

Human phosvitin/casein kinase type II

Molecular cloning and sequencing of full-length cDNA encoding subunit beta

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Phosvitin/casein kinase type II is an ubiquitous, highly conserved enzyme consisting of subunits α , α' and β . Subunit β , presumably serving regulatory functions, was prepared from human placenta and the amino acid sequence of a protease digestion peptide was determined. The deduced nucleotide sequence was employed for the synthesis of a mixture of 20mers as a hybridization probe to screen a λ gt10 HeLa cell cDNA library for clones encoding subunit β . A full-length clone consisting of 1013 bp was isolated and the sequence of both strands determined. The deduced amino acid sequence of the largest open reading frame encodes 215 amino acid residues predicting a maximum M_r of 24925. The nucleotide sequence of the human β subunit shows a similarity of 75% to that of *Drosophila melanogaster* and the deduced amino acid sequence a similarity of 88% and 97% to that of *D. melanogaster* and bovine lung, respectively. No further protein sequence currently known exhibits any notable similarity. Using a 537-bp restriction fragment of the cDNA clone representing 80% of the coding region, a 1-kb subunit- β transcript was detected by Northern hybridization in total RNA prepared from human epithelial cells and placenta as well as from bovine heart suggesting a single transcript of the β -subunit gene and also demonstrating high similarity also between the human and bovine nucleotide sequences. The same restriction fragment was used as the probe to indicate in Southern hybridizations that the corresponding genomic DNA contains at least one intron of roughly 2.5 kb in length and presumably is a single copy gene.

The phosvitin/casein kinase type II (CK II), so named for its ability to phosphorylate acidic model substrates such as phosvitin or casein, is an enzyme with a remarkably broad list of potential substrates and hence of possible cellular functions. CK II phosphorylates a number of nuclear and cytoplasmic proteins intimately involved in transcription and translation of genetic information and phosphorylates metabolic key enzymes (reviewed [1–3]). It also phosphorylates structural proteins (e.g. [4, 5]) and proteins involved in signal transduction (e.g. [6–8]). In addition, substrates have been localized at the outer surface of mammalian cells and in extracellular fluids. Consistently, an ecto-phosvitin/casein kinase type II activity which becomes released under certain conditions has been detected [9–13]. As one would expect of an enzyme playing such versatile and crucial physiological roles, CK II occurs ubiquitously. It has been isolated from a variety of vertebrates and invertebrates (reviewed [1–3]) and detected in plants [14].

Distinguishing biochemical features of CK II are the capability to effectively utilize both ATP and GTP as substrate, the phosphorylation of proteins at serine or threonine residues positioned adjacent N-terminally to clusters of acidic residues,

the stimulation of its enzymatic activity by cationic molecules such as polyamines, the inhibition of its enzymatic activity by anionic molecules such as heparin, and the unresponsiveness to cyclic nucleotides and to Ca^{2+} /calmodulin (reviewed [1–3]). Human CK II preparations [13] are composed, as are most of the CK II preparations of other organisms, of three different subunits, namely, subunits α , α' and β . The subunits of the human enzyme have M_r 43000, 38000, and 28000, respectively, and form fairly stable $\alpha_2\beta_2$, $\alpha\alpha'\beta_2$, and $\alpha'\alpha_2\beta_2$ tetramers. Like CK II of other sources, subunits α and α' of the human CK II are the catalytically active components, with high affinity binding sites for ATP as evidenced by their isolation in an enzymatically active state and by affinity labeling with 8-azido ATP [13, 15].

Subunit β might serve several functions. It has been assumed to play a regulatory role on CK II activity and stability tuned by its autophosphorylation, which is a fast process reaching 1 mol phosphate/mol β subunit [16, 17]. A recent attempt by Agostinis et al. [18], however, failed to correlate the phosphorylation of subunit β with changes in kinase activity. In reconstitution experiments with the separated human CK II subunits, we noticed a marked increase in enzyme activity of α and α' upon addition of subunit β [15]. A similar, although less pronounced, effect had been observed earlier with the bovine CK II subunits [19]. Fascinating potential further roles for subunit β were possibly noted by Szopa and Rose [20]. In their hands, subunit β was obviously able to bind to DNA and to act as an endonuclease, and, moreover, to interfere with RNA polymerase I reactions. In both cases, phosphorylation of subunit β had deactivating character.

Recently, Takio et al. [21] published the amino acid sequence of subunit β of bovine lung CK II, and Saxena et al.

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Abbreviations. CK II, phosvitin/casein kinase type II, ATP: protein phosphotransferase; V8 protease, *Staphylococcus aureus* proteinase

Enzymes. Protein kinases, ATP: protein phosphotransferases (EC 2.7.1.37); EcoRI, PstI, NcoI, nucleic acid endonucleases (EC 3.1.21.4); T7 DNA polymerase (EC 2.7.7.7); T4 polynucleotide kinase (EC 2.7.1.78)

[22] published the isolation and sequencing of a cDNA clone encoding subunit β of *Drosophila melanogaster* CK II. Here we describe the isolation and sequencing of a cDNA clone encoding the complete subunit β of human CK II, the first for vertebrates, and provide some data on its expression and genomic organization.

EXPERIMENTAL PROCEDURES

Construction of the cDNA library

HeLa S3 suspension cells were grown in Spinner bottles as described [13]. Total RNA was isolated from cells (grown to 10^6 /ml) by the guanidinium thiocyanate method [23], and poly(A)-rich RNA selected by two rounds of oligo(dT) chromatography [24]. The poly(A)-rich RNA was used to construct a cDNA library [25]. Blunt end cDNA was synthesized by employing the cDNA synthesis kit from Amersham Buchler (Braunschweig). After methylation of internal *Eco*RI sites, addition of *Eco*RI linkers and sizing over a Bio-Gel A50 column, the cDNAs were ligated into *Eco*RI digested and dephosphorylated λ gt10 DNA. The phage DNA was *in-vitro* packaged using the packaging kit from Promega Biotec (Heidelberg) and plated on competent *Escherichia coli* C600hflA for amplification [25, 26].

Protein purification and amino acid sequencing

Phosvitin/casein kinase II was isolated from human placenta by the procedure described in detail elsewhere for HeLa cell CK II [13], i.e. extraction in the presence of the nonionic detergent NP-40 followed by DEAE-cellulose chromatography, phosphocellulose chromatography and finally affinity chromatography with phosvitin-Sepharose. Subunit β was separated from subunit α and α' by preparative SDS/PAGE and electroeluted with the Biotrap from Schleicher and Schüll (Dassel, FRG). The eluted protein was digested with *Staphylococcus aureus* V8 protease, and the cleavage products were separated on a reversed phase HPLC C_4 column (0.47 cm $\times$ 25 cm; Latek, Heidelberg) with a gradient in the range 0–95% acetonitrile. Sequence analysis of the elution peaks were performed by the automated Edman degradation method on a gas-phase protein sequencer 470A from Applied Biosystems (Weiterstadt, FRG).

Oligonucleotide synthesis

A 20-bp oligonucleotide mixture of 512 different sequences, corresponding to the amino acid sequence of one of the digestion peptides of subunit β , was synthesized with an automated DNA synthesizer from Applied Biosystems (Weiterstadt), purified by HPLC or preparative PAGE, according to current procedures, and sequenced by the Maxam and Gilbert method [27] with the sequencing kit from Sigma (Deisenhofen, FRG).

Oligonucleotide labelling

Approximately 30 pmol oligonucleotides/2 replica filters were labeled with a double molar excess of [γ - ^{32} P]ATP (100 TBq/mmol; Amersham Buchler, Braunschweig) and 25 units polynucleotide kinase from Medac (Hamburg) at 37°C for 30–60 min [25, 27]. The enzyme was then inactivated at 70°C for 10 min. The labeling intensity was determined by spotting aliquots of 1 μ l on Whatman GF/C filters (Maidstone,

England), washing with 10% trichloroacetic acid followed by 70% ethanol, and measuring the radioactivity by liquid scintillation counting. Approximately 30% of the initial radioactivity was incorporated into the oligonucleotides.

Screening of the cDNA library

For a high density screening, approximately 50 000 plaque-forming units were plated on a 150 mm L-broth (1% Peptone, 0.5% yeast extract, 1% sodium chloride) agar plate with 300 μ l competent *E. coli* C600hflA [25, 26]. Two replica filters were prepared with the nylon membrane Hybond-N from Amersham Buchler (Braunschweig) according to [27] except that the filters were soaked in the solutions instead of being placed on wetted paper. Hybridizations were carried out overnight at 45°C in 0.9 M NaCl/0.09 M citrate, 10 $\times$ Denhardt's solution, 0.1% SDS, and approximately 1×10^7 cpm/ml to 2×10^7 of labeled oligonucleotides preceded by a pre-hybridization step of 3–5 h. After hybridization, the filters were washed in 0.9 M NaCl/0.09 M citrate and 0.1% SDS at 45°C with several changes of buffer. The autoradiographs were exposed for 16–24 h at 4°C. For the secondary and tertiary screening, the areas on the plate corresponding to the positive signals on both replica filters were plucked out and overlaid with 1 ml λ dilution buffer and 50 μ l chloroform. After 1 h at room temperature, 0.01- μ l equivalents were plated on a 90 mm L-broth agar plate with 150 μ l competent *E. coli* C600hflA. Replica preparation was performed as in the primary screening. After the tertiary screening, λ gt10 DNA was isolated from plate lysates as described in the filter-supported method [28], *Eco*RI-digested, electrophoresed and transferred to a nylon membrane [29]. The hybridization conditions were the same as in the plaque filter hybridization.

Subcloning of cDNA and sequencing

The cDNA inserts from the original λ gt10 clones were subcloned by a shotgun ligation into Bluescript M13-vector DNA with the totally *Eco*RI-digested DNA [30]. Restriction fragments of these subclones were ligated after insert isolation with GeneClean from Dianova (Hamburg) or electroelution with Biotrap from Schleicher and Schüll (Dassel, FRG) into M13 mp18 vector DNA. *E. coli* JM101 was used as the bacterial host. DNA sequence analysis was performed with recombinant phage M13 mp18 or Bluescript M13 DNA by the dideoxy chain-termination method [31] with modified T7 polymerase from United States Biochemical Corporation (Cleveland, Ohio) and [α - ^{32} P]dATP, or with unmodified T7 polymerase from Pharmacia (Freiburg i.Br.) and a fluorescent label [32]. The DNA templates for sequencing were isolated via glass-fibre membranes [33]. Regions with compressions in the sequencing gel were sequenced with dITP or deaza-dGTP instead of dGTP.

Computer analysis of sequences and employed genetic sequence data banks

Structure analysis of nucleic acid sequences and protein sequences were performed with the Biological Sequence Analysis system of the German Cancer Research Center. The following genetic sequence data banks were employed: the Genetic Sequence Data Bank (Genbank), the EMBL nucleotide library, the Dayhoff library, and the Kyoto library.

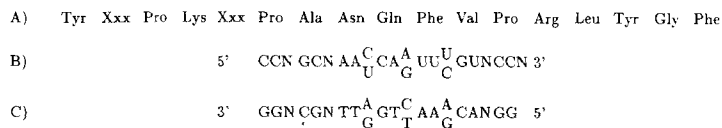


Fig. 1. Amino acid sequence of a cleavage peptide of human phosvitin/casein-kinase-II β subunit and deduced oligonucleotide sequences. (A) Amino acid sequence in the three letter code of a V8-protease digestion peptide sequenced by the automated Edman degradation with an Applied Biosystem 470A sequencer. The sequence analysis was successful over 17 cycles, the amino acids in the second and the fifth cycle could not unambiguously be identified. (B) Deduced oligonucleotide sequence of amino acids 6–12 of the peptide. (C) Hybridization probe. 20-nucleotide-long oligonucleotide mixture of 512 different molecules similar to all possible mRNA sequences

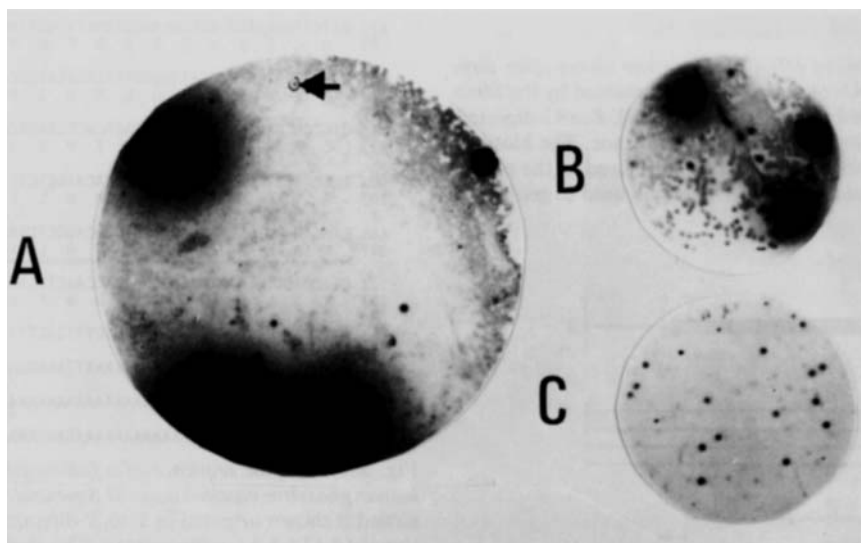


Fig. 2. Plaque hybridization screening of a HeLa cell cDNA. (A) Approximately 50 000 of the 5×10^5 screened clones of the primary screening with the oligonucleotide mixture. The arrow indicates the plaque which showed positive signals on both replica filters. (B) Secondary screening of an aliquot of phages from the marked positive area of the primary screening. (C) Tertiary screening of an aliquot of phages from one of the positive plaques of the secondary screening

Northern blot hybridization

Total RNA isolated by the guanidinium thiocyanate method [23] was separated on a formaldehyde-containing 1.4% agarose gel [34] with ethidium bromide directly added to the loading buffer at a final concentration of 0.1 $\mu\text{g}/\text{ml}$. After photographing, the gel was incubated in 3 M NaCl/0.3 M citrate for 20 min. The transfer was carried out in the same way as in a Southern blot [29]. After transfer, the filter was air-dried and incubated for 2–3 h at 80°C without a washing step. Prehybridization was done at 42°C for 2–4 h in 0.75 M NaCl/0.075 M citrate, 50% formamide, 50 mM sodium phosphate, pH 6.5, 10 $\times$ Denhardt's solution. The hybridization solution was prehybridization solution plus labeled DNA of approximately 1×10^6 cpm/ml to 2×10^6 cpm/ml. The insert DNA was labeled by the multi-priming procedure [35] with 37 kBq/ng DNA. The incorporated radioactivity was determined as described above (oligonucleotide labeling). The hybridization was performed overnight at 42°C. The washing was carried out with 0.3 M NaCl/0.03 M citrate, 0.1% SDS at 60°C with several changes of buffer and the autoradiographs were exposed to X-ray film (X-Omat AR, Kodak) at -80°C with an intensifying screen for several days.

Southern blot hybridization

Genomic DNA was isolated from HeLa S3 cells as a by-product of the RNA isolation. The DNA, positioned on top

of the CsCl cushion after ultracentrifugation, was transferred to a separate tube and diluted with one volume 10 mM Tris/1 mM EDTA [36]. The DNA solution was extracted with phenol/chloroform and precipitated with ethanol. The precipitate was dissolved in 10 mM Tris/1 mM EDTA, digested with restriction enzymes and electrophoresed on a 1% agarose gel (10 $\mu\text{g}/\text{lane}$). After denaturation and neutralization, the transfer [29] was built up with a nylon membrane. After the transfer, the filter was washed in 0.3 M NaCl/0.03 M citrate, air-dried and incubated for 2–3 h at 80°C. The hybridization and washing conditions were the same as for the Northern hybridization except that all solutions contained 0.1% SDS. The autoradiographs were exposed to X-ray film (X-Omat AR, Kodak) at -80°C with an intensifying screen for several days.

RESULTS

Preparation of the hybridization probe

Phosvitin/casein kinase II isolated from human placenta was found to be of the same $\alpha_2\beta_2$, $\alpha\alpha'\beta_2$, and $\alpha'\beta_2$ composition as the HeLa cell enzyme [13]. The subunits were separated by preparative SDS/PAGE and isolated by electroelution. Several attempts to sequence the complete β subunit from its N-terminus were unsuccessful. Therefore, subunit β was cleaved by V8 protease digestion and the resulting peptides were separated by reversed-phase HPLC. One of the

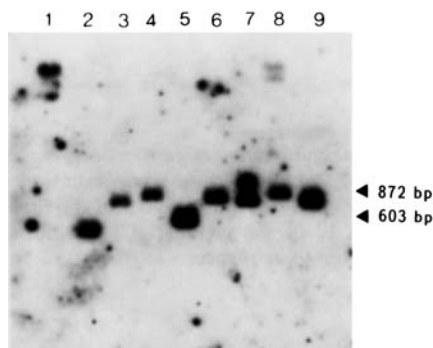


Fig. 3. Southern blot analysis of DNA from positive clones after three cycles of screening. The DNA of nine clones was isolated by the filter-supported method developed in our laboratory [28], *Eco*RI-digested, electrophoresed and transferred to a nylon membrane. The blot was hybridized with the same oligonucleotide mixture used in the plaque hybridization. *Hae*III-digested ϕ X174 DNA was used as molecular mass standard

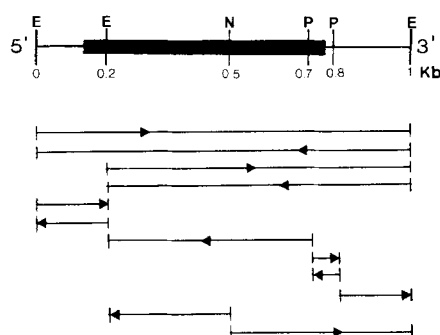


Fig. 4. Restriction map and sequencing strategy of the full-length cDNA encoding human phosvitin/casein-kinase-II β subunit. The restriction map is shown at the top of the figure. The black box represents the translated region for phosvitin/casein-kinase-II β subunit; solid lines represent untranslated regions. Endonuclease cleavage sites indicated are: E, *Eco*RI; N, *Nco*I; P, *Pst*I. The lower part of the figure shows the inserts of M13 mp18 and Bluescript M13 clones which were constructed for sequencing. Each line represents an individual clone. Arrows indicate the direction of sequencing and arrowheads the positions up to which the sequencing of each clone occurred

peptides was successfully sequenced by an automated Edman degradation over 17 cycles (Fig. 1A). The part of the sequence showing the lowest number of possible codons and the least similarity to sequences in genetic sequence data banks was chosen for the construction of the hybridization probe. This was done by deducing the sequence of that part into the corresponding mRNA sequences (Fig. 1B). Accordingly, a 20-bp-long oligonucleotide mixture of 512 different sequences was synthesized (Fig. 1C) and verified by Maxam-Gilbert sequencing [27]. This mixture was employed as the hybridization probe in the cDNA library screening.

Screening of a HeLa cell cDNA library

For the isolation of recombinant clones encoding the human CK II β subunit, 5×10^5 clones of a λ gt10 HeLa cell cDNA library were screened with the oligonucleotide mixture. In the primary screening 15 positive clones were found, nine of which remained positive during two further screening cycles (Fig. 2). Southern blot analysis of isolated and *Eco*RI-digested

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1  ACTCCCCACCCCACTTCGCCTGCCGGTCCGGTCCGGCCCTGCGCTGAGCGGTCG
61  CCGCCGTTCCTGGAAGTAGCAACTTCCCTACCCACCCAGTCTGGTCCCCGTCCAGC
121 CGTGACGTGAAGATGAGCAGCTCAGAGGAGGTGCTGGATTCTCGTCTGTGGGCT
    M S S S E E V S W I S W F C G L
17  R G N E F F C E V D E D Y I Q D K F N L
241 TACTGGACTCAATGAGCAGGTCCCTCACTATCGAAGCTTAGACATGATCTGGACCT
37  T G L N E Q V P H Y R Q A L D M I L D L
301 GGAGCCTGATGAAGAACTGGAAGACAACCCCAACAGAGTGACCTGATTGAGCAGGAGC
57  E P D E E L E D N P N Q S D L I E Q A A
361 CGAGATGCTTTATGGATTGATCCAGCCGCTACATCCTTACCAACCGTGCATCGCCCA
77  E M L Y G L I H A R Y I L T N R G I A Q
421 GATGTTGGAAGTACCAGCAAGGAGACTTTGGTTACTGCTCTGCTGTGTACTGTAGAA
97  M L E K Y Q Q G D F G Y C P R V Y C E N
481 CCAGCAATGCTTCCCATTTGGCCTTTTCCAGACTCCAGGTGAAGCATGGTGAAGCTCTA
117 Q P M L P I G L S D I P G E A M V K L Y
541 CTGCCCCAAGTGCATGGATGTGTACACCCCAAGTCATCAAGACACCACACCGGATGG
137 C P K C M D V Y T P K S S R H H H T D G
601 CGCTACTTCGGCACTGGTTTCCCTCAGATGCTTTCATGGTGATCCCGAGTACCGGCC
157 A Y F G T G F P H M L F M V H P E Y R P
661 CAAGAGACCTGCCAACCAGTTTGTGCCAGGCTCTACGTTTCAAGATCCATCCGATGGC
177 K R P A N Q F V P R L Y G F K I H P M A
721 CTACAGCTGCAGCTCCAAGCCGCCAGCACTTCAAGAGCCAGTCAAGACGATTCCGCTG
197 Y Q L Q L Q A A S N F K S P V K T I R
781 ATTCCTCCCCCACTGTCTGCAGTCTTTCGACTTTCTTTCTTTTTCGCCACCTTTTC
841 AGGAACCTGTATGGTTTTAGTTTAATTAAGGAGTCGTTATTGTGGGGAATATGA
901 AATAAAGTAGAAGAAAGGCCAAAAA
961 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

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Fig. 5. Nucleotide sequence of a full-length cDNA clone encoding the human phosvitin/casein-kinase-II β subunit. The sequence of the coding strand is shown oriented in 5' to 3' direction. A poly(A)-rich addition signal (AATAAA) is underlined. The deduced amino acid sequence of the largest open reading frame is shown below the nucleotide sequence in the single letter code. The amino acid sequence determined by the Edman degradation procedure of the V8-protease digestion peptide shown in Fig. 1 is also underlined. Asterisk indicates deviation from the amino acid sequence of bovine lung [21]

λ gt10 DNA revealed that eight out of these nine clones carried an *Eco*RI cDNA insert, the inserts varying in length between roughly 500 bp and 900 bp (Fig. 3).

DNA sequencing

Four of the longer cDNA inserts (Fig. 3, lanes 6–9) were subcloned into Bluescript M13 and partially sequenced by the dideoxy chain-termination method [31]. The sequences obtained were found to be identical. One of these clones (Fig. 3, lane 6) was therefore selected for total sequence analysis.

Approximately 300–350 nucleotides of the insert could be sequenced starting from both *Eco*RI sites of the Bluescript M13 subclone. The sequence information obtained revealed extremely high similarity to that of the recently published *D. melanogaster* sequence [22] (see also below) and, at the same time, that the 5' *Eco*RI site obviously was not identical to the one generated during the cloning procedure, but was rather an internal *Eco*RI site of the clone. As a consequence, a smaller upstream fragment, not hybridizing with the oligonucleotide probe, must have been lost during the subcloning. To get the entire insert from the original λ gt10 clone, a partial digest with *Eco*RI was thus carried out. The lost fragment was determined to consist of 191 nucleotides (Fig. 4). At this stage it became clear that we were dealing with a full-length cDNA clone of the human CK-II β subunit. The clone was further analyzed by constructing subclones via two internal

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10      20      30      40      50      60      70      80      90      100     110     120
1  ATGAGCAGCTCAGAGGAGGTGCTCTGGATTTCTGGTTCTGTGGGCTCGTGGCAATGAATTTCTTCTGTGAAGTGGATGAAGACTACATCCAGGACAAATTTAATCTTACTGGACTCAAT
***** ***** ***** ***** ***** ***** ***** ***** ***** ***** ***** ***** ***** ***** ***** ***** ***** ***** ***** *****
1  ATGAGCAGCTCCGAGGAAGTCTCTGGGTACCTGGTCTGTGGACTTCTGTGGCAATGAGTTCTTCTGCGAGGTGGATGAGGACTACATACAGGATAAATCAATTTAACTGGTTTAAAT

121  GAGCAGGTCTCTCACTATCGACAAGCTCTAGACATGATCTTGGACCTGGAGCTGATGAAGAAGTGAAGACAAACCCCAACAGAGTGACCTGATTGAGCAGGCAGCCGAGATGCTTTAT
***** **Y *****R*****Y*****R*****R*****Y*****R*****R*****R*****R*****R*****R*****R*****R*****R*****R*****R*****R*****R*****
121  GAGCAGGTACCCAATATCGGCAAGCGTGGACATGATCTTGGACTTGAACCGGAGGACGAGCTCGAGGACAATCCACTGCAGTCCGACATGACCGAGCAGGCCGCGGAGATGCTCTAC

241  GGATTGATCCACGCCGCTACATCTTACCAACCGTGGCATCGCCAGATGTTGGAAAAGTACCAGCAAGGAGACTTTGGTTACTGTCTCTGTGTACTGTGAGAACCAGCCAATGCTT
**Y** ***** **Y** ***** **Y** *****Y*****Y*****R*****R*****R*****R*****R*****R*****R*****R*****R*****R*****R*****R*****R*****
241  GGCTTCATACAGCCAGATATATACTAACAATCGCGGCATCGCTCAATGATCGAGAAATATCAAACTGGCGATTTCGGACATTGTCCACGTGTCTACTGTGAAAGTCAGCCCATGCTG

361  CCCATTGGCCTTTCAGACATCCCAAGTGAAGCCATGGTGAAGCTCTACTGCCCAAGTGCATGGATGTGTACACACCAAGTCAAGACACCATCACACGGATGGCGCTACTTCGGC
** * **Y** *****R*****Y*****R*****R*****R*****R*****R*****R*****R*****R*****R*****R*****R*****R*****R*****R*****R*****R*****
361  CCATTGGGTCTGTCTGGACATCCCGCGCAGGCAATGGTTAAGACCTATTGCCCAAGTGCATGACGTGTACACACCAAAATCGTCGCGTCACCAACCATCCGATGGCGCTATTTCGGC

481  ACTGGTTTCTCTACATGCTCTTCTATGGTGCATCCCGAGTACCGGCCCAAGAGACCTGCCAACCAGTTTGTGCCAGGCTCTACGGTTTCAAGATCCATCCGATGGCTTACCAGCTGCAG
***** **Y** *****R*****Y*****R*****R*****R*****R*****R*****R*****R*****R*****R*****R*****R*****R*****R*****R*****R*****R*****
481  ACTGGATTCCACACATGCTCTTCTATGGTGCATCCCGAATATCGTCCCAAGCGTCTACTAATCAGTTTGTCCAAGGCTATATGGATTAAAAATACACAGCTTAGCTTATCAAATTCAG

601  CTCCAAGCGCCAGCAACTTCAAGAGCCAGTCAAGACGATTCGCTGA
** *****R *****Y*****R*****R*****R*****R*****R*****
601  CTGCAGGCAGCAGCAATTTTAAATGCCACTACGAGCGAAAACTAA

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Fig. 6. Alignment of the coding regions of human and *Drosophila phosvitin/casein-kinase-II β subunits*. The upper sequence represents the human β -subunit cDNA, the lower that of *Drosophila* [22]. The sequences of the coding strands are given in the 5' to 3' direction beginning with the ATG start codons and ending with the TGA and TAA stop codons, respectively. Identical nucleotides are marked by asterisks, purine transitions by R and pyrimidine transitions by Y. The alignment is gap-free over the whole 648 nucleotides of the coding regions including the start and the stop codons

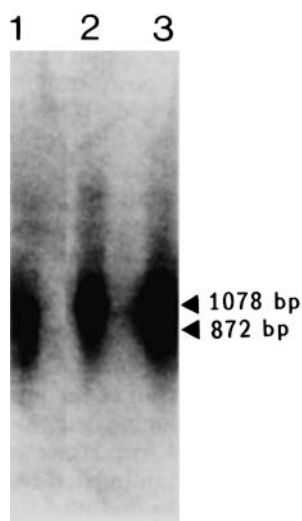


Fig. 7. Comparative Northern blot analysis of human and bovine RNA with human *phosvitin/casein-kinase-II β subunit* probe. 20 μ g each of total RNA prepared from bovine heart (lane 1), human placenta (lane 2), and HeLa S3 cells (lane 3) was separated on a formaldehyde-containing agarose gel, transferred to a nylon membrane and hybridized to a ^{32} P-labeled fragment of the coding region of human CK-II β subunit (537 bp *EcoRI/PstI* fragment indicated in Fig. 3). *HaeIII*-digested ϕ X174 DNA was employed as molecular mass standard

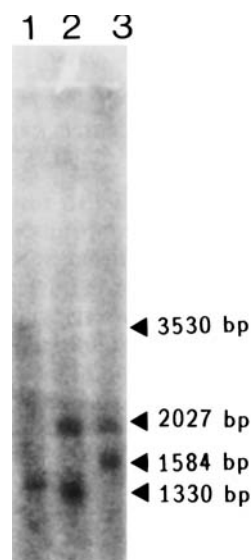


Fig. 8. Southern blot analysis of human genomic DNA with human *phosvitin/casein-kinase-II, β subunit* probe. Genomic DNA was prepared from human epithelial cells in culture (HeLa S3), and approximately 10 μ g digested with *PstI* (lane 1), *EcoRI/PstI* (lane 2), and *EcoRI* (lane 3). The digests were separated by agarose gel electrophoresis, transferred to a nylon membrane and hybridized to the same labeled 537-bp fragment of the coding region of human CK-II β subunit used in the Northern-blot analysis (see legend to Fig. 6). *EcoRI/HindIII*-digested λ DNA was used as molecular mass standard

PstI sites, a *NcoI* site, the internal *EcoRI* site and the flanking *EcoRI* sites (Fig. 4). Sequencing of these subclones provided overlapping sequence information from both stands and finally the clone's complete sequence.

The complete sequence of the full-length cDNA clone turned out to consist of 1013 bp (Fig. 5). The deduced amino acid sequence of the largest open reading frame encoded a polypeptide of 215 amino acid residues starting with the expected codon for methionine. The deduced amino acid sequence included the partial amino acid sequence obtained from the V8 protease digestion peptide by Edman degradation

analysis (see Fig. 1). The 3'-untranslated region comprised 232 bp and terminated in a poly(A)-rich tail. The tail was preceded by an AATAAA poly(A)-rich addition signal at the expected distance upstream from the poly(A)-rich tail.

Comparative sequence analysis

As shown in Fig. 6, the coding region of the sequenced cDNA clone had a similarity of 75% with the coding region

of the *Drosophila* CK II β subunit [22]. The predicted amino acid sequence showed a similarity of 88% with the predicted *Drosophila* amino acid sequence upon gap-free alignment. Although no nucleotide sequencing data are currently available for any mammalian CK II β subunit, the complete amino acid sequence of the β subunit of calf lung CK II has recently been determined [21]. This sequence consists of 209 amino acid residues. When aligned, again in a gap-free manner, the similarity was as high as 97%. Differences were found only at the extreme N-terminal and C-terminal parts (see Fig. 5). At the N-terminus, the deduced human sequence was four amino acid residues longer than the bovine sequence, i.e. three amino acid residues (Met-Ser-Ser) extended the terminal Ser of the bovine sequence and one amino acid (Glu) was added next to it C-terminally. This part of the human sequence, therefore, resembled rather the fruit fly's sequence [22]. At the C-terminus, the human sequence was two amino acids (Ile-Arg) longer than the bovine.

Together, the 215 amino acids of the coding region of the deduced human sequence would amount to a maximum M_r of 24925. This value was considerably below the M_r 28000 estimate obtained by SDS/PAGE and ultracentrifugation of CK II β -subunit preparations [13]. The actual M_r should be even smaller since, in accordance with the bovine lung sequence [21], one or several of the amino acid residues of the N-terminus are presumably removed.

Expression of human phosvitin/casein kinase II β subunit and genomic organization

Employing a 537 bp *EcoRI/PstI* restriction fragment (see Fig. 4) as hybridization probe, a transcript of approximately 1–1.1 kb could be detected in total RNA preparations from both human epithelial cells in culture and human placenta (Fig. 7). The length of the transcripts corresponded perfectly with the length of the sequenced full-length cDNA clone. With the same hybridization probe and under the same conditions, a transcript was clearly detectable as well in total RNA prepared from bovine heart tissue which, however, appeared to be somewhat smaller (Fig. 7). This result confirmed what was to be expected from the comparisons made above, namely, that the degree of similarity between the human and the bovine CK-II β subunit must also be very high at the nucleic acid level.

To find out whether the coding region of the human CK-II β subunit contains introns, a Southern blot analysis of human genomic DNA was carried out. When genomic DNA prepared from HeLa cells was totally digested with *EcoRI* or *PstI* and hybridized with the 537-bp *EcoRI/PstI* restriction fragment of CK-II β subunit, two hybridizing bands were obtained (Fig. 8). Because the fragment employed as hybridization probe carried neither an *EcoRI* nor a *PstI* cleavage site, the genomic sequence corresponding to the fragment must contain at least one intron. This is confirmed by the fact that the combined *EcoRI/PstI* digest of the genomic DNA did not lead to a 537 bp fragment which would be expected to appear if the genomic DNA did not contain an intron within this stretch.

DISCUSSION

The present isolation and sequencing of a full-length cDNA clone of the human CK-II β subunit provides the first sequence information at the nucleic acid level for vertebrates.

The only cDNA sequence available yet has been that of the fruit fly *D. melanogaster* [22]. At the amino acid level, the sole sequence known has been that of bovine lung [21]. Both of these were useful parameters at the different stages of our work. The V8 protease-derived digestion peptide of the human CK-II β subunit sequenced in order to come to a specific hybridization probe, was fully alignable to the respective stretch of the bovine sequence and hence assured us that we had really sequenced a piece of subunit β which was available in only very limited amounts. On the other hand, the *Drosophila* cDNA sequence gradually became an important reference as more and more of the subclones of our human cDNA clone were sequenced. In fact, the strikingly high sequence similarity between the human and the *Drosophila* cDNA finally became one of the identification criteria, the others being the gap-free alignment of the deduced amino acid sequence of the human clone with the amino acid sequence of bovine lung, the partial amino acid sequence of human placenta β subunit obtained by Edman degradation analysis, and the identity of the potential recognition sites for autophosphorylation to that of the bovine sequence and the *Drosophila* sequence [22]. These are serine and/or threonine residues followed by stretches of acidic residues [37].

The deduced amino acid sequence of the human CK-II β subunit varies amazingly little from the bovine sequence. Within cells, the differences found at the N-terminus may be even smaller because of posttranslational and/or cotranslational removal of amino acids. If the N-terminal amino acid of the human protein were, in accordance with the bovine protein, also Ser, the difference would be diminished to a single amino acid residue, namely, Glu. A blocking group has been postulated for the N-terminus of the bovine protein, probably an acetyl group [21]. Some group of this sort also seems to block the N-terminus of the human sequence as suggested by our failure to sequence the complete β subunit from its N-terminus. No further difference seems to exist between the human β and the bovine β subunits along the whole sequence up to the other end of the protein chain. At the C-terminus, two additional amino acids are found in the human sequence, namely, Ile and Arg. However, even this deviation might turn out smaller than implied because, as Takio et al. [21] suspected, it cannot be excluded that the bovine protein does not end with a Thr but with additional amino acid residues, namely, Gly and/or Pro.

Calculating the hydropathy profile according to Hopp and Woods [38] did not show up any significant difference of the deduced human β -subunit sequence to that of the bovine and even the deduced *Drosophila* sequence. Potential secondary structure in subunit β was predicted by the Chou and Fasman method [39] as well as by that of Robson and Suzuki [40]. As already noted for the bovine protein [21], there was little in the way of unusual secondary structure patterns. None of the predictions for α -helical structures, extended chains, β turns and random coils showed significant deviations from the predictions of the bovine β subunit or the deduced *Drosophila* β subunit. Further, neglecting any N-terminal blocking group, the isoelectric point of subunit β was determined by computer analysis and found to be at pH 6.2. The values calculated for bovine and *Drosophila* were exactly the same. It is interesting to recall [21] that the charges carried by subunit β are clustered, negative charges in the N-terminal region and positive charges rather in the C-terminal region, possibly indicating how and why the activity of CK II is so strongly influenced by polyanions and polycations. However, this remains a matter of speculation. Since no similarity with any

known and computer-stored protein exists, there are hardly any serious possibilities for more detailed guesses at the moment.

The appearance of only one band in Northern blot hybridization indicates that there exists only one type of transcript and perhaps only a single copy of the β subunit gene, although expression of additional copies in cell types not examined cannot yet be excluded. The fact that the bovine β -subunit transcript could also be detected with the human cDNA clone as the hybridization probe demonstrates a high similarity within the mammalian β -subunit nucleotide sequences. This, together with the 75% sequence similarity even to the evolutionary distant fruit fly *D. melanogaster* [22], strongly suggests that subunit β had been highly conserved during evolution. The strikingly high similarity of the deduced amino acid sequence with the bovine amino acid sequence [21], i.e. 97% similarity, and even that of the fruit fly, i.e., 88% similarity, confirms this suggestion. Since β -subunit protein occurs in practically every eukaryotic organism (reviewed [1–3]), it is tempting to suppose that it serves crucial cellular functions. Whether this is achieved by regulating the kinase activity of the α subunits of CK II [15–17, 19] or by involvement in any other processes [20] awaits further experimentation.

The pattern of the Southern blot hybridization obtained with two different restriction enzymes in single and combined digestions reveals that the human subunit β gene contains at least one intron. This intron is situated within the coding region, presumably in its central portion. Length and number of the restriction fragments indicate an intron length in the range of 2.5 kb. Although a more precise description of the gene organization awaits further experimentation, all our results are compatible with the existence of only one gene copy/haploid genome for subunit β of the human casein kinase II.

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