# Isolation of cDNA clones coding for the catalytic subunit of mouse cAMP-dependent protein kinase

(polysome immunoadsorption/hybrid select translation/mRNA quantitation)

MICHAEL D. UHLER*, DAVID F. CARMICHAEL*†‡, DAVID C. LEE*§, JOHN C. CHRIVIA*, EDWIN G. KREBS*†, AND G. STANLEY MCKNIGHT*

*Department of Pharmacology and †Howard Hughes Medical Research Institute, Seattle, WA 98195

ABSTRACT     mRNA coding for the catalytic (C) subunit of cAMP-dependent protein kinase (ATP: protein phosphotransferase, EC 2.7.1.37) was partially purified from bovine testis by polysome immunoadsorption and oligo(dT)-chromatography. This enriched mRNA preparation was used to prepare and differentially screen a cDNA library. One of the selected cDNA clones was shown to hybrid-select mRNA coding for a 40-kDa protein that was specifically precipitated with antibodies to the C subunit. This bovine cDNA clone was then used to isolate a series of mouse cDNA clones that are complementary to the entire mouse C subunit mRNA. The mouse clones code for a protein of 351 amino acids that shows 98% homology to the bovine C subunit and hybridize to a single mRNA of 2.4 kilobases in mouse heart and brain. Southern blot analysis of total genomic DNA suggests that there is a single mouse gene coding for the C subunit. mRNA levels for both the C subunit and the type I regulatory subunit in various mouse tissues and cell lines were quantitated and compared by using single-stranded RNA probes prepared with SP6 polymerase.

Many hormones exert their effects on cellular metabolism and gene expression by regulating the intracellular level of cAMP, which in turn regulates the activity of cAMP-dependent protein kinase (ATP: protein phosphotransferase, EC 2.7.1.37) (1–3). The holoenzyme, consisting of two identical regulatory (R) and two catalytic (C) subunits, dissociates when each of the R subunits binds two cAMP molecules (3). The C subunit phosphorylates serine and threonine residues in substrate proteins involved in mediating the biological response to hormonal stimulation. Although many of the effects of cAMP-dependent protein kinase on cellular metabolism involve phosphorylation of substrate proteins by the C subunit (3), there is evidence that the free R subunits also may play a direct role in the regulation of cellular functions (4, 5).

There are at least three types of cAMP-dependent protein kinase: type I, which is the predominant form in bovine skeletal muscle; type II, which is the major form in bovine heart; and a neural type II, which is found in bovine brain (2, 6). The difference between the various types of protein kinase lies in the type of R subunit associated with C subunit. Although three forms of the C subunit have been characterized by isoelectric focusing, C subunits from both types of cAMP-dependent protein kinase have otherwise similar physical and enzymatic properties (1, 7). In addition, the structure of the R and C subunits appears to have been conserved over the course of evolution, since a functional enzyme can be reconstituted using yeast C subunit and bovine R subunit (8).

The C subunit of cAMP-dependent protein kinase has been studied extensively. The complete amino acid sequence of the bovine heart C subunit has been published (9), and some of the amino acid residues that form the C site have been identified by photoaffinity labeling (10, 11). These active-site residues include a lysine at position 72, which occurs in a sequence homologous to the active site in other protein kinases, such as the src gene product (12). Other studies have shown that the C subunit of cAMP-dependent protein kinase is phosphorylated and myristylated (13, 14). Although the importance of phosphorylation and myristylation in modulating the function of the C subunit is not known, a mutation that prevents the myristylation of the src gene product abolishes its transforming activity (15). In order to study the role that cAMP-dependent protein kinase plays in regulating cellular functions in more detail, we isolated cDNA clones for the C subunit and used them in a preliminary characterization of C-subunit gene expression.

## MATERIALS AND METHODS

**Tissues and Cell Culture.** Bull testis and mouse tissues from 12-week-old BALB/c mice were obtained fresh, rapidly frozen, and stored at −70°C prior to use. S49 lymphoma and NIH 3T3 cells were grown as described (16, 17).

**Immunoadsorption of C Subunit Polysomes.** Immunoadsorption of polysomes was as described (18) except that polysomes were incubated with 3 mg of affinity-purified goat anti-C subunit antibody (a gift of Mike Murtaugh, University of Texas), and the protein A-Sepharose (6 ml) was pre-adsorbed with 10 mg of rabbit anti-goat IgG. These modifications were necessary since the protein A-Sepharose did not bind the goat anti-C antibody effectively. Isolation of poly(A)⁺ RNA from the enriched polysomes was performed as described (18).

**Construction of cDNA Libraries.** A bovine testis cDNA library was constructed in pBR322 by using the poly(A)⁺ RNA enriched for the C subunit (18). Phage λgt10 cDNA libraries were constructed with poly(A)⁺ RNA from either S49 lymphoma cells or mouse heart as described elsewhere (19).

**Screening of cDNA and Genomic Libraries.** The enriched bovine testis cDNA library was differentially screened by using ³²P-labeled cDNAs synthesized by reverse transcriptase with either C-enriched or total poly(A)⁺ RNA as template (18). Screening of other cDNA libraries, the bovine genomic library (a gift of Fritz Rottman, University of Illinois), and the mouse genomic library (a gift of Ursula Storb, University of Washington) was performed either at high stringency (42°C) or at low stringency (25°C) in HYB

---

Biochemistry: Uhler *et al.*

*Proc. Natl. Acad. Sci. USA 83 (1986)*     1301

buffer (50% formamide/0.75 M NaCl/0.075 sodium citrate/50 mM Na$_2$HPO$_4$, pH 7.4/5 mM EDTA/0.1% NaDodSO$_4$/0.1% bovine serum albumin/0.1% polyvinylpyrrolidone/0.1% Ficoll/100 $\mu$g of salmon sperm DNA per ml).

**Hybrid-Selection Analysis of Bovine cDNA Clones.** Plasmid DNA was bound to nitrocellulose, and mRNA complementary to the insert was selected by hybridization (18). The mRNA selected was characterized by *in vitro* translation and immunoprecipitation of the translated products (18).

**Isolation of Mouse cDNA Clones Using the Bovine cDNA 128E9.** The bovine cDNA clone 128E9 was presumed to contain sequences complementary to the 3' untranslated sequence of the C subunit mRNA. Since most eukaryotic genes do not contain introns in the 3' untranslated region (20), it was presumed that C subunit coding sequences could be isolated by characterizing genomic sequences 5' of the poly(A)$^+$ addition signal of 128E9. A genomic clone that hybridized to the bovine cDNA clone 128E9 was isolated by screening a bovine genomic library. A 2.0-kilobase (kb) *Bam*HI fragment from the genomic clone was isolated, partially sequenced, and shown to contain sequences coding for the carboxyl terminus of the bovine C subunit (data not shown). The 2.0-kb *Bam*HI fragment hybridized under conditions of low stringency to a small percentage of clones in an S49 mouse lymphoma cDNA library. One of these mouse clones, MC1, was shown by DNA sequencing to contain a 600-base-pair (bp) insert that coded for part of the mouse C subunit. By using the MC1 insert DNA as a hybridization probe, mouse cDNA clone MC4 was obtained from a S49 lymphoma library.

The 5' termination site of clone MC4 corresponds to an internal *Eco*RI site. Sequencing of this cDNA clone showed that this *Eco*RI site corresponded to the codons for Glu-Phe, amino acids 107 and 108 of the C-subunit protein sequence. Since the MC4 insert terminated at this site and the genomic clones for the C subunit had recently been isolated in our laboratory, the genomic *Eco*RI site corresponding to the internal *Eco*RI cDNA site was identified (data not shown). A 75-bp *Sau*3A/*Eco*RI fragment on the 5' side of this *Eco*RI site was isolated from the genomic clone, sequenced, and shown to contain only coding sequence for the C subunit. This genomic fragment was then used to screen for and isolate the cDNA clone MC8 from the mouse heart cDNA library. The 3' termination site of MC8 corresponds to the same *Eco*RI site at the 5' termination sites of MC4.

**Subcloning and Sequencing of cDNA Fragments.** The insert of plasmid 128E9 was excised, labeled with T4 polynucleotide kinase, and sequenced by the chemical method of Maxam and Gilbert (21). All other fragments were subcloned into M13 mp10, M13 mp11, pUC12, or pUC13, and both strands were sequenced by the dideoxy termination method (22).

**Blot-Hybridization Analysis.** RNA or $^{32}$P-labeled size markers (1-kb ladder, Bethesda Research Laboratories) were denatured in 20 mM *N*-morpholinopropanesulfonic acid, pH 7.0/1 mM EDTA/5 mM sodium acetate/2.2 M formaldehyde/50% formamide at 65°C for 15 min. The samples were then loaded on a 1% agarose gel and run in the same buffer without formamide. The gel was then blotted to nitrocellulose, baked at 80°C for 2 hr, and hybridized for 16–20 hr in the HYB buffer at 42°C. Autoradiography was at −70°C for 16 hr, and the size of the hybridizing mRNA was determined with the size markers as standards.

**Southern Blot Analysis of Total Mouse Genomic DNA.** Total genomic DNA from mouse liver was isolated (23), digested with the appropriate restriction enzyme (New England Biolabs), and blotted to nitrocellulose (24). The filters were hybridized at 42°C for 18 hr in HYB buffer containing 10$^6$ cpm of denatured nick-translated (25) *Eco*RI/*Sca* I fragment of MC4 per ml.

**Quantitation of Type I R Subunit (R$^I$) and C Message Levels.** The *Bgl* II/*Apa* I fragment of pMC1 was cloned into pSP64 and M13 mp10 such that transcription from the SP6 promoter gave a single-stranded RNA transcript that hybridized to both the C subunit mRNA and the viral strand of the M13 mp10 subclone. The $^{32}$P-labeled SP6 transcript was synthesized by using SP6 polymerase (Promega Biotec, Madison, WI) and isolated as described (26). Total nucleic acid was isolated by proteinase K digestion and phenol/chloroform extraction (27). Samples of total nucleic acid or dilutions of the M13 mp10 viral DNA were incubated with the SP6 probe in 0.6 M NaCl/20 mM Tris chloride, pH 7.5/10 mM EDTA/0.2% NaDodSO$_4$ at 70°C for 16 hr under paraffin oil. Samples were then digested with 25 $\mu$g of RNase A (Sigma) and 200 units of RNase T1 (Sigma) in 1 ml of 0.3 M NaCl/10 mM Tris chloride, pH 7.5/5 mM EDTA/75 $\mu$g of denatured salmon sperm DNA per ml at 37°C for 30 min. The samples were then made 10% in trichloroacetic acid and filtered onto Whatman GF/C glass fiber filters. Filters were treated with 250 $\mu$l of Soluene 350 (Packard), and the radioactivity was determined after the addition of 4 ml of Omnifluor (New England Nuclear). Molecules per cell were calculated by comparison to M13 standards containing the sense strand of the C-subunit cDNA. DNA content of total nucleic acid samples was determined by a fluorescent dye binding assay (28) or in the case of single-stranded M13 DNA by absorbance at 260 nm. A similar assay was used for quantitation of R$^I$ message levels by using fragments of a mouse cDNA clone (G.S.K., unpublished data) isolated with the bovine R$^I$ cDNA clone described previously (18).

## RESULTS

**Enrichment for C Subunit mRNA.** Polysome immunoadsorption was used to enrich polysomal RNA for C-subunit mRNA. Polysomes were isolated from bovine testis, incubated with a goat antibody specific for the bovine C subunit, and passed over a protein A-Sepharose column that had been treated with rabbit anti-goat IgG antibody. Bound polysomes were eluted, and poly(A)$^+$ RNA was isolated on an oligo(dT) column. Enrichment of C-subunit mRNA was assessed by *in vitro* translation in a rabbit reticulocyte system (Fig. 1). The poly(A)$^+$ RNA preparation from immunoadsorbed polysomes (Fig. 1, lanes 3 and 4) was enriched at least 50-fold over poly(A)$^+$ RNA from total polysomes (Fig. 1, lane 1) for mRNA encoding a 40-kDa protein. In addition, this protein was immunoprecipitated by the antibody specific for the C subunit (Fig. 1, lanes 6 and 7).

**Isolation of a Bovine cDNA Clone Specific for the C Subunit.** A cDNA library was constructed in the *Pst* I site of pBR322 by G·C tailing using the C-subunit-enriched mRNA. This library was then screened for clones complementary to C-subunit mRNA by a differential hybridization method using $^{32}$P-labeled cDNA made against either C-subunit-enriched or total polysomal poly(A)$^+$ RNA. Of the 3600 clones screened, several appeared to hybridize preferentially to cDNA directed against the enriched RNA preparation. These clones then were tested for hybrid-selection of C-subunit mRNA by fixing the plasmid to nitrocellulose filters, hybridizing the filters to total poly(A)$^+$ RNA, and translating the specifically bound RNA in the reticulocyte translation system. One clone was shown to consistently select mRNA coding for a 40-kDa protein that was specifically precipitated with antibody directed against the bovine C subunit (Fig. 1, lane 8). This protein was not detected when pBR322 DNA was used to hybrid-select mRNA (Fig. 1, lane 9). The 260-nucleotide fragment contained in this clone, 128E9, was isolated and partially sequenced but was shown to contain only 3' untranslated sequences and a poly(A)$^+$ addition site.

**Isolation of cDNA Clones Coding for the Entire Mouse C Subunit.** The bovine cDNA clone 128E9 was used to isolate
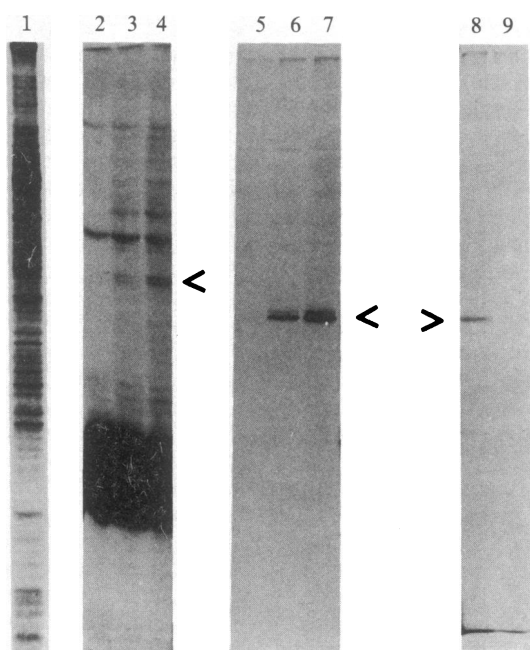
FIG. 1. Enrichment of polysomes for C-subunit mRNA and hybrid selection of C-subunit mRNA. Polysomes were prepared from bovine testis and were immunoadsorbed by using an antibody specific for the bovine C subunit. Total poly(A)$^+$ RNA (500 ng, lane 1), no RNA (lanes 2 and 5), or C-subunit-enriched poly(A)$^+$ RNA (40 ng, lanes 3 and 6; 100 ng, lanes 4 and 7) was translated in the rabbit reticulocyte lysate system with [$^{35}$S]methionine. Translated proteins were loaded directly on a 12% polyacrylamide gel (lanes 1–4) or immunoprecipitated with an anti-C antibody prior to loading (lanes 5–7). Hybridization-selection analysis of cDNA clone 128E9 (lane 8) and control DNA pBR322 (lane 9) was performed by binding plasmid DNA to nitrocellulose, selecting mRNA complementary to the DNA by hybridization, and immunoprecipitating $^{35}$S-labeled translation products prior to polyacrylamide electrophoresis. The bracket indicates a 40-kDa protein that was coded for by mRNA selected by cDNA clone 128E9 and immunoprecipitated by anti-C subunit antibody.

the mouse cDNA clones MC1, MC4, and MC8 as described (Fig. 2). Since no cDNA clone was isolated that spanned the internal EcoRI site, the continuity of nucleotide sequence between the 3' end of MC8 and the 5' end of MC4 is based on the homology of the protein coding regions of these two cDNA clones to the bovine protein sequence and analysis of the mouse genomic clone (unpublished data).

The composite sequence of 2231 nucleotides for the C-subunit mRNA as determined from the cDNA sequences is shown in Fig. 3. The sequence contains a 5' untranslated region of 186 nucleotides, a coding region of 1053 nucleotides, and a 3' noncoding region of 992 nucleotides. The 5' untranslated region contains a particularly high G+C content

(84%), which is one of the highest reported for the 5' noncoding region of a eukaryotic mRNA (29). In addition, there is a potential stem–loop structure that involves the initiator methionine as indicated in Fig. 3. This structure involves 14 contiguous base pairs with 12 perfect matches and 10 G·C base pairs. Other than this stem–loop structure, very little secondary structure was noted.

The coding region predicts a protein of 351 amino acids that is 98% homologous with the bovine C subunit. The mouse cDNA sequence predicts eight amino acid differences between the bovine (9) and mouse C subunits (Fig. 3). The amino acid residues Asn-32, Ala-34, His-39, Glu-44, Met-63, Thr-65, and Ser-348 of the bovine protein sequence are changed to Asp-32, Ser-34, Gln-39, Asp-44, Lys-63, Ser-65, and Thr-348 in the mouse protein sequence. Most of the eight amino acid differences are conservative—i.e., glutamate at position 44 in the bovine sequence is an aspartate in the mouse sequence. The least conservative change is proline at position 124 of the bovine protein to an alanine in the mouse. The lysine residue at position 63 in the mouse is also found in the porcine C subunit (11) but not in the bovine protein.

The long 3' untranslated region in itself is unremarkable; however, when compared with the sequence of the bovine cDNA clone 128E9, regions of high homology were found (Fig. 3). The mouse cDNAs sequenced had neither a poly(A)$^+$ sequence nor the canonical poly(A)$^+$ addition signal sequence (30), whereas the bovine cDNA clone 128E9 had both of these features. Since there is a strong homology between the mouse and bovine 3' untranslated regions, it was estimated that the mouse cDNA is missing only 14 nucleotides at the 3' end in as much as this is the distance from the 3' border of the homologous region to the poly(A)$^+$ tail in the bovine cDNA clone.

**Characterization of a Single C-Subunit mRNA.** Poly(A)$^+$ RNA was isolated from mouse brain and S49 lymphoma cells and subjected to blot-hybridization analysis using the C-subunit of cDNA probes (Fig. 4A). A single RNA species of 2400 nucleotides was found in these RNA samples as well as in mouse heart and mouse AtT-20 anterior pituitary tumor cells (data not shown).

**Southern Analysis of Total Mouse Genomic DNA Suggests a Single C-Subunit Gene.** Total genomic DNA was isolated from mouse liver; digested with the restriction enzymes BamHI, Pvu II, or a combination of BamHI and Bgl II; and subjected to Southern analysis with the nick-translated EcoRI/Sca I fragment of MC4 as a hybridization probe (Fig. 4B, lanes 2–5). This cDNA fragment codes for amino acids 108 through 204 of the C subunit. In all three lanes a single band was detected, suggesting that the C-subunit gene is present at only one copy per haploid genome.

**Comparison of R$^I$ and C-Subunit mRNA Levels in Mouse Tissues.** A single-stranded $^{32}$P-labeled RNA probe generated with the SP6 promoter and polymerase was used to determine the mRNA levels for the C and the R$^I$ subunits in several
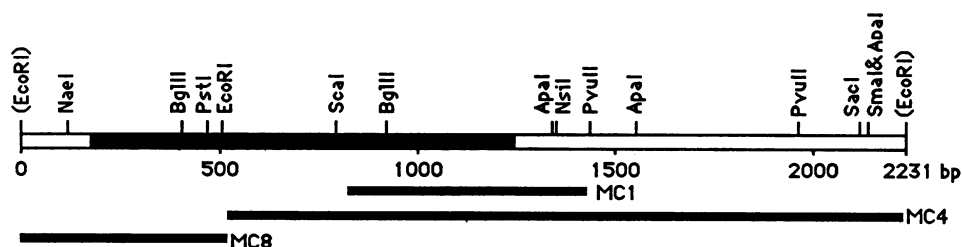


FIG. 2. Restriction map of the composite C-subunit cDNA sequence and location of C-subunit cDNA clones. The top-most bar represents the 2231-base-pair composite cDNA sequence predicted from the individual cDNA clones MC1, MC4, and MC8. Restriction sites are indicated above the bar, as is the protein coding region (solid area in bar below restriction sites). The positions and size of the black bars below the composite DNA restriction map indicate the relative size and location of individual C-subunit cDNA clones. The EcoRI sites in parentheses are derived from the linkers used in cloning.

5'    CCCCCCGCGGCGGCCGCAGAGAGACGCGGGAAGCAGGGGCTGGGCGGGGGTCGTGGCGCCGCAGCCAGCGCAGCCAGCCCCAGGGGCCGCCGCCTCCGCTGCCCAGCGCGCTCCGGGGCCGCCGGCC    113

ACCTTAGCACCCGCCGCGTCGCAGCTCCGGGACTGGCCCCGGCCGCGCACGCCGCCGCG ATG GGC AAC GCC GCC GCC GCC AAG AAG GGC AGC GAG CAG GAG AGC GTG AAA GAG TTC CTA GCC    235
                                                             MET Gly Asn Ala Ala Ala Ala Lys Lys Gly Ser Glu Gln Glu Ser Val Lys Glu Phe Leu Ala

AAA GCC AAG GAA GAT TTC CTG AAA AAA TGG GAA GAC CCC TCT CAG AAT ACA GCC CAG TTG GAT CAG TTT GAT AGA ATC AAG ACC CTT GGC ACC GGC TCC TTT GGG CGA    343
Lys Ala Lys Glu Asp Phe Leu Lys Lys Trp Glu Asp Pro Ser Gln Asn Thr Ala Gln Leu Asp Gln Phe Asp Arg Ile Lys Thr Leu Gly Thr Gly Ser Phe Gly Arg
                                              Asn         Ala              His                              50

GTG ATG CTG GTG AAG CAC AAG GAG AGT GGG AAC CAC TAC GCC ATG AAG ATC TTA GAC AAG CAG AAG GTG GTG AAG CTA AAG CAG ATC GAG CAC ACT CTG AAT GAG AAG    451
Val MET Leu Val Lys His Lys Glu Ser Gly Asn His Tyr Ala MET Lys Ile Leu Asp Lys Gln Lys Val Val Lys Leu Lys Gln Ile Glu His Thr Leu Asn Glu Lys
        Met       Thr

CGC ATC CTG CAG GCC GTC AAC TTC CCG TTC CTG GTC AAA CTT GAA TTC TCC TTC AAG GAC AAC TCA AAC CTG TAC ATG GTC ATG GAG TAT GTA GCT GGT GGC GAG ATG    559
Arg Ile Leu Gln Ala Val Asn Phe Pro Phe Leu Val Lys Leu Glu Phe Ser Phe Lys Asp Asn Ser Asn Leu Tyr MET Val MET Glu Tyr Val Ala Gly Gly Glu MET
                         100                                                                                                    Pro

TTC TCC CAC CTA CGG CGG ATT GGA AGG TTC AGC GAG CCC CAT GCC CGT TTC TAC GCG GCG CAG ATC GTC CTG ACC TTT GAG TAT CTG CAC TCC CTG GAC CTC ATC TAC    667
Phe Ser His Leu Arg Arg Ile Gly Arg Phe Ser Glu Pro His Ala Arg Phe Tyr Ala Ala Gln Ile Val Leu Thr Phe Glu Tyr Leu His Ser Leu Asp Leu Ile Tyr

CGG GAC CTG AAG CCC GAG AAT CTT CTC ATC GAC CAG CAG GGC TAT ATT CAG GTG ACA GAC TTC GGT TTT GCC AAG CGT GTG AAA GGC CGT ACT TGG ACC TTG TGT GGG    775
Arg Asp Leu Lys Pro Glu Asn Leu Leu Ile Asp Gln Gln Gly Tyr Ile Gln Val Thr Asp Phe Gly Phe Ala Lys Arg Val Lys Gly Arg Thr Trp Thr Leu Cys Gly
                                              150                                                                               200

ACC CCT GAG TAC TTG GCC CCC GAG ATT ATC CTG AGC AAA GGC TAC AAC AAG GCT GTG GAC TGG TGG GCT CTC GGA GTC CTC ATC TAC GAG ATG GCT GCT GGT TAC CCA    883
Thr Pro Glu Tyr Leu Ala Pro Glu Ile Ile Leu Ser Lys Gly Tyr Asn Lys Ala Val Asp Trp Trp Ala Leu Gly Val Leu Ile Tyr Glu MET Ala Ala Gly Tyr Pro

CCC TTC TTC GCT GAC CAG CCT ATC CAG ATC TAT GAG AAA ATC GTC TCT GGG AAG GTG CGG TTC CCA TCC CAC TTC AGC TCT GAC TTG AAG GAC CTG CTG CGG AAC CTT    991
Pro Phe Phe Ala Asp Gln Pro Ile Gln Ile Tyr Glu Lys Ile Val Ser Gly Lys Val Arg Phe Pro Ser His Phe Ser Ser Asp Leu Lys Asp Leu Leu Arg Asn Leu
                                              250

CTG CAA GTG GAT CTA ACC AAG CGC TTT GGA AAC CTC AAG GAC GGG GTC AAT GAC ATC AAG AAC CAC AAG TGG TTT GCC ACG ACT GAC TGG ATT GCC ATC TAT CAG AGA    1099
Leu Gln Val Asp Leu Thr Lys Arg Phe Gly Asn Leu Lys Asp Gly Val Asn Asp Ile Lys Asn His Lys Trp Phe Ala Thr Thr Asp Trp Ile Ala Ile Tyr Gln Arg
                                                                                                 300

AAG GTG GAA GCT CCC TTC ATA CCA AAG TTT AAA GGC CCT GGG GAC ACG AGT AAC TTT GAC GAC TAT GAG GAG GAA GAG ATC CGG GTC TCC ATC AAT GAG AAG TGT GGC    1207
Lys Val Glu Ala Pro Phe Ile Pro Lys Phe Lys Gly Pro Gly Asp Thr Ser Asn Phe Asp Asp Tyr Glu Glu Glu Glu Ile Arg Val Ser Ile Asn Glu Lys Cys Gly

AAG GAG TTT ACT GAG TTT TAG GGGTGTGCTTGTGCCCCTTGGGTTCTCTTTCATTTTTTCTTTTTCTTTCTATTTTTTTTCCGGTTGGGGGTGGGAGGGGTTGGATCGGAACAGCCAGAGGGCCCTAGAGTTCCATG    1343
Lys Glu Phe Thr Glu Phe
              Ser       350

CATCTAATTTAACATCCACTCCACACCCCCAGGGTTAAGGAGAGCAGGAAAGCGCTTCCAGATTACTGGGGAAGGGCAACATCAGCTGCTCCCCCTATCCCTTGTTGTCCACCCTTCCCTTCCTGTTTTAATGAATTTCTTAG    1500

CTCCAGCCATACCCAATCTTGCTGGTGTATCCAGGGGCAGGGTACGGAAAGAGGGCCCCAAATTCAGCCTCCTTCCCGACCCTAGCACTGGATACTAAGGATGAACGAACAGTAACGCCAACCTTCCCTTCCATGCAGCCCTA    1643

CCTGGAAAGGGAGATTTTATGACCTGTACAGAGGGCTGCTTGCCAGTGGGTTTTTTTTTTCATTTAAATTAAGTTCCACCAGTGCCTCCCACCCTCCAAATTGTCCCACCCTCCCCAAACACCCTCCTCACTCCCTAAATCAA    1786

TTCTGATGAGACCTGGGTAGCCAACTGACCCTGTCAAGGAAGGAACTGGGCTTGGAATCTCGCCCTGAGCTGCTAGCTCCCGGCCCCCCTTTCCAGTGGTCTCATGCCAATTTGTCCTGTGCATCAGCCCCCTTAAGAAGCCT    1929

CCCCATCCTGGCGCCTCGCTTCTAGCTTAGCTGTCAGCTGTCCATCACCTCTTGCCGTGCGTCCCCACTCACTGCAACCCCAAGTCTGATTGTGCTTTTTCTCTCAATAGAAAGGTGGGGAGCTGCTGGGGAAATTACCCCAT    2072

TTATCCCTGTGTTTATCCCTCGTCGTAACTTCTCCCAAAA--AGGAGGAGCTCTCAGGCCTGGGTGGGGGCCCCGGGTGGACGAGGGGGTCGTCAACCTGTGTGCTTCAAAGGATGAGAC-TTCCTCTTGAACAGTGTGCTGT    2212
        ACATTTGTT----TCCATCATTTCTCCCCATCCTAGGAGGAGCTCTCAGAC                                                TGATTCAACCTGTGTGCTGCGAAGGACGCAACTTTCCTCTTGAACAGTGTGC-GT

TGTAAACATATTTGAAAAC                    3'
TGTAAACATATTTGAAACATACAATAAAGTTTGTA[N]

FIG. 3. Nucleotide sequence of the composite mouse C-subunit cDNA. The number of the last nucleotide in each line is shown at the right, and the translated protein sequence is shown below the nucleotide sequence. Amino acid residues are numbered by assuming a position of −1 for the formylmethionyl residue. The nucleotide sequences that can form a stem–loop structure involving the initiator methionine are underlined. The sequenced regions of 128E9 are shown near the 3' end, and regions of nucleotide homology are underlined in the mouse sequence. The first six and last six nucleotides of the mouse sequence correspond to the EcoRI linkers used in cDNA cloning.

tissues and cell lines (Fig. 5). The level of C-subunit mRNA varied from 10 molecules per cell in skeletal muscle to 25 molecules per cell in liver, heart, and S49 lymphoma cells. The $R^I$-subunit mRNA ranged from 12 molecules per cell in skeletal muscle to 60 molecules per cell in S49 lymphoma cells. In most cases the levels of $R^I$ and C-subunit mRNA were similar for a given tissue, although a slight excess of $R^I$ mRNA over C mRNA was seen in all cases. We have observed a larger excess of $R^I$ subunit in mouse cell lines as shown in Fig. 5 for S49 lymphoma and NIH 3T3 cells.

## DISCUSSION

We report the cloning and sequence of a full-length cDNA for the mouse C subunit of cAMP-dependent protein kinase. The nucleotide sequence predicts an amino acid sequence for the mouse C subunit that is very homologous to the bovine (9) and partial porcine (10) sequences. The amino-terminal glycine found in the bovine C subunit is also conserved in the mouse sequence. This amino acid is myristylated in the bovine protein (14), and a similar amino-terminal residue in the *src* gene product has been shown to be required for myristylation, membrane localization, and transforming activity (15). However, myristylation of the C subunit of cAMP-dependent protein kinase does not appear to localize it to cellular membranes (2). In addition, the phosphorylated

residues of the bovine C subunit (14) are also conserved in the mouse protein. The availability of a full-length cDNA will make possible experiments designed to define the role these modifications play in transduction of the cAMP response.

The third interesting feature of the C-subunit cDNA is a striking homology between the 3' untranslated regions of the mouse and bovine sequences. This type of evolutionary conservation of 3' untranslated sequences is sometimes seen in mRNAs that code for well-conserved proteins such as the actins (31); the function that these sequences serve is unknown.

Southern analysis of total genomic DNA indicates a single gene for the C subunit. Additionally, characterization of phage λ genomic clones isolated by hybridization with the cDNA probes (J.C.C., unpublished data) also indicates a single gene for the C subunit gene. This is in agreement with previous characterization at the protein level that has shown similar physical and enzymatic properties for C subunits isolated from the two types of protein kinase and indicates that heterogeneity of C-subunit protein seen after isoelectric focusing (7) is probably due to posttranslational modification.

Using the mouse C-subunit cDNA to generate probes, we initiated characterization of C-subunit gene expression. We find a single 2.4-kb transcript in mouse brain and heart. We also demonstrated a relative correlation of $R^I$ and C mRNAs
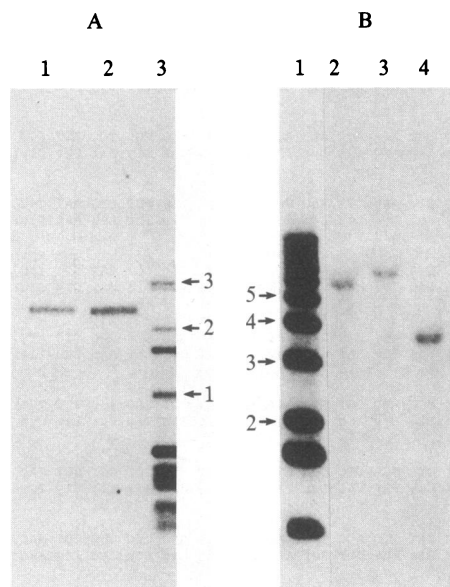
FIG. 4.   Hybridization analysis of C-subunit mRNA and genomic sequences. (*A*) Blot-hybridization analysis of C-subunit mRNA. Poly(A)$^+$ RNA (10 $\mu$g) was isolated from mouse brain (lane 1) and S49 lymphoma cells (lane 2), electrophoresed under denaturing conditions on a 1% agarose gel, and blotted to nitrocellulose. The nitrocellulose blot was hybridized to nick-translated MC4 insert DNA. Lane 3 shows the $^{32}$P-labeled size markers with the size of the bands indicated at right. (*B*) Southern analysis of the C-subunit gene. Size markers (lane 1) or 10 $\mu$g of total genomic DNA isolated from mouse liver that had been digested with *Bam*HI (lane 2), *Pvu* II (lane 3), or a combination of *Bam*HI and *Bgl* I (lane 4) were electrophoresed through a 0.8% agarose gel. The gel was blotted to nitrocellulose, hybridized under conditions of high stringency to the nick-translated *Eco*RI/*Sca* I fragment of MC4, and autoradiographed. The size of some bands in the size markers (lane 1) are indicated in kb; the uppermost band in the size markers corresponds to a size of 12 kb.

in most tissues analyzed. Since many tissues contain the type II R subunit (R$^{II}$) at levels equal to or greater than that of R$^{I}$ subunit (32, 33), the total R-subunit mRNA level is expected to be well in excess of that for the G-subunit mRNA. This is consistent with the finding in S49 lymphoma cells that there may be a pool of free R$^{I}$ protein with a short degradative half-life (34). Presumably this pool of free R$^{I}$ protein ensures that there is negligible free C subunit and, therefore, a low level of basal protein kinase activity from the C subunit in the absence of cAMP. The availability of a full-length C-subunit cDNA will allow a detailed characterization of the role
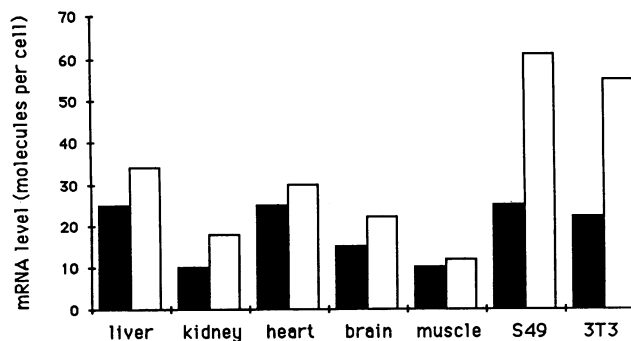


FIG. 5.   C (solid bars) and R$^{I}$ (open bars) mRNA levels in various mouse tissues and cell lines. The levels of both subunit mRNAs for each sample were determined by using an SP6 RNA transcript complementary to each subunit mRNA. Molecules per cell were calculated by comparison to M13 standards.

cAMP-dependent protein kinase plays in regulation of gene transcription and cell growth.

**Note Added in Proof.** Using less stringent hybridization conditions than those shown in Fig. 4, we have recently isolated mouse cDNA and genomic clones that code for a second type of catalytic subunit.

1.  Flockhart, D. A. & Corbin, J. D. (1982) *Crit. Rev. Biochem.* **12**, 133–186.
2.  Lohmann, S. M. & Walter, U. (1984) *Adv. Cyclic Nucleotide Res.* **18**, 63–117.
3.  Krebs, E. G. & Beavo, J. A. (1979) *Annu. Rev. Biochem.* **48**, 923–959.
4.  Nesterova, M. V., Ulmasov, K. A., Abdukarimov, A., Aripdzhanov, A. A. & Severin, E. S. (1981) *Exp. Cell Res.* **132**, 367–373.
5.  Constantinou, A. I., Squinto, S. P. & Jungmann, R. A. (1985) *Cell* **42**, 429–437.
6.  Weldon, S. I., Mumby, M. C. & Taylor, S. S. (1985) *J. Biol. Chem.* **260**, 6440–6446.
7.  Zoller, M. J., Kerlavage, A. R. & Taylor, S. S. (1979) *J. Biol. Chem.* **254**, 2408–2412.
8.  Takai, Y., Yamamura, H. & Nishizuka, Y. (1974) *J. Biol. Chem.* **249**, 530–535.
9.  Shoji, S., Ericsson, L. H., Walsh, K. A., Fischer, E. H. & Titani, K. (1983) *Biochemistry* **22**, 3702–3709.
10.  Bramson, H. N., Thomas, N., Matsueda, R., Nelson, N. C., Taylor, S. S. & Kaiser, E. T. (1982) *J. Biol. Chem.* **257**, 10575–10581.
11.  Zoller, M. J., Nelson, N. C. & Taylor, S. S. (1981) *J. Biol. Chem.* **256**, 10837–10842.
12.  Barker, W. C. & Dayhoff, M. O. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 2836–2839.
13.  Shoji, S., Titani, K., Demaille, J. G. & Fischer, E. H. (1979) *J. Biol. Chem.* **254**, 6211–6214.
14.  Carr, S. A., Biemann, K., Shoji, S., Parmelee, D. C. & Titani, K. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 6128–6131.
15.  Kamps, M. P., Buss, J. E. & Sefton, B. M. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 4625–4628.
16.  Steinberg, R. A., van Daalen Wetters, T. & Coffino, P. (1978) *Cell* **15**, 1351–1361.
17.  Copeland, N. G., Zelenetz, A. D. & Cooper, G. M. (1979) *Cell* **17**, 993–1002.
18.  Lee, D. C., Carmichael, D. F., Krebs, E. G. & McKnight, G. S. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 3608–3612.
19.  Friedman, R. L., Manly, S. P., McMahon, M., Kerr, I. M. & Stark, G. R. (1984) *Cell* **38**, 745–755.
20.  Breathnach, R. & Chambon, P. (1981) *Annu. Rev. Biochem.* **50**, 349–383.
21.  Maxam, A. M. & Gilbert, W. (1980) *Methods Enzymol.* **65**, 499–560.
22.  Messing, J. (1983) *Methods Enzymol.* **101**, 20–78.
23.  Davis, R. W., Thomas, M., Cameron, J., St. John, T. P., Scherer, S. & Padgett, R. A. (1980) *Methods Enzymol.* **65**, 404–411.
24.  Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
25.  Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113**, 237–251.
26.  Melton, D. A., Kreig, P. A., Rebagliati, M. R., Maniatis, T., Zinn, K. & Green, M. R. (1984) *Nucleic Acids Res.* **12**, 7035–7056.
27.  McKnight, G. S., Lee, D. C., Hemmaplardh, D., Finch, C. A. & Palmiter, R. D. (1980) *J. Biol. Chem.* **255**, 144–147.
28.  Labarca, C. & Paigen, K. (1980) *Anal. Biochem.* **102**, 344–352.
29.  Kozak, M. (1984) *Nucleic Acids Res.* **12**, 857–872.
30.  Proudfoot, N. J. & Brownlee, G. G. (1976) *Nature (London)* **263**, 211–214.
31.  Yaffe, D., Nudel, U., Mayer, Y. & Neuman, S. (1985) *Nucleic Acids Res.* **13**, 3723–3737.
32.  Corbin, J. D., Keely, S. L. & Park, C. R. (1975) *J. Biol. Chem.* **250**, 218–225.
33.  Haddox, M. K., Roeske, W. R. & Russell, D. H. (1979) *Biochim. Biophys. Acta* **585**, 527–534.
34.  Steinberg, R. A. & Agard, D. A. (1981) *J. Biol. Chem.* **256**, 11356–11364.