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Short sequence-paper

PKCν, a new member of the protein kinase C family, composes a fourth subfamily with PKCμ[1]

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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 mu (PKCμ) and 77.4% similarity to mouse PKD (the mouse homolog of PKCμ). We designated the new member as protein kinase C nu (PKCν). The PKCν 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.

Keywords: Protein kinase C; Chromosome mapping; 2p21; PKCμ; 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 (PKCα, β1, β2, and γ), require Ca^{2+} to be activated in the presence of phosphatidylserine. The second group, the novel PKCs (PKCδ, ε, η, θ), are Ca^{2+}-independent. The third group, the atypical PKCs (PKCζ and λ) are Ca^{2+}-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

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

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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, PKCμ, 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-myo-inositol-4,5-bisphosphate were found to promote PKCμ/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 PKCμ/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′-GAAAACATAGAAAGACTGGGAGGGATGTGG-3′ (corresponds to nt 2333 to 2362) and 5′-AGACATACAACTCAGCACTTAGACCAGCAG-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′-ATGCAGATTCCTCAAAGCAACAAGTATCTG-3′ (corresponds to nt 2628 to 2599) and 5′-TGACTTTCTTGTTTTGTGGGGAATCTCATC-3′ (corresponds to nt 2411 to 2382) were used with Marathon cDNA amplification kit (Clonetech, USA). The second 5′-RACE experiments were performed using nested primers, 5′-ATTATGAGAGCTGTCCCCATTGTTCTCACC-3′ (corresponds to nt 2076 to 2047) and 5′-TGCTGCTCTTCCTCTTTGTGTGCTTGATGG-3′ (corresponds to nt 1801 to 1772), and the third 5′-RACE experiments were performed using nested primers, 5′-TCACCACAGTAATCACAGAAAGTAGGAGCT-3′ (corresponds to nt 1073 to 1044) and 5′-TAGAGAGTATGTGGACGAATCTGGAAGT-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 PKCμ (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 PKCμ/PKD. This novel PKC and its gene symbol were designated as PKC nu (PKCν) and PRKCN, respectively, according to the proposal by the Human Gene Nomenclature Committee. Comparison of the predicted amino acid sequence of PKCν with PKCμ and PKD sequences revealed that they compose a fourth PKC subgroup (Fig. 2). By hydropathy analysis, PKCμ appeared to harbor an NH_2-terminal hydrophobic sequence (Pro-35 to Ile-55), but PKCν contained no putative transmembranous portion. The cysteine-rich motif, His-X_{12}-Cys-X_2-Cys-X_{10-14}-Cys-X_2-Cys-X_4-His-X_2-Cys-X_7-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 PKCν except Asn-689 (aa 576–832). The PKCν con-

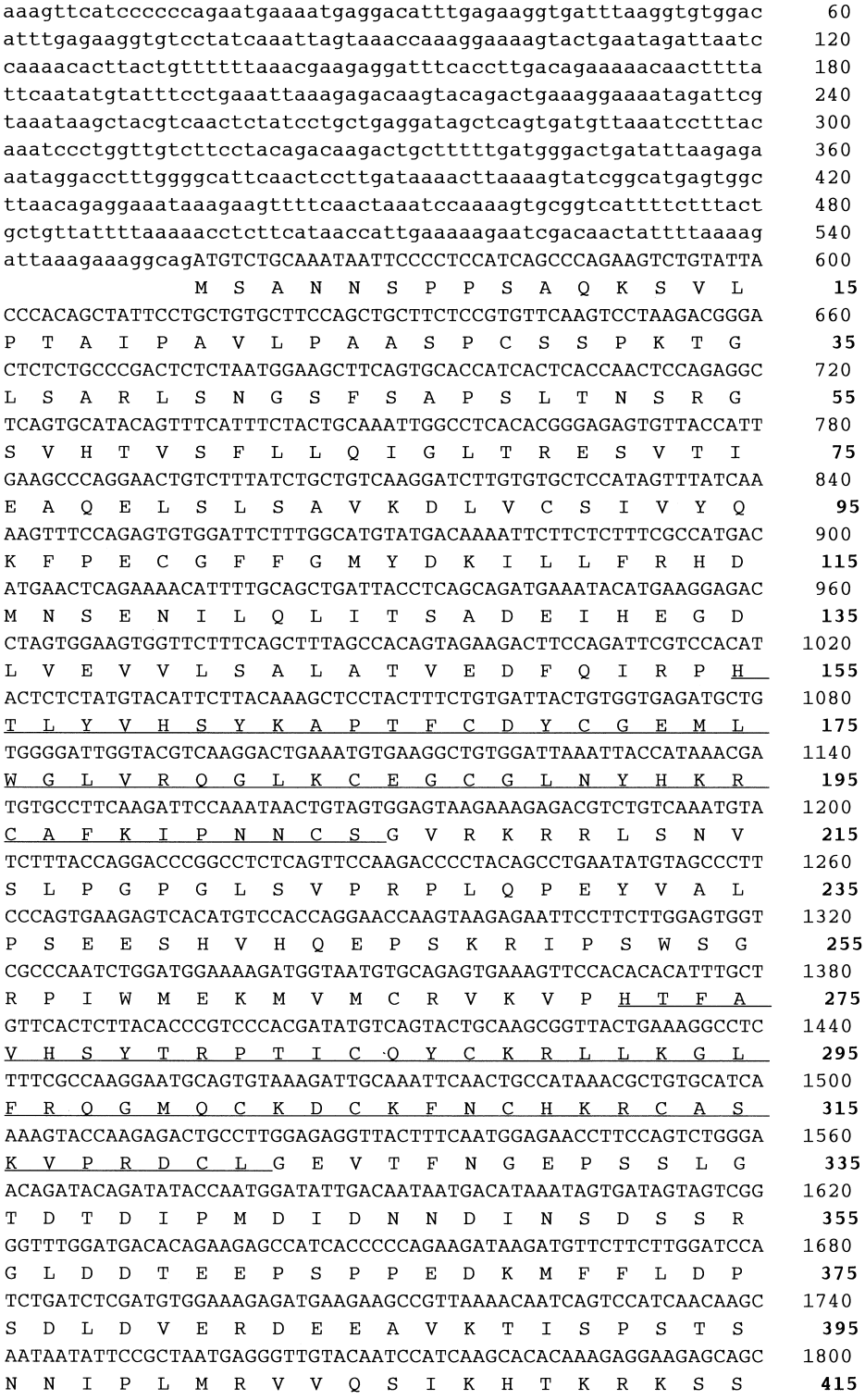

Fig. 1. Nucleotide and deduced amino acid sequences of PKCν. The amino acid sequence is shown below the nucleotide sequence. Asterisks indicate in-frame stop codons. The cysteine-rich domains are underlined and the PH domain is double-underlined.

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ACAATGGTGAAGGAAGGGTGGATGGTCCATTACACCAGCAGGGATAACCTGAGAAAGAGG   1860
T  M  V  K  E  G  W  M  V  H  Y  T  S  R  D  N  L  R  K  R      435
CATTATTGGAGACTTGACAGCAAATGTCTAACATTATTTCAGAATGAATCTGGATCAAAG   1920
H  Y  W  R  L  D  S  K  C  L  T  L  F  Q  N  E  S  G  S  K      455
TATTATAAGGAAATTCCACTTTCAGAAATTCTCCGCATATCTTCACCACGAGATTTCACA   1980
Y  Y  K  E  I  P  L  S  E  I  L  R  I  S  S  P  R  D  F  T      475
AACATTTCACAAGGCAGCAATCCACACTGTTTTGAAATCATTACTGATACTATGGTATAC   2040
N  I  S  Q  G  S  N  P  H  C  F  E  I  I  T  D  T  M  V  Y      495
TTCGTTGGTGAGAACAATGGGGACAGCTCTCATAATCCTGTTCTTGCTGCCACTGGAGTT   2100
F  V  G  E  N  N  G  D  S  S  H  N  P  V  L  A  A  T  G  V      515
GGACTTGATGTAGCACAGAGCTGGGAAAAAGCAATTCGCCAAGCCCTCATGCCTGTTACT   2160
G  L  D  V  A  Q  S  W  E  K  A  I  R  Q  A  L  M  P  V  T      535
CCTCAAGCAAGTGTTTGCACTTCTCCAGGGCAAGGGAAAGATCACAAAGATTTGTCTACA   2220
P  Q  A  S  V  C  T  S  P  G  Q  G  K  D  H  K  D  L  S  T      555
AGTATCTCTGTATCTAATTGTCAGATTCAGGAGAATGTGGATATCAGTACTGTTTACCAG   2280
S  I  S  V  S  N  C  Q  I  Q  E  N  V  D  I  S  T  V  Y  Q      575
ATCTTTGCAGATGAGGTGCTTGGTTCAGGCCAGTTTGGCATCGTTTATGGAGGAAAACAT   2340
I  F  A  D  E  V  L  G  S  G  Q  F  G  I  V  Y  G  G  K  H      595
AGAAAGACTGGGAGGGATGTGGCTATTAAAGTAATTGATAAGATGAGATTCCCCACAAAA   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
GTAAACCTGGAATGTATGTTTGAAACCCCAGAACGAGTCTTTGTAGTAATGGAAAAGCTG   2520
V  N  L  E  C  M  F  E  T  P  E  R  V  F  V  V  M  E  K  L      655
CATGGAGATATGTTGGAAATGATTCTATCCAGTGAGAAAAGTCGGCTTCCAGAACGAATT   2580
H  G  D  M  L  E  M  I  L  S  S  E  K  S  R  L  P  E  R  I      675
ACTAAATTCATGGTCACACAGATACTTGTTGCTTTGAGGAATCTGCATTTTAAGAATATT   2640
T  K  F  M  V  T  Q  I  L  V  A  L  R  N  L  H  F  K  N  I      695
GTGCACTGTGATTTAAAGCCAGAAAATGTGCTGCTTGCATCAGCAGAGCCATTTCCTCAG   2700
V  H  C  D  L  K  P  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
GTGGTAGGAACTCCAGCATACTTAGCCCCTGAAGTTCTCCGGAGCAAAGGTTACAACCGT   2820
V  V  G  T  P  A  Y  L  A  P  E  V  L  R  S  K  G  Y  N  R      755
TCCCTAGATATGTGGTCAGTGGGAGTTATCATCTATGTGAGCCTCAGTGGCACATTTCCT   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
AAGATGAGAAAACGTTACAGTGTTGACAAATCTCTTAGTCATCCCTGGCTACAGGACTAT   3060
K  M  R  K  R  Y  S  V  D  K  S  L  S  H  P  W  L  Q  D  Y      835
CAGACTTGGCTTGACCTTAGAGAATTTGAAACTCGCATTGGAGAACGTTACATTACACAT   3120
Q  T  W  L  D  L  R  E  F  E  T  R  I  G  E  R  Y  I  T  H      855
GAAAGTGATGATGCTCGCTGGGAAATACATGCATACACACATAACCTTGTATACCCAAAG   3180
E  S  D  D  A  R  W  E  I  H  A  Y  T  H  N  L  V  Y  P  K      875
CACTTCATTATGGCTCCTAATCCAGATGATATGGAAGAAGATCCTTAAtcactgagctaa   3240
H  F  I  M  A  P  N  P  D  D  M  E  E  D  P  *                 890
cctaaataaggaaggatttcattttatggactgatattttgctgtgtaacttgttcttcg   3300
tagattgtcatctgcagtgctgcaaagatatgaagaaatatgataacgaataagtgacac   3360
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Fig. 1 (*continued*).

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cagtactgtagttcataatgagtaggtacaggcgggaaactgaataataagaagtcataa   3420
tggaatcaaggtgaagctttttataaacttttttagcctaagcaataactggttttgtat   3480
tttttcttaatccttcactttaatacaataggctcacttaatttgtcttcccatttctct   3540
ttatatatatatatatatatataaaaaaatataaatatatgtttgtttgtttgttttttaa   3600
ggaaaaacaagtcaagctagcatccagttactatatagcttggctaaattatacaagact   3660
tacaagattgattactcgacaggcttgtatttaagagataactgtgaggttaccattatg   3720
tgatgttactataaggacttttaacattggtttaacaaaccatagaggcattgaagggtt   3780
tttcttagatgcctagaaaaagcacactgggctgttttacctttctttttttaggtcaatc   3840
aagactccaaaatagtgattcctaacctttttggagttgctctgctactctgaatatgtt   3900
ctatacagcataaggattgtcaccttctgtgtgttgcaacagcttctaagataattaggg   3960
acaaatgatgttacaaaaggaagagtactgctggtctaagtgctgagttgtatgtct      4017
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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 PLCγ 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 PKCν and PKCμ/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 PCKν 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′-GAAAACATAGAAAGACTGGGAGGGATGTGG-3′ (corresponds to nt 2333 to 2362) and 5′-ATGCAGATTCCTCAAAGCAACAAGTATCTG-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, PKCν 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′-AAGTGATGATGCTGCCTGGGAAATACATGC-3′ (corresponds to nt 3122 to 3152) and 5′-AGACATACAACTCAGCACTTAGACCAGCAG-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 PKCν 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

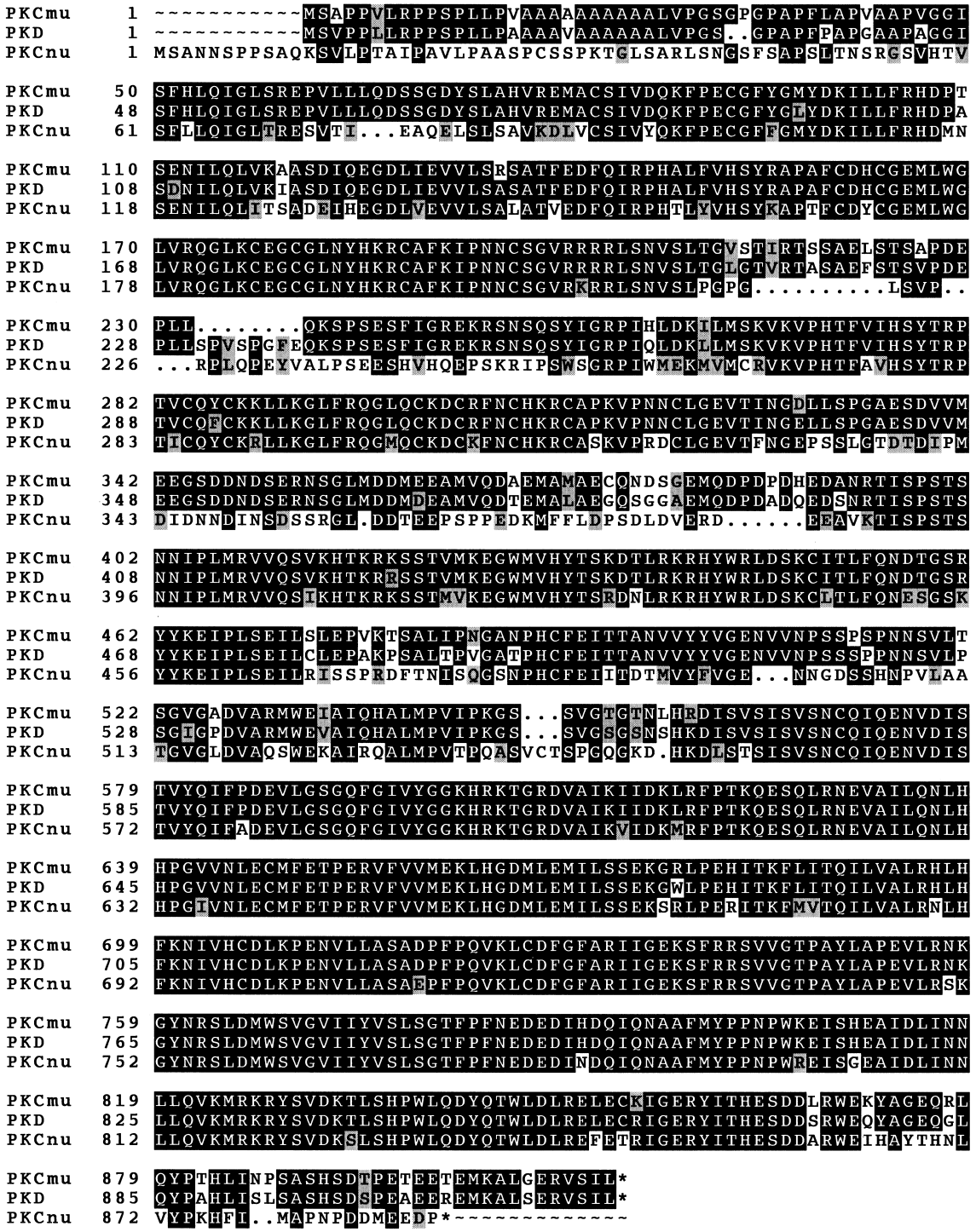

Fig. 2. Amino acid sequence alignment of the PKCν with PKCμ (accession number X75756) and PKD (accession number Z34524) proteins. The amino acid sequences of PKCν, PKCμ, and PKD were analyzed and aligned by GCG software. The predicted amino acids are represented in one-letter designation. The most identical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

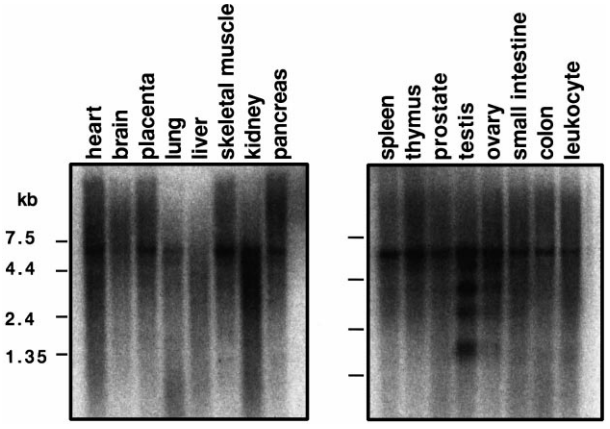

Fig. 3. Northern blot analysis of PKCν. Northern blot filters containing adult human poly(A)$^+$ RNAs (2 μg/lane) were purchased from Clonetech Laboratories (Palo Alto, CA), and hybridization and washing were performed following the manufacturer's instructions. A cDNA fragment corresponding to 3122–3988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

upstream intermediator, 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 PKCν 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.

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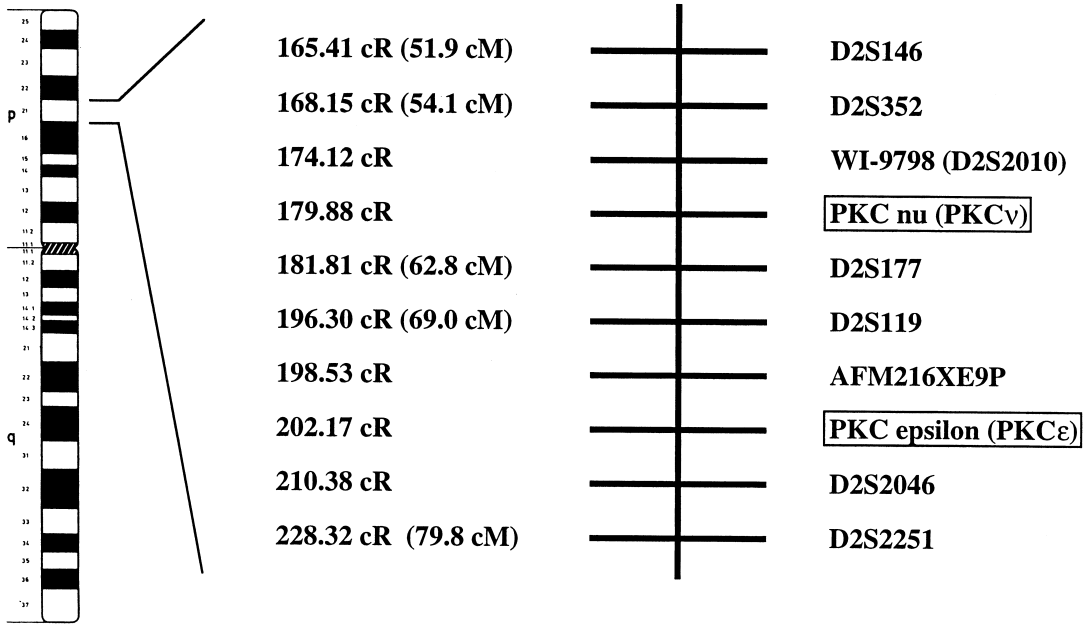

Fig. 4. Chromosomal placement of human PKCν and PKCε genes at a relative distance to framework markers on the WICGR (Whitehead Institute/MIT Center of Genome Research) radiation hybrid map of the human genome. The approximate corresponding cytogenetic location of the gene on 2p21 region. Distances are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

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