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Molecular Cloning of the Human Casein Kinase II α Subunit[†,‡]

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ABSTRACT: A human cDNA encoding the α subunit of casein kinase II and a partial cDNA encoding the rat homologue were isolated by using a *Drosophila* casein kinase II cDNA probe. The 2.2-kb human cDNA contains a 1.2-kb open reading frame, 150 nucleotides of 5′ leader, and 850 nucleotides of 3′ noncoding region. Except for the first 7 deduced amino acids that are missing in the rat cDNA, the 328 amino acids beginning with the amino terminus are identical between human and rat. The *Drosophila* enzyme sequence is 90% identical with the human casein kinase II sequence, and there is only a single amino acid difference between the published partial bovine sequence and the human sequence. In addition, the C-terminus of the human cDNA has an extra 53 amino acids not present in *Drosophila*. Northern analysis of rat and human RNA showed predominant bands of 5.5, 3.1, and 1.8 kb. In rat tissues, brain and spleen had the highest levels of casein kinase II α subunit specific RNA, while skeletal muscle showed the lowest. Southern analysis of human cultured cell and tissue genomic DNA using the full-length cDNA probe revealed two bands with restriction enzymes that have no recognition sites within the cDNA and three to six bands with enzymes having single internal sites. These results are consistent with the possibility that two genes encode the α subunits.

Casein kinase II is a serine/threonine kinase that phosphorylates acidic proteins such as casein or phosvitin. The subunit structure is that of an a_2/b_2 or a-a′/β_2 tetramer, with the α subunit (M_r 40 000) possessing catalytic activity, and the β subunit (M_r 25 000) being autophosphorylated in vitro [for reviews, see Hathaway and Traugh (1982) and Edelman et al. (1987)]. In eucaryotic cells the enzyme is distributed predominantly in the cytosol but also has been found in mitochondria (Damuni & Reed, 1988), nuclei (Hathaway & Traugh, 1983), and coated pits (Bar-Zvi & Branton, 1986). Many enzymes have been identified as potential physiological substrates for casein kinase II, including glycogen synthetase, topoisomerase II, the R subunit of cAMP-dependent protein kinase, RNA polymerase II, acetyl-CoA carboxylase, and the B light chain of clathrin (Hathaway & Traugh, 1982; Edelman et al., 1987; Zandomeni et al., 1986; Haystead et al., 1988; Bar-Zvi & Branton, 1986). The possible role of this enzyme in RNA, DNA, and protein metabolic pathways has been

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reviewed recently (Sommercorn & Krebs, 1987).

Recent work has shown that insulin added to Balb/C 3T3 cells (Klarlund & Czech, 1988) or 3T3L1 adipocytes (Sommercorn et al., 1987) enhances casein kinase II activity within minutes, as measured by the incorporation of [γ-^{32}P]GTP into an acidic peptide substrate Arg-Arg-Arg-Glu-Glu-Glu-Thr-Glu-Glu-Glu (Kuenzel & Krebs, 1985). It is possible that casein kinase II is one of the major protein serine or threonine kinases activated by insulin and therefore is part of the insulin transduction signaling pathway. One approach to examine the potential physiological role of this enzyme in insulin action is to alter the cellular expression of casein kinase II in insulin-sensitive cells and measure the consequences on target enzymes. The first step toward this goal is to isolate and identify the cDNA for mammalian casein kinase II. The *Drosophila* full-length cDNAs for both subunits of casein kinase II have been cloned and sequenced (Saxena et al., 1987). Since the published bovine α amino acid sequence (Takio et al., 1987) is nearly 100% identical with the *Drosophila* α subunit sequence, we chose to screen a human and rat cDNA library for the casein kinase II α cDNA by hybridizing to a radiolabeled *Drosophila* α subunit cDNA. We report here the identification and nucleotide sequencing of a complete human cDNA and a nearly complete rat cDNA.

Materials and Methods

Screening of cDNA Libraries. A full-length *Drosophila* cDNA for the α subunit was obtained from Dr. Claiborne Glover, University of Georgia. *Bam*HI linkers were attached, and the 1.24-kb cDNA was ligated into Bluescribe (Stratagene). A human HepG2 cDNA library (Kwiatkowski et al., 1986) cloned into the λ GT10 vector was plated out at 5×10^5 pfu. The *Drosophila* 1.2-kb insert cDNA was labeled by random priming (Amersham) to a specific activity of $1-2 \times 10^9$ cpm/μg and hybridized to the nitrocellulose filter replicas in 1 M NaCl, 10 mM Tris, pH 7.4, 2 mM EDTA, 5× Denhardt's, 0.1% SDS, and 10% dextran sulfate at 50 °C. Filters were washed in 9 M NaCl/Tris, pH 7.4, at room temperature and then at 45 °C in 0.15 M (1×) NaCl, 10 mM Tris, 2 mM EDTA, and 0.1% SDS. Ultimately, 20 HepG2 clones ranging in size from 1.5 to 2.2 kb were isolated, and cDNAs from three of these were ligated into Bluescribe. Each cDNA was partially restriction mapped and the 2.2-kb cDNA digested with *Pvu*II and *Eco*RI and subcloned into M13 mp 18/19. Both strands were sequenced at least twice by the dideoxynucleotide method (Sanger et al., 1977) with Sequenase (U.S. Biochemical Corp.), [^{35}S]dATPγS and oligonucleotides by using wedge-shaped gradient gels (Biggin et al., 1983). Bionet (Intelligenetics Inc.) was employed to manage the sequencing results.

A rat adipose cDNA library cloned into λ GT10 (obtained from Dr. John Merlie, Washington University) was also screened with the *Drosophila* 1.2-kb α subunit cDNA. a 1.3-kb plaque was isolated and sequenced as above.

RNA Analysis. Total RNA from rat tissue and human term placenta was prepared according to the method of Chirgwin (Chirgwin et al., 1979), pelleted by centrifugation through CsCl, electrophoresed in 2.2 M formaldehyde/agarose gels, transferred to nitrocellulose, and hybridized to ^{32}P-labeled nick-translated rat and human cDNAs, respectively (Rigby et al., 1977). Total RNA from HepG2 cells was prepared according to the method outlined in Davis et al. (1986).

Genomic Analysis. Genomic DNA was extracted from human term placenta and HepG2 cells by the method of Bell (Bell et al., 1981) and digested with restriction enzymes. Samples were electrophoresed on 0.8% agarose gels and following transfer to nitrocellulose were subsequently hybridized to the ^{32}P-labeled nick-translated full-length cDNA. Completeness of restriction digests of genomic DNA was monitored in separate control reactions with plasmid DNA.

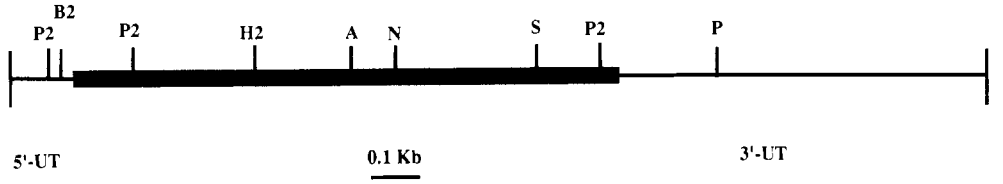
Results

Nucleotide Sequence of the Human Casein Kinase II α Subunit. Screening of the HepG2 cDNA library at low stringency with the 1.2-kb *Drosophila* α subunit cDNA yielded several λ GT10 recombinants with inserts ranging from 1.5 to 2.2 kb. Partial restriction enzyme digests of the three largest inserts (1.9–2.2 kb) with *Dde*I, *Rsa*I, *Hin*fI, and *Pvu*II showed similar sized fragments, suggesting a common mRNA origin. A partial restriction map for the 2.2-kb cDNA is shown in Figure 1a. This cDNA was selected for sequencing by the dideoxynucleotide method (Sanger et al., 1977). The 2.2-kb clone was found to contain 153 nucleotides of 5′ leader sequence, a Met codon at nucleotide 154 followed by an open reading frame of 1173 nucleotides ending in a TAA stop codon, and 852 nucleotides of 3′ noncoding region (Figure 1b). No AATAAA polyadenylation signal consensus sequence or the ATTTA motif, which is involved in mRNA degradation (Shaw & Kamen, 1986), was found. The rat cDNA was found to be nearly complete, but lacked the first 15 nucleotides from the predicted Met translational start site of the human. The coding region of the human cDNA is 93% identical with the rat cDNA, and the short stretch of 3′ noncoding region that has been sequenced is 90% conserved (not illustrated). Although not shown here, the sequence of the *Drosophila* 3′ noncoding region is completely dissimilar from the human and rat 3′ noncoding region.

Species Comparison of the Deduced Amino Acid Sequence of the Casein Kinase II α Subunit. A comparison of the translated amino acid sequences of the human, rat, and *Drosophila* α subunit and a partial bovine sequence (Figure 2) emphasizes the conserved nature of this gene. The first 328 amino acids from the amino terminus are identical between human and rat. The *Drosophila* sequence is 90% identical for the first 325 amino acids and 94% identical if conservative amino acid changes are considered. The partial bovine sequence, which was obtained by amino acid sequencing (Takio et al., 1987), is identical except for a single change of histidine to glutamine at amino acid 238. The C-terminal 67 amino acids of human and rat are completely different from those of *Drosophila*, and it is in this region that the human and rat sequences differ at 6 positions. The additional C-terminal 53 amino acids account entirely for the larger size of the mammalian compared to the *Drosophila* α subunit (Dahmus et al., 1984).

RNA Profile. Northern analysis was performed by hybridizing total RNA prepared from human term placenta and HepG2 cells (Figure 3, lane 1–3). Three bands of different intensities, at 1.8, 3.1, and 5.5 kb, appear on the autoradiograph. In some cases such as lane 2, a fourth band at 1.2 kb was also evident. Washing of the nitrocellulose filters at more stringent conditions did not change the relative proportion of the three RNA species (data not shown). Figure 3, lanes 4–8, shows RNA levels of the α subunit in several tissues of the rat. Brain (lane 4) and spleen (lane 7) have the highest concentration and skeletal muscle (lane 8) has the lowest, which parallel the protein concentration profiles (Singh & Huang, 1985). Regardless of the tissue abundance, all three hybridizing bands show the same relative intensities, i.e., 5.5 kb > 3.1 kb > 1.8 kb.

Genomic Profile. Southern analysis of genomic DNA isolated from term placenta, digested with various restriction



b

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                                   27                                  54
gagcagaggggagacggccgccgccctggccgcttccaccacagtttgaagaaaacaggtctgaaacaaggtcttaccccc
agctgcttctgaacacagtgactgccagatctccaaacatcaagtccagctttgtccgccaacctgtctgacATGTCGGGA
                                                                     METSerGly
                          189                                 216                 243
CCCGTGCCAAGCAGGGCCAGAGTTTACACAGATGTTAATACACACAGACCTCGAGAATACTGGGATTACGAGTCACATGTG
ProValProSerArgAlaArgValTyrThrAspValAsnThrHisArgProArgGluTyrTrpAspTyrGluSerHisVal
                          270                 297                                 324
GTGGAATGGGGAAATCAAGATGACTACCAGCTGGTTCGAAAATTAGGCCGAGGTAAATACAGTGAAGTATTTGAAGCCATC
ValGluTrpGlyAsnGlnAspAspTyrGlnLeuValArgLysLeuGlyArgGlyLysTyrSerGluValPheGluAlaIle
                          351                 378                                 405
AACATCACAAATAATGAAAAAGTTGTTGTTAAAATTCTCAAGCCAGTAAAAAAGAAGAAAATTAAGCGTGAAATAAAGATT
AsnIleThrAsnAsnGluLysValValValLysIleLeuLysProValLysLysLysLysIleLysArgGluIleLysIle
                          432                 459                                 486
TTGGAGAATTTGAGAGGAGGTCCCAACATCATCACACTGGCAGACATTGTAAAAGACCCTGTGTCACGAACCCCCGCCTTG
LeuGluAsnLeuArgGlyGlyProAsnIleIleThrLeuAlaAspIleValLysAspProValSerArgThrProAlaLeu
                          513                 540                                 567
GTTTTTGAACACGTAAACAACACAGACTTCAAGCAATTGTACCAGACGTTAACAGACTATGATATTCGATTTTACATGTAT
ValPheGluHisValAsnAsnThrAspPheLysGlnLeuTyrGlnThrLeuThrAspTyrAspIleArgPheTyrMETTyr
                          594                 621                                 648
GAGATTCTGAAGGCCCTGGATTATTGTCACAGCATGGGAATTATGCACAGAGATGTCAAGCCCCATAATGTCATGATTGAT
GluIleLeuLysAlaLeuAspTyrCysHisSerMETGlyIleMETHisArgAspValLysProHisAsnValMETIleAsp
                          675                 702                                 729
CATGAGCACAGAAAGCTACGACTAATAGACTGGGGTTTGGCTGAGTTTTATCATCCTGGCCAAGAATATAATGTCCGAGTT
HisGluHisArgLysLeuArgLeuIleAspTrpGlyLeuAlaGluPheTyrHisProGlyGlnGluTyrAsnValArgVal
                          756                 783                                 810
GCTTCCCGATACTTCAAAGGTCCTGAGCTACTTGTAGACTATCAGATGTACGATTATAGTTTGGATATGTGGAGTTTGGGT
AlaSerArgTyrPheLysGlyProGluLeuLeuValAspTyrGlnMETTyrAspTyrSerLeuAspMETTrpSerLeuGly
                          837                 864                                 891
TGTATGCTGGCAAGTATGATCTTTCGGAAGGAGCCATTTTTCCATGGACATGACAATTATGATCAGTTGGTGAGGATAGCC
CysMETLeuAlaSerMETIlePheArgLysGluProPhePheHisGlyHisAspAsnTyrAspGlnLeuValArgIleAla
                          918                 945                                 972
AAGGTTCTGGGGACAGAAGATTTATATGACTATATTGACAAATACAACATTGAATTAGATCCACGTTTCAATGATATCTTG
LysValLeuGlyThrGluAspLeuTyrAspTyrIleAspLysTyrAsnIleGluLeuAspProArgPheAsnAspIleLeu
                          999                1026                                1053
GGCAGACACTCTCGAAAGCGATGGGAACGCTTTGTCCACAGTGAAAATCAGCACCTTGTCAGCCCTGAGGCCTTGGATTTC
GlyArgHisSerArgLysArgTrpGluArgPheValHisSerGluAsnGlnHisLeuValSerProGluAlaLeuAspPhe
                         1080                1107                                1134
CTGGACAAACTGCTGCGATATGACCACCAGTCACGGCTTACTGCAAGAGAGGCAATGGAGCACCCCTATTTCTACACTGTT
LeuAspLysLeuLeuArgTyrAspHisGlnSerArgLeuThrAlaArgGluAlaMETGluHisProTyrPheTyrThrVal
                         1161                1188                                1215
GTGAAGGACCAGGCTCGAATGGGTTCATCTAGCATGCCAGGGGGCAGTACGCCCGTCAGCAGCGCCAATATGATGTCAGGG
ValLysAspGlnAlaArgMETGlySerSerSerMETProGlyGlySerThrProValSerSerAlaAsnMETMETSerGly
                         1242                1269                                1296
ATTTCTTCAGTGCCAACCCCTTCACCCCTTGGACCTCTGGCAGGCTCACCAGTGATTGCTGCTGCCAACCCCCTTGGGATG
IleSerSerValProThrProSerProLeuGlyProLeuAlaGlySerProValIleAlaAlaAlaAsnProLeuGlyMET
                         1323                1350
CCTGTTCCAGCTGCCGCTGGCGCTCAGCAGTAAcggccctatctgtctcctgatgcctgagcagaggtgggggagtccacc
ProValProAlaAlaAlaGlyAlaGlnGln
ctctccttgatgcagcttgcgcctggcggggaggggtgaaacacttcagaagcaccgtgtctgaaccgttgcttgtggatt
tatagtagttcagtcataaaaaaaaaaattataataggctgattttctttttttctttttttttaactcgaacttttcata
actcaggggattccctgaaaaattacctgcaggtggaatatttcatggacaatttttttttctccccctcccaaatttagtt
cctcatcacaaaagaacaaagatataaaccagcctcaatcccggctgctgcatttaggtggagacttcttcccattcccacca
ttgttcctccaccgtcccacactttaggggttggtatctcgtgctcttctccagagattacaaaaatgtagcttctcagg
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taaaatctacccaaaatgcagacagatactaaaactctgtcagtagatcatgtcttactgatctaaccctaaatccaactc
atttatacttttatttttagttcagtttaaaatgttgatacctccctcccaggctccttaccttggtcttttccctgttc
atctcccaacatgctgtgctccatagctggtaggagagggaaggcaaaatctttcttagttttctttatctt
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FIGURE 1: Nucleotide sequence of the cDNA for the α subunit of human casein kinase II. (a) Restriction map of the human cDNA. (H2) *Hin*dII; (A) *Acc*I; (P) *Pst*I; (B2) *Bgl*II; (N) *Nco*I; (P2) *Pvu*II; (S) *Sph*I; (5'-UT) 5' untranslated region; (3'-UT) 3' untranslated region. (b) Nucleotide and predicted amino acid sequence. The MET initiation codon is at nucleotide 154, and the termination codon (TAA) is at nucleotide 1327. Underlined amino acids indicate the ATP binding consensus sequence (Hanks et al., 1988).

enzymes and hybridized to the full-length α cDNA, is shown in Figure 4. In general, restriction enzymes that have no recognition sites within the cDNA (*Bam*H1 and *Hin*dIII, lanes 5 and 6) give two hybridizing bands, and enzymes with single internal sites (*Acc*I, *Pst*I, *Bgl*I, *Bgl*II, and *Nco*I, lanes 1–4, and 7, respectively) yield three to six bands. These results are more easily explained by two genes coding for the α subunits, although an alternative explanation of the data, consistent with a single gene, is that additional restriction sites for each enzyme are present within intron regions.

Discussion

The cloning of the rat and human cDNA for an α subunit of casein kinase II has allowed us to address several questions concerning this enzyme. The first is the location and composition of the amino acids that account for the higher mo-

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                                10          20          30          40          50

Human          MSGPVPSRARVYTDVNTHRPREYWDYESHVVEWGNQDDYQLVRKLGRGKYSEV
Rat                 .....................................................
Drosophila       MTL..A........A.K.D......NY..D.....................
Bovine

       60          70          80          90          100         110

FEAINITNNEKVVVKILKPVKKKKIKREIKILENLRGGPNIITLADIVKDPVSRTPALVFEHVNNT
.....................................................................
.......TT..C.........................T.....LAV...........I.......

120         130         140         150         160         170         180

DFKQLYQTLTDYDIRFYMYEILKALDYCHSMGIMHRDVKPHNVMIDHEHRKLRLIDWGLAEFYHPG
.....................................................................
............E..Y.LF.L............................N...................
                                                    ....................

  190         200         210         220         230         240         250

QEYNVRVASRYFKGPELLVDYQMYDYSLDMWSLGCMLASMIFRKEPFFHGHDNYDQLVRIAKVLGT
.....................................................................
.....................................................................
................                                .........Q............

        260         270         280         290         300         310

EDLYDYIDKYNIELDPRFNDILGRHSRKRWERFVHSENQHLVSPEALDFLDKLLRYDHQSRLTARE
.....................................................................
.E..A.L.....D.....H...Q.............D.....................VD......

  320         330         340         350         360         370         380

AMEHPYFYTVVKDQARMGSS-SMPGGSTPVSSANMMSGISSVPTPSPLGPLAGSPVIAAANPLGMP
................-..AG.A.....................................S..I.
..A....LPI.NG.MNPNNQQTAA

390
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FIGURE 2: Comparison of the amino acid sequences of the human, rat, *Drosophila*, and bovine casein kinase II α subunit. Amino acid sequences for human and rat (this work) and *Drosophila* (Saxena et al., 1987) were obtained by reverse translation of nucleotide sequences; the partial bovine sequence was by Edman degradation of peptides (Takio et al., 1987). Dots show homology to the human cDNA.

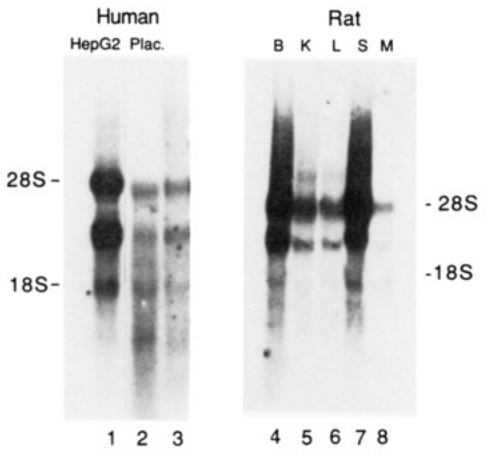

FIGURE 3: Northern blot of total RNA from different human and rat tissues. Total RNA (20 μg) was hybridized at 42 °C in 50% formamide to the 2.2-kb HepG2 cDNA (lanes 1–3) or the rat 1.3-kb cDNA (lanes 4–8), washed at 60 °C in 0.1× SSC, and autoradiographed for 2 days. Ethidium bromide staining of the electrophoresed RNA showed similar intensities of the ribosomal bands in all lanes (not shown). (Lane 1) HepG2 mRNA; (lanes 2, 3) human term placental RNA; (lanes 4–8) RNA from rat brain, kidney, liver, spleen, and muscle, respectively. Markers represent 28S and 18S ribosomal RNA.

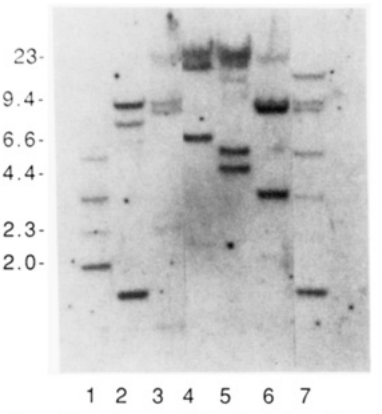

FIGURE 4: Southern blot of genomic DNA isolated from HepG2 cells. High molecular weight DNA was isolated according to the method of Bell et al. (1981); 10 μg was restricted with *Acc*I (lane 1), *Pst*I (lane 2), *Bgl*I (lane 3), *Bgl*II (lane 4), *Bam*HI (lane 5), *Hin*dIII (lane 6), and *Nco*I (lane 7) and hybridized at 42 °C in 50% formamide to the full-length *Eco*RI–*Pst*I 1.57-kb cDNA. The blot was washed in 0.15× SSC, 57 °C, and exposed for 5 days. Molecular weight markers refer to a *Hin*dIII/λ digest.

lecular weight of the mammalian vs *Drosophila* α subunit (Dahmus et al., 1984). We find that the C-terminal 14 amino acids of *Drosophila* are completely different from both the rat and human subunit, and the latter both contain an additional 53 amino acids as well. Interestingly, the α subunits of yeast, which are 42 and 35 kDa, also are larger than *Drosophila* (Padmanabha & Glover, 1987), but in this case the insert in one of the subunits is located near the N-terminus at amino acid 89 (Glover, personal communication). It should be noted that the stretch of 67 amino acids at the C-terminus of the mammalian subunit is very proline rich (15%) compared to an expected average of 3%. Whether this is functionally significant is questionable, since two of these prolines are not conserved between rat and man.

The α subunits of eucaryotic casein kinase II have been proposed to be composed of at least two similar proteins on the basis of amino acid sequencing information and size differences in Western blots (Takio et al., 1987; Sommercorn et al., 1987). The β subunit of mammalian cells shows no analogous amino acid heterogeneity, although two sizes have been found in yeast (Padmanabha & Glover, 1987). Southern genomic blotting experiments presented here support the possibility that two similar genes encode the α subunit. This is consistent with the following: (1) the reported microheterogeneity in the α subunit found by amino acid sequencing of purified bovine casein kinase II (Takio et al., 1987); (2) the presence of three α subunit antibody-reacting bands on a Western blot from mouse (Sommercorn et al., 1987) and bovine (Dahmus et al., 1984); (3) Southern blotting of the *Drosophila* genome showing two genes (Saxena et al., 1987); and (4) the fact that two subunits (α and α') that are heterogeneous in their N-terminal amino acid sequences have been identified in yeast (Padmanabha & Glover, 1987). It should be noted that in rabbit reticulocytes (Hathaway & Traugh, 1982) a single α subunit protein has been found. It is possible that at a particular stage of development only one gene is expressed. Until a second gene encoding the α subunit can be isolated and identified, however, this question must be left open.

Finally, sequencing has emphasized the extremely conserved nature of this enzyme between *Drosophila* and man. Despite the separate evolutionary paths, the amino acid similarity is 94% up to amino acid 328 if conservative replacements are considered. Furthermore, the overall rat/human amino acid homology is 98.5%, and the first 300 amino acids are entirely conserved. The high degree of amino acid sequence identity between these two species suggests that we have cloned an α cDNA homologue from the rat and human cDNA library and that these cDNAs are probably homologous to the cloned *Drosophila* cDNA.

A completely conserved region in all three species is between amino acids 68 and 91, which contains a lysine residue at amino acid 68 that is present in all protein kinases (Hanks et al., 1988) and is the ATP or GTP binding site. A helical wheel representation (Schiffer & Edmundson, 1967) (data not shown) indicates that this region comprises a strong α helix of five turns, with a highly positively charged region that presumably faces out and interacts with negatively charged substrates and a hydrophobic side that faces inward. By comparison, the ATP binding site region of CDC2Hs, which is the human counterpart of the yeast cell division protein CDC28 that is 50–70% similar to the casein kinase II amino acid sequence (Takio et al., 1986), reveals little or no α helix. A survey of the ATP binding region of the catalytic subunit of other serine/threonine kinase families (Hanks et al., 1988) shows no similar helical motifs. The significance of this putative α helical structure in the casein kinase II α subunit is unknown.

The profile of the different sized α subunit RNAs is very similar between rat and human, as well as among the different tissues examined. In all cases, the 3.2- and 5.1- kb species are more abundant than the 1.8-kb mRNA. The uniform distribution makes it unlikely that the different sizes code for tissue-specific isoforms of casein kinase II, as has been observed in the case of protein kinase C (Knopf et al., 1986), although other tissues/developmental stages may show differential expression. One possibility is that the three species represent organelle-specific mRNAs. Present data do not allow us to distinguish whether these bands result from a posttranscriptional modification of a single gene or are the product of two genes.

Registry No. Casein kinase, 52660-18-1; human cDNA, 119618-91-6; human casein kinase α subunit, 119618-93-8.

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