Isolation and Characterization of Human cDNA Clones Encoding the α and the α' Subunits of Casein Kinase II[†,‡]

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ABSTRACT: Casein kinase II is a widely distributed protein serine/threonine kinase. The holoenzyme appears to be a tetramer, containing two α or α' subunits (or one of each) and two β subunits. Complementary DNA clones encoding the subunits of casein kinase II were isolated from a human T-cell λgt10 library using cDNA clones isolated from *Drosophila melanogaster* [Saxena et al. (1987) *Mol. Cell. Biol. 7*, 3409-3417]. One of the human cDNA clones (hT4.1) was 2.2 kb long, including a coding region of 1176 bp preceded by 156 bp (5′ untranslated region) and followed by 871 bp (3′ untranslated region). The hT4.1 clone was nearly identical in size and sequence with a cDNA clone from HepG2 human hepatoma cultured cells [Meisner et al. (1989) *Biochemistry 28*, 4072-4076]. Another of the human T-cell cDNA clones (hT9.1) was 1.8 kb long, containing a coding region of 1053 bp preceded by 171 bp (5′ untranslated region) and followed by 550 bp (3′ untranslated region). Amino acid sequences deduced from these two cDNA clones were about 85% identical. Most of the difference between the two encoded polypeptides was in the carboxy-terminal region, but heterogeneity was distributed throughout the molecules. Partial amino acid sequence was determined in a mixture of α and α' subunits from bovine lung casein kinase II. The bovine sequences aligned with the 2 human cDNA-encoded polypeptides with only 2 discrepancies out of 535 amino acid positions. This confirmed that the two human T-cell cDNA clones encoded the α and α' subunits of casein kinase II. Microsequence data determined from separated preparations of bovine casein kinase II α subunit and α' subunit [Litchfield et al. (1990) *J. Biol. Chem. 265*, 7638-7644] confirmed that hT4.1 encoded the α subunit and hT9.1 encoded the α' subunit. These studies show that there are two distinct catalytic subunits for casein kinase II (α and α') and that the sequence of these subunits is largely conserved between the bovine and the human.

Casein kinase II is a protein kinase that catalyzes the phosphorylation of serine or threonine residues in proteins (i.e., a protein serine/threonine kinase). The enzyme appears to be present in all eukaryotic cells [see reviews by Hathaway and Traugh (1982), Krebs et al. (1988), and Tuazon and Traugh (1990)], implying that it has fundamental cellular functions. Casein kinase II is referred to as a messenger-independent protein kinase because its activity is not affected by factors known to affect the activity of some other protein kinases in the cell (e.g., cyclic nucleotides, Ca^{2+}, or diacylglycerols). The activity of casein kinase II is affected in vitro by a number of substances, including heparin (Hathaway & Traugh, 1982; Meggio et al., 1982), various polyamine compounds (Hara et al., 1981; Cochet & Chambaz, 1983a; Hathaway & Traugh, 1984a; Mamrack, 1989), 2,3-diphosphoglyceric acid (Hathaway & Traugh, 1984b; Gonzatti & Traugh, 1988), certain nucleic acids (Gatica et al., 1989), and a specific protein (Murao et al., 1989). The physiological significance of these effectors is unknown, but there has been speculation that at least some of them may help regulate the activity of casein kinase II in vivo (Hathaway & Traugh, 1984b; Gonzatti & Traugh, 1988).

There is a large list of proteins that can be phosphorylated by casein kinase II in vitro, and a growing number of these appear to be substrates in vivo [see Hathaway and Traugh (1982), Sommercorn and Krebs (1987), Krebs et al. (1988), and Tuazon and Traugh (1990)]. Some of the latter include acetyl-CoA carboxylase (Witters et al., 1983, 1988; Haystead et al., 1988), glycogen synthase (Picton et al., 1982; DePaoli-Roach et al., 1983), c-*myc* protein (Lüscher et al., 1988), the E7 protein of human papillovirus type 16 (Firzlaff et al., 1989), the large T-antigen protein of SV40 (Grasser et al., 1988), the 90-kDa heat shock proteins (Lees-Miller & Anderson, 1989), nucleolin (Caizergues-Ferrer et al., 1987; Schneider et al., 1988), and deoxyribonucleic acid (DNA)[1] topoisomerase II (Ackerman et al., 1985, 1988). The putative cellular substrates listed above plus the many that are not listed include proteins involved in DNA replication, transcription,

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[1] Abbreviations: ATP, adenosine 5′-triphosphate; bp, nucleotide bases; cDNA, complementary deoxyribonucleic acid; CNBr, cyanogen bromide; dATP, deoxyadenosine 5′-triphosphate; dITP, deoxyinosine 5′-triphosphate; DEAE, diethylaminoethyl; DME, Dulbecco-Vogt-modified Eagle's; DNA, deoxyribonucleic acid; DNase, deoxyribonuclease; dpm, disintegrations per minute; FSBA, [*p*-(fluorosulfonyl)benzoyl]adenosine; h, hours; kb, nucleotide kilobases; LB, Luria-Bertani medium; min, minutes; mRNA, messenger ribonucleic acid; pfu, plaque forming unit(s); RNA, ribonucleic acid; RNase, ribonuclease; SDS, sodium dodecyl sulfate; TPCK, L-1-(tosylamino)-2-phenylethyl chloromethyl ketone.

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translation, and the acute regulation of metabolism. This is especially interesting since the activity of casein kinase II is increased in extracts from cells treated with insulin or growth factors (Sommercorn et al., 1987; Klarlund & Czech, 1988; Carroll & Marshak, 1989; Ackerman & Osheroff, 1989), suggesting that casein kinase II is a regulatory component of signal transduction pathways (Krebs et al., 1988).

Purified casein kinase II is generally a tetrameric holoenzyme composed of two α subunits and two β subunits [$\alpha_2\beta_2$; see Edelman et al. (1987) and Tuazon and Traugh (1990) for reviews]. At low salt concentrations, casein kinase II aggregates into much larger, oligomeric, filamentous structures (Glover, 1986; Mamrack, 1989). The β subunit has a molecular weight of 24000–28000 (Edelman et al., 1987; Tuazon & Traugh, 1990). The function of the β subunit is unknown, although it is presumed to a have a regulatory role in the holoenzyme (Takio et al., 1987; Tuazon & Traugh, 1990). Its amino acid sequence has been determined from bovine lung casein kinase II (Takio et al., 1987) and deduced from cDNA clones isolated from *Drosophila* (Saxena et al., 1987), HeLa cultured cells (Jakobi et al., 1989), and HepG2 cultured cells (Heller-Harrison et al., 1989). The amino acid sequence of the β subunit has also been deduced from a human T-cell cDNA clone and is identical with the sequence deduced from the HeLa or HepG2 cDNA clones.[2]

The α subunit has been identified as the catalytic subunit based upon its ability to bind FSBA or 8-azido-ATP, its sequence identity with other protein kinases, and its persistent (but diminished) catalytic activity in the absence of any β subunit (Hathaway et al., 1981; Cochet & Chambaz, 1983b; Feige et al., 1983; Pyerin et al., 1987; Takio et al., 1987; Saxena et al., 1987; Chen-Wu et al., 1988; Meisner et al., 1989; Tuazon & Traugh, 1990). Often, more than one discrete α-like subunit is present in purified casein kinase II; the α subunit has a molecular weight of 40000–44000, and the smaller α' subunit has a molecular weight of 37000–41000 (Edelman et al., 1987; Tuazon & Traugh, 1990). In these cases, the tetrameric holoenzyme structure could be exclusively $\alpha\alpha'\beta_2$ or could be a mixture of two or three possible tetrameric forms: $\alpha_2\beta_2$, $\alpha'_2\beta_2$, and $\alpha\alpha'\beta_2$. Some evidence has suggested that the α subunits are related by posttranslational modification, such as by limited proteolysis of the α subunit to generate the smaller α' subunit (DePaoli-Roach et al., 1981; Hathaway & Traugh, 1982; Hathaway et al., 1983; Meggio & Pinna, 1984; Zandomeni et al., 1988). Other evidence has suggested that α and α' subunits are products of different genes (or alternatively spliced transcripts of the same gene). In yeast *Saccharomyces cerevisiae* and in the bovine, there is immunological and sequence evidence that α and α' subunits are distinct polypeptides (Dahmus et al., 1984; Padmanabha & Glover, 1987; Takio et al., 1987; Litchfield et al., 1990). Other casein kinase II preparations contain only one type of α subunit (i.e., $\alpha_2\beta_2$), with molecular weight values of the α subunit varying from 36000 to 42000 (Tuazon & Traugh, 1990). There are even some purified preparations that have many enzymatic characteristics of casein kinase II that lack a β subunit (Tuazon & Traugh, 1990).

Despite having been identified and studied for 15–20 years, many of the molecular properties and cellular functions of casein kinase II remain obscure. In an effort to discover more about the properties and functions of mammalian casein kinase II, we isolated cDNA clones that encode each of the subunits. This report describes the isolation of two related human T-cell cDNA clones that encode the α and α' subunits of casein kinase II.

[2] F. J. Lozeman and E. G. Krebs, unpublished results.

Experimental Procedures

Materials. General chemicals and reagents were of analytical grade, obtained from commercial suppliers. T4 DNA ligase and the reagent kit for random primer labeling of DNA were from Boehringer Mannheim. Agarose, 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-gal), isopropyl 1-thio-β-D-galactopyranoside (IPTG), nucleic acid molecular size standards, and restriction endonucleases were from Gibco/BRL or New England Biolabs, Inc. Membranes for blotting or intercepting nucleic acid samples (i.e., Nytran, nitrocellulose, and NA-45 DEAE) were from Schleicher and Schuell. Oligo(dT)–cellulose type 7, cesium trifluoroacetate, and Sephadex G-50 Nick columns were from Pharmacia LKB. [α-^{32}P]dATP (3000 Ci/mmol) and [α-^{35}S]dATP (1000 Ci/mmol) were from New England Nuclear. Sequenase enzyme and reagents were obtained from United States Biochemical Corp. M13mp18 and M13mp19 vectors were from Bio-Rad. The plasmid vector pBluescript II SK+ was from Stratagene. Guanidine isothiocyanate was from International Biotechnologies, Inc. Other reagents used for recombinant DNA work that are not listed were molecular biology (or equivalent) grade. The *Drosophila* cDNA clone encoding the α subunit of casein kinase II (λgt11/dm92; Saxena et al., 1987) was provided by Dr. C. V. C. Glover, University of Georgia. The dm92 insert was subcloned into the *Eco*RI site in pBluescript II SK+ for subsequent manipulations. Oligonucleotides were synthesized with an Applied Biosystems 380A DNA synthesizer (Chemical Synthesis Facility, Howard Hughes Medical Institute, University of Washington).

Cultured cell lines were from the American Type Cell Collection. The A431 human carcinoma cells were maintained in monolayer cultures in plastic 150-mm culture dishes (Corning) in DME medium containing high (25 mM) glucose and 10% fetal calf serum. HSB-2 lymphoblasts were propagated to plateau density in Eagle's minimal essential medium with 10% fetal calf serum. Tissues were frozen immediately after collection and stored at −70 °C. Human tissues were obtained from an individual subject immediately after surgery (spleen) or delivery (full-term placenta). Mouse tissues were from male C57 Black 6 hybrid mice.

The composition of 20× SSC, 20× SSPE, and 50× Denhardt's solutions and the deionization and storage of formamide are described in Maniatis et al. (1982). The solution for prehybridization of nitrocellulose membrane plate lifts and genomic Southern blots consisted of 25% formamide, 5× SSPE, 5× Denhardt's solution, 0.1% SDS, and 100 μg/mL denatured herring sperm DNA. The hybridization solution was the same as the prehybridization solution except for the inclusion of ^{32}P-labeled probe. The Northern blot prehybridization solution was composed of 50% formamide, 5× SSPE, 5× Denhardt's solution, 0.1% SDS, and 100 μg/mL denatured herring sperm DNA. The Northern blot hybridization solution consisted of 50% formamide, 5× SSC, 1.5× Denhardt's solution, 0.1% SDS, 100 μg/mL denatured herring sperm DNA and ^{32}P-labeled probe.

Library Screening for α Subunits of Casein Kinase II. The cDNA clones encoding the α subunits of casein kinase II were isolated from a human leukemic T-cell library constructed in λgt10 (Littman et al., 1987). The procedure used was essentialy as in Maniatis et al. (1982). C600 Hfl *Escherichia coli* were infected with approximately 5×10^5 pfu of the cDNA library and plated onto 150-mm-diameter LB + 0.2% maltose agarose plates (25000 plaques/plate). After incu-

bation, duplicate lifts of each plate were made onto nitrocellulose membranes. The membranes were treated briefly to denature and then neutralize the transferred material, then rinsed (3× SSC) and baked at 80 °C for 2 h. The membranes were prehybridized for 2–4 h at 42 °C and then hybridized at the same temperature for 12–16 h with 1–10 ng/mL *Ssp*I fragment of the cDNA clone encoding the α subunit of *Drosophila* casein kinase II (dm92; Saxena et al., 1987). The *Ssp*I fragment was 612 bp in length, encompassing most of the coding region of the *Drosophila* cDNA clone. The *Ssp*I fragment was isolated by agarose gel electrophoresis and band interception onto an NA-45 DEAE membrane (intercepted on and eluted from the membrane according to the manufacturer's suggestions) and labeled with [α-^{32}P]dATP to a specific activity of 10^8–10^9 dpm/μg using the random primer method (Feinberg & Vogelstein, 1983, 1984). Following hybridization, the membranes were washed 3 times in 2× SSC–0.1% SDS at room temperature for 15 min each time and then 1 h in 1× SSC–0.1% SDS at 45 °C. Membranes were exposed to Kodak X-Omat AR film with an intensifying screen at −70 °C as required (as long as 72 h). Positive clones were plaque-purified by three additional rounds of screening (Maniatis et al., 1982) using the conditions described above.

Subcloning and Sequencing of the cDNAs. The procedures used to prepare, analyze, and subclone the λ bacteriophage clones are described by Maniatis et al. (1982, 1989). Briefly, λ bacteriophage DNA of the chosen positive clones was isolated from plate lysates, incubation with DNase I and RNase, precipitation with NaCl and poly(ethylene glycol) (PEG 8000), several rounds of extraction with phenol and chloroform, and ethanol precipitation. The cDNA inserts were analyzed by agarose gel electrophoresis after digestion of the crude preparation of λ bacteriophage DNA with *Eco*RI. Various restriction endonuclease fragments of each of the chosen clones were isolated on and eluted from NA-45 membranes (see above) and then ligated into M13 or pBluescript II SK$^+$ vectors. Aliquots of the ligation reaction mixtures were used to transform *E. coli* Jm109. Both single-stranded DNA, generated from subclones in M13 vectors, and double-stranded DNA from subclones in SK+ vector were used as templates for dideoxynucleotide sequencing (Sanger et al., 1977), according to the instructions provided by the supplier of the Sequenase enzyme and reagents. Oligonucleotides used as primers in the sequencing reactions either were commercially available or were synthesized from a previously determined sequence (see Materials). There were several regions which were difficult to sequence using the standard protocols; these problems were characterized by pauses and compression in the observed sequence. When pauses were encountered, sequencing was performed only with single-stranded DNA template with higher incubation temperatures for the labeling (37 °C) and termination (50 °C) portions of the reactions. When compression was encountered, the labeling reactions were performed with dITP as suggested by the supplier of the Sequenase enzyme and reagents.

The compilation of sequence data was done with Pustell sequence analysis software (International Biotechnologies, Inc.). Comparisons of nucleotide or amino acid sequence to Genbank (Release 61.0) or Protein Identification Resource (Release 21.0) data banks were accomplished with the Intelligenetics Suite of programs (Intelligenetics, Inc., Mountain View, CA) run on a VAX/VMS computer system. Comparisons were made with the FASTDB program.

Genomic Southern Blot Analysis. Human genomic DNA was isolated from venous blood as described by Herrmann and Frischauf (1987). The genomic DNA was digested with restriction endonucleases, electrophoresed on a 1% agarose gel, and transferred to a nitrocellulose membrane (Maniatis et al., 1982). After baking for 2 h at 80 °C, the membrane was prehybridized and hybridized as described above, using specific restriction endonuclease fragments of the human T-cell cDNA clones (see above for the procedures to generate the recombinant plasmids, isolate, and label the fragment). The washing of the membrane was performed as described above, with an extra washing step in 0.2× SSC–0.1% SDS for 30 min at 55 °C.

Northern Blot Analysis. Total cellular RNA was extracted from frozen tissue samples or cultured cell samples as described by Okayama et al. (1987). The pieces of frozen tissue samples were disrupted in guanidine isothiocyanate solution with a Polytron homogenizer (Kinetimatica GmbH). A431 cells were harvested in phosphate-buffered saline from confluent monolayers, and HSB-2 lymphoblasts were harvested from suspension culture. Cells were collected by centrifugation at 500*g* for 10 min. Cell pellets were washed twice in several volumes of phosphate-buffered saline by resuspension and centrifugation (as above) before lysis and denaturation in guanidine isothiocyanate. Poly(A)-rich RNA was prepared from the total RNA samples using oligo(dT)–cellulose (Pharmacia) columns according to the manufacturer's suggestions. RNA samples were electrophoresed on a 1% agarose–0.66 M formaldehyde gel and then transferred to Nytran membrane (Fourney et al., 1988). Membranes were baked at 80 °C and then placed in Northern blot prehybridization solution for 2–4 h at 37 °C. Hybridization was performed for 12–16 h at 37 °C in Northern blot hybridization solution using the specific restriction endonuclease fragments of the human T-cell cDNA clones as probes (see above for the labeling procedure). Hybridized Nytran membranes were washed twice in 6× SSC–0.1% SDS at room temperature for 15 min each, then twice in 1× SSC–0.5% SDS at 37 °C for 15 min each, and finally in 0.2× SSC–0.5% SDS at 48–55 °C for 15 min.

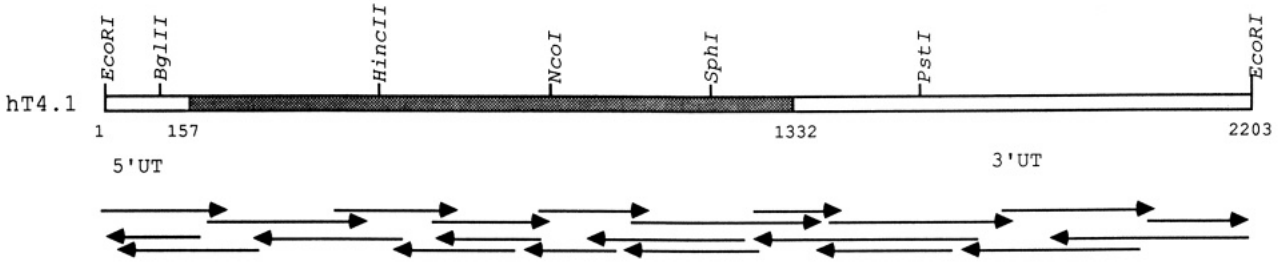
Sequencing of Peptides from Bovine Lung Casein Kinase II α Subunits. The purification of casein kinase II from bovine lung and the subsequent isolation of α subunit peptides is described by Takio et al. (1987). Peptides were generated by cleavage of the α subunits at methionine or lysine residues with cyanogen bromide or *Achromobacter* protease I. Large fragments were subdigested with TPCK-treated trypsin, *Staphylococcus aureus* V8 protease, or chymotrypsin. Amino-terminal amino acid sequence analyses were performed by automated Edman degradation as described by Takio et al. (1987).

Results

Identification and Sequence of cDNA Clones Encoding Casein Kinase II α Subunits. Upon screening of the human T-cell cDNA library with the fragment of the *Drosophila* α subunit cDNA (i.e., dm92), five positive clones were identified. These clones all remained positive through plaque purification. λ bacteriophage DNA was prepared from each of the positive clones. One of the clones had an *Eco*RI insert which was only about 0.35 kb in length. Two of the clones had *Eco*RI inserts of about 2.2 kb; the insert of one of these clones (hT 4.1) was subcloned and sequenced. A schematic of the sequencing, the nucleotide sequence, and the deduced amino acid sequence of the clone are presented in Figure 1.

Clone hT4.1 contained an open reading frame of 1176 bp, initiated at an ATG codon starting at nucleotide 157 and terminated at a TGA codon ending at nucleotide 1332. The open reading frame of the cDNA was preceded by 156 bp,

A



B

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   1  gaattcggAGGGGAGAGCGGCCGCCGCCGCTGCCGCTTCCACCACAGTTTGAAGAAAACAGGTCTGAAACAAGGTCTTACCCCCAGCTGCTTCTGAACACAGTGACTGCC
                7                    CTG
 111  AGATCTCCAAACATCAAGTCCAGCTTTGTCCGCCAACCTGTCTGACATGTCGGGACCCGTGCCAAGCAGGGCCAGAGTTTACACAGATGTTAATACACACAGACCTCGAG
   1                                                  M  S  G  P  V  P  S  R  A  R  V  Y  T  D  V  N  T  H  R  P  R  E

 221  AATACTGGGATTACGAGTCACATGTGGTGGAATGGGGAAATCAAGATGACTACCAGCTGGTTCGAAAATTAGGCCGAGGTAAATACAGTGAAGTATTTGAAGCCATCAAC
  23    Y  W  D  Y  E  S  H  V  V  E  W  G  N  Q  D  D  Y  Q  L  V  R  K  L  G  R  G  K  Y  S  E  V  F  E  A  I  N

 331  ATCACAAATAATGAAAAAGTTGTTGTTAAAATTCTCAAGCCAGTAAAAAAGAAGAAAATTAAGCGTGAAATAAAGATTTTGGAGAATTTGAGAGGAGGTCCCAACATCAT
  59  I  T  N  N  E  K  V  V  V  K  I  L  K  P  V  K  K  K  K  I  K  R  E  I  K  I  L  E  N  L  R  G  G  P  N  I  I

 441  CACACTGGCAGACATTGTAAAAGACCCTGTGTCACGAACCCCCGCCTTGGTTTTTGAACACGTAAACAACACAGACTTCAAGCAATTGTACCAGACGTTAACAGACTATG
  96    T  L  A  D  I  V  K  D  P  V  S  R  T  P  A  L  V  F  E  H  V  N  N  T  D  F  K  Q  L  Y  Q  T  L  T  D  Y  D

 551  ATATTCGATTTTACATGTATGAGATTCTGAAGGCCCTGGATTATTGTCACAGCATGGGAATTATGCACAGAGATGTCAAGCCCCATAATGTCATGATTGATCATGAGCAC
  133   I  R  F  Y  M  Y  E  I  L  K  A  L  D  Y  C  H  S  M  G  I  M  H  R  D  V  K  P  H  N  V  M  I  D  H  E  H

 661  AGAAAGCTACGACTAATAGACTGGGGTTTGGCTGAGTTTTATCATCCTGGCCAAGAATATAATGTCCGAGTTGCTTCCCGATACTTCAAAGGTCCTGAGCTACTTGTAGA
  169  R  K  L  R  L  I  D  W  G  L  A  E  F  Y  H  P  G  Q  E  Y  N  V  R  V  A  S  R  Y  F  K  G  P  E  L  L  V  D

 771  CTATCAGATGTACGATTATAGTTTGGATATGTGGAGTTTGGGTTGTATGCTGGCAAGTATGATCTTTCGGAAGGAGCCATTTTTCCATGGACATGACAATTATGATCAGT
  206   Y  Q  M  Y  D  Y  S  L  D  M  W  S  L  G  C  M  L  A  S  M  I  F  R  K  E  P  F  F  H  G  H  D  N  Y  D  Q  L

 881  TGGTGAGGATAGCCAAGGTTCTGGGGACAGAAGATTTATATGACTATATTGACAAATACAACATTGAATTAGATCCACGTTTCAATGATATCTTGGGCAGACACTCTCGA
  243   V  R  I  A  K  V  L  G  T  E  D  L  Y  D  Y  I  D  K  Y  N  I  E  L  D  P  R  F  N  D  I  L  G  R  H  S  R

 991  AAGCGATGGGAACGCTTTGTCCACAGTGAAAATCAGCACCTTGTCAGCCCTGAGGCCTTGGATTTCCTGGACAAACTGCTGCGATATGACCACCAGTCACGGCTTACTGC
  279  K  R  W  E  R  F  V  H  S  E  N  Q  H  L  V  S  P  E  A  L  D  F  L  D  K  L  L  R  Y  D  H  Q  S  R  L  T  A

1101  AAGAGAGGCAATGGAGCACCCCTATTTCTACACTGTTGTGAAGGACCAGGCTCGAATGGGTTCATCTAGCATGCCAGGGGGCAGTACGCCCGTCAGCAGCGCCAATATGA
  316   R  E  A  M  E  H  P  Y  F  Y  T  V  V  K  D  Q  A  R  M  G  S  S  S  M  P  G  G  S  T  P  V  S  S  A  N  M  M

1211  TGTCAGGGATTTCTTCAGTGCCAACCCCTTCACCCCTTGGACCTCTGGCAGGCTCACCAGTGATTGCTGCTGCCAACCCCCTTGGGATGCCTGTTCCAGCTGCCGCTGGC
  353   S  G  I  S  S  V  P  T  P  S  P  L  G  P  L  A  G  S  P  V  I  A  A  A  N  P  L  G  M  P  V  P  A  A  A  G

1321  GCTCAGCAGTAACGGCCCTATCTGTCTCCTGATGCCTGAGCAGAGGTGGGGGAGTCCACCCTCTCCTTGATGCAGCTTGCGCCTGGCGGGGAGGGGTGAAACACTTCAGA
  389   A  Q  Q  Ter

1431  AGCACCGTGTCTGAACCGTTGCTTGTGGATTTATAGTAGTTCAGTCATAAAAAAAAAAATTATAATAGGCTGATTTTCTTTTTTCTTTTTTTTTTTAACTCGAACTTTTCA
                                                  A                                                  v

1541  TAACTCAGGGGATTCCCTGAAAAATTACCTGCAGGTGGAATATTTCATGGACAAATTTTTTTTTCTCCCCTCCCAAATTTAGTTCCTCATCACAAAAGAACAAAGATAAA

1651  CCAGCCTCAATCCCGGCTGCTGCATTTAGGTGGAGACTTCTTCCCATTCCCACCATTGTTCCTCCACCGTCCCACACTTTAGGGGGTTGGTATCTCGTGCTCTTCTCCAG

1761  AGATTACAAAAATGTAGCTTCTCAGGGGAGGCAGGAAGAAAGGAAGGAAGGAAAGAAGGAAGGGAGGACCCAATCTATAGGAGCAGTGGACTGCTTGCTGGTCGCTTACA

1871  TCACTTTACTCCATAAGCGCTTCAGTGGGGTTATCCTAGTGGCTCTTGTGGAAGTGTGTCTTAGTTACATCAAGATGTTGAAAATCTACCCAAAATGCAGACAGATACTA

1981  AAAACTTCTGTTCAGTAAGAATCATGTCTTACTGATCTAACCCTAAATCCAACTCATTTATACTTTTATTTTTAGTTCAGTTTAAAATGTTGATACCTTCCCTCCCAGGC
      v  v  v  v  v

2091  TCCTTACCTTGGTCTTTTCCCTGTTCATCTCCCAACATGCTGTGCTCCATAGCTGGTAGGAGAGGGAAGGCAAAATCTTTCTTAGTTTTCTTTGTCTTGGCCATTTTGAA
                                                                                                  A   |

2201  TTC
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FIGURE 1: Human casein kinase II α subunit: structure, sequencing strategy, nucleotide sequence, and amino acid sequence of the cDNA clone hT4.1. (A) Structure of the cDNA clone hT4.1 showing several restriction endonuclease sites and the 5′ untranslated region (5′UT), the coding region (the shaded area), and the 3′ untranslated region (3′UT). The arrows under the diagram represent the direction and amount of sequence data determined from individual sequencing reactions by the dideoxy chain termination method. (B) Nucleotide sequence of the hT4.1 cDNA clone is presented with the amino acid sequence deduced from the coding region. The amino acids are depicted by the single-letter abbreviation. The λgt10 cDNA library was constructed with *Eco*RI linkers (Littman et al., 1987). Lowercase letters at the 5′ end of the clone indicate an *Eco*RI linker. The 3′ end of the clone did not appear to have an *Eco*RI linker, so the *Eco*RI site is not in lowercase letters. "Ter" indicates the termination codon at the end of the coding region. The cDNA clone hT4.1 was nearly identical in size and sequence with a cDNA clone isolated from HepG2 cells (Meisner et al., 1989). Differences between the two sequences are indicated as follows: nucleotides that are different in the HepG2 sequence are given below the corresponding underlined nucleotide in the hT4.1 sequence; nucleotide bases that are above the (v) symbol are not in the HepG2 sequence; Â, additional A nucleotide in the HepG2 sequence; the underlined nucleotide above 7 corresponds to nucleotide 7 in the HepG2 sequence; the underlined nucleotide above (|) is the last nucleotide in the HepG2 sequence.

which contained no initiation codons. The open reading frame was followed by a 3′ untranslated end of 871 bp. Neither a polyadenylated tail nor a polyadenylation signal (AATAA; Proudfoot & Brownlee, 1976) was present in the 3′ untranslated end of the cDNA, and the *Eco*RI site at the 3′ end of the clone did not appear to be derived from the *Eco*RI linker used to construct the cDNA library (Figure 1). The lack of a polyadenylation signal, and the presence of an *Eco*RI site probably present in the mRNA, suggested that the cDNA insert was not a full-length representative of the mRNA. The size and nucleotide sequence of hT4.1 were virtually identical with a cDNA clone from a HepG2 library which was recently reported (Meisner et al., 1989). The minor discrepancies between the two clones were substitutions, insertions, or deletions of single nucleotides, present in the 5′ or 3′ noncoding regions of the clones (see Figure 1). These clones encoded an identical polypeptide which was undoubtedly the α subunit of casein kinase II. This conclusion could be made because

the partial amino acid sequence derived specifically from the α subunit (i.e., M_r 45 000) of bovine testis casein kinase II (Litchfield et al., 1990) was identical with the sequence encoded by the human hT4.1 or HepG2 cDNA clones (see Discussion).

The remaining two of the five isolated clones each had two *Eco*RI inserts of similar size, one insert being about 0.5 kb and the other 1.25–1.35 kb. The insert of one of the clones (hT4.2) was subcloned and partially sequenced. This clone was 1752 bp in length, with an open reading frame of 984 bp that was 74% identical with the nucleotide sequence of the corresponding region of the hT4.1 clone (data not shown). However, this clone appeared to be incomplete, since the open reading frame encoding the polypeptide similar to the α subunit was not preceded by a 5′ untranslated region or an initiation codon.

A 479 bp *Eco*RI fragment of the hT4.2 clone was used to screen an additional 8×10^5 individual plaques of the human T-cell cDNA library according to the same procedures used initially with the *Drosophila* cDNA probe (see Experimental Procedures). Seven positive clones were identified and isolated as a result of the screen with the fragment of hT4.2. Restriction endonuclease analysis of the λ bacteriophage DNA from each of these isolated clones indicated three of the seven were similar to clone hT4.2, but longer in the 5′ region of the clones. One of these clones (hT9.1) was subcloned in various pieces into M13 and pBluescript II SK$^+$ vectors and sequenced. A schematic of the sequencing, the nucleotide sequence, and the deduced amino acid sequence of clone hT9.1 are presented in Figure 2. The cDNA clone hT9.1 was 1774 bp in length with a 5′ untranslated region of 171 nucleotides. The ATG initiation codon starting at nucleotide 172 was the first in the clone, and the sequence immediately preceding the ATG corresponded to the consensus eukaryotic initiator sequence (CC^A_GCCAUG) as defined by Kozak (1984). The initiation codon was followed by an open reading frame of 1053 bp. The 3′ untranslated region contained a consensus polyadenylation signal, AATAAA (Proudfoot & Brownlee, 1976), which was 12 bp upstream from the poly(A) tail. It was not possible to sequence the poly(A) tail and surrounding sequence on both strands. The DNA chains synthesized in vitro that contained the poly(A) region [or the poly(T) region of the complement strand] varied in length by one to three bases, and thus made any sequence that followed this region unreadable. This problem may have been caused by heterologous template [i.e., template varying in length by one or more nucleotides in the poly(A) region] generated when template samples were propagated in *E. coli*. Alternatively, the inability to sequence through this region may have been due to stuttering of the polymerase within the poly(A) region during the sequencing reaction. As indicated in Figure 2, the length of the poly(A) tail of the clone was estimated at 80–82 bp.

It was obvious from the nucleotide sequence that the cDNA clones hT4.1 and hT9.1 represent very related, but distinct, mRNA transcripts. The nucleotide sequence of hT9.1 was 76% identical with the aligned sequence of hT4.1 between bp 168 and 1170 of hT4.1. There was little homology between the two clones outside of these regions. The partial amino acid sequence determined specifically from the α' subunit (i.e., M_r 40 000) of bovine testis casein kinase II (Litchfield et al., 1990) was identical with the sequence encoded by hT9.1. Therefore, clone hT9.1 encodes the α' subunit of casein kinase II (see Discussion).

Amino Acid Sequence of Casein Kinase II α Subunits. The predicted molecular weights of the hT4.1- and hT9.1-encoded polypeptides were 45 160 and 41 400, respectively. An alignment of these polypeptides is presented in Figure 3. As seen in Figure 3, most of the differences between the two polypeptides were at the carboxy terminus, with a few positions of heterogeneity scattered throughout the remainder of the polypeptides. The first 330 amino acids of the polypeptide encoded by hT9.1 were 88% identical with the aligned hT4.1-encoded polypeptide and 81% identical with the *Drosophila* α subunit [not shown; see Saxena et al. (1987)].

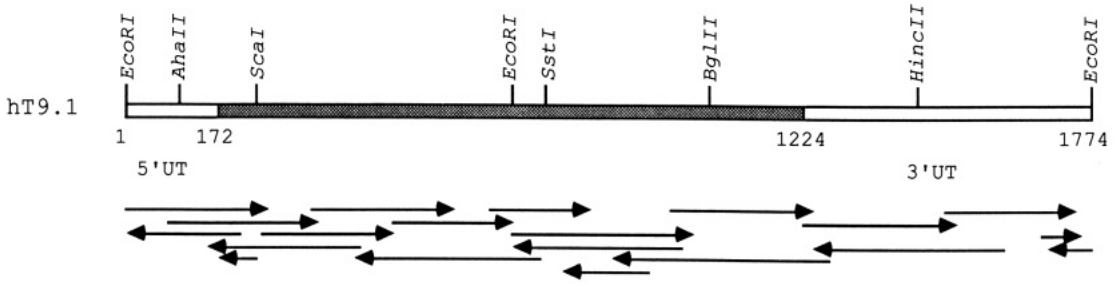
A considerable amount of amino acid sequence was determined from a mixture of the α subunits of bovine lung casein kinase II; a small portion of this sequence has been reported previously (Takio et al., 1987). After separation of the α subunits from the β subunit by size-exclusion chromatography, various proteolytic fragments of the α subunits were generated by CNBr or enzymatic digestion and isolated by a combination of size-exclusion and reverse-phase chromatography. Of the sequence determined by Edman degradation of the various proteolytic fragments, 314 residues could be best aligned with hT4.1-encoded polypeptide (i.e., the human α subunit; Figure 3), and 221 residues could be best aligned with the hT9.1-encoded polypeptide (i.e., the human α' subunit; Figure 3). Only two discrepancies were observed in the alignments despite comparing the sequence from bovine peptides with the sequence deduced from human cDNA clones. These discrepancies are discussed in the next paragraph. The amino terminus of one of the isolated peptides appeared to be blocked, since it was not susceptible to Edman degradation. This peptide was sequenced after mild acid treatment that promoted an acyl N to O shift (Takio et al., 1987), exposing the SGPVPSR sequence (Figure 3). Amino acid analysis verified the lack of additional amino acids in this peptide. Thus, it appears that the initiator methionine was removed in vivo.

There were 29 positions of heterogeneity identified in the mixture of bovine lung sequences after they were aligned with the polypeptides deduced from the human cDNA clones. All but one of these positions of heterogeneity was also observed in the deduced cDNA polypeptides (Figure 3). The exception was at residue 252 (see numbering in Figure 3) where both clones coded for glutamic acid but a bovine peptide corresponding to the α' subunit sequence displayed aspartic acid. This suggests a conservative amino acid change in the α' subunit of human and bovine casein kinase II at this position. Another conservative amino acid change between bovine and human α' subunits appeared at position 229 (Figure 3). Lysine was present in the human α subunit (i.e., encoded by hT4.1) while the human α' subunit (encoded by hT9.1) contained an arginine at this position. Bovine peptides corresponding to either the α or the α' subunit contained only lysine at this position (Figure 3).

Although alone these data did not show which of the cDNA clones encoded the α subunit and which encoded the α' subunit, the nearly perfect identity with the partial bovine lung sequences did show that hT4.1 and hT9.1 encoded bona fide α and α' subunits of casein kinase II.

Nucleic Acid and Protein Sequence Data Bank Comparisons. Comparisons of the nucleotide or deduced amino acid sequences of hT4.1 and hT9.1 were made to recent releases of the GenBank nucleic acid data bank or the Protein Identification Resource protein data bank. As expected, homologies of hT4.1 and hT9.1 nucleotide or amino acid sequences were with the casein kinase II α subunit clones from HepG2 cells (Meisner et al., 1989), *Drosophila* (Saxena et al., 1987), and *S. cerevisiae* CKA1 gene (Chen-Wu et al., 1988). Other proteins or DNA clones identified in the search showed much

A



B

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   1  gaattcggTGTCACCCAGGCTGGAGTGCAGTGGCGCAATCTCAGCTCACTGCAACCTCCACCTCCCTGGTTCAAGCGATTCTCCTGCCTCCTCCGCCCGACGCCCCGCGT

 111  CCCCCGCCGCGCCGCCGCCGCCACCCTCTGCGCCCCGCGCCGCCCCCCGGTCCCGCCCGCCATGCCCGGCCCGGCCGCGGGCAGCAGGGCCCGGGTCTACGCCGAGGTGA
   1                                                                  M  P  G  P  A  A  G  S  R  A  R  V  Y  A  E  V  N

 221  ACAGTCTGAGGAGCCGCGAGTACTGGGACTACGAGGCTCACGTCCCGAGCTGGGGTAATCAAGATGATTACCAACTGGTTCGAAAACTTGGTCGGGGAAAATATAGTGAA
  18    S  L  R  S  R  E  Y  W  D  Y  E  A  H  V  P  S  W  G  N  N  D  D  Y  Q  L  V  R  K  L  G  R  G  K  Y  S  E

 331  GTATTTGAGGCCATTAATATCACCAACAATGAGAGAGTGGTTGTAAAAATCCTGAAGCCAGTGAAGAAAAAGAAGATAAAACGAGAGGTTAAGATTCTGGAGAACCTTCG
  54  V  F  E  A  I  N  I  T  N  N  I  R  V  V  V  K  I  L  K  P  V  K  K  K  K  I  K  R  E  V  K  I  L  E  N  L  R

 441  TGGTGGAACAAATATCATTAAGCTGATTGACACTGTAAAGGACCCCGTGTCAAAGACACCAGCTTTGGTATTTGAATATATCAATAATACAGATTTTAAGCAACTCTACC
  91   G  G  T  N  I  I  K  L  I  D  T  V  K  D  P  V  S  K  T  P  A  L  V  F  E  Y  I  N  N  T  D  F  K  Q  L  Y  Q

 551  AGATCCTGACAGACTTTGATATCCGGTTTTATATGTATGAACTACTTAAAGCTCTGGATTACTGCCACAGCAAGGGAATCATGCACAGGGATGTGAAACCTCACAATGTC
 128    I  L  T  D  F  D  I  R  F  Y  M  Y  E  L  L  K  A  L  D  Y  C  H  S  K  G  I  M  H  R  D  V  K  P  H  N  V

 661  ATGATAGATCACCAACAGAAAAAGCTGCGACTGATAGATTGGGGTCTGGCAGAATTCTATCATCCTGCTCAGGAGTACAATGTTCGTGTAGCCTCAAGGTACTTCAAGGG
 164  M  I  D  H  Q  Q  K  K  L  R  L  I  D  W  G  L  A  E  F  Y  H  P  A  Q  E  Y  N  V  R  V  A  S  R  Y  F  L  G

 771  ACCAGAGCTCCTCGTGGACTATCAGATGTATGATTATAGCTTGGACATGTGGAGTTTGGGCTGTATGTTAGCAAGCATGATCTTTCGAAGGGAACCATTCTTCCATGGAC
 201   P  E  L  L  V  D  Y  Q  M  Y  D  Y  S  L  D  M  W  S  L  G  C  M  L  A  S  M  I  F  R  R  E  P  F  F  H  G  Q

 881  AGGACAACTATGACCAGCTTGTTCGCATTGCCAAGGTTCTGGGTACAGAAGAACTGTATGGGTATCTGAAGAAGTATCACATAGACCTAGATCCACACTTCAACGATATC
 238    D  N  Y  D  Q  L  V  R  I  A  K  V  L  G  T  E  E  L  Y  G  Y  L  K  K  Y  G  I  D  L  D  P  H  F  N  D  I

 991  CTGGGACAACATTCACGGAAACGCTGGGAAAACTTTATCCATAGTGAGAACAGACACCTTGTCAGCCCTGAGGCCCTAGATCTTCTGGACAAACTTCTGCGATACGACCA
 274  L  G  Q  H  S  R  K  R  W  E  N  F  I  H  S  E  N  R  H  L  V  S  P  E  A  L  D  L  L  D  K  L  L  R  Y  D  H

1101  TCAACAGAGACTGACTGCCAAAGAGGCCATGGAGCACCCATACTTCTACCCTGTGGTGAAGGAGCAGTCCCAGCCTTGTGCAGACAATGCTGTGCTTTCCAGTGGTCTCA
 311   Q  Q  R  L  T  A  K  E  A  M  E  H  P  Y  F  Y  P  V  V  K  E  Q  S  Q  P  C  A  D  N  A  V  L  S  S  G  L  T

1211  CGGCAGCACGATGAAGACTGGAAAGCGACGGGTCTGTTGCGGTTCTCCCACTTTTCCATAAGCAGAACAAGAACCAAATCAAACGTCTTAACGCGTATAGAGAGATCACG
 348    A  A  R  Ter

1321  TTCCGTGAGCAGACACAAAACGGTGGCAGGTTTGGCGAGCACGAACTAGACCAAGCGAAGGGCAGCCCACCACCGTATATCAAACCTCACTTCCGAATGTAAAAGGCTCA

1431  CTTGCCTTTGGCTTCCTGTTGACTTCTTCCCGACCCAGAAAGCATGGGGAATGTGAAGGGTATGCAGAATGTTGTTGGTTACTGTTGCTCCCCGAGCCCCTCAACTCGTC

1541  CCGTGGCCGCCTGTTTTTCCAGCAAACCACGCTAACTAGCTGACCACAGACTCCACAGTGGGGGGACGGGCGCAGTATGTGGCATGGCGGCAGTTACATATTATTATTTT

1651  AAAAGTATATATTATTGAATAAAAGGTTTTAAAAGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

1761  AAAAAAccgaattc
```

FIGURE 2: Human casein kinase II α′ subunit: structure, sequencing strategy, nucleotide sequence, and amino acid sequence of the cDNA clone hT9.1. (A) Structure of the cDNA clone hT9.1 showing several restriction endonuclease sites and the 5′ untranslated region (5′UT), the coding region (the shaded area), and the 3′ untranslated region (3′UT). The arrows under the diagram represent the direction and amount of sequence data determined from individual sequencing reactions by the dideoxy chain termination method. It was not possible to determine the sequence 3′ of the poly(A) region regardless of the strand used as the template in the reactions. Therefore, the sequence on either side of the poly(A) region could not be confirmed by sequence data from the complement strand. It was estimated that the poly(A) region was 80–82 nucleotides in length. (B) Nucleotide sequence of the hT9.1 cDNA clone is presented with the amino acid sequence deduced from the coding region. The amino acids are depicted by the single-letter abbreviation. The λgt10 library was constructed with *Eco*RI linkers (Littman et al., 1987). Lowercase letters at the 5′ and 3′ ends of the clone indicate *Eco*RI linkers. "Ter" indicates the termination codon at the end of the coding region. The consensus polyadenylation signal sequence (Proudfoot & Brownlee, 1976) is underlined.

less identity. They included amino acid or DNA sequences of other protein kinases and, in particular, the protein encoded by the cell division control gene *CDC*28 in *S. cerevisiae* (Lorincz & Reed, 1984) or its homologues in *Schizosaccharomyces pombe* (*cdc*2; Hindley & Phear, 1984) and human (*cdc*2-hs; Lee & Nurse, 1987). The homology of casein kinase II α subunit with *CDC*28 and *cdc*2 proteins was noted previously by Takio et al. (1987).

Detection of α Subunit mRNA in Various Tissues and Cultured Cell Lines. Northern blot analysis was performed on poly(A)-rich RNA isolated from two different human cultured cell lines and from three mouse tissues using the hT4.1 (α subunit) and hT9.1 (α′ subunit) cDNA clones (or portions of the clones) as probes. In mouse brain, thymus, and testis samples, the hT4.1 clone hybridized to RNA with sizes of about 4.6 and 3.1 kb (Figure 4A); a band of about 1.8 kb was visible with a longer exposure or if more poly(A)-rich RNA (20 μg) was analyzed (data not shown). In RNA from the human cultured cell lines, bands of about 4.5, 2.8, and 1.7 kb were detected (Figure 4B,C). The same results were obtained

```
                                                    10        20        30
Human α subunit from cDNA hT4.1:                    MSGPVPSRARVYTDVNTHRPREYWDYESHV
Human α' subunit from cDNA hT9.1:                   MPGPAAG******AE**SL*S*******A**

         40        50        60        70        80        90        100
VEWGNQDDYQLVRKLGRGKYSEVFEAINITNNEKVVVKILKPVKKKKIKREIKILENLRGGPNIITLADI
PS*******************************R*****************V*********T***K*I*T

        110       120       130       140       150       160       170
VKDPVSRTPALVFEHVNNTDFKQLYQTLTDYDIRFYMYEILKALDYCHSMGIMHRDVKPHNVMIDHEHRK
******K********YI**********I***F********L*********K***************QQK*

        180       190       200       210       220       230       240
LRLIDWGLAEFYHPGQEYNVRVASRYFKGPELLVDYQMYDYSLDMWSLGCMLASMIFRKEPFFHGHDNYD
**************A*****************************************R******Q****
                                                          (K)

        250       260       270       280       290       300       310
QLVRIAKVLGTEDLYDYIDKYNIELDPRFNDILGRHSRKRWERFVHSENQHLVSPEALDFLDKLLRYDHQ
***********EE**G*LK**H*D***H******Q*******N*I****R*********L**********
           (D)

        320       330       340       350       360       370       380
SRLTAREAMEHPYFYTVVKDQARMGSSSMPGGSTPVSSANMMSGISSVPTPSPLGPLAGSPVIAAANPLG
Q****K*********P***E*SQPCADNAVLSSGLTAARtga

        390
MPVPAAAGAQQtga
```

FIGURE 3: Alignment of the polypeptides deduced from the human cDNA clones hT4.1 and hT9.1 with segments of amino acid sequences determined from bovine lung casein kinase II. The amino acid sequences (one-letter code) deduced from the two cDNA clones were aligned without gaps; an asterisk indicates that the amino acid residue coded by hT9.1 (α' subunit) was the same as for hT4.1 (α subunit). The end of each polypeptide encoded by the respective cDNA clone is indicated by "tga". The numbering above the sequence relates to the hT4.1-encoded amino acid sequence. Note that this numbering is also used when referring to the hT9.1-encoded sequence. Underlined amino acid sequence represents residues observed by automated Edman degradation of various peptides from the mixture of the α and α' subunits of bovine lung casein kinase II (see Experimental Procedures). Some bovine lung sequences aligned best to the hT4.1-encoded polypeptide (shown by underlining of the first row of sequence); others aligned best to the hT9.1-encoded polypeptide (shown by underlining of the second row of sequence). Two differences between the bovine lung sequences and the sequence deduced from the hT9.1 clone are shown by circled letters at residues 229 and 252.

with poly(A)-rich RNA (5–10 μg) from human spleen or from full-term human placenta with the hT4.1 cDNA clone as the probe (data not shown), indicating that the cultured human cells express the same RNA transcripts as at least two different tissues. The smallest RNA (1.7–1.8 kb) had the least prominent signal in each of the samples, suggesting that it was present in the lowest concentration or that it had the lowest homology with the hT4.1 cDNA clone. The 2.8-kb RNA was more prominent than the 4.5-kb RNA in HSB-2 cells, but in the other samples, those bands were about equal in intensity.

Multiple hybridizing bands were also evident by Northern blot analysis when a portion of the hT9.1 cDNA clone was used as the probe. Bands of about 5.6, 3.2, and 2.1 kb were observed in the three mouse tissues, the 2.1-kb band being the most intense (Figure 4A). The hT9.1 clone hybridized to RNA of 5.7, 4.5, 2.8, 2.0, and 1.0 kb in the A431 sample (Figure 4B) and 5.7, 3.0, 2.0, and 1.6 kb in the HSB-2 sample (Figure 4C). In the A431 sample, the 4.5- and 2.8-kb bands were present with hybridization with either cDNA clone (Figure 4B). This suggests either that A431 cells contain independent 4.5- and 2.8-kb RNAs both for α and for α' subunits or that the 4.5- and 2.8-kb RNAs can be recognized by either cDNA clone (i.e., the cDNA clones cross-react with the 4.5- and 2.8-kb RNAs in A431 cells). The former explanation seems more plausible, since cross-reaction of the two cDNA clones was not obvious in any of the mouse tissues or in the HSB-2 cells.

The amount of RNA recognized by the hT9.1 clone was greater in mouse testis than in thymus or brain (Figure 4A), especially for the 2.1-kb transcript. This was not true of the transcripts recognized by the hT4.1 clone (Figure 4A). When the same blots were hybridized with a cDNA clone encoding the β subunit of casein kinase II, the concentration of the major transcript was also greatest in testis, followed by thymus and then brain (data not shown). This is in general agreement with studies on the enzymatic activity of casein kinase II, since its activity in extracts of testis is greater than in other tissues (Nakajo et al., 1986; Krebs et al., 1988).

Analysis of the α Subunits of Casein Kinase II by Genomic Southern Blot. The entire hT4.1 (α subunit) cDNA clone and a *Sca*I–*Bgl*II fragment of the hT9.1 (α' subunit) clone (nucleotides 242–1068) were labeled with [α-^{32}P]dATP and used to probe a Southern blot of human genomic DNA which had been digested with various restriction endonucleases. The analysis was performed separately on the genomic DNA from two different individuals. The results of the analysis of the DNA from one of the individuals are presented in Figure 5. The results with the DNA from the other individual were identical. There were no *Eco*RI, *Hin*dIII, or *Bam*HI sites within the hT4.1 cDNA clone (see Figure 1), but there were at least two hybridizing bands in the lanes of genomic DNA digested with these restriction endonucleases (lanes 1, 4, and 5; Figure 5a). There were single sites for *Pst*I and *Bgl*II in the hT4.1 cDNA, and, as expected, there was more than one hybridizing band in each of the lanes (lanes 2 and 3, Figure 5a). The autoradiogram of the same blot (washed stringently to remove previous probe) hybridized with the hT9.1 fragment also indicated at least two hybridizing bands in each lane

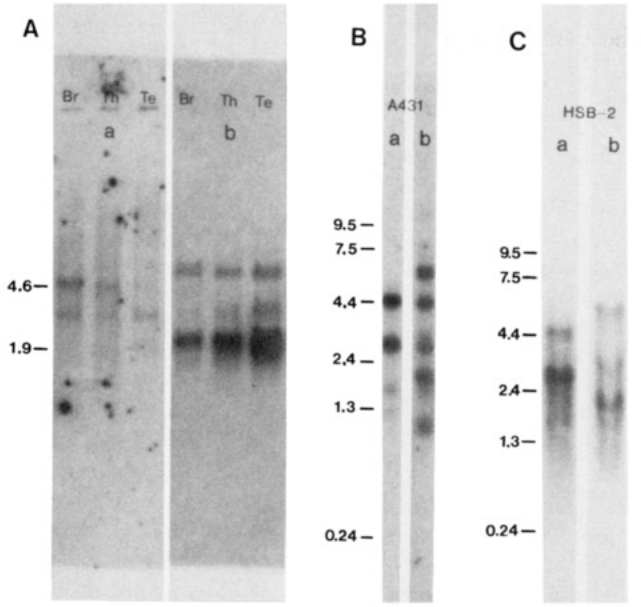

FIGURE 4: Northern blot analysis of mouse and human RNA with the cDNA clones hT4.1 and hT9.1. Poly(A)-rich RNA was separated by agarose–formaldehyde gel electrophoresis and transferred to charged nylon membrane. The membranes were then prehybridized, hybridized in the presence of the appropriate ^{32}P-labeled cDNA fragment, and washed (see Experimental Procedures). Kodak X-Omat AR film was exposed to washed membranes with an intensifying screen at −70 °C for 5 days. (a) Membrane blots were hybridized with the entire hT4.1 (human α subunit) cDNA clone (*Eco*RI fragment, 2197 nucleotides). (b) Membrane blots were hybridized with a *Sca*I–*Sst*I (538 nucleotides) fragment of the hT9.1 (human α' subunit) cDNA clone. (A) Ten micrograms of poly(A)-rich RNA from mouse brain (Br), mouse thymus (Th), and mouse testis (Te). (B) Ten micrograms of poly(A)-rich RNA from A431 cultured cells. (C) Five micrograms of poly(A)-rich RNA from HSB-2 cultured cells. The positions of RNA molecular size standards (see Experimental Procedures; kb) or mouse 28S and 18S rRNA (4.6 and 1.9 kb) are indicated beside the sample lanes.

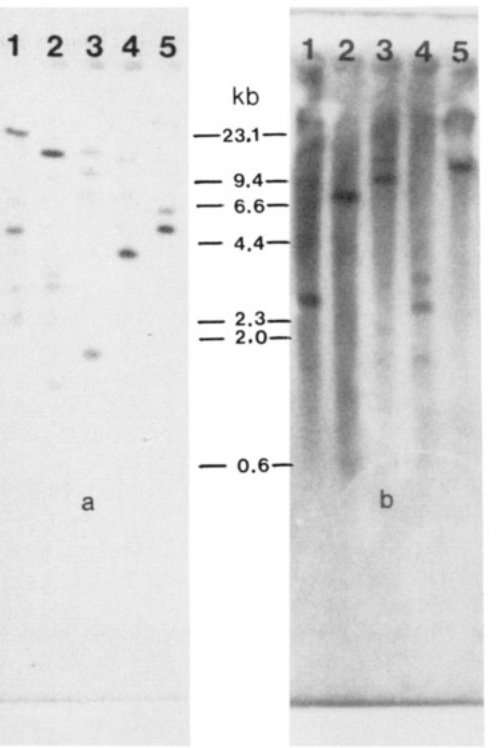

FIGURE 5: Southern blot analysis of human genomic DNA with the cDNA clones hT4.1 and hT9.1. Genomic DNA from human whole blood was prepared, digested, electrophoresed, and blotted to nitrocellulose membrane as described under Experimental Procedures. The conditions for prehybridization, hybridization, and washing of the membrane blot are also given under Experimental Procedures. Kodak X-Omat AR film was exposed to the washed membrane blot with an intensifying screen at −70 °C for 5 days. Ten micrograms of genomic DNA was used for each restriction enzyme digest: lane 1, *Eco*RI; lane 2, *Pst*I; lane 3, *Bgl*II; lane 4, *Hin*dIII; lane 5, *Bam*HI. The membrane blot was hybridized either with the entire hT4.1 cDNA clone (a) or with a *Sca*I–*Bgl*II (827 nucleotides) fragment of the hT9.1 cDNA clone (b). The same nitrocellulose membrane blot was used for analysis with each of the cDNA probes. Hybridized probe was removed by a stringent wash (twice, 15 min each in 0.2× SSC and 0.1% SDS at 95 °C). Removal of the probe was verified by autoradiography for 3 days. The positions of DNA standard size markers (kb) are indicated beside the samples lanes.

(Figure 5b). There were no *Pst*I, *Bgl*II, *Hin*dIII, or *Bam*HI sites within the fragment of the hT9.1 cDNA clone used, but there were two or more hybridizing bands in each of the lanes of genomic DNA digested with these restriction endonucleases (lanes 2–5, Figure 5b; the very large ~23-kb band was not very evident in this autoradiogram but was more evident in the autoradiogram from the other sample of genomic DNA). There was an *Eco*RI site within the fragment of hT9.1, and bands of ~2.5, 8.5, and 23 kb were evident in the lane containing *Eco*RI-digested genomic DNA (lane 1, Figure 5b). The hT9.1 fragment did not recognize the same sizes of digested genomic DNA as the hT4.1 cDNA clone, indicating that there was little cross-hybridization of the two clones under the conditions used for the analysis. (Bands of equal size were faintly evident in some cases.)

It was obvious from the autoradiograms that the two cDNA clones recognized different genomic DNA restriction endonuclease fragments, indicating that the cDNA clones represent two different gene products. The analysis was consistent with a single copy of a gene corresponding to each of the two cDNA clones, each containing one or more introns (with the pertinent restriction endonuclease sites) to explain the multiple and large hybridizing bands. An alternative but more unlikely explanation of the multiple hybridizing bands is that there were additional copies of each gene which were highly related to the respective cDNA clones.

Discussion

Two distinct clones were isolated from a human T-cell cDNA library using a fragment of a *Drosophila* cDNA clone encoding the α subunit of casein kinase II. These two cDNA clones encode distinct, but homologous, polypeptides. The sequences of the polypeptides encoded by the two human cDNA clones are nearly identical with the partial amino acid sequences determined from a mixture of α and α' subunits from purified bovine lung casein kinase II (Figure 3). The isolation of the two human cDNA clones confirms previous immunological and sequence data, which suggested that the α and α' subunits of bovine casein kinase II were separate gene products, or products of alternatively spliced transcripts (Dahmus et al., 1984; Takio et al., 1987; Litchfield et al., 1990). The analysis of genomic DNA with the two cDNA clones (Figure 5) indicates that the α and α' subunits of human casein kinase II are products of distinct genes.

From the data presented in this report, it was not possible to conclude which of the cDNA clones encodes the α subunit and which encodes the α' subunit. This was accomplished by comparing the sequences deduced from the human cDNA clones to sequences determined exclusively from the α or the α' subunit of bovine testis casein kinase II (Litchfield et al., 1990). Protein microsequencing techniques were used to generate the specific amino acid sequences from the α and the α' subunits. Among others, the sequences VLGTEDLYDYIDK and YNIELDPRFNDILGRHSRK were determined

from the bovine α subunit. These sequences are identical with residues 248–260 and 261–279 in the hT4.1-encoded polypeptide (Figure 3), confirming that hT4.1 encodes the α subunit. The sequences VLGTDELYGYLK and YHIDLDPHFNDILGQHSRK were determined from the bovine α' subunit. This confirms that the hT9.1 clone encodes the α' subunit of casein kinase II, since these peptide sequences are identical (except for one conservative change at residue 252) with residues 248–259 and 261–279 in the hT9.1-encoded polypeptide (see numbering in Figure 3). The conclusions based upon the partial bovine sequence are supported by immunoblot studies with antiserum against a peptide with a sequence EDLYDYIDKYNIEL, corresponding to residues 252–265 in the hT4.1-encoded polypeptide; this antipeptide antiserum specifically recognizes the α subunit of bovine testis casein kinase II (Litchfield et al., 1990).

Despite the evidence that α and α' are distinct subunits, the proteolytic sensitivity of α and α' often makes it difficult to distinguish one from another on SDS–polyacrylamide gel electrophoresis. In the purification of bovine testis casein kinase II, limited proteolysis of the α subunit generates a polypeptide which is difficult to distinguish from the legitimate α' subunit.[3] Therefore, caution should be exercised when interpreting the subunit composition of purified casein kinase II by SDS–polyacrylamide gel electrophoresis.

The α subunit is the catalytic subunit of casein kinase II, based upon enzymatic activity in the absence of the β subunit, affinity labeling with ATP analogues, and sequence features common to other protein kinases (see references in the introduction). The α' subunit also has the sequence and primary structural features common to other protein kinases. In fact, all of the sequence features of protein kinases that have been compiled by Hanks et al. (1988) are conserved between the α and α' subunits.

The basic region that follows the critical nucleotide binding lysine (KPVKKKKIKR; residues 71–80 in the hT4.1-encoded polypeptide) is a sequence feature in casein kinase II α subunits from numerous organisms (Padmanabha et al., 1990) including the human α and α' subunits (Figure 3). This region could be responsible for interaction with the substrates of casein kinase II [see Tuazon and Traugh (1990)], considering that the major substrate specificity requirement is for acidic residues in the first few positions (and especially at the +3 position) immediately following the phosphorylated serine or threonine (Meggio et al., 1984; Marin et al., 1986; Kuenzel et al., 1987). This basic region may also be involved in the interaction with the β subunit. The sequence DLEPDEELED (residues 51–60 of the bovine lung β subunit; Takio et al., 1987) contains seven acidic residues and is highly conserved between organisms (Saxena et al., 1987; Takio et al., 1987; Jakobi et al., 1989; Heller-Harrison et al., 1989). Thus, it is possible that an interaction between this acidic region in the β subunit and the basic region in the α and α' subunits is partly responsible for the interaction of the subunits within the holoenzyme. However, it is unlikely that this is the only motif responsible for the subunit interactions since high salt concentration is not sufficient to dissociate the holoenzyme (Meggio & Pinna, 1984). The effects of polyanionic and polycationic compounds on the activity and the autophosphorylation of casein kinase II (Maenpaa, 1977; Hathaway & Traugh, 1982; Meggio & Pinna, 1984; Mamrack, 1989) are consistent with these proposed roles for the highly charged motifs KPVKKKKIKR and DLEPDEELED in substrate recognition, activity, and subunit interaction.

Recent studies have suggested another intriguing function for the basic KPVKKKKIKR region in the α and α' subunits. Several investigators have noted that nuclear proteins contain specific sequence motifs which direct the protein to the nucleus. The motifs are short segments of basic amino acids (Kalderon et al., 1984; Richardson et al., 1986; Lyons et al., 1987; Dang & Lee, 1989). Dang and Lee (1989) determined that a helix-breaking residue (proline or glycine) followed by at least three basic residues in the next seven to nine carboxy-terminal residues was an effective nuclear targeting sequence. The KPVKKKKIKR sequence certainly falls into this description, implying that the α and α' subunits could be targeted to the nucleus by this motif. Casein kinase II is found in the nucleus where it may consist of both an α and an α' subunit (Thornburg & Lindell, 1977; Thornburg et al., 1979; Inoue et al., 1984; Qi et al., 1986). However, others report that nuclear casein kinase II contains only a single α subunit (Rose et al., 1981; Goueli et al., 1986).

The molecular weights of the α and α' subunits predicted from the human cDNA clones are 45 160 and 41 400, which agree well with the molecular weight values of 45 000 and 40 000 as determined by SDS–polyacrylamide gel electrophoresis of bovine testis or lung casein kinase II (Litchfield et al., 1990). This is an indication that there are no major co- or posttranslational modifications of the α and α' subunits, i.e., proteolytic processing. There is some evidence for minor co- or posttranslational modifications. Isoelectric variants of both the α and the α' subunits from rat liver nuclei and bovine testis casein kinase II have been observed by two-dimensional gel electrophoresis (Qi et al., 1986; Litchfield et al., 1990). Some of the isoelectric variation within each subunit appears to be due to phosphorylation since treatment with alkaline phosphatase changed the isoelectric pattern (Qi et al., 1986). The bovine lung α subunit appears to be blocked on a seryl residue (see Results), as is the β subunit of bovine lung casein kinase II (Takio et al., 1987). A blocked seryl residue is consistent with the data reviewed by Arfin and Bradshaw (1988) which suggest that eukaryotic proteins translated with a penultimate serine residue will lose the initiator methionine and that the serine residue will be blocked. Interestingly, proteins with proline in the penultimate position (like the α' subunit) are usually not blocked (Arfin & Bradshaw, 1988).

The existence of distinct, separately encoded α and α' catalytic subunits raises the possibility of specialized properties and cellular functions. Those different properties may be conferred by the carboxy terminus of the two polypeptides, since this is the region of greatest sequence heterogeneity. Specialization could involve differential regulation of activity, substrate selectivity, subcellular localization, association with the β subunit, filament formation, or other properties of casein kinase II. It is not clear whether the α and α' subunits can exist independently of the β subunit in vivo. There have been reports that casein kinase II purified from some sources is composed only of an α subunit (Baydoun et al., 1980; Renart et al., 1984) and that in vitro the α subunit has some catalytic activity in the absence of β subunit (Cochet & Chambaz, 1983b; Tuazon & Traugh, 1990). However, given the current understanding of casein kinase II, any different properties or functions conferred by the α and α' subunits would be manifest in the holoenzyme. There are three possible isoforms: $\alpha_2\beta_2$, $\alpha\alpha'\beta_2$, and $\alpha'_2\beta_2$. The ratio of these isoforms would be dependent upon the expression of the individual subunits, and on the relative abilities of each of the subunits to form complexes with the others. From the Northern blot analysis of

[3] D. W. Litchfield and E. G. Krebs, unpublished results.

RNA from A431 or HSB-2 cells (Figure 4), the mRNAs for both the α subunit and the α′ subunit are present. Some preparations of casein kinase II purified from homogeneous cultured cell populations contain both α and α′ subunits (Pyerin et al., 1987). These studies indicate that there are examples of α and α′ being expressed simultaneously in the same cell. There are also some reports of only one size of α subunit in casein kinase II (Boivan & Galand, 1979; Hathaway & Traugh, 1982; Cochet & Chambaz, 1983b; Glover et al., 1983; Meggio & Pinna, 1984) which suggests that there are examples of selective expression (or possibly only one gene in some species) of these subunits. Cochet et al. (1981) reported evidence suggesting distinct isoforms of the holoenzyme. The isoforms were distinct on the basis of elution from phosphocellulose resin, but not on the basis of subunit composition, substrate specificity, catalytic activity, or other parameters measured. However, the α subunits from both isoforms appeared much smaller than α subunits from other purified preparations of casein kinase II from the same organism (the bovine), suggesting proteolysis during purification. In our hands, analyses of casein kinase II holoenzyme rapidly purified from bovine testis have failed to resolve isoforms by size- or ion-exchange chromatography.[4]

Both the α subunit and the α′ subunit cDNA clones recognized multiple RNAs in samples of poly(A)-rich RNA (i.e., presumably mRNA). There are also multiple mRNAs for the β subunit of casein kinase II (Heller-Harrison et al., 1989). These β subunit multiple mRNAs appear to differ in the 3′ noncoding region. A similar situation may exist for the α and α′ subunits of casein kinase II. Messenger RNAs that have different noncoding regions could theoretically have different stabilities, different rates of nucleocytoplasmic transport, or different rates of translation, thereby affecting expression of the given subunit. It is also possible that the mRNAs may have different coding regions, for example, coding regions that encode signal or localization sequences that may or may not be proteolytically removed after being translated and processed through the appropriate transport pathway. Multiple mRNAs could be products of differential processing of a single RNA transcript, which can be generated by alternative splicing and/or the presence of more than one polyadenylation site [see Adams et al. (1986)]. The multiple mRNAs for the β subunit of casein kinase II in HepG2 cells appear to be the result of more than one polyadenylation site (Heller-Harrison et al., 1989). Alternatively, some of the mRNAs could be the products of different, but highly related, genes. This remains a possibility because the analysis of genomic DNA cannot distinguish between the presence of a single gene with introns or the presence of an additional, highly related gene(s) for each subunit. Further analysis is required to determine the differences between the multiple mRNAs of the α and α′ subunits.

To our knowledge, the only other organism (besides bovine and human) in which α and α′ subunits have been isolated and characterized is in the yeast *Saccharomyces cerevisiae* (Padmanabha & Glover, 1987; Chen-Wu et al., 1988; Padmanabha et al., 1990). The α and α′ subunits of *S. cerevisiae* (products of the CKA1 and the CKA2 genes, respectively) are 60% identical, with most of the difference in primary structure between the subunits due to a 38-residue insertion in the α subunit. The primary structure of the α and α′ subunits in *S. cerevisiae* and in the human (or bovine) suggests that the α and α′ genes in each organism arose by independent gene duplication events (Padmanabha et al., 1990). This is reflected in the degree of identity of the *S. cerevisiae* subunits to the human subunits: CKA1 vs human α, 69%; CKA1 vs human α′, 71%; CKA2 vs human α, 64%; CKA2 vs α′, 64% [calculated after alignments of domains I through XI as compiled by Hanks et al. (1988)]. This sequence alignment and identity comparison do not reveal an obvious relationship of the two α subunits and the two α′ subunits. Instead, the two yeast subunits resemble each human subunit to a similar extent.

The functions of the yeast α and α′ subunits have been addressed by genetic analysis. Disruption of the genes (by gene replacement) that encode the α (CKA1 gene) and α′ (CKA2 gene) subunits is lethal for the resulting *S. cerevisiae* strain (Padmanabha et al., 1990). However, disruption of the CKA1 gene alone or the CKA2 gene alone is not lethal; the strains with disrupted CKA1 or CKA2 are normal with respect to growth on rich medium, resistance to heat shock, resistance to nitrogen starvation, and ability to mate (Chen-Wu et al., 1988; Padmanabha et al., 1990). The α and α′ subunits could have subtly different functions that were not detected in the limited analysis of the strains, but apparently, the α′ subunit can adequately compensate for the absence of the α subunit, and vice versa (Chen-Wu et al., 1988; Padmanabha et al., 1990). Therefore, in *S. cerevisiae*, the properties of the α and α′ subunits are sufficiently similar that either subunit can accomplish the essential cellular functions. Further experiments are required before similar conclusions can be made about the properties and cellular functions of the α and α′ subunits in mammalian cells.

[4] D. W. Litchfield and E. G. Krebs, unpublished results.

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Registry No. DNA (human clone hT4.1 casein kinase II α-subunit messenger RNA complementary), 128495-02-3; casein kinase II (human clone hT4.1 α-subunit precursor reduced), 128495-03-4; DNA (human clone hT9.1 casein kinase II α′-subunit messenger RNA complementary), 128495-04-5; casein kinase II (human clone hT9.1 α′-subunit precursor reduced), 128495-05-6; casein kinase II, 52660-18-1; casein kinase (human clone hT4.1 isoenzyme II deblocked α-subunit protein moiety reduced), 128495-06-7; casein kinase (human clone hT9.1 isoenzyme II α′-subunit protein moiety reduced), 128495-07-8.

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Modification of Bases in DNA by Copper Ion-1,10-Phenanthroline Complexes†

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ABSTRACT: Damage to the bases in DNA by the cupric ion-1,10-phenanthroline complex was investigated. Ten base products in DNA were identified and quantitated by the use of gas chromatography/mass spectrometry with selected-ion monitoring. DNA damage by the cupric ion-1,10-phenanthroline complex required the presence of a reducing agent such as ascorbic acid or mercaptoethanol. Products identified were typical hydroxyl radical induced products from the pyrimidines and purines in DNA, well-known from previous studies using various hydroxyl radical producing systems such as ionizing radiation, hypoxanthine/xanthine oxidase, or hydrogen peroxide in the presence of transition metal ions. Product formation was not significantly inhibited by typical scavengers of hydroxyl radical such as mannitol and sodium formate, but there was partial inhibition by dimethyl sulfoxide. Catalase substantially decreased formation of base products, and added hydrogen peroxide stimulated it, indicating the hydrogen peroxide dependency of DNA base damage. Superoxide dismutase afforded only a partial reduction in product yields in systems containing ascorbic acid. On the basis of the types of base products formed, the hydrogen peroxide dependency of product formation, and a previous report suggesting that DNA damage is due to a diffusible species [Williams, L. D., Thivierge, J., & Goldberg, I. H. (1988) *Nucleic Acids Res. 16*, 11607-11615], we propose that DNA base damage is caused by hydroxyl radical.

In the presence of H_2O_2, a 2:1 complex of the chelating agent 1,10-phenanthroline (OP)[1] with Cu^+ ions [$(OP)_2Cu^+$] produces strand cleavage of DNA after binding to the minor groove (Sigman et al., 1979; Sigman, 1986). The nuclease activity of the $(OP)_2Cu^+$ complex has been widely used for structural studies upon DNA (Sigman, 1986; Marshall et al., 1981; Pope & Sigman, 1984; Veal & Rill, 1988). In addition, measurement of DNA damage in the presence of an excess of DNA and of OP has been employed as a sensitive assay to measure the availability of copper ions in human body fluids (Gutteridge, 1984; Gutteridge et al., 1985; Evans et al., 1989). During experimentation, Cu^{2+} ions are often mixed with the OP so that a reducing agent has to be added to reduce Cu^{2+} ions to Cu^+ ions and form the DNA-cleaving species. The reducing agents that have been employed in vitro to facilitate

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[1] Abbreviations: OP, 1,10-phenanthroline; O_2^-, superoxide radical; •OH, hydroxyl radical; GC/MS-SIM, gas chromatography/mass spectrometry with selected-ion monitoring; 5-OH-5-MeHyd, 5-hydroxy-5-methylhydantoin; 5-OH-Hyd, 5-hydroxyhydantoin; 5-OHMeUra, 5-(hydroxymethyl)uracil; 5,6-diOH-Cyt, 5,6-dihydroxycytosine; FapyAde, 4,6-diamino-5-formamidopyrimidine; FapyGua, 2,6-diamino-4-hydroxy-5-formamidopyrimidine; 8-OH-Ade, 8-hydroxyadenine; 8-OH-Gua, 8-hydroxyguanine; ME, mercaptoethanol; SOD, copper-zinc superoxide dismutase; asc, ascorbic acid; TBA, thiobarbituric acid; Me_2SO, dimethyl sulfoxide.

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