome 19p13.3 by somatic hybrid analysis and fluorescence in-situ hybridization, *J. Hum. Genet.* 43 (1998) 209–211.
- [19] N. Seki, Y. Nimura, M. Ohira, T. Saito, S. Ichimiya, N. Nomura, A. Nakagawara, Identification and chromosome assignment of a human gene encoding a novel phosphatidylinositol-3 kinase, *DNA Res.* 4 (1997) 355–358.
- [20] N. Seki, A. Hayashi, M. Abe, R. Araki, A. Fujimori, R. Fukumura, A. Hattori, S. Kozuma, M. Ohira, T. Hori, T. Saito, Chromosomal assignment of the gene for human DNA-PKcs interacting protein (KIP) on chromosome 15q25.3–q26.1 by somatic hybrid analysis and fluorescence in situ hybridization, *J. Hum. Genet.* 43 (1998) 275–277.
- [21] J.L. Zugaza, J. Sinnott-Smith, J. Van Lint, E. Rozengurt, Protein kinase D (PKD) activation in intact cells through a protein kinase C-dependent signal transduction pathway, *EMBO J.* 15 (1996) 6220–6230.
- [22] X. Chen, J.A. Knauf, R. Gonsky, M. Wang, E.H. Lai, S. Chisoe, J.A. Fagin, J.R. Korenberg, From amplification to gene in thyroid cancer: a high-resolution mapped bacterial-artificial-chromosome resource for cancer chromosome aberrations guides gene discovery after comparative genome hybridization, *Am. J. Hum. Genet.* 63 (1998) 625–637.