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Primary structures of human protein kinase C βI and βII differ only in their C-terminal sequences

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Two types of cDNA clones encoding human protein kinase C (PKC) were isolated from a spleen cDNA library using rabbit protein kinase C βI/βII cDNA as a hybridization probe. Nucleotide sequence analyses of these cDNA inserts revealed complete primary structures of two distinct types of human protein kinase C βI and βII which differ only in their C-terminal 50 or 52 amino acid residues. It was concluded that there exist four distinct types of PKC, PKC α, βI, βII and γ, in human as well as rabbit, and that the corresponding sequences are strictly conserved among mammalian species.

Protein kinase C; cDNA sequence; Alternative splicing

1. INTRODUCTION

Protein kinase C (PKC) has been suggested to be involved in various cellular functions [1]. We have recently identified rabbit cDNA clones encoding four PKC types, α, βI, βII and γ, and shown that the brain PKC preparation is a mixture of these four types of PKC ([2]. Ohno, S. et al., in preparation). cDNA clones for multiple PKC types have been also identified for rat (βI, βII and γ) [3,4], bovine (α, βII and γ) and human (α, βII and γ) [6]. In order to examine for the presence of a human homologue of rabbit or rat PKC βI, we searched a human spleen cDNA library using rabbit PKC βI/βII cDNA as a hybridization probe. We have identified two distinct types of cDNA clones encoding human PKC βI and βII.

2. EXPERIMENTAL

The human spleen λgt10 cDNA library is described in [7]. A fragment of rabbit cDNA for PKC βII (nucleotide positions 774–1963, see [2]) was ^{32}P-labeled and used as a hybridization probe. Screening of the cDNA library was done as described [8]. DNA sequencing was carried out by subcloning suitable overlapping DNA fragments from the recombinant cDNA clones into pUC or BKS (Stratagene Cloning Systems) plasmids followed by primed DNA synthesis on the denatured DNA template [9] in the presence of dideoxynucleotide triphosphates [10].

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3. RESULTS AND DISCUSSION

Screening of the human spleen cDNA library using rabbit PKC βI/βII cDNA fragment as a hybridization probe identified two distinct types of cDNA clones whose representatives are H707 (major type) and H106 (minor type). The complete nucleotide sequences of the cDNA inserts of H707 and H106 were determined. The restriction map and the nucleotide sequences of these cDNA inserts, as well as the predicted amino acid sequences, are shown in fig.1. The two cDNAs, H707 and H106, share identical nucleotide se-

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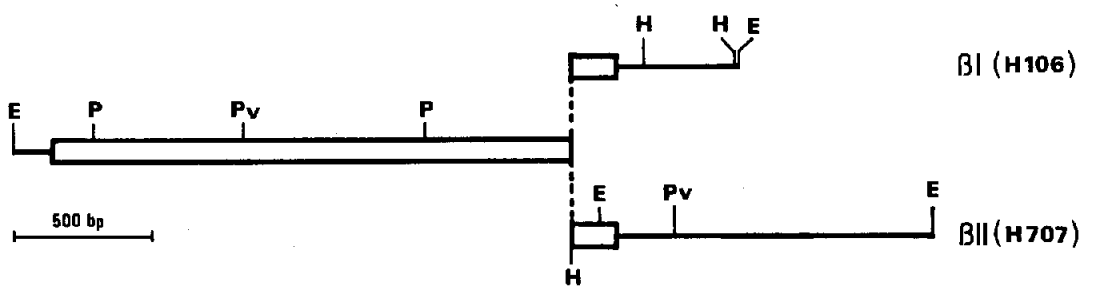

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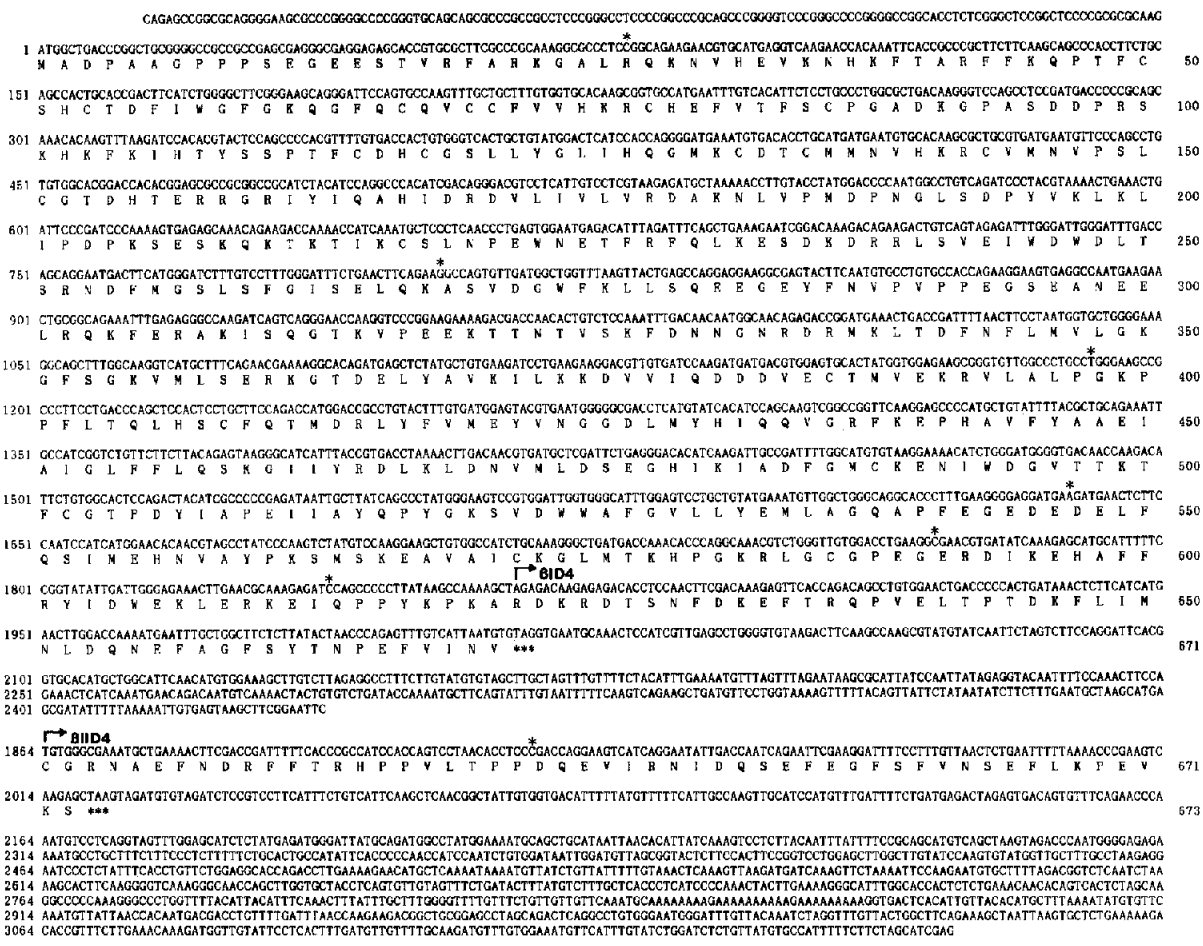

Fig.1. cDNAs for human PKC βI and βII. (a) Restriction maps of cDNA inserts for H106 (βI) and H707 (βII). E, *Eco*RI; H, *Hin*dIII; P, *Pst*I; Pv, *Pvu*II. (b) Nucleotide and deduced amino acid sequences for βI (H106) and βII (H707). Nucleotides are numbered starting at A of the presumptive translation initiation codon. (***) Termination codon. Nucleotides that differ from those in [6] are indicated by single asterisks. Amino acids are numbered starting at Met-1. Arrows indicate the beginning of the divergent regions (D4) for βI and βII. The sequences of βII, different from βI, are shown separately after the βI sequences.

quences from the 5′-end to the nucleotide position at 2000 (fig.1b), suggesting that their mRNAs are derived from a single gene by an alternative splicing mechanism. As will be described below, H707 and H106 are derived from mRNAs for PKC βII and βI, respectively. We have recently isolated a human gene for PKC βI/βII where the 3′-portions of the mRNAs for PKC βI and βII, corresponding to the D4 regions [2] and 3′-flanking sequences, comprise individual exons, clearly indicating that the mRNAs for PKC βI and βII are derived from a single gene on chromosome 16 [6] by alternate use of their 3′-exons (Kubo et al., in preparation).

Although the nucleotide sequence of H707 is different in 7 positions from the previously described cDNA sequence for human PKC βII [6] isolated from a brain cDNA library (see fig.1b), the differences are silent and the predicted amino acid sequences are completely identical. Thus, H707, which comprises a 2019 bp open reading frame, corresponds to βII.

H106 contains an open reading frame of 2013 bp with 135 bp 5′-flanking and 417 by 3′-flanking sequences. (From the analysis of a genomic clone containing the 3′-portion of βII PKC, a putative poly(A) site is located 80 bp downstream from the 3′-end of H106.) The open reading frame of H106 predicts a protein of 671 amino acid residues. The sequence shows 99% identity to PKC βI from rabbit [2] and rat [3], indicating that H106 encodes PKC βI and that the primary sequence of the protein is highly conserved among mammalian species (fig.2).

Isolation of cDNA clones for PKC βI and βII from a spleen cDNA library confirms our previous observation that PKC βI and βII are expressed in spleen as well as in brain [2]. The presence of the fourth type of human PKC, PKC βI, indicates that the presence of all four types of PKC is a common feature in mammals.

It is noteworthy that the sequence of the C-terminal 50 or 52 amino acid residues of PKC βI and βII (D4 region), the region that contains the only differences between PKC βI and βII and in which the sequence diverged significantly among the four types of PKC [2], is completely conserved among human, rabbit, and rat. This suggests that the primary structure of each PKC type, including the D4 region, is essential to the individual function of each PKC type. Furthermore, comparison of the 5′- and 3′-untranslated sequences among human PKC βI/βII cDNAs and those of other mammals reveal extensive conservation of the sequences in these regions. For example, between human and rabbit, the 5′-flanking region has more than 89% homology, and the 3′-region has 94.7%

Fig.2. Comparison of amino acid sequences of PKC βI and βII from various species. Only the amino acids different from those for human are shown. Only the D4 regions are shown for βII sequences. Sequences for rabbit, rat, and bovine are from [2,3, 6], respectively.

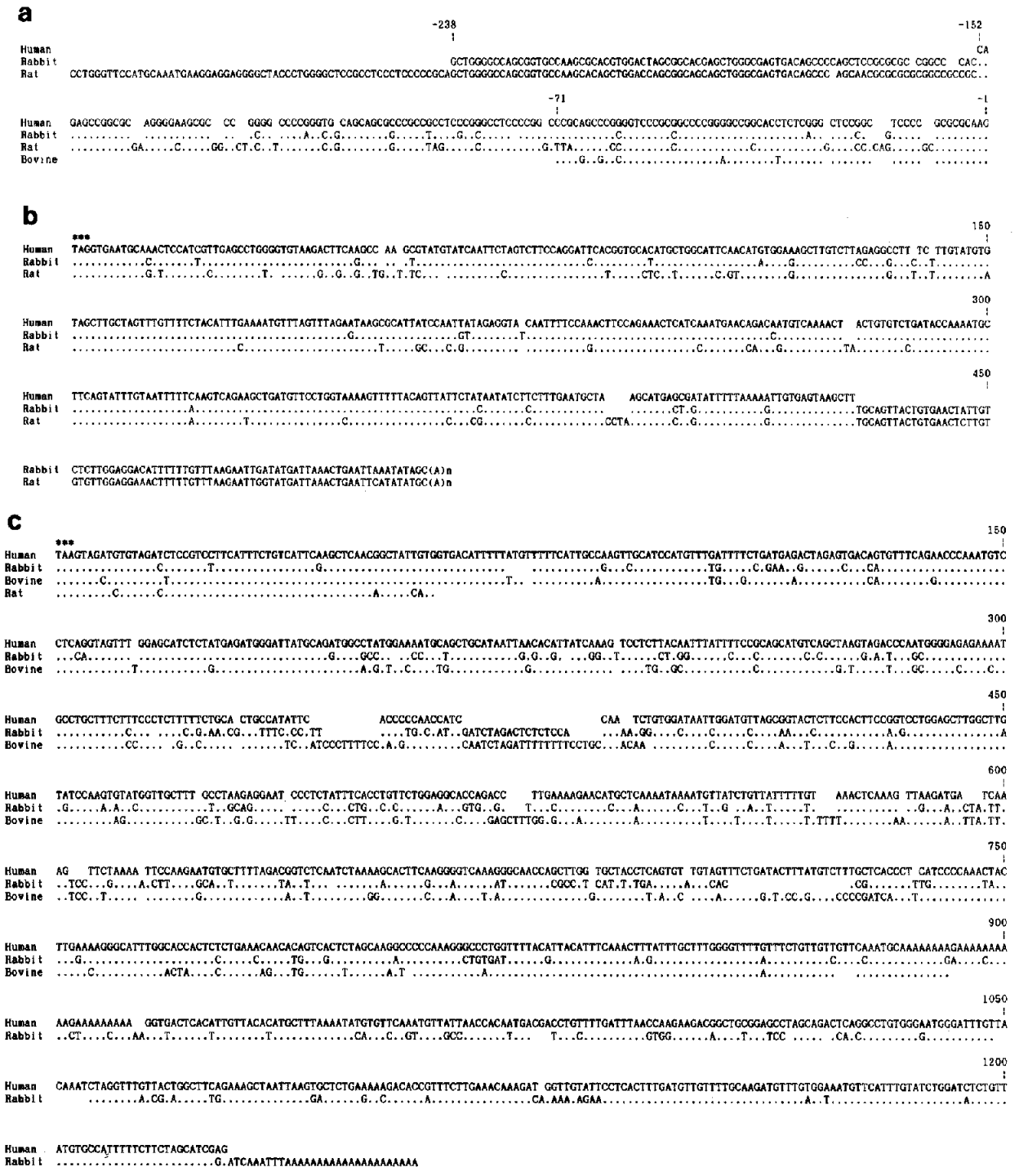

Fig.3. Comparison of the nucleotide sequences of the 5′ (a)- and 3′ (b and c)-flanking sequences among cDNAs for PKC βI and βII. Only nucleotides different from those for human are shown. Gaps are introduced to maximize the homology. Sequences for rabbit, rat, and bovine are from [2,3, 6], respectively.

and 73.3% homology for βI and βII, respectively (fig.3). This suggests that the untranslated sequences of these PKC mRNAs might play important roles in translational activity, stability, or other functions specific to these mRNAs.

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