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Identification of three additional members of rat protein kinase C family: δ-, ε- and ζ-subspecies

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Three types of cDNA clone of the protein kinase C family termed δ, ε and ζ were newly identified by molecular cloning and sequence analysis. These members have a common structure that is closely related to, but clearly different from the other four known members of the family which have α-, βI-, βII- and γ-sequences, although the ζ-cDNA available at present does not contain a complete reading frame for a protein kinase C molecule. The diverse heterogeneity of the enzyme seems to be an important factor in determining the mode of response of many tissues and cell types to a variety of external stimuli.

Protein kinase C; cDNA; (Rat brain)

1. INTRODUCTION

Various kinds of extracellular signals which act by stimulating inositol phospholipid hydrolysis now appear to operate through a bifurcating intracellular signal pathway which involves Ca^{2+} mobilization and protein kinase C activation [1–3]. Recent molecular cloning analysis has shown that protein kinase C is a family of multiple subspecies having closely related structures [4–11]. Thus far, the complete structures of four subspecies, α-, βI-, βII- and γ-sequences, have been clarified by sequence analysis of the cDNAs obtained from the brain libraries [4–8,10,11]. In the brain, three major distinct types of protein kinase C can be resolved upon chromatography on a hydroxyapatite column [12–14] and the correspondence of these subtypes to the cDNA clones has been determined by comparison with the enzymes that were separately expressed in COS 7 cells transfected with the respective cDNA-containing plasmids [13,14]. The subspecies of protein kinase C thus identified appear to show a slightly different mode of activation, kinetic properties and substrate specificities [16,17]. In the present study an additional three subspecies of the protein kinase C family will be described. The cDNAs encoding these subspecies were isolated from the rat brain cDNA library, and referred to hereafter as δ, ε and ζ.

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2. MATERIALS AND METHODS

2.1. *Isolation of cDNA clones*

Rat brain cDNA libraries constructed in λgt10 were screened using nitrocellulose filters with a mixture of inserts of λCKRα5, λCKR107 and λCKRγ1 as probes which were specific to α-, βII- and γ-cDNAs, respectively [7,13]. Two hybridization conditions of low- and high-stringency were employed [18]. The cDNA clones which were positive only under the low-stringency condition were selected.

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2.2. *DNA sequence analysis*

Nucleotide sequence analysis was performed by subcloning suitable overlapping restriction fragments from the recombinant cDNA clones into M13 series phage DNA, followed by primed DNA synthesis on single-stranded DNA templates in the presence of dideoxynucleotide triphosphates [6].

3. RESULTS AND DISCUSSION

3.1. *cDNA cloning and sequence analysis*

The rat brain cDNA library which was constructed in λgt10 as described in [7] was employed. For isolation of the new class of cDNA clones of protein kinase C, the plaque screening was made under low- and high-stringency hybridization conditions with a mixture of restriction endonuclease fragments as probes. These probes were prepared from each of the rat brain protein kinase C cDNA clones, α, βII and γ, previously isolated [7,13]. Three cDNA clones, λCKRδ5, λCKRϵ41 and λCKRζ3, were obtained by this procedure, which could hybridize with the probes only under the low-stringency condition. Southern blot analysis indicated that the three cDNA clones were closely related to, but distinct from the previously isolated cDNA clones. The three clones did not cross-hybridize with one another.

The λCKRδ5 clone contained δ-cDNA in its insert, composed of a total of 2891 nucleotides, which has an open reading frame encoding a sequence of 673 amino acids with a molecular mass of 77517 Da. The second clone, λCKRϵ41, contained ϵ-cDNA in its insert, composed of a total of 2686 nucleotides. The reading frame encodes 737 amino acids with a molecular mass of 83474 Da. The third clone, λCKRζ3, contained ζ-cDNA which appeared to encode an additional protein kinase C subspecies. This clone did not contain a full length of the coding region for a protein kinase C molecule, since the open reading frame continues to the 5′-terminal direction, and a potential initiation codon of ATG is not found in the terminal region. Housey et al. [19] have reported a partial nucleotide sequence of cDNA termed RP16, which is related to but distinct from the known cDNA clones of protein kinase C. The RP16 cDNA clone has a nucleotide sequence nearly identical to a part of λCKRϵ41. The detailed nucleotide sequences of δ-, ϵ- and ζ-cDNA clones and their expression as well as the enzymatic properties of the new members of the family will be described elsewhere.

3.2. *Structural characteristics*

The protein kinase C subspecies encoded by α-, βI-, βII- and γ-cDNAs isolated from the rat brain cDNA library all consist of a single polypeptide with four conserved (C1–C4) and five variable (V1–V5) regions as schematically shown in fig.1. The detailed nucleotide sequences are described [20]. The two conserved regions C3 and C4 located in the carboxyl-terminal half appear to be essential for the catalytic activity, and the region C3 contains an ATP-binding sequence which is found in many protein kinases. The conserved regions C1 and C2 in the amino-terminal half presumably compose the regulatory domain that interacts with calcium, phospholipid and diacylglycerol. The region C1 contains a tandem repeat of a cysteine-rich sequence.

In comparison, the new members of the protein kinase C family reported herein show closely related, but different structures. The protein kinase C subspecies encoded by δ-, ϵ- and ζ-cDNAs all contain the characteristic cysteine-rich sequences in the amino-terminal half of the enzyme molecules. Fig.2 shows a comparison of the amino acid sequences in the cysteine-rich region commonly present among the protein kinase C subspecies so far identified. The sequence $CX_2CX_{13(14)}$-$CX_2CX_7CX_7C$, where C is cysteine and X represents any amino acid, is conserved in all subspecies. The sequence agrees with the consensus sequence of a 'cysteine-zinc DNA-binding finger', which is seen in a variety of proteins involved in transcriptional regulation [21]. The sequences of

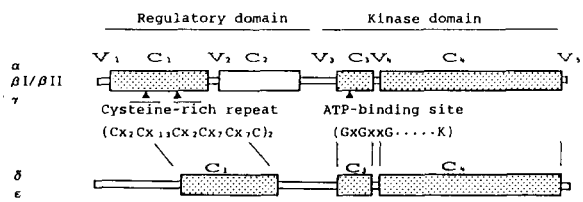

Fig.1. Schematic representation of the structures of two groups of protein kinase C family. C1–C4 (thick boxes) and V1–V5 (thin boxes) indicate conserved and variable regions, respectively.

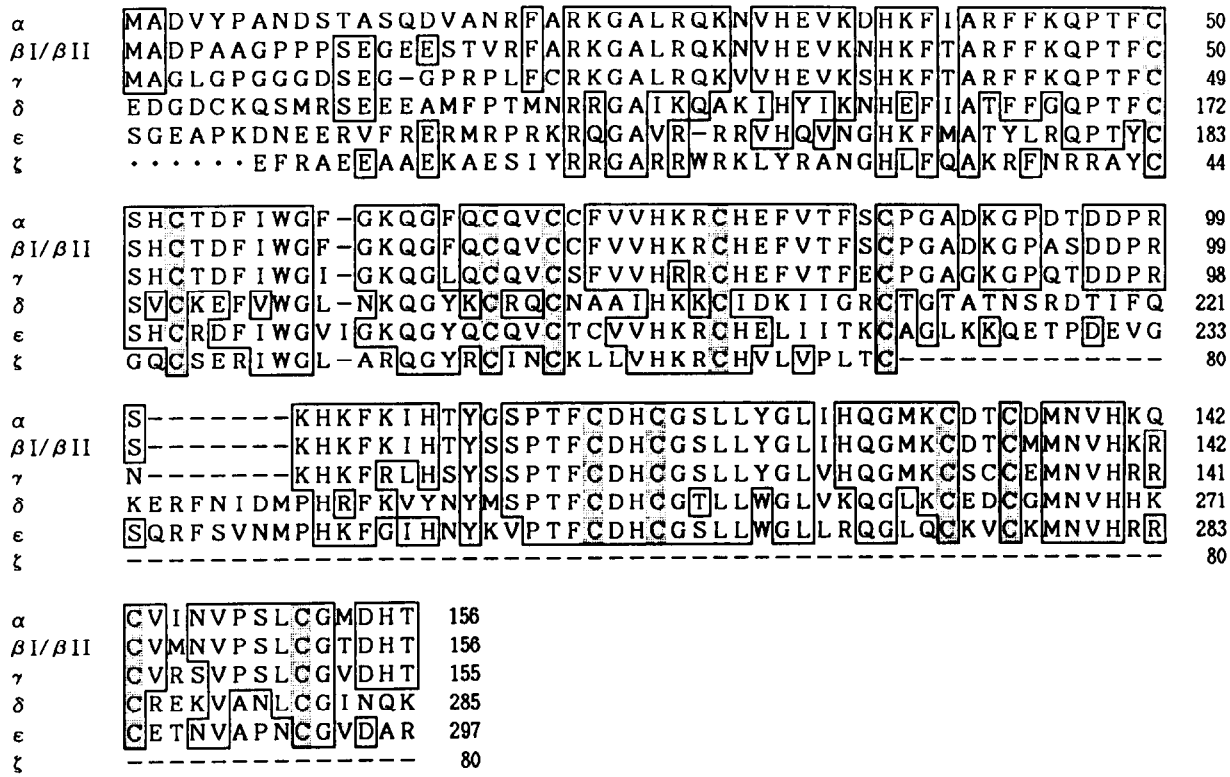

Fig.2. Comparison of amino acid sequences of the cysteine-rich regions among protein kinase C subspecies. Amino acids are numbered at the right. Residues commonly present among the subspecies are boxed. Hyphens are introduced for optimal alignment. Cysteine residues universally present in all protein kinase C subspecies are shaded.

the kinase domain, C3 and C4, are highly homologous to one another, and show more similarity among the protein kinase C family than any other protein kinases. The conserved region C2, that is present in α-, βI-, βII- and γ-subspecies, is absent in the new members which have δ-, ϵ- and ζ-sequences (fig.1).

The results outlined above suggest the existence of several additional subspecies of protein kinase C. The new members of the protein kinase C family predicted by the cDNA clone analysis have a common structure similar to that of the previously known members, but have clearly different regulatory domains. The enzymatic properties and the distributions of the new members of the enzyme family are not known at present. However, it is becoming more evident that the protein kinase C family consists of a large number of subspecies which show subtle individual characteristics with slightly different properties. Their functional roles in physiological responses of tissue and cell types to various external stimuli may be clarified by further investigation.

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