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Structure and sequence of an intronless gene for human casein kinase II-α subunit

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Using sixteen different primers based on the cDNA sequence of the human casein kinase II-α subunit, different fragments of this gene were amplified by PCR from human genomic DNA. The sizes of these fragments were identical to amplified cDNA, which suggests the existence of an intronless genomic gene. The amplification was carried out on whole blood genomic DNA from three different individuals. The total sequence of the amplified casein kinase II-α gene showed more than 99% homology to the cDNA. The gene contains a noninterrupted open reading frame, as expected for the homolog cDNA. Although the gene sequence is complete, four point mutations were found. Since there are no interruptions of the open reading frame, this intronless gene might be expressed.

Structure intronless gene; Sequence intronless gene; Casein kinase II-α subunit

1. INTRODUCTION

Casein kinase II (CKII) is a serine/threonine protein kinase widely distributed among species and it is independent of any known physiological regulators such as, cAMP Ca^{2+}/calmodulin and phospholipids. The gene structure, expression regulation and enzymatic activity of CKII have been widely studied particularly in relation to its involvement in cell growth (reviewed in [1]). Although the physiological role of CKII is still unclear it has been shown to phosphorylate a wide range of different substrates, in vivo and in vitro, which are involved in key regulatory processes. Among these substrates are several oncogenes and antioncogenes [2–4], transcription factors [5,6] and enzymes that participate in DNA and RNA synthesis [7,8].

CKII is an heterodimer composed of two very similar but distinct catalytic subunits (α/α′) and two regulatory subunits (β). The cDNA [9–12] and gene [13,14] sequences for the three subunits have been characterized in several species. The cDNAs for human CKII, α, α′ and β subunits [15–17] have been described as well as the gene sequence for the β subunit [14]. Mapping of human chromosomes through in situ hybridization have localized the human CKII-α DNA sequence to two loci [18], indicating the presence of at least two different genes.

In this report, we present the sequence of an intronless gene for human CKII-α which shows no interruptions in an open reading frame. There are four differences in the amino acid sequences of the cDNA and the gene, although none occur in conserved regions for protein kinase, catalytic and/or ATP binding system [19]. In contrast to the (pseudo)gene recently described by Wirkner et al. [20], which contained a stop codon corresponding to amino acid position 296, the findings presented here indicate that this intronless gene may code for a physiologically relevant CKII.

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2. MATERIALS AND METHODS

Oligonucleotides based on the human cDNA sequence of CKII-α were synthesized with an Applied Biosystems DNA synthesizer. A *Bam*HI restriction site was added to each 5′ end in order to subclone the PCR amplified fragments into the M13mp18 vector. The primers (P) were homologous to the cDNA sequence at the indicated positions: P0: −69/−50; P1: 0/17; P3: +112/+129; P5: +318/+334; P7: +568/584; P9: +819/+835; P11: +879/895; P13: +1033/+1049; P14: +1159/+1141; P15: +1211/+1193. Primers, P2, P4, P6, P8, P10 and P12, correspond to primers P3, P5, P7, P9, P11 and P13, respectively, but in the opposite directions (Fig. 1).

2.1. *DNA isolation from whole blood*

Ten ml of whole blood were collected from three different individuals, in tubes containing 500 μl of 0.5 M EDTA as an anticoagulant. After centrifugation at 2500 rpm for 15 min, the plasma was discarded and solution A (0.32 M sucrose, 10 mM Tris buffer pH 7.5, 5 mM $MgCl_2$, 1% Triton X-100) was added to a final volume of 25 ml. After mixing gently the tubes were kept on ice for 30 min, and centrifuged as before. After centrifugation, 21 ml of the supernatant was discarded, and the tubes were refilled with solution A, to a final volume of 25 ml. After incubating the tubes on ice for 20 min, they were centrifuged at 2500 rpm for 15 min, after which time the supernatant was discarded. The pellet was resuspended in 1.5 ml of solution B (10 mM Tris, pH 7.5, 400 mM NaCl, 2 mM EDTA) supplemented with 0.7% SDS and 500 μg of proteinase K. After overnight incubation at 37°C, 500 μl of 5 M NaCl were added by vortexing for 15 s. The tubes were centrifuged, the supernatant recovered and the DNA was precip-

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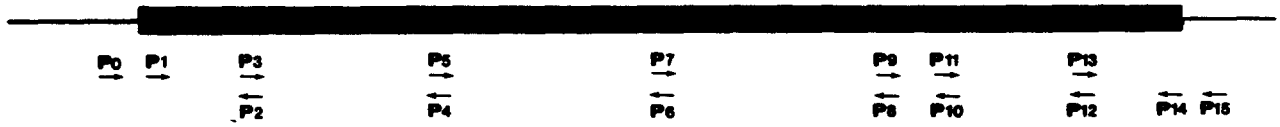

Fig. 1. Primer localization in the human CKII-α subunit cDNA sequence. The localization of all the primers used is indicated.

itated by adding 2 vols. of ethanol (95%). The DNA was collected by centrifugation, resuspended in TE buffer and quantified.

2.2. *PCR amplification*

PCR amplification was carried out using Taq DNA polymerase (Promega Co.) with the following program: step 1, 95°C – 5 min (1 cycle); step 2, 95°C – 1 min; 52°C – 1 min; 72°C – 1 min (28 cycles); step 3, 72°C – 10 min (1 cycle).

2.3. *DNA sequencing*

After amplification, the DNA fragments were digested with *Bam*HI restriction endonuclease and subcloned into a M13mp18 vector. The sequencing was performed using the dideoxy chain termination method [21] using Sequenase version 2.0 (US Biochemicals) and [^{35}S]α-dATP (New England Nuclear). All of the fragments were amplified twice to check possible Taq DNA polymerase errors. Amino acid and nucleotide analysis of the sequences obtained were performed with the PCgene software.

2.4. *Southern blot analysis*

Human genome DNA (5 μg) was digested with *Pvu*II restriction endonuclease, fractionated on 1% agarose gels and hybridized as described [22]. DNA fragments were transferred onto Nytran nylon filters and hybridized with ^{32}P-labeled probes. DNA probes were the products of PCR amplifications of the CKII-α subunit gene, prepared using different pairs of primers. These probes contained the 5′ third, the middle and the 3′ third of the gene. After amplification the DNA fragments were purified through electrophoresis on 3% NuSieve-agarose gels, followed by electroelution onto DEAE membrane as described [22].

3. RESULTS

3.1. *DNA amplification of the CKII-α subunit gene*

Sixteen different oligonucleotides based on the cDNA sequence of the human CKII-α subunit were used to amplify different segments of the genomic homolog gene. The primers were combined in pairs in different ways. Fig. 2 shows the analysis of some of the fragments obtained (lanes 1–7). All of them correspond closely to the expected sizes for an intronless gene. None of the fragments were found to be larger than the corresponding cDNA amplified products. Also the amplification of the whole gene with primers P0 and P15 (lane 8) shows a 1.3 kb fragment which is identical to the size of the cDNA.

3.2. *Genomic profile of the CKII-α subunit by Southern blotting*

In order to analyze the entire structure of the gene, four different probes containing segments of the CKII-α gene were utilized in a Southern blot analysis. These probes corresponded to DNA fragments amplified with primers P0/P4; P5/P8; P9/P15 and the whole gene amplified with primers P0/P15. The published cDNA for

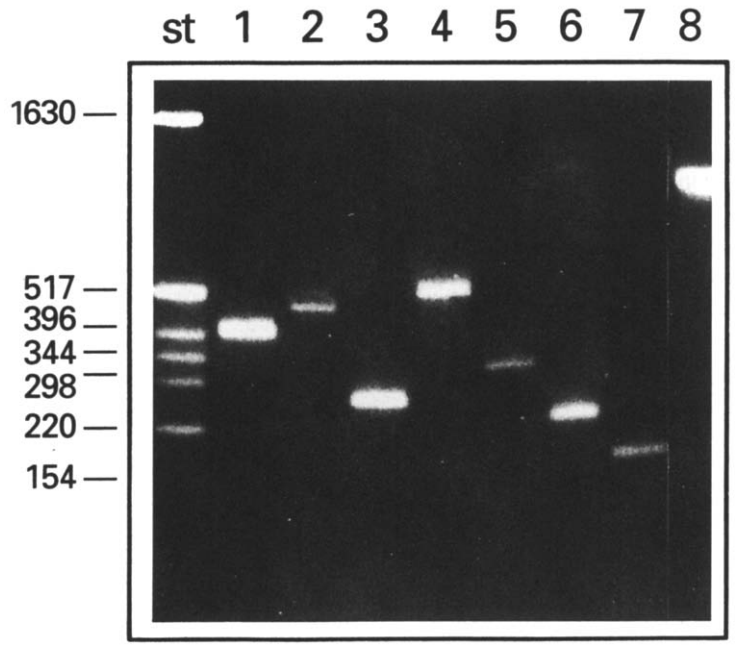

Fig. 2. DNA amplification of the human CKII-α gene: 300 ng of DNA were submitted to PCR amplification with different pairs of oligonucleotide primers. The primers used and the size of the DNA fragments obtained are indicated. Lane 1 = P0/P4 (403 bp); lane 2 = P3/P6 (476 bp); lane 3 = P5/P6 (266 bp); lane 4 = P5/P8 (518 bp); lane 5 = P7/10 (327 bp); lane 6 = P9/12 (230 bp); lane 7 = P13/15 (178 bp); lane 8 = P0/P15 (1.3 kb).

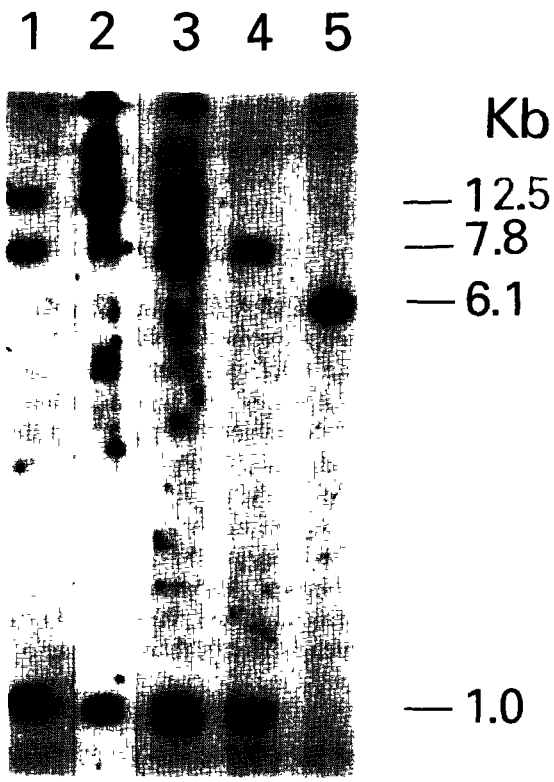

Fig. 3. Southern blot analysis of CKII-α genes. Genomic DNA was digested with *Pvu*II, electrophoresed and blotted to a nylon membrane as described under Materials and Methods. The hybridization was carried out with four different probes as indicated. The membrane was exposed to X-Omat-AR Kodak film for 3 days. Lane 1 = P0/P15; lane 2 = P0/P4; lane 3 = P5/P8; lane 4 = P9/P15; and lane 5 = CKII-α′ 3′ end.

CKII-α subunit has two *Pvu*II restriction sites in the coding sequence, located at positions +120 and +1153, and one site in the 5′ noncoding region at position −70 (the cDNA for CKII-α′ subunit has no *Pvu*II restriction sites within its coding region). The expected hybridizing bands for an intronless CKII-α gene are 1.03 kb and 190 bp. Of these, only the 1.03 kb band would be detected under the electrophoretic conditions used.

To perform the Southern analysis, human genomic DNA was digested with *Pvu*II restriction endonuclease, electrophoresed, transferred to a nylon membrane, and hybridized with the four described probes, as shown in Materials and Methods. As shown in Fig. 3, lane 1, there are three main hybridizing bands of 12.5 kb, 7.8 kb and 1.0 kb, when the probe (P0/P15) representing the entire cDNA sequence is used. When the hybridization is carried out with probe P0/P4 only two bands appeared (lane 2). In the case of probe P9/P15 (lane 4) two bands are shown, but the larger fragment (7.8 kb) is different in size from the one in lane 2 (12.5 kb). As these two probes carry the 5′ and 3′ coding sequences, respectively, the results shown indicate that these two hybridizing bands correspond to the beginning (12.5 kb) and the end (7.8 kb) of a CKII-α subunit gene with intron(s).

The fact that the gene shows an approximate size of 20 kb, suggests that it has introns. The small band (1.0 kb) hybridizes with all of the probes, indicating that this is the one that carries the intronless gene.

A similar Southern analysis was done using as the probe a fragment from the CKII-α′ subunit gene. In this case (lane 5) only one hybridizing band of 6.1 kb was obtained, which is different in size from the CKII-α ones. Since this probe contains the 3′ third of the CKII-α′ gene, this result suggests that there is only one CKII-α′ subunit gene.

3.3. *Sequencing analysis of DNA fragments*

The amplified DNA fragments, from the three individuals, were subcloned into the M13mp18 vector and sequenced. The resulting sequences (Fig. 4) show a 99% homology to the CKII-α cDNA. No stop codon mutations were found inside the coding sequence. The initiation and termination codons were located as expected from the cDNA. No deletion or insertion mutations were found. However, four nucleotide point mutations that resulted in four amino acid differences, appeared within the coding region at amino acid positions 128, 256, 287 and 351 (Fig. 5). These mutations would result in changes of Leu to Phe, Asp to Gly, Ser to Arg, and Met to Val, respectively, of which only the Met to Val change is conservative.

4. DISCUSSION

We have described a human CKII-α gene that does not contain introns in its structure. The size of the DNA fragments obtained after PCR amplification suggested the presence of an intronless gene. In the human genome, two CKII-α genes have been mapped by in situ hybridization and assigned to two different loci: 11p15.5-p15.4 and 20p13 [18]. We have established through Southern blot analysis the approximate size of these two genes, showing that one of them has the same size of the cDNA (1.2 kb). The second one has an approximate size of 20 kb. The DNA sequence of the gene described showed a 99% homology to the cDNA. Two nucleotide changes which would not lead to changes in amino acid composition were found. Four other nucleotide differences occurred in the corresponding cDNA coding region, which would lead to amino acid differences at positions 128 (Leu to Phe), 256 (Asp to Gly), 287 (Ser to Arg), and 351 (Met to Val). However, none of these amino acids are conserved in CKII-α subunits [23] and/or protein kinases [19], and all of them are located in sequences not involved in the catalytic or ATP binding domains [19].

While this manuscript was in preparation, Wirkner et al. [20] described a (pseudo)gene from a human placenta genomic library. This (pseudo)gene contained a stop

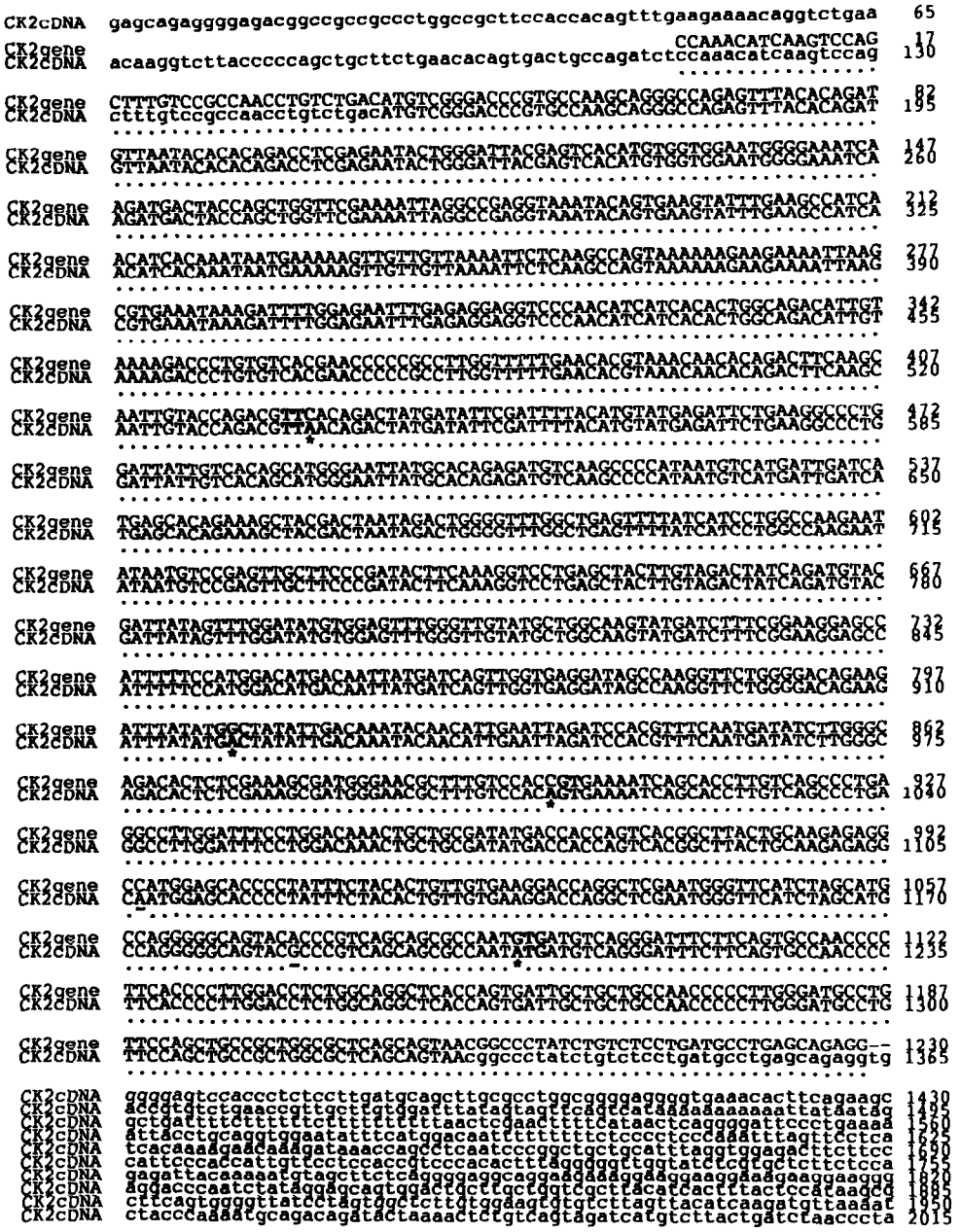

Fig. 4. Comparison of an intronless CKII-α gene DNA sequence with the human cDNA. Sequence of the PCR amplified DNA fragments. All of the fragments were amplified at least twice, purified as described under Materials and Methods and sequenced. The nucleotide identity with the cDNA sequence is indicated by dots. Nucleotide changes that would result in a different amino acid indicated by *. Nucleotides changes that would not lead into amino acid differences are indicated by dashes.

codon in the position corresponding to amino acid 296 of the cDNA open reading frame. The important difference between the sequence reported by Wirkner et al. and that shown in this work is that our gene does not possess a stop codon in the coding region and thus would not lead to a truncated protein. It should be noted that we have sequenced DNA fragments from three different individuals, in order to avoid Taq DNA polymerase errors and specific polymorphisms. In these sequences the results clearly indicate the presence of the same codon (GAG) at position 296. This discrepancy might obviously be explained by the existence of a mutation in the individual from which the placenta gene was obtained by Wirkner et al. [20]. It will be interesting to examine the expression of CKII-α in different human tissues in order to determine if in fact this gene is expressed. It is noteworthy that the hypothetical (pseudo)gene possesses a promoter-like region upstream from the potential initiation codon which includes two TATA boxes and one CAAT box [20]. In

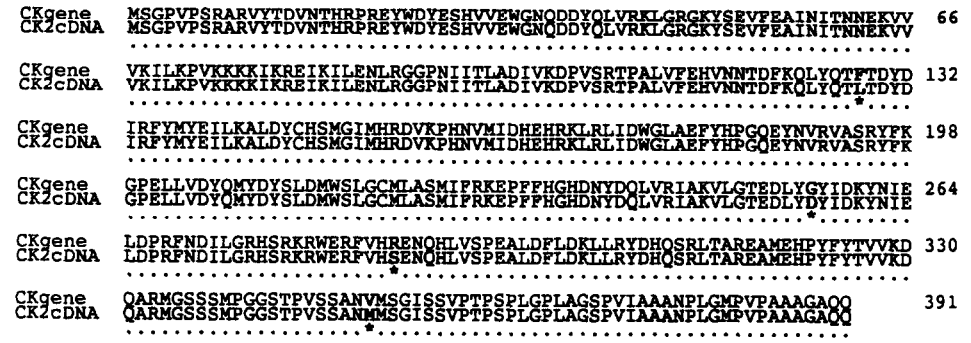

Fig. 5. Comparison of the resulted amino acid sequences from the intronless gene and the cDNA for the human CKII-α subunit. The amino acid sequences from the cDNA and the intronless gene are shown. The four amino acid changes are indicated by *.

conclusion, the facts that a stop codon is not present in the gene of the individuals analyzed in this report, and that the gene is 99% identical to the cDNA, suggest that this gene might code for a physiologically relevant CKII-α.

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