Cloning and Characterization of a cDNA Encoding the β Subunit of Human Casein Kinase II†

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ABSTRACT: Previous studies [Summercorn et al. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 8834–8838; Klarlund & Czech (1988) *J. Biol. Chem. 263*, 15872–15875] have indicated that Balb/c 3T3 cells and 3T3-L1 adipocytes incubated with insulin show increased casein kinase II activity within minutes, implicating this serine/threonine kinase as an early step in an insulin signaling pathway. We recently reported the isolation of a cDNA encoding an α subunit of human casein kinase II [Meisner et al. (1989) *Biochemistry 28*, 4072–4076] as an initial step toward examining the regulation of this enzyme. We now describe a HepG2 cell casein kinase II β subunit cDNA of 2.57 kb containing 96 bases of 5′ untranslated sequence, 645 bases of open reading frame, and 1832 bases of 3′ untranslated sequence with two polyadenylation consensus signal sequences and two poly(A) stretches. The open reading frame of the human β subunit cDNA was 77% and 87% identical with the *Drosophila* sequence at the nucleotide and amino acid levels, respectively, and 99% identical with the bovine amino acid sequence. RNA analysis of HepG2 cell RNA utilizing HepG2 β subunit cDNA fragments as probes revealed one major band migrating at 1.2 kb and two minor bands migrating at 3.0 and 4.2 kb. Results from DNA analysis of HepG2 genomic DNA, consistent with results utilizing *Drosophila* genomic DNA, suggest the presence of a single gene for the β subunit of casein kinase II.

Casein kinase II is a serine/threonine protein kinase that appears to be widely distributed in eukaryotic cells [for reviews see Hathaway and Traugh (1982, 1983) and Edelman et al. (1987)]. Studies of the purified enzyme demonstrate that casein kinase II exists as the oligomeric structures $\alpha_2\beta_2$ or $\alpha\alpha'\beta_2$ with the α and α′ subunits (M_r 37 000–44 000) possessing the catalytic site and the β subunit (M_r 24 000–28 000) possessing a site for autophosphorylation. The chief characteristics of this enzyme are the following: (1) both ATP and GTP can be utilized as phosphate donor (Hathaway & Traugh, 1982); (2) the enzyme is potently inhibited by anions such as heparin and activated by cations such as spermine (Hathaway & Traugh, 1983); (3) localization is predominantly cytosolic, although nuclear (Hathaway & Traugh, 1983), mitochondrial (Damuni & Reed, 1988), coated pit (Bar-Zvi & Branton, 1986), and microsomal (Edelman et al., 1987) forms of the enzyme have been described; (4) its substrates contain several acidic amino acid residues located carboxy terminal to the phosphorylated serine or threonine (Carmichael et al., 1982; Meggio et al., 1984; Pinna et al., 1984; Kuenzel & Krebs, 1985; Holmes et al., 1986; Marin et al., 1986; Kuenzel et al., 1987). Renaturation studies performed on bovine lung casein kinase II (Cochet & Chambaz, 1983) demonstrate that optimal activity is achieved when α and β subunits are present in a 1:1 molar stoichiometry, suggesting that the β subunit might play a role in modulating α subunit catalytic activity. Autophosphorylation of the β subunit is one possible mechanism for the regulation of casein kinase II activity because activation of several protein kinases, e.g., the multifunctional Ca^{2+} calmodulin-dependent protein kinase (Lou et al., 1986) and phosphorylase kinase (King et al., 1983; Hallenbeck et al., 1983; Ramachandran et al., 1987), has been shown to be associated with an autophosphorylation event.

The mode of regulation and physiological importance of casein kinase II are not yet understood. Casein kinase II has been implicated to play a role in both nucleic acid and protein synthesis as well as in other cellular processes because it phosphorylates such diverse substrates as DNA topoisomerase II (Ackerman et al., 1988), RNA polymerases I (Dahmus, 1981) and II (Dahmus, 1981; Stetler & Rose, 1982), translational initiation factors (Hathaway et al., 1979), glycogen synthase (DePaoli-Roach et al., 1979), and acetyl-CoA carboxylase (Witters et al., 1983). It has been shown that phosphorylation of acetyl-CoA carboxylase by casein kinase II occurs in response to insulin treatment (Brownsey & Denton, 1981; Witters et al., 1983; Brownsey et al., 1984); however, the phosphorylation has no apparent effect on function. More recent studies (Sommercorn et al., 1987; Klarlund & Czech, 1988) have indicated that insulin treatment of Balb/c 3T3 cells and 3T3-L1 adipocytes can rapidly increase casein kinase II activity when assayed with a specific peptide substrate. One approach to examine the possible role of casein kinase II in insulin-mediated signal transduction is to generate cell lines that exhibit altered levels of expression of this enzyme. Toward this goal, we recently reported the cloning and sequencing of cDNAs encoding an α subunit of both human and rat casein kinase II (Meisner et al., 1989). In the present paper, we describe the cloning and sequencing of a 2.57-kb cDNA encoding the β subunit of human casein kinase II.

Materials and Methods

Isolation and Sequencing of a HepG2 Casein Kinase II β cDNA Clone. A cDNA coding for the β subunit of casein kinase II from *Drosophila melanogaster* (Saxena et al., 1987) was a gift from Dr. Claiborne Glover, University of Georgia. *Bam*HI linkers were attached to this cDNA, and the 0.97-kb cDNA was ligated into Bluescribe (Stratagene). A human HepG2 cDNA library cloned into λ-gt10 (Kwiatkowski et al., 1986) was screened (1×10^6 recombinants) with the 0.97-kb

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Drosophila probe labeled by nick translation (specific activity = 5×10^8 cpm/μg). Hybridization of replicate nitrocellulose filters (Schleichter and Schuell) was for 24 h at 37 °C in 25% formamide, 1× Denhardt's solution, 5× saline sodium citrate (SSC) (1× SSC = 105 mM NaCl and 15 mM sodium citrate), 0.02 mg/mL salmon testes DNA, 20 mM Tris, pH 7.4, and 10% dextran sulfate. Filters were washed twice in 2× SSC and 0.1% SDS at room temperature followed by a single wash in 1× SSC and 0.1% SDS for 30 min at 52 °C. Sixty-five clones were isolated, ranging in size from approximately 0.8 to 2.4 kb. Initially, three clones of 0.8, 1.0, and 2.4 kb were subcloned into pGem4z (Promega) and partially mapped with restriction enzymes (Boehringer Mannheim). It was determined that the 2.4-kb cDNA contained an internal *Eco*RI restriction site. Digestion with *Eco*RI yielded 1.28- and 1.12-kb cDNA fragments which were subcloned into M13MP18 (New England Biolabs). Suitably sized restriction fragments from the 1.28-kb cDNA fragment (a 0.52-kb *Eco*RI–*Pvu*II fragment and a 0.76-kb cDNA *Pvu*II–*Eco*RI fragment) were subcloned into M13mp18/19 digested with *Eco*RI and *Hin*dII. Both strands were sequenced at least twice by the dideoxynucleotide method utilizing Sequenase (U.S. Biochemical Corp.) and wedge-shaped gradient gels. Bionet (Intelligenetics) was utilized in analyzing the sequencing data.

By comparing nucleotide sequence data of our isolated 2.4-kb cDNA with the published nucleotide sequence for the *Drosophila* casein kinase II β subunit (Saxena et al., 1987), we determined that we were lacking 5′ coding sequence. A 39-bp oligonucleotide corresponding to nucleotides 30–69 of the published nucleotide sequence for the *Drosophila* casein kinase II β subunit was synthesized. Dot blot analysis of our cloned HepG2 cDNAs using this oligonucleotide labeled with [γ-^{32}P]ATP (Amersham) and T4 polynucleotide kinase (New England Biolabs) demonstrated that all clones previously isolated lacked the 5′ sequence.

A second HepG2 library (Clontech) was then differentially screened with both this oligonucleotide probe labeled with [γ-^{32}P]ATP as described above and with the 0.52-kb *Eco*RI–*Pvu*II fragment of our isolated human HepG2 2.4-kb cDNA clone. Hybridization of one set of replicate nitrocellulose filters with the oligonucleotide probe was for 24 h at 37 °C in 10× Denhardt's solution, 5× SSC, 0.02 mg/mL salmon testes DNA, 20 mM Tris, pH 7.4, and 5% dextran sulfate. Filters were washed twice in 2× SSC and 0.1% SDS at room temperature followed by a single wash in 1× SSC and 0.1% SDS for 15 min at 47 °C. The second set of replicate filters was hybridized with the nick-translated 0.52-kb HepG2 cDNA probe for 24 h at 37 °C in 25% formamide, 1× Denhardt's solution, 5× SSC, 0.02 mg/mL salmon testes DNA, 20 mM Tris, pH 7.4, and 10% dextran sulfate. Filters were washed twice in 2× SSC and 0.1% SDS at room temperature followed by a single wash in 0.2× SSC and 0.1% SDS for 15 min at 52 °C. Plaques that hybridized to both probes were selected, and the resultant clones were screened with both probes and purified, and *Eco*RI-digested phage DNA was analyzed. A 155-bp cDNA fragment was isolated from a 2.55-kb cDNA clone and subcloned into *Eco*RI-digested pGem4z, and double-stranded sequencing was performed by the dideoxynucleotide method utilizing DNA polymerase Klenow fragment (New England Biolabs). The 155-bp cDNA fragment was also subcloned into *Eco*RI-digested MP18, and both strands were sequenced at least twice by the dideoxynucleotide method utilizing Sequenase as described above. In addition, detailed restriction analysis of the 1.28- and 1.12-kb cDNA *Eco*RI fragments isolated from the same clone showed it to be identical with the 2.4-kb cDNA obtained from the initial HepG2 library.

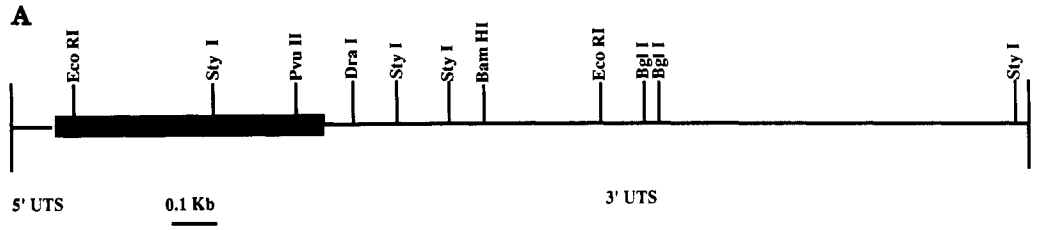
RNA Analysis. Total RNA from cultured HepG2 cells was prepared according to the method outlined in Davis et al. (1986). Equal amounts of RNA, as determined both by spectrophotometric analysis and by staining with ethidium bromide, were electrophoresed in agarose gels containing 2.2 M formaldehyde, transferred to nitrocellulose, and hybridized at 42 °C for 24 h with ^{32}P-labeled nick-translated HepG2 cDNAs in a solution containing 50% formamide, 5× SSC, 1× Denhardt's solution, 5% dextran sulfate, and 0.2 mg/mL salmon sperm DNA. The specific activity of each probe was approximately 3×10^8 cpm/μg DNA, and the concentration of each probe during the hybridization step was 3×10^6 cpm/mL. Two short washes were performed at room temperature in 2× SSC, followed by a final wash in 0.1× SSC and 0.1% SDS at 60 °C for 45 min. Filters were then subjected to autoradiography.

DNA Analysis. High molecular weight genomic DNA was prepared from cultured HepG2 cells by the method outlined in Davis et al. (1986) and digested with restriction enzymes. Samples were electrophoresed on 0.8% agarose and following transfer to nitrocellulose were subsequently hybridized at 42 °C for 24 h to ^{32}P-labeled nick-translated HepG2 cDNA (specific activity = 5×10^8 cpm/μg) in a solution containing 30% formamide, 5× SSC, 1× Denhardt's solution, 10% dextran sulfate, and 0.2 mg/mL salmon sperm DNA. Two short washes were performed at room temperature in 2× SSC, followed by a final wash in 0.1× SSC and 0.1% SDS at 57 °C for 30 min. Filters were then subjected to autoradiography.

Results

Nucleotide Sequence of the HepG2 Casein Kinase II β Subunit. To isolate recombinant clones coding for the β subunit of human casein kinase II, two different HepG2 cDNA libraries were screened with both a full-length 0.97-kb cDNA encoding the β subunit of *Drosophila* casein kinase II and a 39-bp oligonucleotide sequence encoding amino acids 3–15 of the β subunit of *Drosophila* casein kinase II (Saxena et al., 1987). Partial restriction mapping of isolated clones ranging in size from 0.8 to 2.57 kb was performed. The partial restriction map and the nucleotide sequence of a cDNA clone designated H2β 2.57 are shown in Figure 1. The 2.57-kb cDNA contains 96 nucleotides of 5′ untranslated sequence which is 68% G-C rich, a MET initiation codon at nucleotide 97 followed by 645 nucleotides of open reading frame encoding a polypeptide of 215 amino acids with a predicted M_r of 24 900. The 3′ untranslated sequence is 1832 nucleotides in length and contains two polyadenylation signal sequences: Located 121 nucleotides after the TGA termination codon is an AATAAA sequence followed 18 nucleotides downstream by a poly(A) stretch. A second, longer poly(A) stretch, preceded 12 nucleotides upstream by the variant polyadenylation signal sequence ATTAAA (Wickens & Stephenson, 1984), is located 1640 nucleotides downstream from the first poly(A) stretch. The nucleotide sequence of the open reading frame of H2β 2.57 is 77% identical with the nucleotide sequence obtained for the *Drosophila* casein kinase II β subunit (Saxena et al., 1987).

Figure 2 shows a line comparison of the predicted amino acid sequence for the cloned human β subunit cDNA with the published amino acid sequence of the β subunit from bovine lung casein kinase II (Takio et al., 1987) and the predicted amino acid sequence for the *Drosophila* β subunit cDNA (Saxena et al., 1987). These results confirm that the cDNA encodes the β subunit of casein kinase II as there are only two



B

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        10         20         30         40         50         60         70
tccgggcctg gcctgtacgg tcgccgccgt tccctggaag tagcaacttc cctaccccac cccagtcctg
        80         90        100        110        120        130        140
gtccccgtcc agccgctgac gtgaagATGA GCAGCTCAGA GGAGGTGTCC TGGATTTCCT GGTTCTGTGG
                           M  S  S  S  E  E  V  S  W  I  S  W  F  C  G
       150        160        170        180        190        200        210
GCTCCGTGGC AATGAATTCT TCTGTGAAGT GGATGAAGAC TACATCCAGG ACAAATTTAA TCTTACTGGA
  L  R  G  N  E  F  F  C  E  V  D  E  E  Y  I  Q  D  K  F  N  L  T  G
       220        230        240        250        260        270        280
CTCAATGAGC AGGTCCCTCA CTACCGACAA GCTCTAGACA TGATCTTGGA CCTGGAGCCT GATGAAGAAC
  L  N  E  Q  V  P  H  Y  R  Q  A  L  D  M  I  L  D  L  E  P  D  E  E
       290        300        310        320        330        340        350
TGGAAGACAA CCCCAACCAG AGTGACCTGA TTGAGCAGGC AGCCGAGATG CTTTATGGAT TGATCCACGC
L  E  D  N  P  N  Q  S  D  L  I  E  Q  A  A  E  M  L  Y  G  L  I  H  A
       360        370        380        390        400        410        420
CCGCTACATC CTTACCAACC GTGGCATCGC CCAGATGTTG GAAAAGTACC AGCAAGGAGA CTTTGGTTAC
  R  Y  I  L  T  N  R  G  I  A  Q  M  L  E  K  Y  Q  Q  G  D  F  G  Y
       430        440        450        460        470        480        490
TGTCCTCGTG TGTACTGTGA GAACCAGCCA ATGCTTCCCA TTGGCCTTTC AGACATCCCA GGTGAAGCCA
  C  P  R  V  Y  C  E  N  Q  P  M  L  P  I  G  L  S  D  I  P  G  E  A
       500        510        520        530        540        550        560
TGGTGAAGCT CTACTGCCCC AAGTGCATGG ATGTGTACAC ACCCAAGTCA TCAAGACACC ATCACACGGA
M  V  K  L  Y  C  P  K  C  M  D  V  Y  T  P  K  S  S  R  H  H  T  D
       570        580        590        600        610        620        630
TGGCGCCTAC TTCGGCACTG GTTTCCCTCA CATGCTCTTC ATGGTGCATC CCGAGTACCG GCCCAAGAGA
  G  A  Y  F  G  T  G  F  P  H  M  L  F  M  V  H  P  E  Y  R  P  K  R
       640        650        660        670        680        690        700
CCTGCCAACC AGTTTGTGCC CAGGCTCTAC GGTTTCAAGA TCCATGCGAT GGCCTACCAG CTGCAGCTCC
  P  A  N  Q  F  V  P  R  L  Y  G  F  K  I  H  A  M  A  Y  Q  L  Q  L
       710        720        730        740        750        760        770
AAGCCGCCAG CAACTTCAAG AGCCCAGTCA AGACGATTCG Ctgattccct cccccacctg tcctgcagtc
Q  A  A  S  N  F  K  S  P  V  K  T  I  R
       780        790        800        810        820        830        840
tttgacttttt ccttttcttt ttgccaccct ttcaggaacc ctgtatggtt tttagtttaa attaaaggag
       850        860        870        880        890        900        910
tcgttattgt ggtgggaata tgaaataaag tagaagaaaa ggccatgaaa aaaaaaaaaa aaaaaaaaaa
       920        930        940        950        960        970        980
aaaggtttgt gttctcgacg ccttggtagt tggcataggc taaagaaaag ggatctcagc cccgaggaag
       990       1000       1010       1020       1030       1040       1050
ggtcaccctc ctagagatag ctactacccc gtctcaggag accctggtat ttctagagca cgctttgctt
      1060       1070       1080       1090       1100       1110       1120
tcaccaaacc caaggaggtg acaggaggag cccccgcaca ggacctaaga atgctgtgac cagaagatgg
      1130       1140       1150       1160       1170       1180       1190
gatcgcggaa cagcagcagt gcaggatccg gtccggagac ccctccgagg gcttgccccg aagaggggct
      1200       1210       1220       1230       1240       1250       1260
ggcctgcgtc ggagtgagga agaggaagaa gaggatgaag atgtggatct ggccccaggta ctggcctatc
      1270       1280       1290       1300       1310       1320       1330
tcctccgcag aggccaagtg aggttggtgc agggaggagg tgcagcaaat ttacaattca ttcaggccct
      1340       1350       1360       1370       1380       1390       1400
cttggactca gaggaagaga atgacagagc ttgggatggt cgtcttgggg atcgatacaa cccacctgtg
      1410       1420       1430       1440       1450       1460       1470
gatgctaccc ctgacacccg ggagctggaa ttcctggagt tgtgaacgcc gcggactccg gagccgcaca
      1480       1490       1500       1510       1520       1530       1540
aaccagggct cgccatgaag ccaggattca gtcccccgtg ggggtggctt tggcggccga gggggctttg
      1550       1560       1570       1580       1590       1600       1610
gtgaccgtgg tggtcgtgga ggccgagggg gctttggcgg gggccgaggt cgaggcggag gctttagagg
      1620       1630       1640       1650       1660       1670       1680
tcgtggacga ggaggaggtg gaggcggcgg cggcggtgga ggaggaggaa gaggtggagg cttccattct
      1690       1700       1710       1720       1730       1740       1750
ggtggcaacc ggggtcgtgg tcggggagga aaaagaggaa accagtcggg gaagaatgtg atggtggagc
      1760       1770       1780       1790       1800       1810       1820
cgcatcggac tgagggtgtc ttcatttgtc gaggaaagga gatgcactgg tcaccaagaa cctggtcctg
      1830       1840       1850       1860       1870       1880       1890
ggaatcaagt tatggagaga agagagtctc gattcggaag gagatgacaa aattgagtac cgagcctgga
      1900       1910       1920       1930       1940       1950       1960
acccctccg ctccaagcta gcagcagcaa tcctgggtgg tgtggaccag atccacatca aaccgggggc
      1970       1980       1990       2000       2010       2020       2030
taaggttctc tacctcgggg ctgcctcggg caccacggtc tcccatgtct ctgacatcgt tggtccggat
      2040       2050       2060       2070       2080       2090       2100
ggtctagtct atgcagtcga gtttccccac cgctctggcc gtgacctcat taacttggcc aagaagagga
      2110       2120       2130       2140       2150       2160       2170
ccaacatcat tcctgtgatc gaggatgctc gacacccaca caaataccgc atgctcatcg caatggtgga
      2180       2190       2200       2210       2220       2230       2240
tgtgatcttt gctgatgtgg cccagccaga ccagaccccg aatgtggccc tgaaagccca caccttcctg
      2250       2260       2270       2280       2290       2300       2310
cgctaatgga ggacactttg tgatttccat taaggccaac tgcattgact ccacagccta gcggtcaggc
      2320       2330       2340       2350       2360       2370       2380
cgtgtttgcc tccgaagtga aaaagatgca acaggagaac atgaagccgc aggagcagtt gaccctgag
      2390       2400       2410       2420       2430       2440       2450
ccatatgaaa gagaccatgc cgtggtcgtg ggagtgtaca ggccaccccc caaggtgaag aactgaagtt
      2460       2470       2480       2490       2500       2510       2520
cagcgctgtc aggattgcga gagatgtgtg ttgatactgt tgcccgtgtg ttttctaat taaaagactc
      2530       2540       2550       2560       2570
ctccgtcaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaa
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FIGURE 1: Nucleotide sequence of a cDNA clone encoding the β subunit of human casein kinase II. (A) Restriction map of H2β 2.57. 5′ UTS, 5′ untranslated sequence; 3′ UTS, 3′ untranslated sequence. The boldface region indicates the open reading frame. (B) Nucleotide sequence of the coding strand of H2β 2.57. The predicted amino acid sequence of the largest open reading frame is shown below the nucleotide sequence. The Met start codon (ATG) is at nucleotide position 97, while the termination codon (TGA) is at nucleotide position 742.

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                    10         20         30         40         50         60
Human       MSSSEEVSWISWFCGLRGNEFFCEVDEDYIQDKFNLTGLNEQVPHYRQALDMILDLEPDE
Bovine           S.....
Drosophila  .........VT.....................................N.............ED

                    70         80         90        100        110        120
Human       ELEDNPNQSDLIEQAAEMLYGLIHARYILTNRGIAQMLEKYQQGDFGYCPRVYCENQPML
Bovine      ....................................................................
Drosophila  ......L...MT.........................I....T....H.......S....

                   130        140        150        160        170        180
Human       PIGLSDIPGEAMVKLYCPKCMDVYTPKSSRHHHTDGAYFGTGFPHMLFMVHPEYRPKRPA
Bovine      ....................................................................
Drosophila  .L............T.....I.....................................T

                   190        200        210
Human       NQFVPRLYGFKIHAMAYQLQLQAASNFKSPVKTIR
Bovine      .............P...................
Drosophila  .............SL...I.....A...M.LRAKN
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FIGURE 2: Comparison of the amino acid sequences of the β subunit of casein kinase II from human, bovine, and *Drosophila*. The predicted amino acid sequence of human casein kinase II β subunit was aligned with the published partial amino acid sequence of bovine lung casein kinase II β subunit (Takio et al., 1987) and with the predicted amino acid sequence for *Drosophila* casein kinase II β subunit published by Saxena et al. (1987). A possible autophosphorylation site in the human sequence is indicated by underline (see text).

amino acid differences between our predicted amino acid sequence and the bovine amino acid sequence when the two sequences are aligned in a gap-free manner. The amino acid change from P (bovine) to A (human) at the carboxy-terminal end of the β subunit is a conservative one; overall, the sequences are 99% identical. The human predicted amino acid sequence is 88% identical with the *Drosophila* sequence, but 19 of the 26 amino acid changes are conservative, indicating a high degree of conservation for this polypeptide.

RNA analyses of HepG2 cell total RNA performed at high stringency utilizing various 5′ and 3′ fragments of H2β 2.57 cDNA as probes demonstrated three hybridizing species with the relative intensities 1.2 kb ≫ 3.0 kb = 4.2 kb as shown in Figure 3. When cDNA fragments containing the terminal 1.15-kb 3′ untranslated sequence (lanes 4 and 5) were utilized as probes, bands with the identical sizes described above were

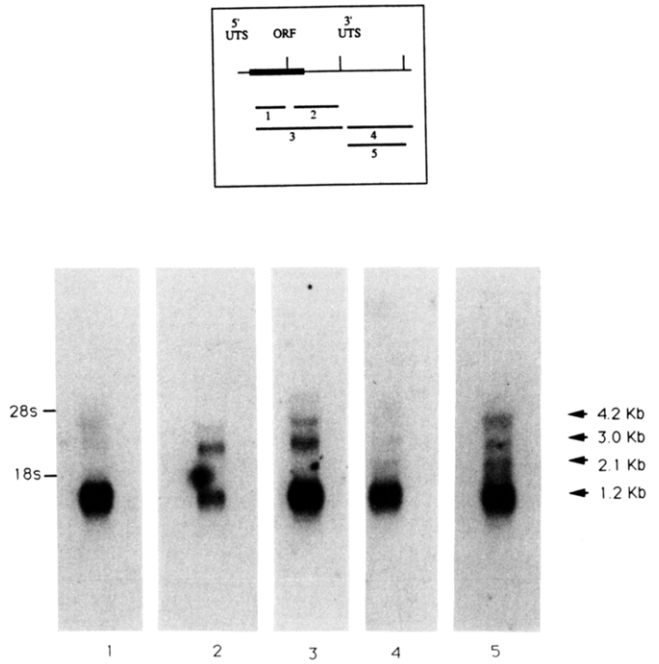

FIGURE 3: RNA analysis of human RNA. Twenty micrograms of total RNA prepared from HepG2 cultured cells was electrophoresed in formaldehyde agarose gels, transferred to nitrocellulose, and hybridized at 42 °C for 24 h in a solution containing the different probes and 50% formamide as fully stated under Materials and Methods. Two washes in 2× SSC at room temperature were followed by a single wash at 60 °C in 0.1× SSC and 0.1% SDS for 45 min. Blots were autoradiographed for 36 h. Lanes represent identical nitrocellulose strips incubated with different fragments of the H2β 2.57 human β subunit cDNA. Lanes 1–3 utilize probes that contain either only open reading frame sequence or open reading frame sequence and some 3′ untranslated sequence. Lanes 4 and 5 utilize probes that contain only 3′ untranslated sequence (see insert). The 28S and 18S ribosomal RNA molecular weight markers are indicated. (Lane 1) 520-bp *Eco*RI–*Pvu*II fragment derived from the 1.25-kb cDNA fragment; (lane 2) 720-bp *Pvu*II–*Eco*RI fragment derived from the 1.25-kb cDNA fragment; (lane 3) 1.25-kb *Eco*RI–*Eco*RI fragment; (lane 4) 1.15-kb *Eco*RI–*Eco*RI fragment [poly(A) stretch intact]; (lane 5) 1.15-kb *Eco*RI–*Sty*I fragment [poly(A) stretch removed].

observed as well as a fourth band migrating at 2.1 kb. Since this 2.1-kb species is absent when all three 5′ probes containing ORF[1] were utilized but is present with 3′ probes, it probably represents hybridization of some portion of the 3′ probe to sequences in another mRNA. Removal of the poly(A) stretch from the 1.15-kb cDNA fragment containing 3′ untranslated sequence by restriction endonuclease digestion with *Sty*I did not alter the pattern or intensity of hybridization of the bands (Figure 3, lanes 4 and 5). Hence, probes derived from both the 5′ and 3′ terminal regions of H2β 2.57 hybridize with similar intensities to an abundant mRNA migrating at 1.2 kb.

DNA analysis of HepG2 genomic DNA demonstrated the presence of two to four major hybridizing bands with restriction enzymes that do not cut the HepG2 β cDNA internally (Figure 4, lanes 1–4), while restriction enzymes that cut once within the cDNA demonstrated the presence of one to four bands (Figure 4, lanes 5–8). Enzymes that cut within the cDNA multiple times showed hybridization of probe to two to four bands (lanes 9 and 10). These results are consistent with Southern results obtained for the *Drosophila* β subunit (Saxena et al., 1987) and suggest the presence of a single copy of the gene for the HepG2 β subunit, although more direct evidence is required to document this conclusion.

[1] Abbreviations: ORF, open reading frame; UTS, untranslated sequence.

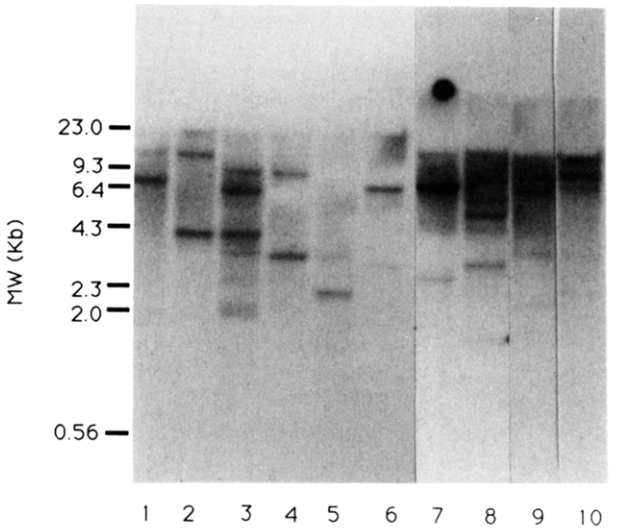

FIGURE 4: DNA analysis of HepG2 genomic DNA. High molecular weight DNA was prepared by the method of Davis et al. (1986), digested with restriction endonucleases, and electrophoresed as stated under Materials and Methods. Hybridization was for 24 h at 42 °C in a solution containing 30% formamide and the 1.25-kb *Eco*RI–*Eco*RI cDNA fragment of H2β 2.57. Two washes in 2× SSC at room temperature were followed by a single wash at 57 °C in 0.1× SSC and 0.1% SDS for 30 min. Blots were autoradiographed for 18 h. The number in parentheses following the name of each restriction enzyme denotes the number of times that enzyme cuts within the cDNA. (Lane 1) *Hin*dIII digest (0); (lane 2) *Bgl*II digest (0); (lane 3) *Acc*I digest (0); (lane 4) *Sac*I digest (0); (lane 5) *Pvu*II digest (1); (lane 6) *Bam*HI digest (1); (lane 7) *Hin*dII digest (1); (lane 8) *Nco*I digest (1); (lane 9) *Eco*RI digest (2); (lane 10) *Ava*I digest (5).

Discussion

We have described the cloning and sequencing of a cDNA that encodes the β subunit of human casein kinase II. Our sequencing results predict the β subunit of the casein kinase II enzyme present in human hepatoma cells to be a polypeptide of 215 amino acids with a M_r of 24 900. This M_r is in agreement with the reported M_r for the β subunit of casein kinase II isolated from bovine lung (M_r = 24 200), and our predicted amino acid sequence shows 99% identity with the published bovine amino acid sequence and 96.7% homology with the predicted amino acid sequence of the *Drosophila* β subunit if conservative amino acid changes are taken into account.

The Northern results shown in Figure 3 demonstrate that the most abundant mRNA species migrating at 1.2 kb hybridizes to a similar degree with probes that were generated from both 5′ and 3′ portions of H2β 2.57. It is interesting that the intensity of the 1.2 kb mRNA appears reduced when the 0.76-kb *Pvu*II–*Eco*RI cDNA fragment is utilized as probe (Figure 3, lane 2). This fragment contains the portion of the open reading frame coding for the 17 carboxy-terminal amino acids of the β subunit, the termination codon, and 688 nucleotides of 3′ untranslated sequence including the first poly(A) consensus signal sequence and poly(A) stretch. These data suggest that a transcriptional processing event may occur in HepG2 cells, which results in a deletion of a portion of the 3′ untranslated sequence flanking the termination codon.

It is noteworthy that two cDNA clones that were isolated for the *Drosophila* β subunit (Saxena et al., 1987), Dm98 and Dm107, each contained a poly(A) consensus signal sequence that was followed downstream by a poly(A) stretch. In Dm98, the variant signal sequence AATATA (Tosi et al., 1981) was located 26 nucleotides upstream of a poly(A) stretch and 294 nucleotides downstream of the TAA termination codon, while Dm107 contained two poly(A) signal sequences. The more

3′ of these signals was located at the expected distance upstream of a poly(A) stretch and was 720 nucleotides downstream of the TAA termination codon. Although the paper describing the *Drosophila* sequence did not include RNA analysis and did not report the existence of clones containing both poly(A) stretches preceded by signal sequences, it is possible that a clone similar to H2β 2.57 exists in the *Drosophila* cDNA library and that a similar transcriptional processing event occurs with *Drosophila* mRNAs.

Multiple polyadenylation sites have also been described for other cellular genes. The murine α amylase (Amy-1^A) gene is a single copy gene containing two poly(A) stretches preceded by the poly(A) signal sequences AATAAA and AATATA (Tosi et al., 1981). RNA analysis of mouse salivary gland and liver mRNAs showed the existence of a major mRNA species and a 20-fold less abundant minor mRNA species that accumulate in both tissues at similar ratios. Results from genomic sequencing and S1 nuclease mapping demonstrated transcriptional readthrough can occur past a primary polyadenylation site to a secondary site and that Amy-1^A utilizes alternative polyadenylation sites. In addition, the murine μ immunoglobulin heavy chain gene contains two polyadenylation sites which have been hypothesized to be involved in regulating synthesis of the membrane-bound and secreted immunoglobulin isoforms (Rogers et al., 1980). One possibility is that the 3′ untranslated sequence of the HepG2 and *Drosophila* casein kinase II β subunits containing the two polyadenylation sites is involved in regulating the production of the different subcellular forms of the enzyme.

It was previously shown that antiserum raised against casein kinase II prepared from calf thymus cross-reacts with both the α and β subunits of *Drosophila* casein kinase II (Dahmus et al., 1984). Conversely, antiserum generated against *Drosophila* casein kinase II cross-reacts with the α and β subunits of calf thymus enzyme, and casein kinase II from these two species demonstrated almost identical structural and functional characteristics (Dahmus et al., 1984). Antibodies raised against the *Drosophila* β subunit cross-react with a 41-kDa polypeptide from yeast that exhibits autophosphorylation and copurifies with the casein kinase II activity (Padmanabha & Glover, 1987). In addition, a comparison of the predicted amino acid sequences of cloned human, rat, and *Drosophila* α subunit cDNAs shows a 98.5% identity between human and rat and a 90% identity between human and *Drosophila* (Meisner et al., 1989). Hence, the results from these studies as well as the present work indicate that both subunits of casein kinase II have been highly conserved in evolution.

Interestingly, a casein kinase II activity has been purified from *Dictyostelium discoideum* (Renart et al., 1984) which displays functional properties identical with those described for casein kinase II isolated from other sources (e.g., activation by cations, inhibition by anions, utilization of ATP and GTP as phosphate donor, and phosphorylation of serine and threonine residues in acidic substrates). Unlike casein kinase II described from other eukaryotic sources, the enzyme consisted of a single polypeptide of M_r = 38 000 that was autophosphorylated. It is unknown, however, how the amino acid sequence of this autophosphorylation site compares with the sequence of the autophosphorylation site in β subunits of other previously described casein kinase II enzymes. It is possible that the β subunit, with its capacity for autophosphorylation, arose because more complex regulation of casein kinase II is required in higher order eukaryotes.

A possible autophosphorylation site that is conserved between the human and *Drosophila* enzyme is indicated by underline in Figure 2. This sequence, S-E-E, is a prototype casein kinase II recognition site that has been identified in the protein substrates α and β casein (Pinna et al., 1979; Hathaway & Traugh, 1983). The serine at the amino terminus of this sequence has also been underlined as studies utilizing synthetic peptides as substrates for casein kinase II (Meggio et al., 1984; Pinna et al., 1984; Kuenzel & Krebs, 1985; Marin et al., 1986; Kuenzel et al., 1987 have demonstrated the general sequence S(T)-X-X-E to be a requirement for peptide phosphorylation. In vivo phosphorylation experiments followed by peptide mapping/HPLC should provide conclusive evidence as to whether this site is autophosphorylated in vivo.

It has been concluded from renaturation studies that the presence of both the α and β subunits in a 1:1 stoichiometry is required for optimal casein kinase II activity (Cochet & Chambaz, 1983). Thus, it is possible that the autophosphorylation of the β subunit plays a role in regulating this activity. Previous studies attempting to correlate phosphorylation states of casein kinase II with alterations in the activity of the native enzyme suggest that intramolecular autophosphorylation has no apparent effect on activity (Agostinis et al., 1987). Phosphorylation by other kinases, e.g., casein kinase I (Ahmed & Goueli, 1988), or autophosphorylation on sites other than the in vitro autophosphorylation site results in increases in casein kinase II activity (Agostinis et al., 1987). Information regarding the role of autophosphorylation of the β subunit in the regulation of casein kinase II activity and the identification of the in vivo autophosphorylation site in the β subunit are critical to our understanding of the physiological role of this enzyme. In addition, the recent observations that casein kinase II activity is rapidly increased in response to insulin (Sommercorn et al., 1987; Klarlund & Czech, 1988) raise further important physiological questions regarding the role of casein kinase II in an insulin-mediated signal transduction pathway. The availability of cDNA clones encoding both the α and β subunits of human casein kinase II should facilitate experiments designed to address these questions.

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Phenylalanine-to-Tyrosine Singlet Energy Transfer in the Archaebacterial Histone-like Protein HTa†

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Appendix: Analysis of Energy Transfer and Fluorescence Lifetime Data

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ABSTRACT: The Archaebacterium *Thermoplasma acidophilum* has a histone-like protein (HTa) abundantly associated with its deoxyribonucleic acid. Each native tetrameric complex of HTa contains 20 phenylalanine residues, 4 tyrosine residues, and no tryptophan. When the protein was excited by radiation at 252 nm, which is a wavelength absorbed predominantly by phenylalanine, the fluorescent emission was mostly from tyrosine. According to the excitation spectrum for this tyrosine fluorescence, the cause was energy transfer from phenylalanine, which occurred with about 50% efficiency. When the tyrosine residues were removed enzymatically, the exicted-state lifetime of the phenylalanine residues nearly doubled. Because of energy transfer, the tyrosine emission had two apparent fluorescence decay lifetimes; one lifetime (3.9 ns) was that of tyrosine while the second (12.1 ns) corresponded to the excited state of phenylalanine.

The histone-like protein HTa[1] is abundantly associated with the DNA of the Archaebacterium *Thermoplasma acidophilum* (Searcy, 1975). It functions to protect DNA from thermal denaturation (Stein & Searcy, 1978). In contrast to other known bacterial histone-like proteins (Pettijohn, 1988), HTa remains stably bound to DNA in salt concentrations up to 10-fold higher than is physiological (Searcy, 1976). Thus, the nucleoprotein has been easily isolated and studied (Searcy & Stein, 1980).

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[1] Abbreviations: HTa, the histone-like protein of *Thermoplasma acidophilum*; SDS, sodium dodecyl sulfate.

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