

SHORT COMMUNICATION

Structure of the Gene Encoding the Murine Protein Kinase CK2 β Subunit

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The mouse protein kinase CK2 β subunit gene (*Csnk2b*) is composed of seven exons contained within 7874 bp. The exon and intron lengths extend from 76 to 321 and 111 to 1272 bp, respectively. The lengths of the murine coding exons correspond exactly to the lengths of the exons in the human CK2 β gene. Both genes contain a first untranslated exon. Also, the promoter regions from the human and murine CK2 β gene share some common features, e.g., they contain neither a TATA nor a CAAT box, exon 1 is flanked by a cluster of CpG dinucleotides and recognition sequences for the *HpaII* restriction endonuclease, and several blocks of sequence in the 5' flanking region are conserved between mouse and human. Despite all of these common features, one of the most striking differences found concerns the human CK2 α subunit binding domain at position -170 to -239 of the human gene. This domain has no counterpart in the murine gene. Hence, regulation of transcription of the CK2 β gene by the catalytic CK2 α subunit as was described by Robitzki *et al.* (*J. Biol. Chem.* 268: 5694-5703, 1993) for the human gene cannot be considered a general regulatory mechanism. © 1995 Academic Press, Inc.

Protein kinase CK2 is a serine/threonine protein kinase that phosphorylates numerous substrates *in vivo* and *in vitro* and is implicated in the regulation of proliferation (1, 7, 13). It consists of a tetrameric $\alpha_2\beta_2$ or $\alpha\alpha'\beta_2$ complex. The function of the CK2 β subunit was partly characterized using recombinant CK2 β subunit protein. It was shown that the CK2 β subunit confers activity, specificity, and stability on the catalytic α subunit (4). Compared to other protein kinases, the β subunit is unique in this respect.

Recent data (12) suggest that 80% of CK2 subunits do not form the tetrameric complex and were instead found to bind to subcellular complexes yet to be identified. It was also reported that CK2 β is produced far in excess of the α subunit and that the additional β pro-

tein is rapidly degraded (5). Furthermore it has been shown that in 10 of 21 renal carcinomas asymmetric distribution of CK2 β subunit occurs (11). Studies in *Schizosaccharomyces pombe* have shown that the overexpression of CK2 β leads to inhibition of cell growth and cytokinesis with formation of multiseptate cells (9). Disruption of the CK2 β gene caused a cold-sensitive phenotype and abnormalities in cell shape. Overexpression of CK2 α did not lead to any visible alterations in the phenotype. Taking everything into consideration we postulate a role for CK2 β both in the CK2 holoenzyme and in other regulatory mechanisms in the cell. It is possible that the presence of abnormally high levels of CK2 β is deleterious to the cell and may be one of the events leading to a malignant phenotype. A prerequisite, however, is knowledge of how CK2 β gene expression is regulated in normal cells. As a first step in this direction we have elucidated the structure of the murine CK2 β gene (*Csnk2b*) with special attention to putative promoter sequences.

A mouse genomic library in Lambda FIXII (Stratagene) was screened with the cDNA insert of the human CK2 β clone pBB-4 (2). A positive clone λ GM β 6 was isolated and further characterized by restriction mapping and subcloning using standard procedures (10) (Fig. 1). The subclones β 151, β 131, β 105, and β 96 were sequenced by using the nested deletion procedure (3). Gaps within the obtained sequence were filled by sequencing with internal primers. All *XbaI* and *EcoRI* restriction cutting sites were sequenced by using internal primers. The overlapping clones β 74 or β 194 served as templates in a second control sequence analysis. A T7 sequencing kit (Pharmacia) was used, and sequences were aligned with the DNASIS program (Pharmacia).

The complete sequence encompasses 7874 bp (EMBL Accession No. X80685) and contains seven exons and six introns. The sizes of the exons were 321 (No. 1), 83 (2), 103 (3), 116 (4), 76 (5), 190 (6), and 231 (7). Those of the introns were 594 (1), 589 (2), 1181 (3), 388 (4), 111 (5), and 1272 (6). The exon/intron boundaries conform to the GT/AG rule (6). The lengths of the coding exons 2-7 correspond exactly to the lengths of the exons in the human CK2 β gene (14). The first exon is

Sequence data from this article have been deposited with the EMBL/GenBank Data Libraries under Accession No. X80685.

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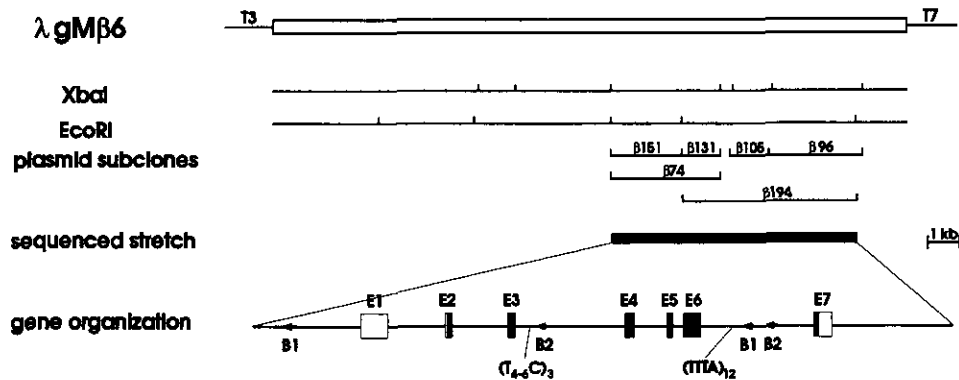


FIG. 1. Recombinant λ gM β 6 clone and derived subcloned genomic fragments. A partial restriction map of genomic DNA fragments that were analyzed to obtain the sequences of the murine CK2 β genes is shown. Six primary subclones were obtained from the genomic clone λ gM β 6 after digestion with *Xba*I or *Eco*RI. The phage clone λ gM β 6 is shown to scale. The size of the clone and subclones is indicated by the scale bar. The shaded box indicates the sequenced area. The intron/exon organization and repetitive elements are shown below. The exons are indicated as E1 through E7; coding exon regions are in black.

untranslated, as it is in human. However, major differences in intron length between the mouse and the human gene have been found, mainly as a result of insertion of retrotransposons. The two largest introns in the mouse gene (3 and 6) contain simple sequence repeats and B1 and/or B2 elements. These elements are widespread in the mouse genome and are often found in introns and intergenic regions, but their significance is not understood.

A more refined comparison between the CK2 β genes from mouse and human is shown by the Harr plot analysis in Fig. 2. Clusters of related sequences are found in the exons, but there are also good matches in the untranslated 5' and 3' areas and in introns 1, 4, and 5. The overall identity in the coding exon regions between the two sequences is 91–95%. The first untranslated exon is 71% and the 5'- and 3'-flanking regions are 55 and 62% identical, whereas introns have about 50% or less similarity. Intron 1 is an exception; it is 66% identical and contains a stretch from position 1809 to 1937 in the mouse sequence, which shows 73% identity to the corresponding human sequence.

Figure 3 shows an alignment of the exon 1 and the promoter region with the human sequence. As in the case for the human sequence, three transcription start sites were determined (data not shown). The first transcription start site of the murine CK2 β gene is 19 bp downstream of that determined for the human gene. The CK2 β gene promoters from mouse and human share some more common features: lack of a TATA box and a CAAT box, clusters of CpG dinucleotides and *Hpa*II recognition sequences around exon 1, and a GC content of more than 60% (Fig. 3B). Several putative transcription factor binding sites are located at identical or similar positions in both genes. Two sequence stretches at positions -338 to -401 and -564 to -612 in the mouse gene share 92 and 98% identity with the human counterpart. Despite all of these similarities between the mouse and the human CK2 β gene pro-

moter, we did not find a CK2 α binding domain in the mouse promoter as was described for the human promoter (8). The corresponding sequence stretch in the mouse promoter is only 38.5% identical to the human CK2 α binding domain. In particular the GC boxes, which were shown to be required for binding of the CK2 α subunit, are lacking in the mouse sequence. Rob-

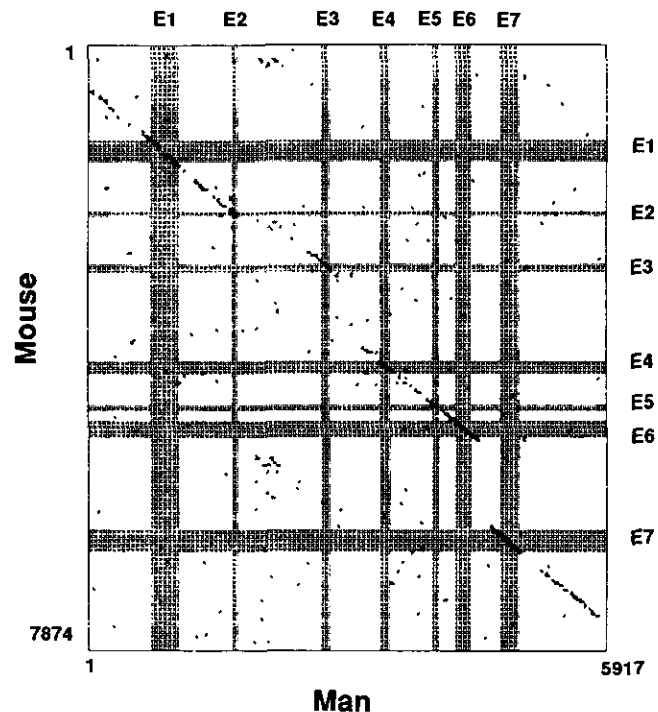


FIG. 2. Harr plot analysis. We used the Harr plot function of the DNASIS program (Pharmacia). The Harr plot is a dot matrix homology plot in which a block of DNA sequence is analyzed for matching bases in another sequence. The block size was 20 nucleotides, and the number of matching bases was 15. The complete mouse and human sequences were compared. The size for the human gene is shown on the abscissa and that for the mouse on the ordinate. The exon areas are stippled. The exons are indicated as E1 through E7.

A

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M agcctgtcggttgggtcctgcttttacataacgccccacaaatgcccttcg..... -564
H gatctgtcggttgggtcctacttttacataacgccccacaaatgcccttcgcttctcaacgtggccccgcctccaag -603
                                     NF1
M .....CACCC
                                     ccacctccggttcagaagactctgaggacacagtgaggagacctgtgggatgccg -506
H cccattttctggagccaggaatccactctgtgggttaggaaagccctcaggaggc.ggagggaaacctgtggaatgccg -524

M aggagctgtgagatgaagtaac.atcacgg..ttaccctcaccactgctctgagcagggttgaag..aacagtcaggg -431
H agaagccgtgtaataaataacgggtcaagccctggccccctcaccaattactctgaccagggttcgaaggtcacacttagag -444
                                     NF1
M tcatcagcacaaactctctaccccagctctgaggagatggagaagtacaaagggacaaagcggcgagagaatggctggcccc -351
H .....cctaaagggaaatggagaagtgcgaagggacga.....gcagaatggctggcacc -394
                                     Pu-stretch
M acctcaggttagctcgacgggtccaggtttctcagcgagctgacagctcagcgcatcccaagcacacccgacacaa -271
H acctcaggttagcgactgggacgttccagttctca.....caccgccccccccccccacccaagtcctacgcac -323
                                     SP1
                                     SP1-RC/AP2/CACCC
M g.....tcctcaccacaactctgcgatgcgggagcccgagcgca.....ctctgtgcggggcc -216
H ggagccaagccgacactctccctcatgaggcaggagcccgaggaaacagtcacggcgtaagggtctctgaggcgac -243
                                     SP1-RC
M tg.....gcgagagaacaggatcccggtgggttggcctggcacagggaagaggagaaaaaagc.. -155
H tgattcgcactaggggcccaacaggaataaggacccagcgatttggccgaggataggccagtcacctgggcagcagcgc -163
                                     GC-box
                                     α-binding domain
M .....aaagccaactgagggagcaccgcagggatgacaatcagcccaggagacctac...ccggcagcttagc - 91
H cgcgcgcggactagaggggaacgtgagggagagctgc.ggaagagatccagcctggctccctccttcccgccctaaagt - 84
                                     GCF-RC
                                     Pu-stretch
                                     SP1-RC
M gaggct.....aacaagactatatttccaggttctctggtcgggaagacccccctgctcggcttccgcttc - 25
H cagcctcttcacccagtgagcacaaaactgtattgcccagactcccgggcccca..acgccatactggcttccgcttc - 6
                                     GCF-RC
                                     GCF-RC/SP1-RC
M cgggtggcgcctcggttcgccccgcCCCCAG..GCCTTCCTCTAGGGCCTCGCGGCTTAGAGAGTCATGCGCGGGTCTCT - 54
H cgggtgCCTTCTCGTTGTGCCCGCCCGCAAGCGCCCTCCTCGGGCCTTCGTGACAGCCAG.GTCGTGCGCGGGTCATCC - 74
                                     GCF-RC/SP1-RC
                                     GCF-RC
M TGGGATTGGTAGTTTGTCTTCCCCCATTTTCGTCAAGTTTCTCTCTGCGACGGGACTACATCTCCCGGCGTCCACGGAGG - 134
H TGGGATTGGTAGTTTCG.CTTTCTCTCATTTAGCCAGTTTCTTCTCTACCGGGGACTCCGTGTCCCGGCATCCACCGCGG - 153
                                     CP1/CP2/NF1
                                     Py-stretch
                                     Py-stretch
M TAGGGGGCGGTTCTTAGCGCCTGC.CGTTGCCCTTTTCTCACCCAAATCCTCACAGTTTCCAGTGCGCCCCCCCCCTCC - 213
H CACCTGAC...CCTTGGCGCTTGGTGTGCTCCCTCTT...CCCCACCTCCCTAATTTCCA....CTCCCCACCCC - 220
                                     GCF
                                     CACCC
                                     AP2/CACCC
M TACTTCGTCTGCTGCGGGCGGGTCCGGCTTTTTCGCGTGTAGTGGTCTCTGCGGTTCTTGGGAAGCA.CAGCTCCCTTCC - 292
H CACTTCGCTGCCGCGGTCCGGTCCGCGGCTGCGGTGTAGCGGTGCGCGGCTTCCCTGGAAGTAGCAACTTCCTTACC - 300
                                     GCF
                                     CACCC
M CCGCCCCAGTCCAGTCCCGTCCGGCCG 321
H CCACCCCAATCCTGGTCCCGTCCAGCCG 329

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B

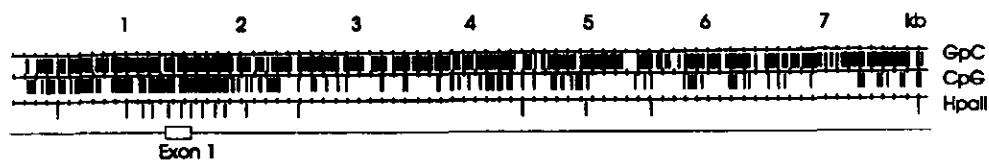


FIG. 3. (A) Alignment of the promoter sequences from the human and murine *CK2β* gene. The upper sequence shows the murine sequence (M) and the lower sequence the corresponding human sequence (H). Gaps (indicated by dots) have been introduced for optimal alignment. Alignment was performed using the BestFit program of HUSAR (German Cancer Center, Heidelberg). Putative transcription factor binding sites are underlined and labeled (purine stretch (Pu), pyrimidine stretch (Py), reverse complement (RC), SP1, CP1/CP2, GCF, AP2, NF1). The *CK2α* binding domain in the human sequence is underlined, and the GC boxes are thickly underlined. From the first transcription start site on, both sequences are in capital letters. The transcription start sites are in boldface. (B) Distribution of GpC, CpG dinucleotides, and *HpaII* sites in the mouse sequence.

itzki *et al.* (8) suggested that the *CK2α* subunit protein could activate human *CK2β* gene transcription by binding to the α binding domain and thus control the *CK2*

subunit stoichiometry in the cell. In mouse, this mechanism does not hold true and is obviously not a general regulatory mechanism.

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