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(54) Title: IDENTIFICATION OF NOVEL SPLICE VARIANTS OF THE HUMAN CATALYTIC SUBUNIT Cβ OF cAMP-DEPENDENT PROTEIN KINASE AND THE USE THEREOF

(57) Abstract: The present invention demonstrates that the Cβgene encodes at least 6 different gene products, designated Cβ1, Cβ2, Cβ3, Cβ4, Cβ4ab and Cβ4abc. As is the case with the murine and bovine splice variants, all the human Cβ splice variants vary in the N-terminal part preceding the part encoded by exon 2. Homologues to all Cβ splice variants identified in mouse and bovine were identified in human (Cβ1, Cß β2, Cβ3, and Cβ4) in addition to two novel Cβ splice variants (Cβ4ab and Cβ4abc), that have previously not been identified in any other species. The present invention includes in this respect genomic DNA- and cDNA sequences encoding said splice variants and comprises the nucleotide sequences shown in SEQ ID NO: 1, 2, 3, 4, 5 and 6 respectively. Wherein the said proteins are new splice variants of the Cβ protein. The present invention is further directed to vectors comprising said cDNA sequences. The invention also includes proteins characterised by the specific amino acid Cß β splice variant proteins shown in SEQ ID NO: 7, 8 and 9 respectively. The invention includes further use of the said Cβ splice variant proteins and DNA sequences in preparation of pharmaceuticals for diagnostic- and therapeutic purposes.

IDENTIFICATION OF NOVEL SPLICE VARIANTS OF THE HUMAN CATALYTIC SUBUNIT Cβ OF cAMP-DEPENDENT PROTEIN KINASE AND THE USE THEREOF

FIELD OF THE INVENTION

The present invention relates to genomic- and complementary DNA sequences encoding the 6 different gene products, designated Cβ1, Cβ2, Cβ3, Cβ4, Cβ4ab and Cβ4abc which are novel splice variants of Cβ. The present invention also relates to vectors comprising said DNA sequences and is also directed to said proteins in diagnosis and treatment.

BACKGROUND OF THE INVENTION

Cyclic 3', 5'-adenosine monophosphate (cAMP) is a key intracellular signalling molecule, which main function is to activate the cAMP-dependent protein kinases (PKA) [1]. PKA consists of a heterotetramere, with a regulatory (R) subunit dimer and two catalytic (C) subunits. The holoenzyme is activated when four molecules of cAMP bind to the R subunit dimer, two to each R subunit, releasing two free active C subunits [2]. In man, four different R subunits (RIα, RIβ RIIα, RIIβ), and four different C subunits (Cα, Cβ, Cγ and PrKX) have been identified [3]. The Cα and Cβ subunits are expressed in most tissues, while the Cγ subunit, which is transcribed from an intron-less gene and represents a retroposon derived from the Cα subunit [4], is only expressed in human testis [5]. PrKX is an X chromosome-encoded protein kinase, and was recently identified as a PKA C subunit since it is inhibited by both PKI and RIα and the RIα/PrKX complex is activated by cAMP [6].

Splice variants of both Cα and Cβ have been identified. The splice variants of Cα have been termed Cα1 (previously named Cα [7]), Cα2 [8] and Cα-s [9]. Originally Cα2 was isolated from interferon-treated cells and identified as a C-terminally truncated Cα1 subunit. However,

recently a novel Cα2 splice variant was reported [10]. The novel Cα2 variant was shown to be identical to the previously identified Cα splice variant, Cα-s. Moreover, Cα-s which was originally isolated and characterized from ovine sperm [9], has later been cloned from a human testis cDNA library and identified in human sperm [11]. Both Cα-s/Cα2 are encoded with a truncated N-terminal end when compared to Cα1. The variable parts of Cα1 and Cα-s are located upstream of exon 2 in the murine Cα gene, implying that the variation in the N-terminal end of the Cα1 and Cα-s/Cα2 are due to alternative use of different first exons. In bovine, two splice variants of Cβ have been identified, termed bovine Cβ1 [12] and bovine Cβ2 [13]. The bovine splice variants contain variable N-terminal ends in which the non-identical sequences are most probably encoded by different forms of exon 1. Bovine Cβ2 is expressed at low levels in most tissues with the highest expression in the spleen, thymus, and kidney and to some extent brain. Furthermore, in the mouse, three splice variants of Cβ have been identified and are designated mouse Cβ1, mCβ2 and mouse Cβ3 [14]. Whereas mouse Cβ1 is ubiquitously expressed, mouse Cβ2 and mouse Cβ3 have so far only been identified in the brain. The mouse Cβ1 and bovine Cβ1 are similar in the entire sequence, demonstrating that they represent orthologe protein sequences. However, neither mouse Cβ3 nor mouse Cβ4 were similar to bovine Cβ2 in the N-terminal part, indicating that their N-terminals are encoded by unrelated exons. Previous to this study, only a single splice variant of human Cβ had been identified (Cβ1), homologous to mouse Cβ1 and bovine Cβ1.

SUMMARY OF THE INVENTION

The present invnetion demonstrate that the Cβ gene encodes at least 6 different gene products, designated Cβ1, Cβ2, Cβ3, Cβ4, Cβ4ab and Cβ4abc. As is the case with the murine and bovine splice variants, all the human Cβ splice variants vary in the N-terminal part preceding

the part encoded by exon 2. Homologues to all Cβ splice variants identified in mouse and bovine were identified in human (Cβ1, Cβ2, Cβ3 and Cβ4) in addition to two novel Cβ splice variants (Cβ4ab and Cβ4abc), that have previously not been identified in any other species. The present invention includes in this respect genomic DNA- and cDNA sequences encoding said splice variants and comprises the nucleotide sequences shown in SEQ ID NO: 1,2,3,4,5 and 6 respectively. Wherein the said proteins are new splice variants of the Cβ protein. The present invention is further directed to vectors comprising said cDNA sequences. The invention also includes proteins characterised by the specific amino acid Cβ splice variant proteins Cβ2, Cβ4ab and Cβ4abc shown in SEQ ID NO: 7,8 and 9. The invention includes further use of the said Cβ splice variant proteins and DNA sequences in preparation of pharmaceuticals for diagnostic- and therapeutic purposes.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: **A: Identification of cDNAs encoding human Cβ splice variants.** Schematic representation of the protein-encoding sequences of the various Cβ splice variants found in human. Human cDNAs from total fetus and brain were amplified using primers complementary to the Cβ cDNA, subcloned and sequenced. The resulting cDNAs were identical to the previously published Cβ cDNA (Cβ1) downstream of nucleotide 46 (constant region). However, five novel cDNA sequences, designated Cβ2, Cβ3, Cβ4, Cβ4ab and Cβ4abc, could be identified based on differences in the 5'-ends of the sequences (variable region).

Figure 2: **A: Structure of the human genomic region encoding the novel Cβ splice variants.** Primers were made based on exon 2 and the most 5'-end of the different Cβ cDNAs, and used to amplify human genomic DNA by PCR. Two overlapping PCR products of 14 and

17 kb, respectively, were identified and mapped by Southern blotting and hybridization to oligonucleotides corresponding to the different cDNAs. As derived from the 14 and 17 kb PCR products, exon 1-2 1-3, 1-4 and exon a, b and c are located 31, 14.1, 14, 8.1, 5.4 and 4.4 kb upstream of exon 2. Based on restriction mapping of the PAC clone RPCI-6-228E23, exon 1-1 is located approximately 60 kb upstream of exon 1-2. Exon 1-1 is specific for the splice variant, which encodes CB1. The exons are indicated as vertical lines. The introns are drawn to scale as indicated. **B: Nucleotide sequence of genomic regions encoding novel splice variants of Cβ.** Protein encoding sequences are in capital letters, intron and 5'-untranslated sequences are in lower case letters. Translation initiation codons are underlined. Only the 5'-end of exon 2 is included. **C: Schematic representation of how the various human Cβ exons 5'to exon 2 may be spliced.** The upper panel describes a potential model in which four variants of exon 1 designated exon 1-1, 1-2, 1-3 and 1-4 may alternatively splice with exon 1 to encode the splice variant specific sequence in Cβ1, Cβ2, Cβ3 and Cβ4. The lower panel describes a model in which the exons a, b and c may splice with exon 1-4 and 1-3 upstream of exon 2 to encode the splice variant-specific sequences in Cβ4ab, Cβ4abc and Cβ3ab.

Figure 3: **Deduced amino acid sequence of Cβ splice variants.** The amino acid sequences of the amino terminal parts of Cβ1 and five new splice variants, designated Cβ2, Cβ3, Cβ4, Cβ4ab and C4βabc according to the cDNA clones shown in figure 1A. The amino acid sequences are shown in the one letter code and demonstrate that six novel Cβ exons give rise to five different cDNAs as a result of alternative promoter use and alternative splicing. The myristylation motive G-N previously identified in Cβ1 is boxed. A PKA autophosphorylation motive that has previously been identified in Cβ1, is underlined and Ser10 which is potentially

phosphorylated, is labeled by an asterisk. Note that there is a PKA autophosphorylation motif, encoded by exon a, present in Cβ4ab and Cβ4abc.

Figure 4: **Tissue distribution of different Cβ splice variants.** Northern blots containing various human tissues were hybridized using probes specific for Cβ1, Cβ2, Cβ4, exon a+b and a probe common to all Cβ splice variants (Cβ common). For comparison, the same blots were hybridized using a GAPDH cDNA (GAPDH). All Cβ mRNAs had the same apparent length (4.4 kb).

Figure 5: **A**: **Species distribution of Cβ2**. A Southern blot containing EcoRI digested genomic DNA from various species was hybridized using a DNA probe corresponding to exon 1-2 (Cβ2 specific). A single hybridizing band identifying genomic sequence homologous to human exon 1-2 was identified in mammalians such as monkey, dog, rabbit and human except mouse and rat. **B: Cβ2 is not expressed in the mouse.** A Northern blot containing total RNA (20 μg pr. lane) isolated from wild type (+/+) mouse brain and spleen (lane 1 and 3), brain and spleen of mice ablated (-/-) for Cβ1 (lane 2 and 4) and human peripheral blood leukocytes (lane 5) was probed with a Cβ probe expected to recognize all known Cβ splice variants (Cβ Common, upper panel) and a Cβ probe specific for the Cβ2 splice variant (Cβ2, lower panel). Messenger RNA recognized by the two probes is indicated as 4.4 kb.

DETAILED DESCRIPTION OF THE INVENTION

The present invnetion demonstrate that the human Cβ gene encodes five novel Cβ splice variants, designated Cβ2, Cβ3, Cβ4, Cβ4ab and Cβ4abc, in addition to the previously identified splice variant Cβ1 [12]. All the Cβ splice variants contained a unique N-terminal end, and showed tissue specific expression. As we found no evidence of an additional exon upstream of exon 1-1 and all the cDNA characterized had unique 5'-ends, it is reasonable to assume that the exon 1-1, 1-2, 1-3 and 1-4 each contain a separate promoter, and that the resulting mRNA products are due to alternative use of different promoters. Despite this, we can not rule out the possibility that two or more of these splice variants share a common promoter used to alternatively splice the different exons. Furthermore, we found two Cβ variants, Cβ4ab and Cβ4abc, that were the results of alternative splicing of either exon a and b, or exon a, b and c, between exon 1-4 and exon 2. The presence of the corresponding mRNA was confirmed by hybridizing a Northern blot with a probe complimentary to the sequences found in exons a and b. This probe and the probe specific for Cβ4 bound to an RNA with the same apparent length located in human brain. The location of the exons a, b and c may suggest that they generate splice variants of Cβ in addition to those demonstrated here. Indeed, a short cDNA from human infant brain have been sequenced and demonstrated to contain a combination of exons 1-3, a, b and 2 (Accession no. AA351487, see Fig. 2C). We were unable to produce such a cDNA, which could be due to low level expression of Cβ3 in adult brain.

The two splice variants Cα1 and Cβ1 are highly conserved in the parts encoded by exon 1, differing in only 2 of the first 16 amino acids [7;12]. It is therefore tempting to suggest that this region serve a specific role in the function of these splice variants. Thus, the fact that we have identified several Cβ splice variants with variable N-terminal ends could suggest that the N-terminal domain might reflect specific functional features associated with each splice

variant. This is supported by studies of the mouse Cβ1 KO mouse, which displayed impaired hippocampal plasticity [16]. However, to what extent N-terminal differences influence catalytic activity is not known since it was shown that the N-terminally truncated Cβ splice variants in mouse, Cβ2 and Cβ3 were catalytically active, an activity that was inhibited both by PKI and the R subunit *in vivo* [14]. In addition, a study by Herberg et al [17] showed that deleting amino acids 1-14 in the Cα isoform did not influence catalytic activity, demonstrating that the N-terminal specific for the Cα1/Cβ1 is not necessary for catalytic activity.

The N-terminal of Cα1 and Cβ1 contain two sites for post-translational modification, a myristylation site and an autophosphorylation site [5;18;19]. In Cα1, Cβ1 and Cβ3 the N-terminal amino acid is G (Gly) which has been shown as an absolute requirement for myristylation [20]. Despite this, it was previously demonstrated in the mouse that Cβ3 does not undergo myristylation *in vivo* [14]. This phenomena may be explained based on a recent study, demonstrating that the amino acid C-terminal to G must be N if myristylation shall occur. This because deamination of N to yield D is an absolute requirement [21]. Because the amino acid C-terminal to G is L in both mouse and human Cβ3, it explains why mouse Cβ3 is not myristylated and suggests that the human Cβ3 may not be myristylated *in vivo*.

The fact that several human Cβ splice variants (Cβ2, Cβ3, Cβ4, Cβ4ab and Cβ4abc) lack the ability to become myristylated *in vivo,* question the role of this post translational modification. Based on the Cα crystal structure it appears that the myristyl group serves to fill and shade a hydrophobic pocket in the large lobe [22], suggesting that this N-terminal modification serves to solubilize the C subunit. This is supported by two independent observations. Firstly, expression of an N-terminally truncated form of Cα1 revealed a C subunit tightly associated with the particulate fraction [23]. Secondly, the Cα-s/Cα2 which is a naturally occuring N-terminally truncated splice variant is tightly associate with sub cellular

structures in both ovine- [9;24] and human [11] sperm. This taken together with a recent report, which demonstrated that the myristyl group serves to increase the lipofilic properties of the C subunit when binding the RII- but not the RI subuni t[25], suggests that the N-terminal amino acids of Cα1 together with myristylation serves to influence C subunit solubility. Thus, the sequence similarity between Cα1 and Cβ1 and the difference in solubility of Cα1 and Cα-s/Cα2, may imply comparable difference in solubility between Cβ1 and the truncated Cβ forms.

Previously a consensus autophosphorylation motif (-KKGS^{10} -) was identified in Cα1 and Cβ1 [12;26], that is phosphorylated when Cα1 is expressed in bacteria [18;23]. In the study by Yonemoto et al. (1993) mutation of S^{10} yielded an insoluble enzyme that appeared inactive. Thus, the N-terminal domain may also have implications for catalytic activity by an unknown mechanism. However, like the human Cβ2, Cβ3, Cβ4, the mouse Cβ2 and Cβ3 lack S^{10}, yet these splice variants are soluble and catalytically active *in* vivo [14]. This suggests that the human homologues most probably are active and may imply that S^{10} phosphorylation is not crucial for C subunit catalysis. Interestingly, we identified a potential autophosphorylation site (-RKSS^{6}-) in Cβ4ab and Cβ4abc that was encoded by exon a. To what extent this site represents a true autophosphorylation site that will influence Cβ4ab and Cβ4abc properties, remains to be seen.

The human Cβ2 splice variant was similar to the previously identified bovine Cβ2 splice variant, but we have been unable to identify a similar splice variant in mice. Interestingly, the human Cβ2 splice variant is expressed only in peripheral tissues, while no detectable Cβ2 mRNA signal is found in human brain. However, no Cβ can be detected outside the brain in mice lacking the Cβ1 splice variant [14;16]. In addition, we were unable to detect any signal

when hybridizing mouse DNA using a human Cβ2 specific probe. Thus, it is likely that mice do not contain a homologue of the human and bovine Cβ2 splice variants.

Interestingly, Cβ2 is the most atypical of the Cβ splice variants. This subunit is encoded with an extended N-terminal domain, which do not resemble any of the other Cβ splice variants. The unique domain together with the fact that Cβ2 lacks the myristylation- as well the autophosphorylation site, and that Cβ2 is the only Cβ splice variant not identified in the brain, may suggest specific and unique features associated with this splice variant in other tissues that will await further studies.

The inventors suggest that tissue-specific expression of various Cβ splice variants when complexed with R subunits may imply novel PKA holoenzymes with specific functional features that may be important as mediators of cAMP effects.

The present invention includes in this respect genomic DNA- and cDNA sequences encoding splicevariants Cβ1, Cβ2, Cβ3, Cβ4, Cβ4ab and Cβ4abc and comprises the nucleotide sequence shown in SEQ ID NO: 1,2,3,4,5 and 6 respectively. Wherein the said proteins are new splice variants of the Cβ protein. The present invention is further directed to vectors comprising said cDNA sequences. The invention also includes proteins characterised by the specific amino acid Cβ splice variant proteins; Cβ2, Cβ4ab and Cβ4abc shown in SEQ ID NO: 7,8and 9 respectively. The invention includes further use of the said Cβ splice variant proteins and DNA sequences in preparation of pharmaceuticals for diagnostic- and therapeutic in order to identify, characterize and produce pharmacological compositions .

Cβ2 is an enzyme that is expressed in lymphoid cells, whereby its function is to mediate the regulatory effects of cAMP on T cell activation. Thus, altered levels, location and/or activity of Cβ2 will according to the inventors results, have impact on the regulation and normal function

of receptors and enzymes which are important for T cell activation and are regulated by cAMP. This knowledge can be used to diagnose hyperreactive and dysfunctional T cells associated with various immune diseases.

1) ***Malfunctioned T cells:*** I is well known that T cells isolated from patients suffering from T cell-dependent common variable immune deficiency (CVI) and acquired immune deficiency syndrome (AIDS) do not respond to antigen. Furthermore, T cells isolated from patients suffering from certain types of rheumatoid arthritis and other auto immune diseases are hyper sensitive to foreign antigens. In both cases these situations evoke abnormal immune responses that may involve malfunctioned Cβ2. This may either be monitored as constitutively activated Cβ2, sub-normal activity or dislocation of Cβ2.

1.1) ***Improving T cell dysfunction:*** Present invention makes it possible to identify, characterize and produce pharmacological compositions after high through put screening that specifically will inhibit the enzymatic activity of Cβ2. These compositions should be developed such that they can be introduced orally or intra venously to enter the blood system reaching the dysfunctional T cells.

Furthermore, dislocation of Cβ2 protein from the T cell membrane will short cut the regulatory effects of Cβ2 on relevant receptors. Thus, the present invention makes it possible to identify, characterize and produce pharmacological composition after high through put screening that will specifically and irreversibly block Cβ2 interaction with the T cell membrane. These compositions should be developed such that they can be introduced orally or intra venously to enter the blood system reaching the T cell.

1.2) Down regulation of hyper active T cells: Present invention makes it possible to identify, characterize and produce pharmacological compositions after high through put screening that specifically will activate the enzymatic activity of Cβ2. These compositions should be

developed such that they can be introduced orally or intra venously to enter the blood system reaching the dysfunctional T cells.

1.3) ***Kits for diagnosing Cβ2 mutations:*** T cell malfunction caused by mal function or - localization of Cβ2 enzyme activity may be caused by mutation(s) in the Cβ2 protein. Present invention makes it possible to develop kits, which would diagnostically facilitate if mutated Cβ2 is present. Such kits should be developed with Cβ2 specific DNA probes.

Present invention makes it possible to develop a method for inspection and screening of patient T cells for the presence and location of Cβ2 comprising:

a) collection and washing in buffer of isolated peripheral blood T lymphocytes according to [27];

b) preparing for identification of Cβ2 protein by immunofluorescence, T cells are let to settle onto poly L-lysine coated cover slips following detergent-dependent lysis;

c) incubation with primary antibody (Ab), either irrelevant Ab or Cβ2 specific Ab, Ab overshoot will be removed by washing buffer and T cells incubated with secondary anti-IgG Ab conjugated with a fluorescent;

d) inspection of T cells under fluorescent microscopy.

Present invention makes it further possible to develop a method of screening patient T cells for membrane associated Cβ2 catalytic activity comprising:

a) collection and washing in buffer of isolated peripheral blood T lymphocytes according to [27];

b) preparation of T cells by lysing in detergent buffer;

b) monitoring Cβ2 specific catalytic activity by established assay, Cβ1 activity is used as an internal control to determine relative activity.

Present invention makes it also possible to screen patients for mutations in the Cβ2 gene and mRNA comprising:

a) collection and washing in buffer of isolated peripheral blood T lymphocytes according to [27];

b) isolation of total RNA and genomic DNA according to established methods followed by RT-PCR using Cβ2 specific primers according to cDNA sequence of Cβ2 specific nucleotides or the Cβ2 specific exon, designated exon 1-2.

Materials and methods.

General protocols

Complementary DNA probes were radiolabeled using the Megaprime random priming kit and α-[32P]dCTP (Amersham) as instructed by the manufacturers to a specific activity of at least 1 x 10^9 cpm. Synthetic oligonucleotides were radiolabeled using T4 polynucleotide kinase (Pharmacia) and γ-[32P]ATP as instructed by the manufacturer.

DNA was either sequenced manually using Thermo Sequenase radioabeled terminator cycle sequencing kit (Amersham, Buckinghamshire, UK) or by Medigenomix (Martinsried, Germany). Sequences were analyzed using the Wisconsin University GCG program package (UWGCG) and the basic local alignment and search tool (BLAST) [15].

Identification of cDNAs

The 5'-end of human Cβ cDNA was amplified from human total fetus and brain Marathon RACE-ready cDNAs (Clontech) using the Advantage KlenTaq Polymerase Mix (Clontech) as described by the manufacturer. Amplification was performed using adapter primer 1 (Clontech) and four different primers complementary to the human Cβ cDNA sequence (5'-CAACCCAAAGAGAAGTAAGAAAGTGGTCTA-3',

5'- TTGGTTGGTCTGCAAAGAATGGGGGATAGC-3',

5'-TTTTCTCATTCAAAGTATGCTCTATTTGC-3' and

5'- AGAATAATGCCGGACTTGAAGATTTTGAAA-3').

Five cycles were performed with 45 sec 94 °C, 2 min 72 °C, five cycles 45 sec 94 °C, 2 min 70 °C, 25 cycles 45 sec 94 °C, 2 min 68 °C, and a final extension of 10 min at 72°C. The resulting products were separated by gel electrophoresis, subcloned to pCR2.1TOPO (Invitrogen) as instructed by the manufacturer and sequenced.

Amplification of Cβ gene fragments.

A genomic fragment was amplified using an oligonucleotide corresponding to exon 1-3 (5'-GTTTAGGTGCAATCATTCTGCTGTTTG-3') and a primer complementary to sequences in exon 2 (5'- AAAAAGTCTTCTTTGGCTTTGGCTAGA-3'). Another genomic fragment was amplified using a primer corresponding to exon 1-2 (5'-TGGCAGCTTATAGAGAACCACCTT-3') and a primer complementary to sequence found in exon 1-3 (5'- CAATCCCATGTTGAACCTGGCA-3'). PCR reactions were performed using the Boehringer-Mannheim Expand Long Template PCR kit as instructed by the manufacturer using buffer 2. PCR was performed using human genomic DNA (Boehringer-Mannheim) as template with 1 min at 92 °C, 30 cycles of 10 sec94 °C, 30 sec 60 °C and 10 min (extended with 20 sec per cycle from cycle 11 to cycle 30) 68 °C, and a final incubation of 7 min at 68 ° C. Products were separated by agarose gel electrophoresis and analyzed by Southern blotting using radiolabeled cDNAs and synthetic oligonucleotides corresponding to the different exons.

Screening of PAC library and subcloning of exon-containing sequences.

The human P1-derived Artificial Chromosome (PAC) library, RPCI-6 was screened and the isolated bacterial clone was grown in liquid culture and plasmid DNA was isolated using ion-exchange columns as described by the manufacturer (Qiagen, Hilden, Germany). Exon-containing DNA restriction fragments were identified by Southern blotting using radio labeled

cDNAs and synthetic oligonucleotides. Exon-containing fragments were excised from the gel and subcloned to the pZERO2.1 vector (Invitrogen) as instructed by the manufacturer.

Generation of splice variant specific probes, Northern blotting and Southern blotting.

DNA fragments corresponding to the splice variant-specific parts of the cDNAs were amplified by PCR. The following primers were used for the different splice variants:

Cβ1: 5'-GCTCTCCACCTCGCTGCCTTTCTT-3'and primer

5'-CCAGCCCCCCTTCCCTTCCCTGAC-3',

Cβ2: primer 5'-TGGCAGCTTATAGAGAACCACCTT-3' and primer

5'-ATTGATCTGTCCATAAGGCAGTAT-3',

Cβ3: primer 5'-TCACAGCTAGCAGTAAGAGCTG-3' and primer

5'-CAATCCCATGTTGAACCTGGCA-3',

Cβ4:primer 5'-TCTCCAGTGTGTGTGTTTACAC-3' and primer

5'-ATGATGAAAACCAACCTTTCCA-3'.

The primers were used for amplification of the fragments from cloned RACE-products using Taq DNA polymerase (Perkin-Elmer) as described by the manufacturer. For generation of a probe specifically recognizing exon a and b, the primers

5'-GATATTTCTGAAGAGGAGCAAGCAGATGCATCTGATGATTTGCGTG-3' and 5'-CACGCAAATCATCAGATGCATCTGCTTGCTCCTCTTCAGAAATATC-3' were annealed, phosphorylated and ligated. A 1.5 kb fragment of Cβ cDNA [5] was used for recognizing the parts of the Cβ mRNA common to all splice variants. Two similar Northern blots containing RNA from various human sources were purchased from Clontech. One blot was hybridized using a probe specific for Cβ2, while the other blot was probed in succession with probes specific for Cβ3, Cβ4, exon a and b, and the 1.5 kb Cβ cDNA. Both blots were hybridized using GAPDH cDNA as control. As an almost identical pattern of hybridization was

obtained using GAPDH on both blots, only one GAPDH blot is shown (Fig. 4). All probes were hybridized in ExpressHyb hybridization solution (Clontech) as described by the manufacturer. A Southern blot containing EcoRI-digested DNA from various species (Clontech) and Southern blots containing human and mouse DNA digested with various enzymes were hybridized using the probe specific for Cβ2. The filters were prehybridized in 5 X Denhardt's solution, 5 X SSC, 50 mM sodium phosphate buffer, pH 6.8, 0.1 % SDS, 250 μg/ml single stranded salmon sperm DNA, and 50 % (v/v) formamide at 42 °C for 3 h, and hybridized for 16 h in a similar solution containing the radiolabeled Cβ common or Cβ2 probe. The membranes were washed four times in 2 X SSC, 0.1 % SDS for 5 min at room temperature, followed by two washes using 0.5 X SSC, 0.1 % SDS at 50°C for 30 min. Autoradiography was performed at -70°C using Amersham Hyperfilm MP and intensifying screens.

In order that this invention may be better understood, the following examples are set forth. These examples are for the purposes of illustration only, and are not to be construed as limiting the scope of the invention in any manner.

Examples

Example 1

Identification of exons encoding novel splice variants of human Cβ.

The 5'-ends of human Cβ cDNAs were amplified from human brain and total fetus RACE-ready cDNA using four different oligonucleotide primers complementary to the previously published human Cβ cDNA sequence, in combination with an anchor primer. The resulting PCR products were subcloned, sequenced and compared to the previously published human C

β cDNA sequence which is now designated Cβ1 (Fig. 1). All clones sequenced were shown to lack the 46 first protein-encoding nucleotides in the human Cβ1 cDNA sequence. Instead 5 novel stretches of protein encoding sequences were identified (Fig. 1, variable region). Each of the clones contained a translation initiation codon and one or more in-frame upstream stop codons. The five novel cDNA sequences were designated Cβ2, Cβ3, Cβ4, Cβ4ab and Cβ4abc.

All the Cβ cDNAs were similar from nucleotide 47 and down stream in the Cβ1 cDNA, which corresponds to the start of exon 2 in the murine Cβ gene. The identification of novel protein-encoding sequences upstream of exon 2, indicated the presence of several different exons upstream of exon 2. Thus, human genomic DNA was amplified using a combination of primers corresponding to exon 2 (antisense orientation) and the 5'-ends of the different novel cDNAs (sense and antisense orientation) in different combinations. A 17 kb PCR product was the result of an amplification using a primer corresponding to the 5'-end of Cβ2 cDNA (sense orientation) and the 5'-end of Cβ3 (antisense orientation). Furthermore, a 14 kb PCR product was the result of an amplification using a primer corresponding to the 5'-end of Cβ3 cDNA (sense orientation) and a primer corresponding to exon 2 (antisense orientation). These clones enabled us to physically map six novel exons in the Cβ gene that were designated 1-2, 1-3, 1-4, a, b and c, and which were located 31, 14.1, 14, 8.1, 5.4 and 4.4 kb upstream of exon 2, respectively (Fig. 2A). Furthermore, a PAC library was screened using the 5' ends of Cβ1 and Cβ2 cDNAs as probes. One of the clones identified, RPCI-6-228E23, contained both exon 1-2 and an exon containing the entire splice variant-specific part of the Cβ1 cDNA, which we termed exon 1-1.This PAC clone was selected for detailed restriction mapping using CpG cutters. The digested PAC DNA was separated by pulsed-field gel electrophoresis (PFGE), transferred to Southern blot membranes and hybridized with exon 1-1 and 1-2, as well as Sp6 and T7 oligonucleotide probes. These results revealed a distance of

approximately 60kb between exon 1-1 and 1-2 (Fig. 2A). All nucleotide sequences found in the different Cβ cDNAs could be identified in a continuous stretch of human genomic DNA, thereby supporting the notion that these cDNAs are products of the same gene. Exon 1-1 was shown to be homologous to the previously identified exon 1A of the murine Cβ gene. As shown in Fig. 2B, exon 1-2 contains the entire Cβ2 specific sequence, and exon 1-3 contains the sequence specific for Cβ3 which is homologous to the previously identified exon 1B in the mouse Cβ gene. Finally, exon 1-4 was shown to contain the sequence specific for the human Cβ4 splice variant, and to be homologous to the murine exon 1C, which encodes the N-terminal end in the murine Cβ2 splice variant. Based on the Cβ4ab and Cβ4abc cDNA sequences, the exons a, b and c (Fig. 2B), were demonstrated to be alternatively spliced in between exon 1-4 and exon 2, with either exons 1-4, a, b and 2 or exons1-4, a, b, c and 2 (Fig. 2C, lower panel). These cDNA sequences represent novel Cβ splice variants not identified in any other species.

Example 2

Deduced amino acid sequence of novel Cβ splice variants.

The N-terminal parts of the deduced amino acid sequences of the previously published Cβ1-sequence and the 5 novel Cβ splice variants are illustrated in Fig. 3 (upper and lower panels). The splice variants were identical starting from the sequence encoded by exon 2 (amino acid 17 in Cβ1) to the C-terminus, while the N-termini varied both in length and sequence composition. The Cβ2 splice variant contains a 63 amino acid sequence substituting the first 16 amino acids in Cβ1, and is homologous to the previously identified bovine Cβ2 [13]. Furthermore, the human Cβ3 splice variant contains four amino acids in the N-terminal substituting the first 16 amino acids in Cβ1, and is similar to the previously identified murine

Cβ3 [14]. The human Cβ4 contains three amino acids substituting the first 16 amino acids in Cβ1, and is similar to the murine Cβ2 [14]. Finally, the splice variants Cβ4ab and Cβ4abc contain 18 and 21 amino acids, respectively, that substitute the first 16 amino acids of Cβ1. These splice variants show no homology to the N-terminus of any other C subunits identified thus far.

Example 3

Tissue distribution of Cβ splice variants.

To examine the tissue distribution of Cβ splice variants, exon specific DNA probes and a DNA probe common to all Cβ splice variants were hybridized to two similar Northern blots containing RNA from various human tissues. For comparison the blots were hybridized to a cDNA encoding glycer-aldehyde 3-phosphate dehydrogenase (GAPDH). In Fig. 4 (panel Cβ1) we show that Cβ1 is predominantly expressed in brain and kidney with low level expression in several other tissues as well. Cβ2 is expressed at high levels in thymus, spleen and kidney in addition to a weak signal in other tissues (Fig. 4, panel Cβ2). In contrast to Cβ2 the exon 1-4 and exon a and b containing mRNAs appeared to be present exclusively in brain (Fig. 4, panels Cβ4 and exon a+b). Finally, probing the Northern blot with a probe common to all the Cβ splice variants, we observed ubiquitous expression of Cβ with the strongest signal in brain and a somewhat weaker signal in spleen and thymus, when compared to the GAPDH signal (Fig. 4, panel Cβ common). Hybridization using a DNA fragment corresponding to the Cβ3 specific cDNA resulted in an almost undetectable signal in the brain and no detectable signals in any other tissues (data not shown).

Example 4

The human Cβ2 splice variant is not present in the mouse.

Previously we have identified three splice variants of Cβ in the mouse, Cβ1, Cβ2 and Cβ3 [14]. Based on the present work, it is apparent that mouse Cβ2 is not homologous to either bovine or the human Cβ2. Instead, mouse Cβ2 is homologous to what we now have designated human Cβ4. Thus, we investigated whether a Cβ splice variant similar to human Cβ2 was present in the mouse genome. A Zoo-blot containing genomic DNA isolated from human, monkey, rat, mouse, dog, cow, rabbit, chicken and yeast was hybridized using a DNA fragment corresponding to exon 1-2 of human Cβ. In Fig. 5 (panel A, lanes 1 to 9) we show that a DNA fragment was detected using Cβ2 specific probe in man, monkey, dog, cow, and rabbit. In contrast, the Cβ2 specific probe did not recognize any fragments in the rat and mouse suggesting that the Cβ2 specific exon is not present in the murine genome. To further substantiate this observation we isolated total RNA from human, wild type mice and mice that are ablated (knockout, KO) for exon 1A of the Cβ gene [16]. The RNA was isolated from immune tissues and brain since we observed high level expression of Cβ2 in human thymus, spleen and peripheral blood leukocytes and high level of the other Cβ splice variants in the brain (Fig. 4). The Northern blots were probed with a Cβ cDNA probe (expected to recognize all known Cβ splice variants) and a Cβ2 specific probe (see material and methods). In Fig. 5B (upper panel) we demonstrate that Cβ is present in the brain of wild type and Cβ exon 1 KO (lanes 1 and 2) and in human peripheral blood leukocytes (lane 5). The mouse spleen did not contain Cβ mRNA (lanes 3 and 4). When probing the same filter with the Cβ2 specific probe

(Fig. 5, lower panel) Cβ2 message was only detected in human peripheral blood leukocytes (lane 5) whereas all the mouse tissues were negative for Cβ2 mRNA (lanes 1 to 4).

References.

[1] Butcher,R.W., Ho,R.J., Meng,H.C., & Sutherland,E.W. (1965) Adenosine 3',5'-monophosphate in biological materials. II. The measurement of adenosine 3',5'-monophosphate in tissues and the role of the cyclic nucleotide in the lipolytic response of fat to epinephrine. *J. Biol. Chem.*, **240,** 4515-4523.

[2] Doskeland,S.O., Maronde,E., & Gjertsen,B.T. (1993) The genetic subtypes of cAMP-dependent protein kinase--functionally different or redundant? *Biochim. Biophys. Acta,* **1178,** 249-258.

[3] Skalhegg,B.S. & Tasken,K. (1997) SPECIFICITY IN THE cAMP/PKA SIGNALING PATHWAY. DIFFERENTIAL EXPRESSION, REGULATION, AND SUBCELLULAR LOCALIZATION OF SUBUNITS OF PKA. *Front Biosci.*, **2,** d331-d342.

[4] Reinton,N., Haugen,T.B., Orstavik,S., Skalhegg,B.S., Hansson,V., Jahnsen,T., & Tasken,K. (1998) The gene encoding the C gamma catalytic subunit of cAMP-dependent protein kinase is a transcribed retroposon. *Genomics*, **49,** 290-297.

[5] Beebe,S.J., Oyen,O., Sandberg,M., Froysa,A., Hansson,V., & Jahnsen,T. (1990) Molecular cloning of a tissue-specific protein kinase (C gamma) from human testis--representing a third isoform for the catalytic subunit of cAMP-dependent protein kinase. *Mol. Endocrinol.*, **4,** 465-475.

[6] Zimmermann,B., Chiorini,J.A., Ma,Y., Kotin,R.M., & Herberg,F.W. (1999) PrKX is a novel catalytic subunit of the cAMP-dependent protein kinase regulated by the regulatory subunit type I. *J. Biol. Chem.*, **274,** 5370-5378.

[7] Showers,M.O. & Maurer,R.A. (1988) Cloning of cDNA for the catalytic subunit of cAMP-dependent protein kinase. *Methods Enzymol.*, **159,** 311-318.

[8] Thomis,D.C., Floyd-Smith,G., & Samuel,C.E. (1992) Mechanism of interferon action. cDNA structure and regulation of a novel splice-site variant of the catalytic subunit of human protein kinase A from interferon-treated human cells. *J. Biol. Chem.*, **267,** 10723-10728.

[9] San Agustin,J.T., Leszyk,J.D., Nuwaysir,L.M., & Witman,G.B. (1998) The catalytic subunit of the cAMP-dependent protein kinase of ovine sperm flagella has a unique amino-terminal sequence. *J. Biol. Chem.*, **273,** 24874-24883.

[10] Desseyn,J.L., Burton,K.A., & McKnight,G.S. (2000) Expression of a nonmyristylated variant of the catalytic subunit of protein kinase A during male germ-cell development. *Proc. Natl. Acad. Sci. U. S. A*, **97,** 6433-6438.

[11] Reinton,N., Orstavik,S., Haugen,T.B., Jahnsen,T., Tasken,K., & Skalhegg,B.S. (2000) A novel isoform of human cyclic 3',5'-adenosine monophosphate-dependent protein kinase, calpha-s, localizes to sperm midpiece. *Biol. Reprod.*, **63,** 607-611.

[12] Uhler,M.D., Chrivia,J.C., & McKnight,G.S. (1986) Evidence for a second isoform of the catalytic subunit of cAMP- dependent protein kinase [published erratum appears in J Biol Chem 1987 Apr 15;262(11):5431]. *J. Biol. Chem.*, **261,** 15360-15363.

[13] Wiemann,S., Kinzel,V., & Pyerin,W. (1991) Isoform C beta 2, an unusual form of the bovine catalytic subunit of cAMP-dependent protein kinase. *J. Biol. Chem.*, **266,** 5140-5146.

[14] Guthrie,C.R., Skalhegg,B.S., & McKnight,G.S. (1997) Two novel brain-specific splice variants of the murine Cbeta gene of cAMP-dependent protein kinase. *J. Biol. Chem.*, **272,** 29560-29565.

[15] Altschul,S.F., Madden,T.L., Schaffer,A.A., Zhang,J., Zhang,Z., Miller,W., & Lipman,D.J. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.*, **25,** 3389-3402.

[16] Qi,M., Zhuo,M., Skalhegg,B.S., Brandon,E.P., Kandel,E.R., McKnight,G.S., & Idzerda,R.L. (1996) Impaired hippocampal plasticity in mice lacking the Cbeta1 catalytic subunit of cAMP-dependent protein kinase. *Proc. Natl. Acad. Sci. U. S. A*, **93,** 1571-1576.

[17] Herberg,F.W., Zimmermann,B., McGlone,M., & Taylor,S.S. (1997) Importance of the A-helix of the catalytic subunit of cAMP-dependent protein kinase for stability and for orienting subdomains at the cleft interface. *Protein Sci.*, **6,** 569-579.

[18] Yonemoto,W., McGlone,M.L., & Taylor,S.S. (1993) N-myristylation of the catalytic subunit of cAMP-dependent protein kinase conveys structural stability. *J. Biol. Chem.*, **268,** 2348-2352.

[19] Clegg,C.H., Ran,W., Uhler,M.D., & McKnight,G.S. (1989) A mutation in the catalytic subunit of protein kinase A prevents myristylation but does not inhibit biological activity. *J. Biol. Chem.*, **264,** 20140-20146.

[20] Carr,S.A., Biemann,K., Shoji,S., Parmelee,D.C., & Titani,K. (1982) n-Tetradecanoyl is the NH2-terminal blocking group of the catalytic subunit of cyclic AMP-dependent protein kinase from bovine cardiac muscle. *Proc. Natl. Acad. Sci. U. S. A*, **79,** 6128-6131.

[21] Jedrzejewski,P.T., Girod,A., Tholey,A., Konig,N., Thullner,S., Kinzel,V., & Bossemeyer,D. (1998) A conserved deamidation site at Asn 2 in the catalytic subunit of mammalian cAMP-dependent protein kinase detected by capillary LC-MS and tandem mass spectrometry. *Protein Sci.*, **7,** 457-469.

[22] Zheng,J., Knighton,D.R., Xuong,N.H., Taylor,S.S., Sowadski,J.M., & Ten Eyck,L.F. (1993) Crystal structures of the myristylated catalytic subunit of cAMP- dependent protein kinase reveal open and closed conformations. *Protein Sci.*, **2,** 1559-1573.

[23] Yonemoto,W., McGlone,M.L., Grant,B., & Taylor,S.S. (1997) Autophosphorylation of the catalytic subunit of cAMP-dependent protein kinase in Escherichia coli. *Protein Eng*, **10,** 915-925.

[24] San Agustin,J.T. & Witman,G.B. (1994) Role of cAMP in the reactivation of demembranated ram spermatozoa. *Cell Motil. Cytoskeleton*, **27**, 206-218.

[25] Gangal,M., Clifford,T., Deich,J., Cheng,X., Taylor,S.S., & Johnson,D.A. (1999) Mobilization of the A-kinase N-myristate through an isoform-specific intermolecular switch. *Proc. Natl. Acad. Sci. U. S. A*, **96**, 12394-12399.

[26] Uhler,M.D., Carmichael,D.F., Lee,D.C., Chrivia,J.C., Krebs,E.G., & McKnight,G.S. (1986) Isolation of cDNA clones coding for the catalytic subunit of mouse cAMP-dependent protein kinase. *Proc. Natl. Acad. Sci. U. S. A*, **83**, 1300-1304.

Patent Claims.

1.

The genomic DNA sequence encoding the Cβ1, Cβ2, Cβ3, Cβ4, Cβ4ab and Cβ4abc splice variant proteins respectively comprising the nucleotide sequence of SEQ ID NO: 1, wherein said proteins are new splice variants of the catalytic sub unit of c-AMP dependent protein kinase termed Cβ.

2.

The cDNA sequence encoding the Cβ1, Cβ2, Cβ3, Cβ4, Cβ4ab and Cβ4abc splice variant proteins respectively comprising the nucleotide sequences of SEQ ID NOs: 2,3,4,5 and 6, wherein said proteins are new splice variants of the catalytic sub unit of c-AMP dependent protein kinase termed Cβ.

3.

The vectors comprising the DNA sequences according to claims 1 or 2.

4.

The specific amino acid sequences of SEQ IDNOs 7,8 and 9 of Cβ2, Cβ4ab and Cβ4abc respectively.

5.

A protein encoded by the nucleotide sequences according to claims 1 or 2.

6.

A protein encoded by the specific DNA sequences according to claims 1 or 2 comprising the specific amino acid sequence of SEQ ID NO: 7,8 and 9.

7.

A kit comprising Cβ2 specific DNA probes.

8.

The use of the Cβ2, Cβ4, Cβ4ab and Cβ4abc proteins for the preparation of pharmaceuticals.

9.

The use of Cβ2 protein for the preparation of a medicament for inhibition of the enzymatic activity of Cβ2.

10.

The use of Cβ2 protein for the preparation of a medicament that will specifically and irreversibly block Cβ2 interaction.

11.

The use of Cβ2 protein for the preparation of a medicament that will activate the enzymatic activity of Cβ2.

12.

The use of the DNA sequences which is complementary to the Cβ1, Cβ2, Cβ3, Cβ4, Cβ4ab and Cβ4abc DNA according to claims 1 or 2 for the preparation of an anti sense drug.

13.

A Method for inspection and screening of patient T cells for the presence and location of Cβ2 comprising:

a) collecting and washing in buffer of isolated peripheral blood T lymphocytes;

b) preparing for identification of Cβ2 protein by immunofluorescence, T cells are let to settle onto poly L-lysine coated cover slips following detergent-dependent lysis;

c) incubating with primary antibody (Ab), either irrelevant Ab or Cβ2 specific Ab, Ab overshoot will be removed by washing buffer and T cells incubated with secondary anti-IgG Ab conjugated with a fluorescent;

d) Inspection of T cells under fluorescent microscopy.

14.

A method of screening patient T cells for membrane associated Cβ2 catalytic activity comprising:

a) collecting and washing in buffer of isolated peripheral blood T lymphocytes;

b) preparing of T cells by lysing in detergent buffer;

b) monitoring Cβ2 specific catalytic activity by established assay, Cβ1 activity is used as an internal control to determine relative activity.

15.

A method for screening of patients for mutations in the Cβ2 gene and mRNA comprising:

a) collecting and washing in buffer of isolated peripheral blood T lymphocytes;

b) isolating of total RNA and genomic DNA according to established methods followed by RT-PCR using Cβ2 specific primers according to cDNA sequence of Cβ2 specific nucleotides or the Cβ2 specific exon, designated exon 1-2.

16.

A product produced by the method, according to claim 13, 14 and 15.

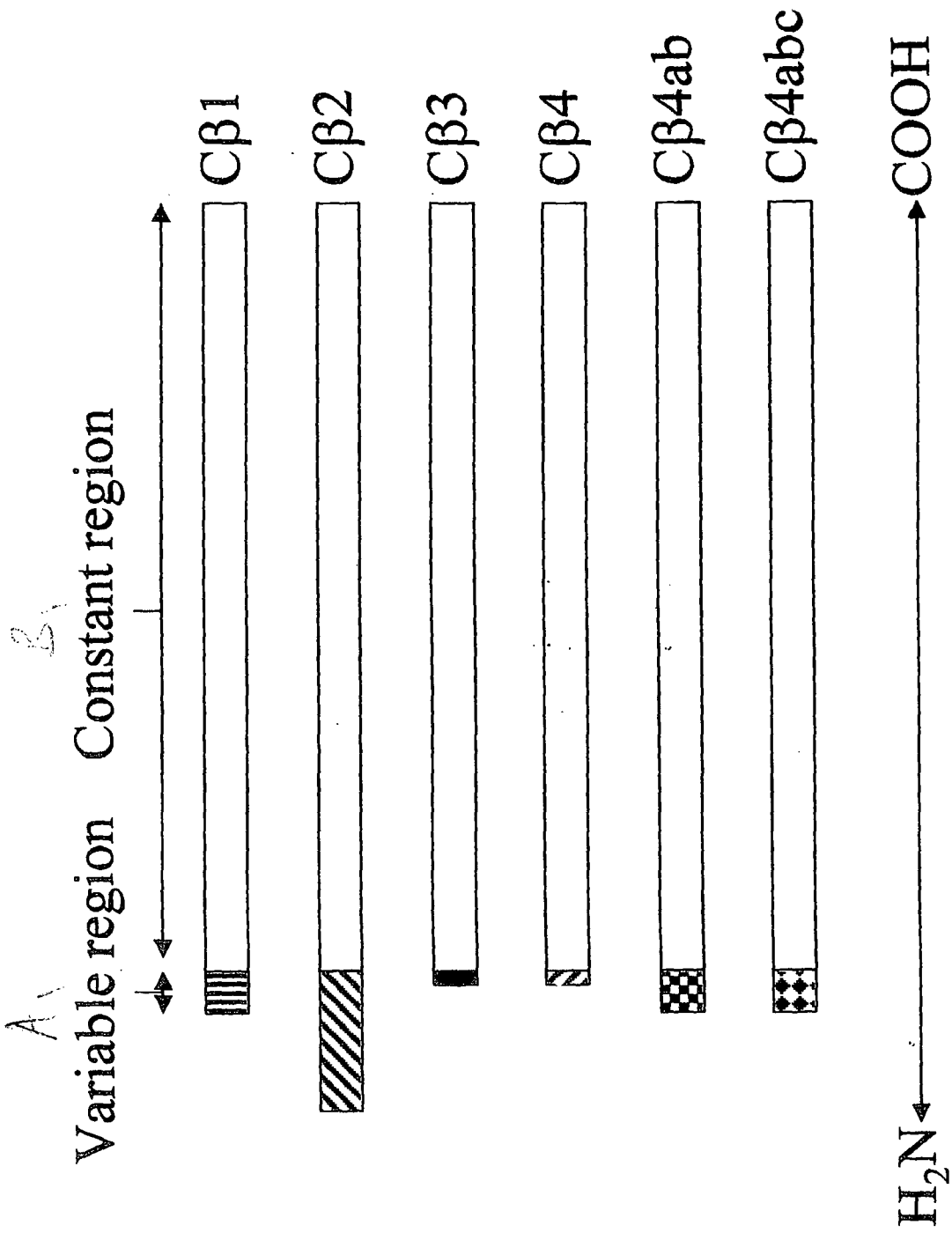
17.

A test system for screening for inhibitory- or activating molecules of the Cβ2 protein.

18.

The product from the screening method according to claim 17.

FIGURE 1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Constant region
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Cβ2
Cβ3
Cβ4
Cβ4ab
Cβ4abc
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H2N

A

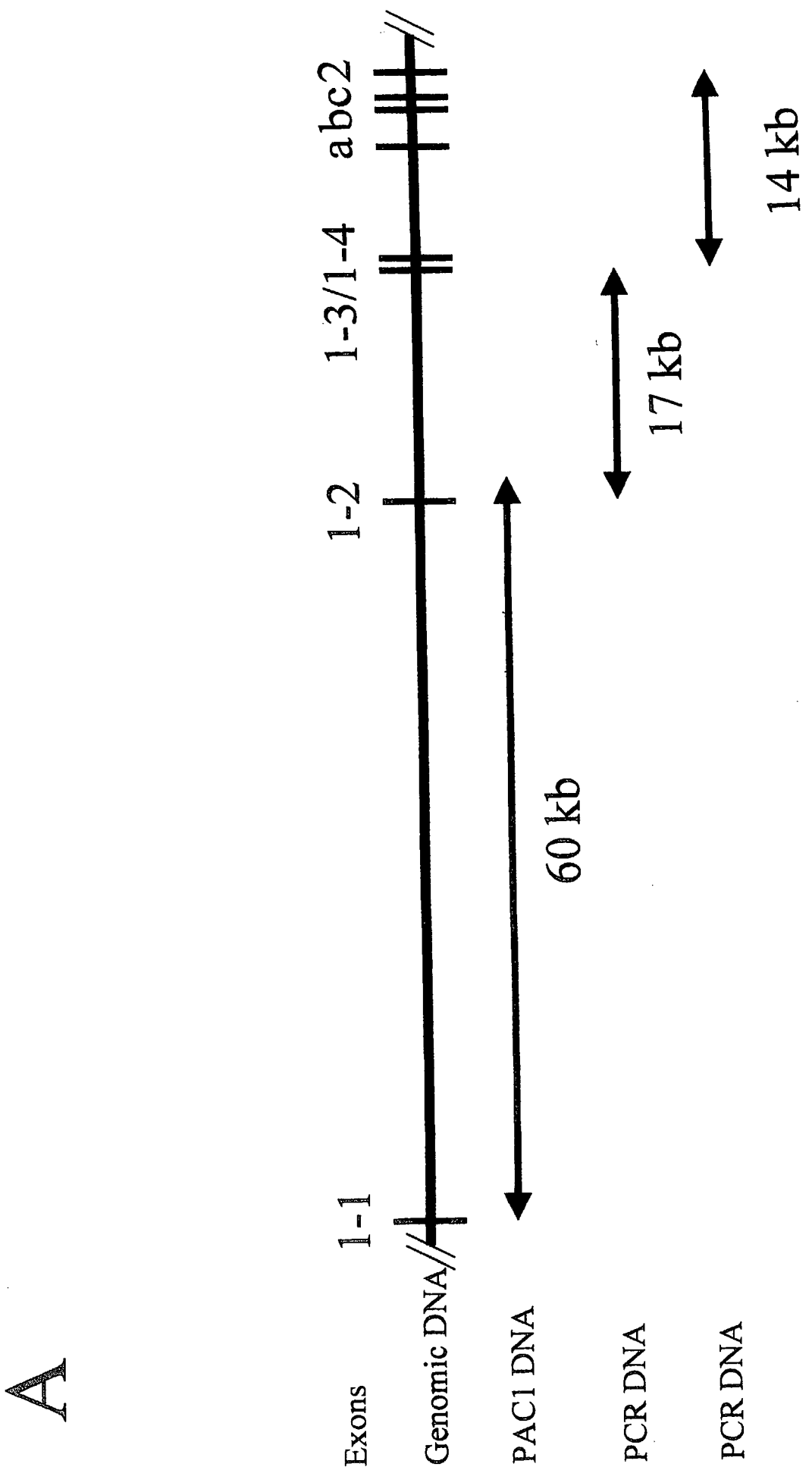
Exons
1-1
1-2
1-3/1-4
abc2
Genomic DNA//
//
PAC1 DNA
60 kb
PCR DNA
17 kb
PCR DNA
14 kb

FIGURE 2B

B

Exon 1-1:

ccagccccccttcccttccctgacccctttcttgccatcgccccagac**ATGGGGAACGCGGCGACCG**
CCAAGAAAGGCAGCGAGgtggagagcg

Exon 1-2:

agctttatatttaatgctctcattagcctatatattaatattaaaacacccaaacataaagccttt
taggcagatattgcaagtttttaaaatcctcaactctagctgaaaagtgttttgctaagaaaagct
cagtaatgtgctgttttatattaacaggaaacagaacagcagtagtggtttgaataccctgcaaac
aggaagtttgacacatgcatagctcttagcttctgtgtaagaagttgtgagctccttctggaaaca
tttgcagttacattaagtaaagtgtaaatgcacatga**ATGGCAGCTTATAGAGAACCACCTTGTAA**
CCAGTATACAGGTACAACTACAGCTCTTCAGAAATTGGAAGGTTTTGCTAGCCGGTTATTTCATAG
ACACTCTAAAGGTACTGCACATGATCAGAAAACAGCTCTGGAAAATGACAGCCTTCATTTCTCTGA
ACATACTGCCTTATGGGACAGATCAAgtaagttttg

Exon 1-3 and 1-4:

tgtttttaggcagagttcagtggttcgtcacaaataaatgtttcttaatttgttgtttatgactgc
tcgatttccagagccatgaaatcattgtttttgataattctgttcagcttcatagttgtttcttag
gaagatttcctgacttacttccacatcaccaaaagtcctgcctcacatggcaactgttaaaatggc
aagttcacgtgctgaagttctacttaacaaggaaccattctatagattctttgtaccattttggta
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attgaaagacgtttaggtgcaatcattctgctgtttgctccttgccaggttcaac**ATGGGATTGT**g
tgagtatttgaagaaaacagcaatttttttcatatctttgaaagatgtaaaaagcgtagattagtgc
ttaaatttaagaaatctggtaatttataatcatgtggctctaaaataaaaaggtattttatttgtc
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ctgcatgctccagtgtgtgtgtttacaccatcggttcttctccctctagagattagcataactccc
tttgctgttggattgttattttgagcaatatgttttggaaaggttggttttcatc**ATGAGTG**gtaa
gtatgc

Exon a

cttgatcaag**CACGCAAATCATCAGATGCATCTG**gtaggaaaac

Exon b

tggacacaag**CTTGCTCCTCTTCAGAAATATCTG**gtaggcaagt

Exon c

gaacatgtag**ATTCCTTTG**gtatgctcat

Exon 2

atattttcag**TGAAAGAGTT**...

FIGURE 2C

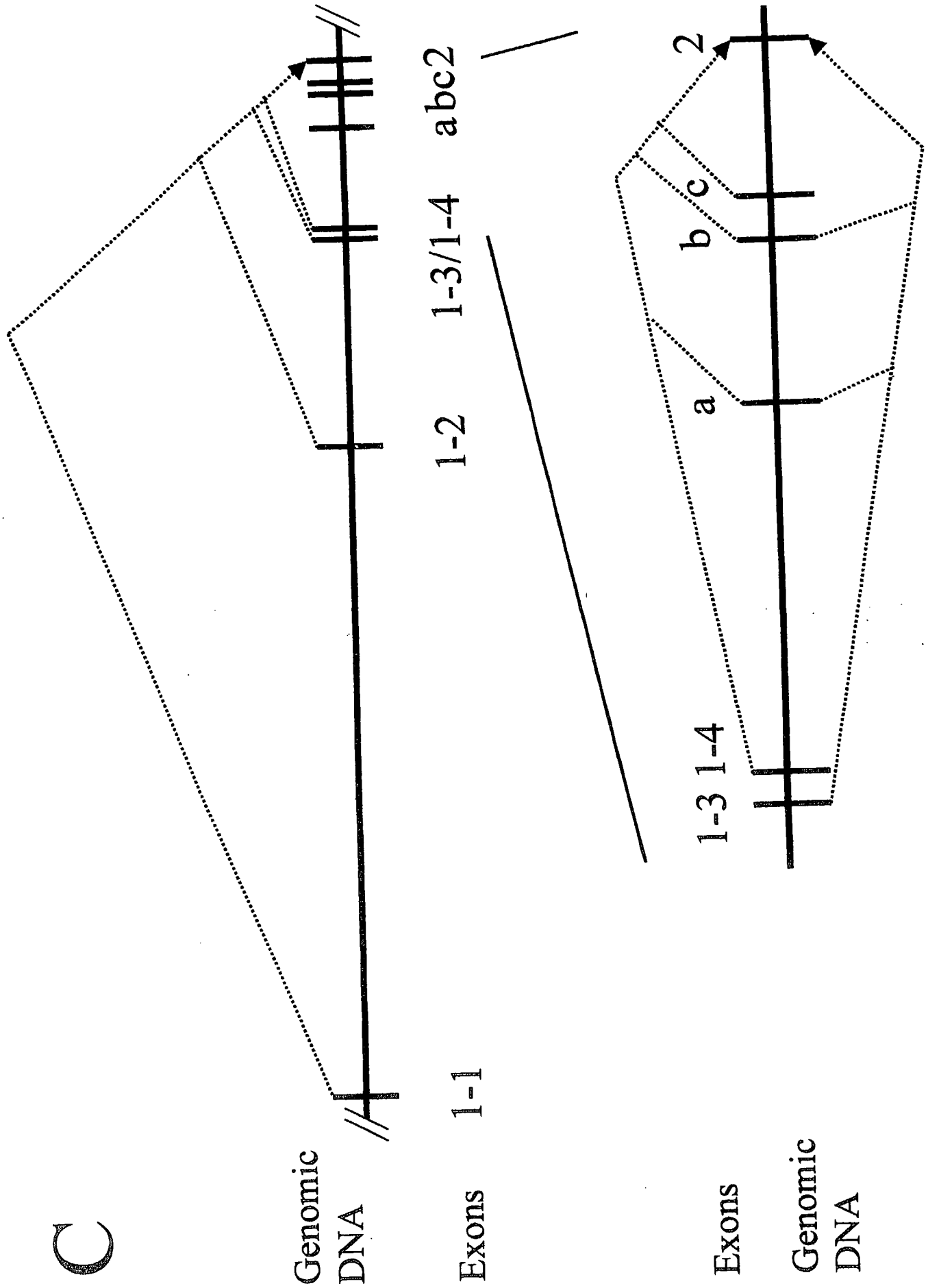
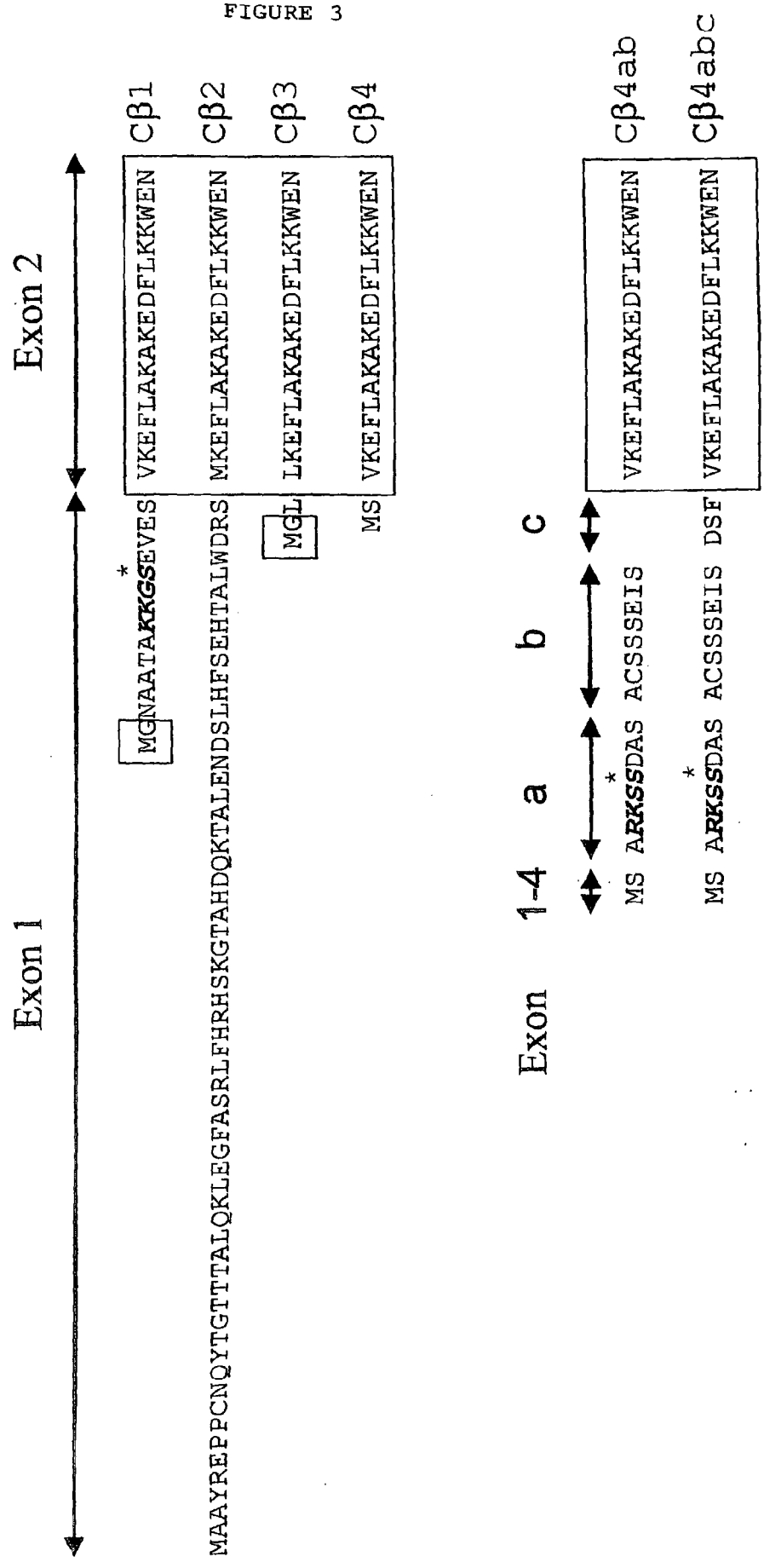

C
Genomic DNA
Exons
1-1
1-2
1-3/1-4
abc2
Exons
1-3 1-4
a
b
c
2
Genomic DNA

FIGURE 3


Exon 1
Exon 2
Cβ1
Cβ2
Cβ3
Cβ4
Cβ4ab
Cβ4abc
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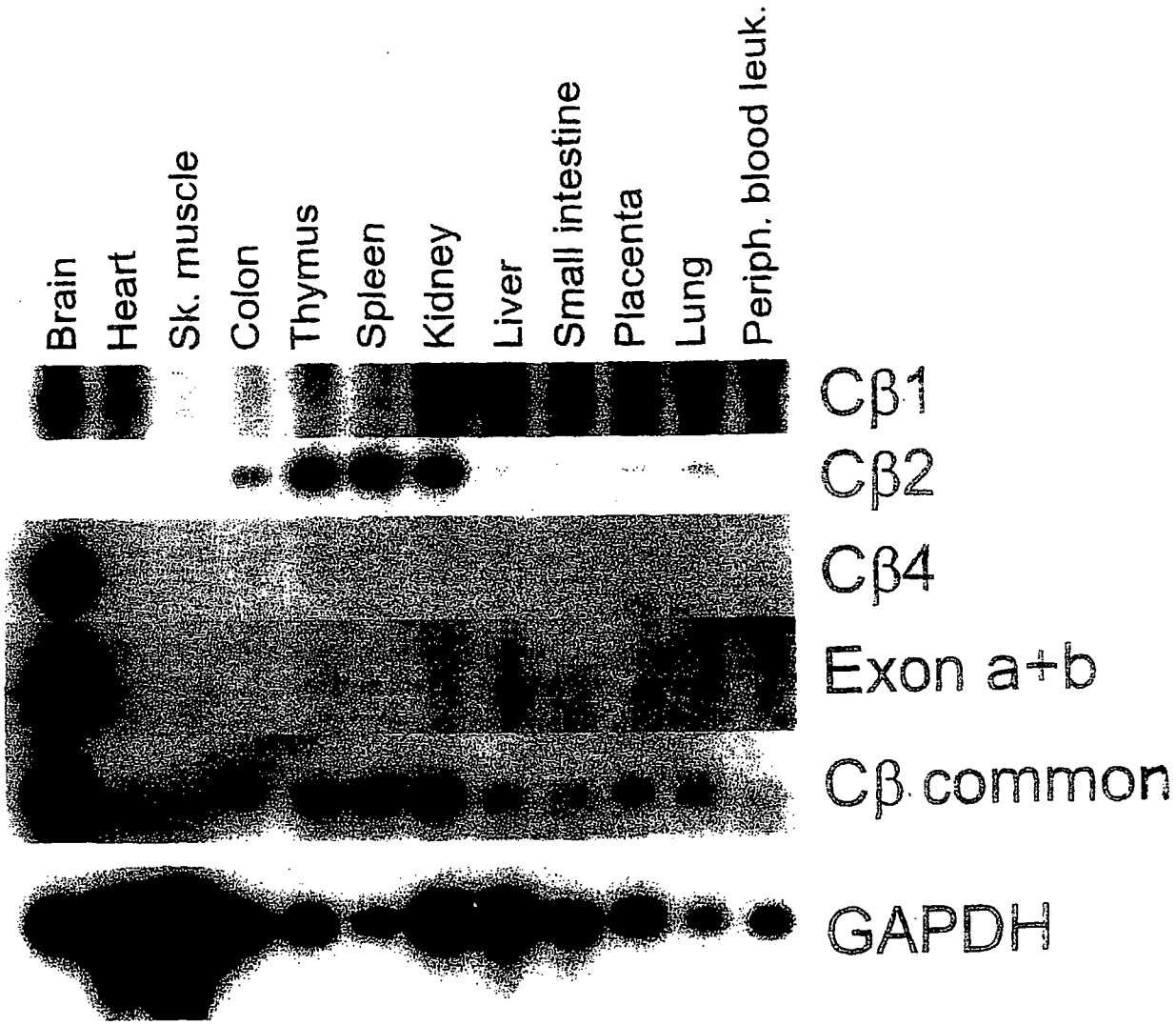
MGNAATAKKGSEVES
MGL
MS
VKEFLAKAKEDFLKKWEN
MKEFLAKAKEDFLKKWEN
LKEFLAKAKEDFLKKWEN
VKEFLAKAKEDFLKKWEN
Exon 1-4 a b c
MS ARKSSDAS ACSSSEIS
MS ARKSSDAS ACSSSEIS DSF

FIGUR 4


Brain
Heart
Sk. muscle
Colon
Thymus
Spleen
Kidney
Liver
Small intestine
Placenta
Lung
Periph. blood leuk.
Cβ1
Cβ2
Cβ4
Exon a+b
Cβ common
GAPDH

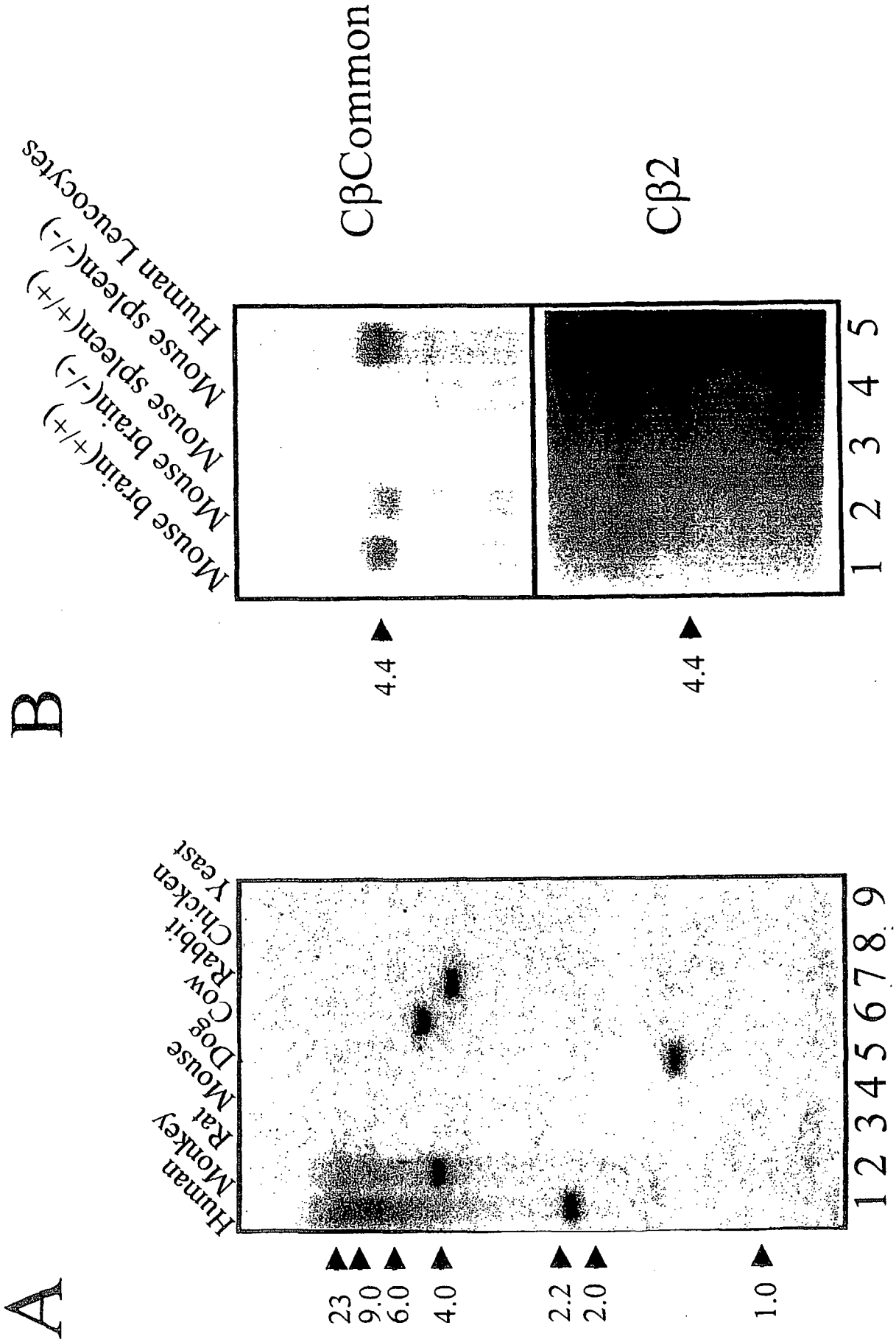


FIGURE 5A

FIGURE 5B

SEQUENCE LISTING

SEQ ID NO: 1

<110> Sigurd Ørstavik, Nils Reinton, Eirik Frengen, Bjørn Tore Langeland, Tore Jahnsen og Bjørn S. Skålhegg

<120> **Identification of novel splice variants of the human catalytic subunit Cβ of cAMP-dependent protein kinase and the use thereof**

<160>9
<210>1
<211>
<212>genomic DNA
<213>Homo sapiens

<400>

Genomic sequence of exons and exon intron bouderies located upstream of exon 2 in the human Cβ gene.

Exon 1-1:

ccagcccccc ttcccttccc tgacccctte ttgccatcgc cccagacATG
GGGAACGCGG CGACCGCCAA GAAAGGCAGC GAGGTGGAGA GCGgtgagtt gaaggccg

Exon 1-2:

agctttatat ttaatgctct cattagccta tatattaata ttaaaacacc
caaacataaa gccttttagg cagatattgc aagtttttaa aatcctcaac
tctagctgaa aagtgttttg ctaagaaaag ctcagtaatg tgctgtttta
tattaacagg aaacagaaca gcagtagtgg tttgaatacc ctgcaaacag
gaagtttgac acatgcatag ctcttagctt ctgtgtaaga agttgtgagc
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aATGGCAGCT TATAGAGAAC CACCTTGTAA CCAGTATACA GGTACAACTA
CAGCTCTTCA GAAATTGGAA GGTTTTGCTA GCCGGTTATT TCATAGACAC
TCTAAAGGTA CTGCACATGA TCAGAAAACA GCTCTGGAAA ATGACAGCCT
TCATTTCTCT GAACATACTG CCTTATGGGA CAGATCAAgt aagttttg

Exon 1-3 and 1-4:

tgtttttagg cagagttcag tggttcgtca caaataaatg tttcttaatt
tgttgtttat gactgctcga tttccagagc catgaaatca ttgtttttga
taattctgtt cagcttcata gttgtttctt aggaagattt cctgacttac
ttccacatca ccaaaagtcc tgcctcacat ggcaactgtt aaaatggcaa
gttcacgtgc tgaagttcta cttaacaagg aaccattcta tagattcttt
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caccatgcta cccagtcagc ccaccttgct atacaatctg tcatcttagt
cctgtttcat gtgaggaatt ttacatttct gcaataattg ccagtaactt
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SEQ ID NO:2

<110> Sigurd Ørstavik, Nils Reinton, Eirik Frengen, Bjørn Tore Langeland, Tore Jahnsen og Bjørn S. Skålhegg

<120> **Identification of novel splice variants of the human catalytic subunit Cβ of cAMP-dependent protein kinase and the use thereof**

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<213>Homo sapiens

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SEQ ID NO:3

<110> Sigurd Ørstavik, Nils Reinton, Eirik Frengen, Bjørn Tore Langeland, Tore Jahnsen og Bjørn S. Skålhegg

<120> **Identification of novel splice variants of the human catalytic subunit Cβ of cAMP-dependent protein kinase and the use thereof**

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<213>Homo sapiens

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<110> Sigurd Ørstavik, Nils Reinton, Eirik Frengen, Bjørn Tore Langeland, Tore Jahnsen og Bjørn S. Skålhegg

<120> **Identification of novel splice variants of the human catalytic subunit Cβ of cAMP-dependent protein kinase and the use thereof**

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SEQ ID NO:5

<110> Sigurd Ørstavik, Nils Reinton, Eirik Frengen, Bjørn Tore Langeland, Tore Jahnsen og Bjørn S. Skålhegg

<120> **Identification of novel splice variants of the human catalytic subunit Cβ of cAMP-dependent protein kinase and the use thereof**

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<110> Sigurd Ørstavik, Nils Reinton, Eirik Frengen, Bjørn Tore Langeland, Tore Jahnsen og Bjørn S. Skålhegg

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  51  CATAACTCCC TTTGCTGTTG GATTGTTATT TTGAGCAATA TGTTTTGGaA
 101  AGGTTGGTTT TCATCATGAG TGCACGCAAA TCATCAGATG CATCTGCCTG
 151  CTCCTCTTCA GAAATATCTG ATTCCTTTGT GAAAGAGTTT CTAGCCAAAG
 201  CCAAAGAAGA CTTTTTGAAA AAATGGGAGA ATCCAACTCA GAATAATGCC
 251  GGACTTGAAG ATTTTGAAAG GAAAAAAACC CTTGGAACAG GTTCATTTGG
 301  AAGAGTCATG TTGGTAAAAC ACAAAGCCAC TGAACAGTAT TATGCCATGA
 351  AGATCTTAGA TAAGCAGAAG GTTGTTAAAC TGAAGCAAAT AGAGCATACT
 401  TTGAATGAGA AAAGAATATT ACAGGCAGTG AATTTTCCTT TCCTTGTTCG
 451  ACTGGAGTAT GCTTTTAAGG ATAATTCTAA TTTATACATG GTTATGGAAT
 501  ATGTCCCTGG GGGTGAAATG TTTTCACATC TAAGAAGAAT TGGAAGGTTC
 551  AGTGAGCCCC ATGCACGGTT CTATGCAGCT CAGATAGTGC TAACATTCGA
 601  GTACCTCCAT TCACTAGACC TCATCTACAG AGATCTAAAA CCTGAAAATC
 651  TCTTAATTGA CCATCAAGGC TATATCCAGG TCACAGACTT TGGGTTTGCC
 701  AAAAGAGTTA AAGGCAGAAC TTGGACATTA TGTGGAACTC CAGAGTATTT
 751  GGCTCCAGAA ATAATTCTCA GCAAGGGCTA CAATAAGGCA GTGGATTGGT
 801  GGGCATTAGG AGTGCTAATC TATGAAATGG CAGCTGGCTA TCCCCCATTC
 851  TTTGCAGACC AACCAATTCA GATTTATGAA AAGATTGTTT CTGGAAAGGT
 901  CCGATTCCCA TCCCACTTCA GTTCAGATCT CAAGGACCTT CTACGGAACC
 951  TGCTGCAGGT GGATTTGACC AAGAGATTTG GAAATCTAAA GAATGGTGTC
1001  AGTGATATAA AAACTCACAA GTGGTTTGCC ACGACAGATT GGATTGCTAT
1051  TTACCAGAGG AAGGTTGAAG CTCCATTCAT ACCAAAGTTT AGAGGCTCTG
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1101 GAGATACCAG CAACTTTGAT GACTATGAAG AAGAAGATAT CCGTGTCTCT

1151 ATAACAGAAA AATGTGCAAA AGAATTTGGT GAATTT**TAA**A GAGGAACAAG

1201 ATGACATCTG AGCTCACACT CAGTGTTTGC ACTCTGTTGA GAGATAAGGT

1251 AGAGCTGAGA CCGTCCTTGT TGAAGCAGTT ACCTAGTTCC TTCATTCCAA

1301 CGACTGAGTG AGGTCTTTAT TGCCATCATC CGTGTGCGCA CTCTGCATCC

1351 ACCTATGTAA CAAGGCACCG CTAAGCAAGC ATTGTCTGTG CCATAACACA

1401 GTACTAGACC ACTTTCTTAC TTCTCTTTGG GTTGTCTTTC TCCTCTCCTA

1451 CATCCATTTC TTCCTTTTCA ATTTCATTGG TTTTCTCTAA ACAGTGCTCC

1501 ATTTTATTTT GTTGGTGTTT CAGATGGGCA GTGTTATGGC TACGTGATAT

1551 TTGAAGGGAA GGATAAGTGT TGCTTTCAGT AGTTATTGCC AATATTGTTG

1601 TTGGTCAATG GCTTGAAGAT AAACTTTCTA ATAATTATTA TTTCTTTGAG

1651 TAGCTCAGAC TTGGTTTTGC CAAAACTCTT GGTAATTTTT GAAGATAGAC

1701 TGTCTTATCA CCAAGGAAAT TTATACAAAT TAAGACTAAC TTTCTTGGAA

1751 TTCACTATTC TGGCAATAAA TTTTGGTAGA CTAATACAGT ACAGCTAGAC

1801 CCAGAAATTT GGAAGGCTGT AGATCAGAGG TTCTAGTTCC CTTTCCCTCC

1851 TTTTATATCC TCCTCTCCTT GAGTAATGAA GTGACCAGCC TGTGTAGTGT

1901 GACAAACGTG TCTCATTCAG CAGGAAAAAC TAATGATATG GATCATCACC

1951 CAGATTCTCT CACTTGGTAC CAGCATTTCT GTAGGTATTA GAGAAGAGTT

2001 CTAAGTTTTC TAAACCTTAA CTGTTCCTTA AGGATTTTAG CCAGTATTTT

2051 AATAGAACAT GATTAATGAA AGTGACAAAT TTTAAATTTT CTCTAATAGT

2101 CCTCATCATA AACTTTTTAA AGGAAAATAA GCAAACTAAA AAGAACATTG

2151 GTTTAGATAA ATACTTATAC TTTGCAAAGT CAAAAATGGC TTGATTTTTG

2201 GAAACAATAT AGAGGTATTC ATATTTAAAT GAGGGTTTAC ATTTGTTTTG

2251 TTTTGTAACC GTTAAAAAGA AGTTGTTTCC AGCTAATTAT TGTGGTGTAC

2301 TATATTTGTG AGCCTAGGGT AGGGGCACTG CTGCAACTTC TGCTTTCATC

2351 CCATGCCTCA TCAATGAGGA AAGGGAACAA AGTGTATAAA ACCTGCCACA

2401 ATTGTATTTT AATTTTGAGG TATGATATTT TCAGATATTT CATAATTTCT

2451 AACCTCTGTT CTCTCAGTAA ACAGAATGTC TGATCGATCA TGCAGATACA

2501 ATGTTGGTAT TTGAGAGGTT AGTTTTTTTC CTACACTTTT TTTTGCCAAC

2551 TGACTTAACA ACATTGCTGT CAGGTGGAAA TTTCAAGCAC TTTTGCACAT

2601 TTAGTTCAGT GTTTGTTGAG AATCCATGGC TTAACCCACT TGTTTTGCTA

2651 TTTTTTTCTT TGCTTTTAAT TTTCCCCATC TGATTTTATC TCTGCGTTTC

2701 AGTGACCTAC CTTAAAACAA CACACGAGAA GAGTTAAACT GGGTTCATTT

2751 TAATGATCAA TTTACCTGCA TATAAAATTT ATTTTTAATC AAGCTGATCT

2801 TAATGTATAT AATCATTCTA TTTGCTTTAT TATCGGTGCA GGTAGGTCAT

2851 TAACACCACT TCTTTTCATC TGTACCACAC CCTGGTGAAA CCTTTGAAGA

2901 CATAAAAAAA ACCTGTCTGA GATGTTCTTT CTACCAATCT ATATGTCTTT

2951 CGGTTATCAA GTGTTTCTGC ATGGTAATGT CATGTAAATG CTGATATTGA

3001 TTTCACTGGT CCATCTATAT TTAAAACGTG C

SEQ ID NO:7

<110> Sigurd Ørstavik, Nils Reinton, Eirik Frengen, Bjørn Tore Langeland, Tore Jahnsen og Bjørn S. Skålhegg

<120> **Identification of novel splice variants of the human catalytic subunit Cβ of cAMP-dependent protein kinase and the use thereof**

<160>9
<210>
<211>
<212>amino acid
<213>Homo sapiens

<400> **Proteinsequence Cβ2**

Met-Ala-Ala-Tyr-Arg-Glu-Pro-Pro-Cys-Asn-Gln-Tyr-Thr-Gly-Thr-Thr-Thr-Ala-Leu-Gln-Lys-Leu-Glu-Gly-Phe-Ala-Ser-Arg-Leu-Phe-His-Arg-His-Ser-Lys-Gly-Thr-Ala-His-Asp-Gln-Lys-Thr-Ala-Leu-Glu-Asn-Asp-Ser-Leu-His-Phe-Ser-Glu-His-Thr-Ala-Leu-Trp-Asp-Arg-Ser-Met-Lys-Glu-Phe-Leu-Ala-Lys-Ala-Lys-Glu-Asp-Phe-Leu-Lys-Lys-Trp-Glu-Ser-Pro-Ala-Gln-Asn-Thr-Ala-His-Leu-Asp-Gln-Phe-Glu-Arg-Ile-Lys-Thr-Leu-Gly-Thr-Gly-Ser-Phe-Gly-Arg-Val-Met-Leu-Val-Lys-His-Lys-Glu-Thr-Gly-Asn-His-Tyr-Ala-Met-Lys-Ile-Leu-Asp-Lys-Gln-Lys-Val-Val-Lys-Leu-Lys-Gln-Ile-Glu-His-Thr-Leu-Asn-Glu-Lys-Arg-Ile-Leu-Gln-Ala-Val-Asn-Phe-Pro-Phe-Leu-Val-Lys-Leu-Glu-Phe-Ser-Phe-Lys-Asp-Asn-Ser-Asn-Leu-Tyr-Met-Val-Met-Glu-Tyr-Val-Pro-Gly-Gly-Glu-Met-Phe-Ser-His-Leu-Arg-Arg-Ile-Gly-Arg-Phe-Ser-Glu-Pro-His-Ala-Arg-Phe-Tyr-Ala-Ala-Gln-Ile-Val-Leu-Thr-Phe-Glu-Tyr-Leu-His-Ser-Leu-Asp-Leu-Ile-Tyr-Arg-Asp-Leu-Leu-Lys-Pro-Glu-Asn-Leu-Leu-Ile-Asp-Gln-Gln-Gly-Tyr-Ile-Gln-Val-Thr-Asp-Phe-Gly-Phe-Ala-Lys-Arg-Val-Lys-Gly-Arg-Thr-Trp-Thr-Leu-Cys-Gly-Thr-Pro-Glu-Tyr-Leu-Ala-Pro-Glu-Ile-Ile-Leu-Ser-Lys-Gly-Tyr-Asn-Lys-Ala-Val-Asp-Trp-Trp-Ala-Leu-Gly-Val-Leu-Ile-Tyr-Glu-Met-Ala-Ala-Gly-Tyr-Pro-Pro-Phe-Phe-Ala-Asp-Gln-Pro-Ile-Gln-Ile-Tyr-Glu-Lys-Ile-Val-Ser-Gly-Lys-Val-Arg-Phe-Pro-Ser-Ser-His-Phe-Ser-Ser-Asp-Leu-Lys-Asp-Leu-Leu-Arg-Asn-Leu-Leu-Gln-Val-Asp-Leu-Thr-Lys-Arg-Phe-Gly-Asn-Leu-Lys-Asn-Gly-Val-Asn-Asp-Ile-Lys-Asn-His-Lys-Trp-Phe-Ala-Thr-Thr-Asp-Trp-Ile-Ala-Ile-Tyr-Gln-Arg-Lys-Val-Glu-Ala-Pro-Phe-Ile-Pro-Lys-Phe-Lys-Gly-Pro-Gly-Asp-Thr-Ser-Asn-Phe-Asp-Asp-Tyr-Glu-Glu-Glu-Glu-Ile-Arg-Val-Ser-Ile-Asn-Glu-Lys-Cys-Gly-Lys-Glu-Phe-Ser-Glu-Phe

SEQ ID NO:8

<400> **Protein sequence Cβ4ab**

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Leu-Tyr-Met-Val-Met-Glu-Tyr-Val-Pro-Gly-Gly-Glu-Met-Phe-Ser-
His-Leu-Arg-Arg-Ile-Gly-Arg-Phe-Ser-Glu-Pro-His-Ala-Arg-Phe-
Tyr-Ala-Ala-Gln-Ile-Val-Leu-Thr-Phe-Glu-Tyr-Leu-His-Ser-Leu-
Asp-Leu-Ile-Tyr-Arg-Asp-Leu-Leu-Lys-Pro-Glu-Asn-Leu-Leu-Ile-
Asp-Gln-Gln-Gly-Tyr-Ile-Gln-Val-Thr-Asp-Phe-Gly-Phe-Ala-Lys-
Arg-Val-Lys-Gly-Arg-Thr-Trp-Thr-Leu-Cys-Gly-Thr-Pro-Glu-Tyr-
Leu-Ala-Pro-Glu-Ile-Ile-Leu-Ser-Lys-Gly-Tyr-Asn-Lys-Ala-Val-
Asp-Trp-Trp-Ala-Leu-Gly-Val-Leu-Ile-Tyr-Glu-Met-Ala-Ala-Gly-
Tyr-Pro-Pro-Phe-Phe-Ala-Asp-Gln-Pro-Ile-Gln-Ile-Tyr-Glu-Lys-
Ile-Val-Ser-Gly-Lys-Val-Arg-Phe-Pro-Ser-Ser-His-Phe-Ser-Ser-
Asp-Leu-Lys-Asp-Leu-Leu-Arg-Asn-Leu-Leu-Gln-Val-Asp-Leu-Thr-
Lys-Arg-Phe-Gly-Asn-Leu-Lys-Asn-Gly-Val-Asn-Asp-Ile-Lys-Asn-
His-Lys-Trp-Phe-Ala-Thr-Thr-Asp-Trp-Ile-Ala-Ile-Tyr-Gln-Arg-
Lys-Val-Glu-Ala-Pro-Phe-Ile-Pro-Lys-Phe-Lys-Gly-Pro-Gly-Asp-
Thr-Ser-Asn-Phe-Asp-Asp-Tyr-Glu-Glu-Glu-Glu-Ile-Arg-Val-Ser-
Ile-Asn-Glu-Lys-Cys-Gly-Lys-Glu-Phe-Ser-Glu-Phe

SEQ ID NO:9

Protein sequence Cβ4abc

Met-Ser-Ala-Arg-Lys-Ser-Ser-Asp-Ala-Ser-Ala-Cys-Ser-Ser-Ser-
Glu-Ile-Ser-Asp-Ser-Phe-Val-Met-Lys-Glu-Phe-Leu-Ala-Lys-Ala-
Lys-Glu-Asp-Phe-Leu-Lys-Lys-Trp-Glu-Ser-Pro-Ala-Gln-Asn-Thr-
Ala-His-Leu-Asp-Gln-Phe-Glu-Arg-Ile-Lys-Thr-Leu-Gly-Thr-Gly-
Ser-Phe-Gly-Arg-Val-Met-Leu-Val-Lys-His-Lys-Glu-Thr-Gly-Asn-
His-Tyr-Ala-Met-Lys-Ile-Leu-Asp-Lys-Gln-Lys-Val-Val-Lys-Leu-
Lys-Gln-Ile-Glu-His-Thr-Leu-Asn-Glu-Lys-Arg-Ile-Leu-Gln-Ala-
Val-Asn-Phe-Pro-Phe-Leu-Val-Lys-Leu-Glu-Phe-Ser-Phe-Lys-Asp-
Asn-Ser-Asn-Leu-Tyr-Met-Val-Met-Glu-Tyr-Val-Pro-Gly-Gly-Glu-
Met-Phe-Ser-His-Leu-Arg-Arg-Ile-Gly-Arg-Phe-Ser-Glu-Pro-His-
Ala-Arg-Phe-Tyr-Ala-Ala-Gln-Ile-Val-Leu-Thr-Phe-Glu-Tyr-Leu-
His-Ser-Leu-Asp-Leu-Ile-Tyr-Arg-Asp-Leu-Leu-Lys-Pro-Glu-Asn-
Leu-Leu-Ile-Asp-Gln-Gln-Gly-Tyr-Ile-Gln-Val-Thr-Asp-Phe-Gly-
Phe-Ala-Lys-Arg-Val-Lys-Gly-Arg-Thr-Trp-Thr-Leu-Cys-Gly-Thr-
Pro-Glu-Tyr-Leu-Ala-Pro-Glu-Ile-Ile-Leu-Ser-Lys-Gly-Tyr-Asn-
Lys-Ala-Val-Asp-Trp-Trp-Ala-Leu-Gly-Val-Leu-Ile-Tyr-Glu-Met-
Ala-Ala-Gly-Tyr-Pro-Pro-Phe-Phe-Ala-Asp-Gln-Pro-Ile-Gln-Ile-
Tyr-Glu-Lys-Ile-Val-Ser-Gly-Lys-Val-Arg-Phe-Pro-Ser-Ser-His-
Phe-Ser-Ser-Asp-Leu-Lys-Asp-Leu-Leu-Arg-Asn-Leu-Leu-Gln-Val-
Asp-Leu-Thr-Lys-Arg-Phe-Gly-Asn-Leu-Lys-Asn-Gly-Val-Asn-Asp-
Ile-Lys-Asn-His-Lys-Trp-Phe-Ala-Thr-Thr-Asp-Trp-Ile-Ala-Ile-
Tyr-Gln-Arg-Lys-Val-Glu-Ala-Pro-Phe-Ile-Pro-Lys-Phe-Lys-Gly-
Pro-Gly-Asp-Thr-Ser-Asn-Phe-Asp-Asp-Tyr-Glu-Glu-Glu-Glu-Ile-
Arg-Val-Ser-Ile-Asn-Glu-Lys-Cys-Gly-Lys-Glu-Phe-Ser-Glu-Phe

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NO 00/00445

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C12N 9/12 // C12Q 1/48
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C12N, C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	The Journal of Biological Chemistry, Volume 266, No 8, 1991, Stefan Wiemann et al, "Isoform CBeta2, an Unusual Form of the Bovine Catalytic Subunit of CAMP-independent Protein Kinase", page 5140 - page 5146, & Swissprot. Accession no. P24256, 01.03.1992, 93.5% identity in 400 aa overlap with SEQ ID No 7 --	1-18
X	The Journal of Biological Chemistry, Volume 272, No 47, 1997, Chris R. Guthrie et al, "Two Novel Brain-specific Splice Variants of the Murine C Beta Gene of caMP-dependent Protein Kinase", page 29560 - page 29565 --	1-18

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 May 2001	30 -05- 2001
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Yvonne Siösteen/BS Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NO 00/00445

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Molecular Endocrinology, Volume 4, No 3, 1990, Stephen J. Beebe et al, "Molecular Cloning of a Tissue-Specific Protein Kinase (C-Gamma) from Human Testis - Representing a Third Isoform of the Catalytic Subunit of caMP-Dependent Protein Kinase", page 465 - page 475, & EMBASE/GENBANK/DDBJ, accession no M34181, 23.06, 1990, 100% identity in 2852 bp overlap with SEQ ID No 2	1-18
A	The Journal of Biological Chemistry, Volume 261, No 33, 1986, Michael D. Uhlert et al, "Evidence for a Second Isoform of the Catalytic Subunit of cAMP-dependent Protein Kinase", page 15360 - page 15363	1-18
A	The Journal of Biological Chemistry, Volume 261, No 35, 1986, Mark Ol Showers et al, "A Cloned Bovine cDNA Encodes an Alternate Form of the Catalytic Subunit of cAMP-dependent Protein Kinase", page 16288 - page 16291	1-18
A	Proc. Natl. Acad. Sci., Volume 89, 1992, Sven Beushausen et al, "Catalytic subunit of Aplysia neutronal cAMP-dependent protein kinase with two different N termini" page 1641 - page 1645	1-18
A	The Journal of Biological Chemistry, Volume 273, No 38, 1998, Jovental T. San Agustin et al, "The Catalytic Subunit of the cAMP-dependent Protein Kinase of Ovine Sperm Flagella Has a Unique Amino-terminal Sequence", page 24874 - page 24883	1-18

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NO00/00445

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see next sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

The International Searching Authority considers that the present application contains the following six inventions which relate to different splice variants of the catalytic subunit of cAMP-dependent protein kinase:

1. The splice variant c-beta1 according to part of claims 1-3,5,6 and 12.
2. The splice variant c-beta2 according to part of claims 1-6, 8, 12,16 and claims 7,9-11,13-18.
3. The splice variant c-beta3 according to part of claims 1-3,5 and 12.
4. The splice variant c-beta4 according to part of claims 1-3,5,8 and 12.
5. The splice variant c-beta4ab according to part of claims 1-6,8 and 12.
6. The splice variant c-beta4abc according to claims 1-6,8 and 12.

The invention listed as Groups 1-6 do not relate to a single inventive concept under PCT Rule 13.1 because they lack the same or corresponding novel special technical features for the following reasons: The claims are not so linked by a special technical feature so as to form a single inventive concept. Because c-beta splice variants of the catalytic subunit of cAMP-dependent protein kinase was already known from the prior art (see description page 2, line 19 and Guthrie et al, The journal of Biological Chemistry, Vol 272, No47,1997, page 29560-29565) it cannot form basis of unity of invention.

As inventions 2-6 could be searched without effort justifying additional fee, this Authority did not invite payment of any additional fee for these inventions. Thus, inventions 1-6 have been searched.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NO 00/00445

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Advances in Second Messenger and Phosphoprotein Advanced, Volume 31, 1997, Kjetil Taskén et al, "Structure, Function and Regulation of Human cAMP-Dependent Protein Kinases" page 191 - page 204 -- --------	1-18

专利名称(译)	CAMP依赖性蛋白激酶人催化亚基Cβ新型裂解变异体的鉴定及其应用		
公开(公告)号	EP1242585A1	公开(公告)日	2002-09-25
申请号	EP2000987849	申请日	2000-12-22
[标]申请(专利权)人(译)	ORSTAVIK SIGURD REINTON NILS FRENGEN的Eirik 朗厄兰岛比约恩撕 JAHNSEN TORE LHEGG BJ RN S		
申请(专利权)人(译)	ORSTAVIK，SIGURD REINTON，NILS FRENGEN，的Eirik JAHNSEN，撕		
当前申请(专利权)人(译)	ORSTAVIK，SIGURD REINTON，NILS FRENGEN，的Eirik JAHNSEN，撕		
[标]发明人	ORSTAVIK SIGURD REINTON NILS FRENGEN EIRIK LANGELAND BJ RN TORE JAHNSEN TORE SK LHEGG BJ RN S		
发明人	ORSTAVIK, SIGURD REINTON, NILS FRENGEN, EIRIK LANGELAND, BJ RN TORE JAHNSEN, TORE SK LHEGG, BJ RN S.		
IPC分类号	G01N33/48 A61K31/7088 A61K38/00 A61K38/45 A61K45/00 A61K48/00 A61P37/04 A61P43/00 C12N9/00 C12N9/12 C12N9/99 C12N15/09 C12Q1/02 C12Q1/48 C12Q1/68 G01N21/78 G01N33/15 G01N33/50 G01N33/53 G01N33/569		
CPC分类号	C12N9/1205 A61K38/00 C12Q1/485 C12Y207/11011 G01N33/56972		
优先权	19996423 1999-12-23 NO		
外部链接	Espacenet		

摘要(译)

本发明证明Cβ基因编码至少6种不同的基因产物，命名为Cβ1，Cβ2，Cβ3，Cβ4，Cβ4ab和Cβ4abc。与鼠和牛剪接变体的情况一样，所有人Cβ剪接变体在由外显子2编码的部分之前的N末端部分中变化。在人类中鉴定了在小鼠和牛中鉴定的所有Cβ剪接变体的同源物。（C beta 1，Css beta 2，C beta 3和C beta 4）以及之前未在任何其他物种中鉴定的两种新型Cβ剪接变体（C beta 4ab和C beta 4abc）。在这方面，本发明包括编码所述剪接变体的基因组DNA-和cDNA序列，并且分别包含SEQ ID NO：1,2,3,4,5和6中所示的核苷酸序列。其中所述蛋白质是Cβ蛋白质的新剪接变体。本发明还涉及包含所述cDNA序列的载体。本发明还包括特征在于分别如SEQ ID NO：7,8和9中所示的特定氨基酸Cssβ剪接变体蛋白的蛋白质。本发明还包括所述Cβ剪接变体蛋白和DNA序列在制备用于诊断和治疗目的的药物中的进一步用途。