Structure and characterization of the genes for murine choline/ethanolamine kinase isozymes α and β[1]

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Abstract Choline/ethanolamine kinase (CK/EK) is the first enzyme in phosphatidylcholine/phosphatidylethanolamine biosynthesis in all animal cells. The highly purified CKs from mammalian sources and their recombinant gene products so far were all shown to have EK activity also, indicating that both activities reside on the same protein. CK/EK in most animal cells exists as several isoforms, for two of which (α and β) their cDNAs have been cloned from both the rat and mouse, and they are found to be separate gene products. The physiological significance for the existence of more than one CK/EK enzyme, however, remains to be clarified. In this study, we isolated mouse genes encoding both types of CK/EK isozyme and determined their entire structure. The 5′-flanking promoter regions were found to have quite different features from each other, indicating that their expression could be under distinct control. Comparison of the nucleotide sequence between the corresponding coding exons showed the best homology (75%) residing on exon VIII. A search of the database resulted in the possible existence of 17 different origins of eukaryotic CK and/or EK, each of which presumably contained the entire amino acid sequence. Multialignment of their putative amino acid sequences led to an identification of the novel consensus sequence possibly required for the expression of either CK or EK activity, which corresponded to the sequence within exons VII and VIII of CK/EK-α and -β genes from the mouse. This sequence was localized in close proximity to the C-terminal region of the general (Brenner's) phosphotransferase concensus sequence which was also completely conserved in all of the putative eukaryotic CK/EK proteins. The results demonstrated that, while both CK/EK-α and -β genes were composed of 11 major exons, the size of their genes was quite different: 40 kb for CK/EK-α, whereas it was only 3.5 kb for CK/EK-β.—Aoyama, C., N. Yamazaki, H. Terada, and K. Ishidate. **Structure and characterization of the genes for murine choline/ethanolamine kinase isozymes α and β. *J. Lipid Res.* 2000.** 41: **452–464.**

Supplementary key words choline kinase • ethanolamine kinase • gene structure • isozymes (isoforms) • carnitine palmitoyltransferase I (M-CPTI) • phosphatidylcholine biosynthesis • phosphatidylethanolamine biosynthesis

Choline kinase (CK) (ATP: choline phosphotransferase, EC 2.7.1.32) and ethanolamine kinase (EK) (ATP: ethanolamine O-phosphotransferase, EC 2.7.1.82) catalyze the phosphorylation of choline/ethanolamine by ATP yielding phosphocholine/phosphoethanolamine. This step commits choline/ethanolamine to the CDP-choline/CDP-ethanolamine pathway for the biosynthesis of phosphatidylcholine (PC)/phosphatidylethanolamine (PE) in all animal cells. Although the CTP: phosphocholine (phosphoethanolamine) cytidylyltransferase, the second enzyme in the pathway, is commonly referred to as the rate-limiting step in PC (PE) biosynthesis (1, 2), considerable evidence indicates that CK is also a slow step and can be regulatory for PC biosynthesis in a number of situations where increased (decreased) CK activity results in a similarly increased (decreased) rate of PC biosynthesis (2, 3).

CK has been purified to apparent homogeneity from rat kidney (4), liver (5), and brain (6) and the purified preparations were all shown to have significant EK activities. These as well as other (7, 8) investigations suggested also that CK/EK does not exist in one particular active form but exists in several isoforms in rat tissues. Uchida and Yamashita (9) were the first to report a cloning of the mammalian CK cDNA from rat liver cDNA library. The cloned cDNA had an ORF encoding a protein of 435 amino acids with a calculated molecular size of 50 kDa

Abbreviations: CK, choline kinase; EK, ethanolamine kinase; PC, phosphatidylcholine; PE, phosphatidylethanolamine; ORF, open reading frame; M-CPTI, carnitine palmitoyltransferase I (muscle type); DIG, digoxigenin; 5′-RACE, rapid amplification of cDNA 5′-end; XRE, xenobiotic responsive element; PAH, polycyclic aromatic hydrocarbon; CCl_4, carbon tetrachloride; pfu, plaque-forming unit.

[1] The nucleotide sequence data reported in this paper have been submitted to the DDBJ/EMBL/GenBank nucleotide sequence databases with the Accession Numbers of AB030616 (exon I), AB030617 (exon II), AB030618 (exon II′), AB030619 (exon III), AB030620 (exon IV–VIII), AB030621 (exon IX–XI) for CK/EK-α and AB030615 (complete cds.) for CK/EK-β.

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(tentatively termed CK/EK-α1 in this paper[3]). Expression in *E. coli* of this cDNA resulted in high production of not only CK but also significant amount of EK activities in the cell lysate which otherwise lacking these activities. Subsequently, Uchida (10) isolated another CK/EK cDNA from the same library in which an extra 54 b internal sequence was inserted into that of CK-α1 probably through an alternative splicing, thus predictedly encoding 453 amino acids with a molecular size of 52 kDa (termed CK/EK-α2). A human homolog to the rat α2 cDNA has previously been isolated by Hosaka et al. (11) from a glioblastoma cDNA library. On the other hand, we have recently isolated a cDNA clone for a 42 kDa (estimated by SDS-PAGE) rat kidney CK/EK encoding a protein of 394 amino acids with a calculated molecular size of 45 kDa (12). The predicted amino acid sequence showed only 57–59% homology to either CK/EK-α1 or -α2, indicating that the 42 kDa rat kidney CK/EK (termed CK/EK-β) must be a product of a gene distinct from that for CK/EK-α. Most recently, the mouse cDNA homologs to both CK/EK-α(α1/α2) and -β have been cloned in our laboratory (13).

The most intriguing characteristic feature of CK/EK in mammals could be its inducibility in various experimental systems (for review, see refs. 14–16). Although the exact physiological meaning of the inducibility has not been fully understood, the induction of CK/EK by several means suggests that the enzyme plays an important role in the long-term regulation of the CDP–choline pathway and may be involved in the coordinate regulation of PE biosynthesis. Alternatively, the induction of CK/EK could be associated with the increased generation of phosphocholine/phosphoethanolamine which has been proposed recently as an essential event in the activation of MAP-kinase cascade during certain growth factor-induced mitogenic stimulation (2, 16, 17). The latter mechanism appears to be independent of the rate of net PC/PE biosynthesis.

In this study, we have isolated mouse genes for both CK/EK-α and -β and determined their entire structures. The 5′-flanking region of approximately 4 kb each has been sequenced and searched for the possible *cis*-elements. Comparison of the nucleotide sequence between the corresponding coding exons of CK/EK-α and -β has led us to determine finally a novel consensus amino acid sequence which must be critical for the expression of not only CK but also EK activity in all eukaryotic cells. In addition, the gene for CK/EK-β was shown to be located only 300 b (human) or 560 b (mouse and rat) (N. Yamazaki, Y. Shinohara, K. Kajimoto, M. Shindo, and H. Terada, unpublished observations) upstream of the gene for an isozyme (muscle-type) of carnitine palmitoyltransferase I (M-CPTI) (18, 19).

[3] We suggest that the previous nomenclatures for the 50 kDa and 52 kDa CK/EK isoforms (CKR1 and CKR2 for the rat enzyme), both of which have been shown to be derived from the same gene with an alternative splicing, should be denoted hereafter together as CK/EK-α, or α1 and α2, respectively, for individual isoforms. Then, another isozyme, 42 kDa CK/EK should be denoted as CK/EK-β, as this form was shown to be a product of separate gene from that of CK/EK-α.

EXPERIMENTAL PROCEDURES

Materials

All restriction endonucleases with their reaction buffers and other enzymes were purchased from TaKaRa and TOYOBO, Japan. A PCR DIG Probe Synthesis Kit, DIG Easy hyb, DIG Wash and Block Buffer Set, Anti-digoxigenin-AP Fab Fragment and CSPD were from Boehringer Mannheim. The DNA preparation kit was obtained either from Qiagen (Lambda System for phage DNA) or Promega (Wizard Minipreps for plasmid or PCR-amplified DNAs). The *Taq* polymerases (r*Taq* and Ex*Taq*) were from TaKaRa, Japan.

Isolation and characterization of the mouse CK/EK-α genomic clones

Two DIG-labeled PCR products amplified from a mouse CK/EK-α cDNA (13) by sets of primers (probe-1: 5′-CTGGAGC AGTTTATCCC-3′ and 5′-CCAAGCTTCCTCTTCTG-3′, and probe-2: 5′-GCCTACCTGTGGTGTAAGGA-3′ and 5′-TTCATAC CATGAAATGTGGCC-3′) were used for screening a mouse 129/SV genomic DNA library in λFIXII (STRATAGENE, Cat. No. 946305). Three phage clones containing the part of a CK/EK-α gene, one (clone 2-1) by probe-1 and the other two (clones 17-1 and 28-1) by probe-2, were obtained. These three clones were further confirmed by Southern blot analysis using the corresponding DIG-labeled probes (probe-1 located in the 3′-region 709-1463 and probe-2 in the 5′-region 421-812, of the CK/EK-α cDNA) in a DIG Easy hyb solution, following the protocol of the manufacturer. After subcloning of restriction fragments into pT7Blue (NOVAGEN) or pBluescript II (STRATAGENE), most of their sequences were determined and mapped. The nucleotide sequence was analyzed by ABI PRIZM 377 DNA sequencer using a Dye Terminator Cycle Sequencing Kit (Applied Biosystems). While we could map exon I through exon II′ and exon IV through exon XI, exon III could not be found in any of these clones. Then, we examined genomic PCR (Ex *Taq*) using a whole DNA isolated from 129/SV ES cells as a template (upper primer: 5′-CTGGAGCAGTTTATCCC-3′ and lower primer: 5′-TCTTC AGTGTCCAATCGCC-3′) and finally mapped the exon III at 1.3 kb upstream of the exon IV. In similar fashions, the nucleotide distance between exon I and exon II (upper primer: 5′-GGCT GCCACAAATAATCCTC-3′ and lower primer: 5′-GCTGT CAAAGTACAGGGAAG-3′) as well as exon II′ and exon III (upper primer: 5′-CTGGCACCCACATTCTTGTTC-3′ and lower primer: 5′-TGACCTCTCTGCAAGAATGGC-3′) was estimated by a genomic PCR analysis.

Isolation of the mouse genomic clones for M-CPTI and their characterization

Two positive clones (MG3 and MG5) were isolated from a ddy mouse genomic library constructed in λFIXII by screening with a mouse M-CPTI cDNA probe (−1 to 466 where a putative translation start codon ATG was numbered as +1; N. Yamazaki, Y. Shinohara, K. Kajimoto, M. Shindo, and H. Terada, unpublished observations). A multipriming method (*Redi* prime DNA Labeling System from Amersham) with [α-^{32}P]dCTP was used instead of the DIG-PCR labeling method for the preparation of a probe. A library was constructed with partially Sau3AI-digested mouse (25-weeks male) liver DNA fragments into λFIXII Partial Fill-in vector (STRATAGENE) according to the manufacturer's protocol, then the ligated products were packaged in vitro with a Gigapack III XL Packaging Extract (STRATAGENE). A titer of approximately 5.4×10^5 pfu was obtained. Both MG3 and MG5 were found to have an entire M-CPTI gene and its 5′-flanking region. As we had been aware of the presence of a CK/EK-β gene in short

upstream of the M-CPTI gene in either human (18) or rat (19), we first tried to confirm by Southern blot whether or not these clones actually had a CK/EK-β gene in their inserts. Two DIG-labeled PCR products amplified either from a mouse CK/EK-β cDNA (13) by a set of primers (5′-region probe: 5′-CTATCAGTGGTGCCGG GAGT-3′ and 5′-CGCTCACGGGACAAACGTTC-3′, 446–526 of the cDNA) or from a rat CK/EK-β cDNA (12) by another set of primers (internal and 3′-region probe: 5′-TTGTTTGGGAC CATGGAGCG-3′ and 5′-TTCTGCTGGAAGTAGAACTG-3′, which correspond to 846–1432 of the rat CK/EK-β cDNA) were used as probes for Southern blot. A rat cDNA clone 3–1 (12) was used as a PCR template for the preparation of the internal and 3′-region probe because a mouse cDNA clone m2–1 (13) was found to have several intron sequences in its structure. After subcloning of restriction fragments into appropriate plasmid vectors as described above, their sequences were determined thoroughly and mapped. Finally, it was found that both MG3 and MG5 contained the entire sequence of mouse CK/EK-β gene and clone MG5 had a sequence of the longer 5′-flanking region.

5′-RACE for the mouse kidney mRNA

Total RNA was extracted from freshly isolated mouse kidney with ISOGEN (Nippon Gene Inc., Japan). Then, mRNA was purified with an Oligo (dT)-cellulose column (mRNA Purification Kit from Pharmacia Biotech). One microgram of the purified mRNA was used for every 5′-RACE reaction according to the manufacturer's (GIBCO BRL) protocol. For the reverse transcriptase reaction, 5′-TTCATACCATGAAATGTGGCC-3′ (CK/EK-α) or 5′-CGCTCACG GGACAAACGTTC-3′ (CK/EK-β) was used as a primer. The tailing reaction to a 3′-end of the resulting 1st strand cDNA, oligo (dA) was used instead of oligo (dC) for CK/EK-α because it was found that the 5′-region of its cDNA had an extremely GC-rich sequence. The first PCR was performed with a set of 5′-RACE Abridged Anchor Primer carrying oligo (dT) or oligo (dG) and a gene specific primer, 5′-TCTTCAGTGTCCAATCGCC-3′ (CK/EK-α) or 5′-ATC CGCCTATCAGCTCTGC-3′ (CK/EK-β). The second nested PCR was then carried out with a set of primers, the Abridged Universal Amplification Primer and either 5′-TCCTTACACCACAGGTAGGC-3′ (CK/EK-α) or 5′-AGGCAAAGAGGGACGGCAG-3′ (CK/EK-β). The amplified DNA band on agarose gel was cut off and extracted with Wizard Minipreps resin (Promega), then subjected to TA cloning (pT7Blue Vector from NOVAGEN and Ligation High form TOYOBO, Japan) and sequenced.

Cap Site hunting

Cap Site cDNA™ from the mouse brain was obtained from Nippon Gene, Japan and the first PCR was performed with a set of primers, 1CR2 (included in the Kit) and CK/EK-β gene-specific primer 5′-CGCTCACGGGACAAACGTTC-3′. The second nested PCR was performed with 2RC2 (in the Kit) and 5′-ATG ACGTGGGCTCCGTTAGG-3′ as primers. The amplified DNA fragment was purified as described above, then subjected to TA cloning and sequenced.

RESULTS

Highly conserved domains among the putative amino acid sequences of CK/EK-α and CK/EK-β from the mouse, rat, and human

The entire amino acid sequences deduced from the cloned cDNA of mouse CK/EK-α (13) and CK/EK-β (13), rat CK/EK-α (9, 10) and CK/EK-β (12), and human CK/EK-α (11) are aligned in **Fig. 1A**, together with that of the hypothetical human CK/EK-β which has been assumed from the genome sequence. We found that the genomic DNA sequence for the putative human CK/EK-β reported in the DNA database (Accession No. U62317) had a sequencing error in its coding exon I which resulted in a frameshift to deduce an incorrect N-terminal amino acid sequence. The human CK/EK-β sequence corrected by our hand (Accession Nos. AB029885 and AB029886 in the DDBJ/EMBL/GenBank) is shown in Fig. 1A. All of CK/EK-α isozymes from mouse, rat, and human have been shown to have α1 and α2 isoforms, the latters of which have extra 18 amino acid sequences (Fig. 1B) inserted at the position indicated by an arrowhead in Fig. 1A.

The similarity scores of the entire amino acid sequences for CK/EK-α (α1) are: 97.9% between mouse and rat, 87.5% between mouse and human, and 88.4% between rat and human. The scores for CK/EK-β are: 95.9% between mouse and rat, 86.0% between mouse and human, and 85.6% between rat and human. On the other hand, the similarity scores of the entire amino acid sequences between α (α1) and β isozymes are 59.7% for both the mouse and rat and 60.6% for the human. These results clearly indicated that CK/EK-α and -β must be separate gene products in all of these animal species. In addition, the multialignment data for the putative entire amino acid sequences of CK/EK-α and -β from the mouse, rat, and human provided evidence for the presence of several highly conserved sequence domains expanding from N-terminal to C-terminal regions (d-1 to d-8 in Fig. 1A). These domains were supposed to be essential parts of catalytic and/or regulatory functions in CK/EK. One of the most highly conserved domains (d-6) contained a Brenner's (20) phosphotransferase consensus sequence (**H**x**Dh**xxx**Nhhh**.... ..**D**, where **h** stands for large hydrophobic amino acids, FLIMVWY), thus indicating this portion possibly to be involved in the catalytic function. As shown in Fig. 1B, an additional highly conserved domain existed in the internal 18 amino acid sequence of the α2 isoforms. Although the physiological meaning for the existence of two isoforms of CK/EK-α (α1 and α2) as well as the difference in their catalytic functions are presently unknown, the highly conservative nature of this inserted sequence probably indicates an additional regulatory function with the α2 isoform.

Structure of the mouse gene for CK/EK-α

A 129/SV strain mouse genomic library in λFIXII was screened with mouse CK/EK-α1 cDNA probes. Three independent phage clones, 17-1, 28-1, and 2-1 were isolated and most parts of their nucleotide sequences were determined. As shown in **Fig. 2**, clones 17-1 had only exon I which contained the putative translation start codon, and clone 28-1 contained exon II and the alternatively spliced exon II′, the latter of which encodes the inserted 18 amino acids in the α2 isoform. Clone 2-1 was found to include the coding sequences for exon IV through exon XI plus 3′-noncoding region. The sequence corresponding to exon III, however, could not be found in either clone 28-1 or 2-1. Further analysis by genomic PCR with 129/SV

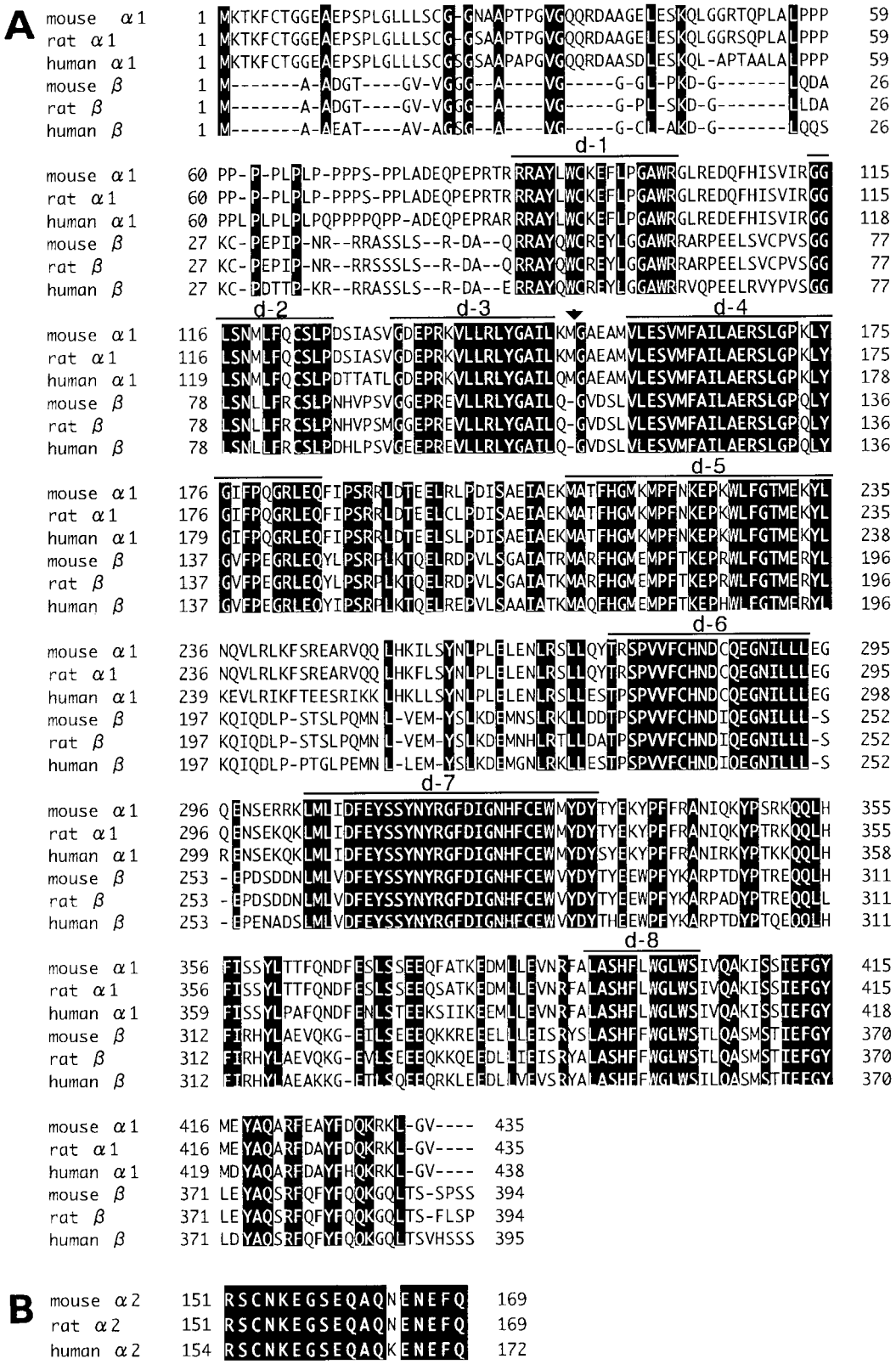

Fig. 1. Comparison of the putative amino acid sequences of CK/EK-α and CK/EK-β from the mouse, rat, and human. Origins of cDNAs are as follows: mouse CK/EK-α and -β, Aoyama et al. (13); rat CK/EK-α, Uchida and Yamashita (9, 10); rat CK/EK-β, Aoyama et al. (12); human CK/EK-α, Hosaka et al. (11); and human CK/EK-β, Yamazaki (Accession No. AB029885 in DDBJ/EMBL/GenBank). The isoforms α1 and α2 have been shown to be the splicing variants from CK/EK-α gene, in which an extra 18 amino acid sequence (corresponding to exon II′) is incorporated (the site is indicated by an arrowhead) with α2. The highly conserved domains (tentatively defined as the regions of more than 75% matching in at least 10 amino acids with no consecutive mismatch) among six mammalian sequences are numbered from d-1 to d-8. A Brenner's (20) phosphotransferase consensus sequence (**H**x**Dh**xxx**Nhhh**... ...**D**, where **h** stands for large hydrophobic amino acids, FLIMVWY) is located in d-6.

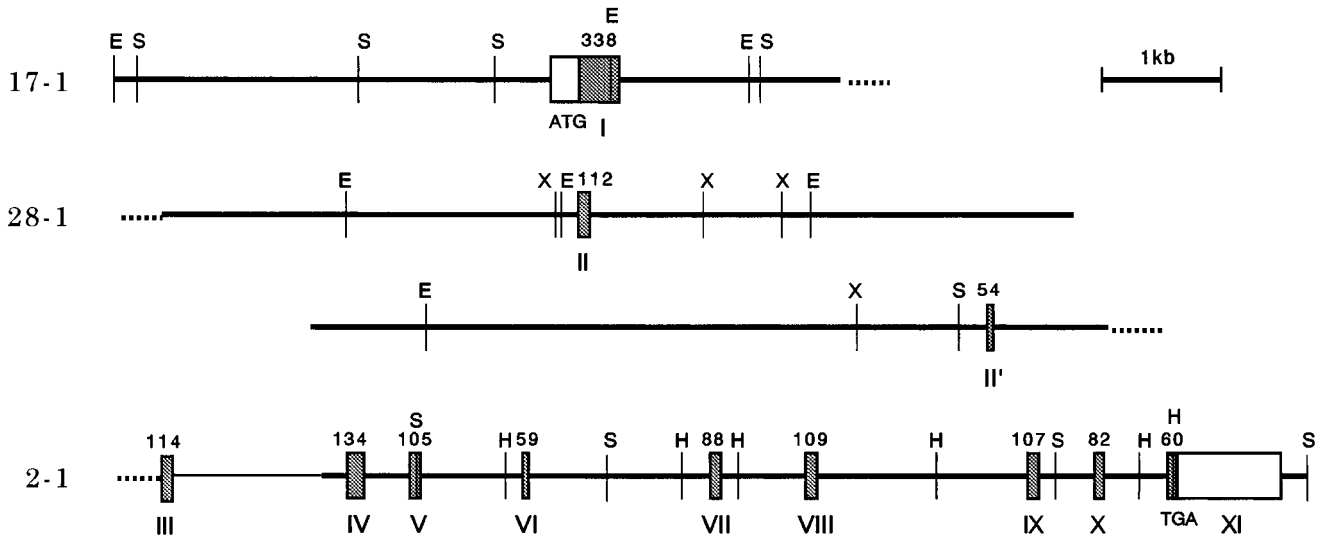

Fig. 2. Structure of the mouse gene for CK/EK-α. The 129/SV mouse genome library (λFIXII) was screened with mouse CK/EK-α cDNA probes. Three independent phage clones (17-1, 28-1, and 2-1) were isolated and the most parts of them were sequenced. The boxes (open, non-coding; and hatched, coding) and thick lines indicate exons and introns, respectively. The thick dotted lines and thin line indicate the parts not involved in the isolated clones and both the size of them and the location of exon III were determined by genomic PCR as 129/SV ES cells-derived DNA as a template. Exon II′ has been shown to be spliced out with an α1 isoform but retained with an α2 isoform. The nucleotide size in each coding exon is indicated above the box and sites of several restriction enzymes (E, EcoRI; S, SacI; X, XbaI; and H, HindIII) are also indicated.

ES cells-derived DNA as a template revealed that exon III (114 b) is located approximately 1.3 kb upstream of exon IV. Similar genomic PCRs also revealed the nucleotide distance between clone 17-1 and 28-1, and that between clone 28-1 and exon III. Thus, the overall results demonstrated that the mouse gene for CK/EK-α is composed of 11 major exons and 1 extra alternatively spliced exon (exon II′). While the structure from exon III to exon XI was relatively compact in size, the first four exons including exon II′ were shown to be split by extremely large introns (Fig. 2 and **Table 1A**). The entire size of mouse CK/EK-α gene was thus estimated to be approximately 40 kb. The exon/intron organization of the CK/EK-α gene is shown in Table 1A. A 5′-splice site sequence (CA**A**gt) of the alternatively spliced intron 2′ was found not in good match for its general consensus sequence MA**G** preceding gt (21). Another unmatched 5′-splice site sequence (AA**A**gt) was seen with an intron 4. We have identified that some mouse and rat cDNA clones, though not very frequently, had an intron 4 that remained unsplit (data not shown), which might be the result of its weak 5′-splice site signal.

Nucleotide sequence in the 5′-flanking promoter region of mouse CK/EK-α gene and locations of the putative *cis*-elements

The 5′-flanking promoter region of CK/EK-α gene together with the possible *cis*-elements are shown in **Fig. 3**. The putative transcription start sites estimated by 5′-RACE (mouse kidney) are also indicated. While a TATA box was not found in the proximity of the start site, several CCAAT and GC boxes (SP-1 binding sites) were found in the very proximal region, showing a house-keeping nature of CK/EK-α gene. On the other hand, a number of regulatory as well as tissue-specific transcription enhancer elements were also found. Especially, two sites for xenobiotic responsive element (XRE), which has been shown to exist in the promoter region of a number of polycyclic aromatic hydrocarbon (PAH)-inducible genes (22–26), were found in the proximal region. As we reported earlier (27), the activity of CK/EK in the liver was highly inducible by treatment of rats with several PAHs. The possible function of these XREs in CK/EK-α gene expression is now under investigation with mouse Hepa-1 cells. We also reported that the expressions of both CK/EK-α and -β were extremely high in testis when compared to other rat tissues examined (13). Although it is not yet clear at present that the same is true for the mouse, it should be noted that there are several sites for both testis-specific (SRY) (28) and tissue-specific (GATA-1) (29) transcription factors in the proximal promoter region of CK/EK-α gene.

Structure of the mouse gene for CK/EK-β and its comparison to the hypothetical human CK/EK-β gene structure

Through the isolation and sequencing of cDNAs for the rat and mouse CK/EK-β, we found that the gene for murine CK/EK-β is localized in short upstream of the M-CPTI gene. The human gene homologous to the murine CK/EK-β has also been reported in EMBL/GenBank/DDBJ database by the Human Genome Project to be located shortly upstream of the M-CPTI gene. We had previously isolated ddy mouse genome clones MG3 and MG5, both of which were found to contain the entire M-CPTI gene and its 5′-flanking region. By sequencing the upstream region of these clones, we obtained the entire structure of CK/EK-β gene and its 5′-flanking promoter

TABLE 1. Exon/intron organizations of the mouse CK/EK-α (A) and CK/EK-β (B) genes

Intron No.	5′-Splice Site	Approximate Intron Size	3′-Splice Site
		kb	
(A)CK/EK-α			
1	CATCAG**gt**cagt	11.4	gtattctcttgc**ag**GGGTGG
2	AAGATG**gt**gagt	9.8	tttctcattcat**ag**AGGTCC
2′	TTT**CAA**gtaagt	8.0	gccatctgttttag**GGGGCT**
3	ATCCCG**gt**aaga	1.3	attcctgtttcc**ag**AGCCGG
4	GGA**AAA**gtaagt	0.4	ccttcctatggc**ag**ATACCT
5	CCTGAG**gt**gagt	0.9	ctttctttttcc**ag**GTCATT
6	AAGAAG**gt**aaga	1.6	caaatctcttgt**ag**GTAATA
7	TTACAG**gt**aatg	0.8	gtcctctctttc**ag**GGGATT
8	CAACAG**gt**agga	1.8	aattctttttta**ag**CTCCAT
9	CAACAG**gt**aagc	0.5	gtttgtgattgc**ag**ATTTGC
10	TACATG**gt**aagt	0.6	tcggtctcttcc**ag**GAATAT
		Intron Size	
(B)CK/EK-β		*b*	
1	CGTGAG**gt**cagg	219	gtgtggatctgc**ag**CGGAGG
2	CTGCAG**gt**gagg	221	acatattttatc**ag**GGTGTA
3	CTC**CCA**gtaaga	169	gttactgggtac**ag**AGCCGG
4	GGAGCG**gt**gagt	181	tgggcggcttcc**ag**GTACCT
5	CCTCAG**gt**gaag	118	ctctacctctac**ag**GAAGTT
6	AGGAAG**gt**agga	83	ccccattcccct**ag**GAAACA
7	CTACAG**gt**aagt	122	ctctatcctccc**ag**GGGCTT
8	CAGCAG**gt**atat	133	tccattgatctc**ag**CTCCAT
9	CAGTCG**gt**gaga	431	ccctcgttccct**ag**GTACTC
10	TACTTG**gt**aagt	148	gattccttcctt**ag**GAGTAT
(C) General consensus sequence			
	MAG**gt**ragt		$(y)_n$nyag**G**

Capital and small letters indicate parts of exon and intron sequences, respectively (M, A or C; R, A or G; Y, pyrimidine nucleotides; N, any nucleotides).

region. The result demonstrated that the mouse CK/EK-β gene is composed also of 11 exons splitted by 10 short introns with its entire size of only about 3.5 kb (**Fig. 4A**). The coding exon sizes and their organization were found to be quite similar to those of CK/EK-α gene (Fig. 2 and **Table 2**) except for the small difference in sizes of the first and final coding exons. In addition, the alternatively spliced exon II′ was not found in the CK/EK-β gene.

The hypothetical human CK/EK-β gene structure originally reported in the EMBL/GenBank/DDBJ Data Bank (Accession No. U62317) by the Human Genome Project and recently corrected by our hand (Accession No. AB029885) is depicted in Fig. 4B. Although the transcription start site has not been estimated for the human gene, the sizes of coding exons as well as their organization were shown to be matching completely between the mouse and putative human CK/EK-β genes except for the last exon (XI) where it was 72 b in the mouse whereas it was 75 b in the human.

The exon/intron organization of the mouse CK/EK-β gene is shown in Table 1B. When compared to those for the mouse CK/EK-α gene (Table 1A), intron sizes were found to be extremely small for CK/EK-β. The sequence of 5′-splice site of intron 3 (C**CA**gt) was shown to be not in good match for its consensus sequence (M**AG**gt). We have found that not a few mouse (13) as well as rat (12) cDNA clones (as much as 30%) had an intron 3 that remained unsplit, and this could cause a truncated form of CK/EK-β protein presumably with no kinase activity. At present, however, whether or not this indicates the possible existence of a regulatory mechanism in the splicing step of CK/EK-β expression is not clear. From our observation for both CK/EK-α and -β gene expressions, it should be noted that **G** preceding gt at the 5′-splice site of introns seems to be critical for the efficient intron-splitting event.

Nucleotide sequence in the 5′-flanking promoter region of mouse CK/EK-β gene

The 5′-flanking promoter region of mouse CK/EK-β gene is shown in **Fig. 5**, together with locations of the possible *cis*-elements. Several transcription start sites were estimated by either 5′-RACE (mouse kidney) or cap site cDNA (mouse brain) analyses. A functional TATA box was not present at the proximity of the putative transcription start sites, although a TATA-like sequence was found around 135 b upstream of the start sites. Instead, two CCAAT boxes (both in reverse orientation) and one GC box (SP-1 binding site) were found in close proximity to the initiation sites, suggesting certain house-keeping nature of the CK/EK-β gene. The existence of a number of SRY binding sites in the proximal region may implicate our previous observation (13) for the extremely high expression of CK/EK-β mRNA in murine testis. Unlike the CK/EK-α gene, there appeared no XRE in the 5′-flanking proximal region of CK/EK-β gene.

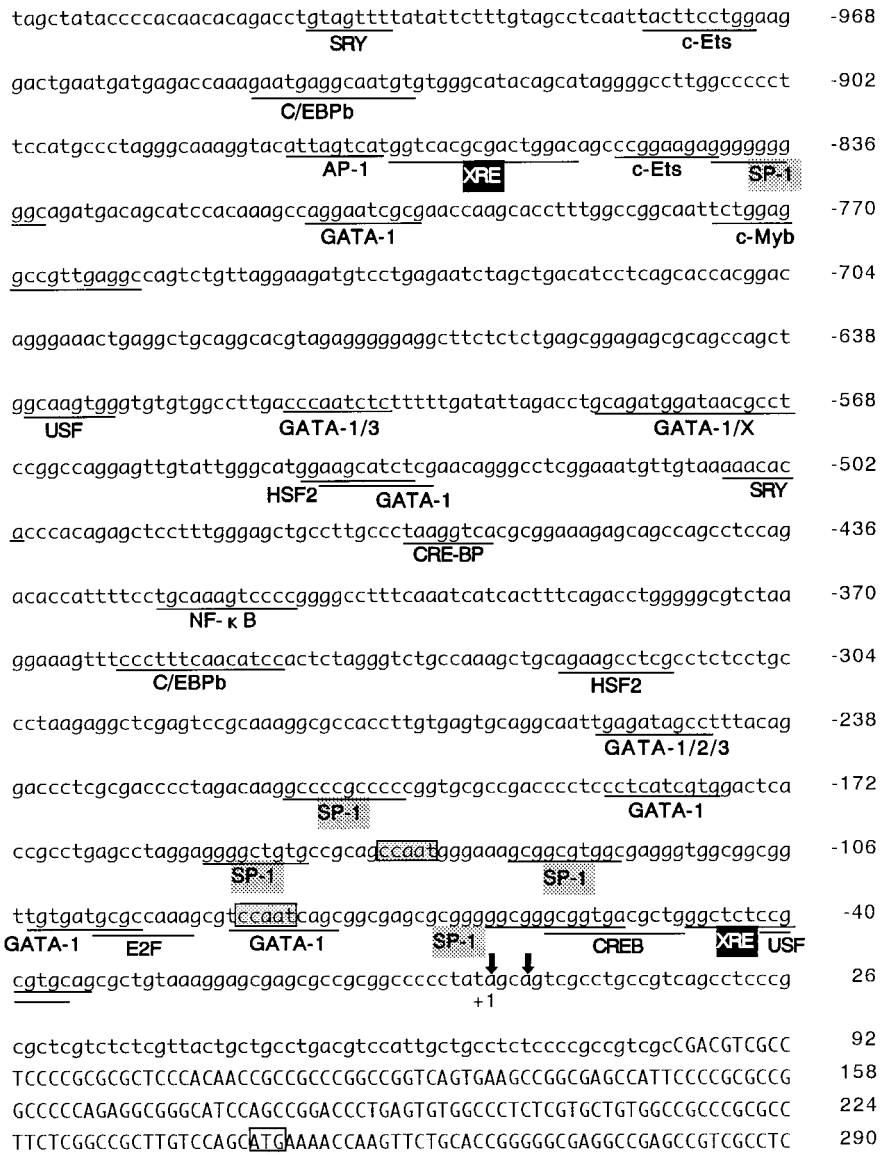

Fig. 3. Nucleotide sequence of the 5′-flanking promoter (proximal) region of mouse CK/EK-α gene and locations of the putative *cis*-elements. The putative transcription start sites estimated by 5′-RACE (mouse kidney) are indicated by arrows. A nucleotide of the more 5′-site is numbered as +1 and the preceding nucleotides are negatively numbered as indicated in the right margin. The capital letters indicate the sequence derived from the cDNA (13) and the putative translation start codon in exon I is boxed. The consensus sequences of typical transcription factor-binding elements by TFSEARCH are also boxed (CCAAT) or indicated with underlines.

Comparison of the nucleotide sequence in the corresponding exons between CK/EK-α and CK/EK-β genes

As both the size of coding exons and their organization have been shown to bear close resemblance to each other between the mouse CK/EK-α and -β genes, sequence similarity was compared next in every coding exon and the results are shown in Table 2. The highest similarity (74.5% homology) was shown to reside on exon VIII, the sequence of which corresponds to the C-terminal half of domain-7, one of the most highly conserved amino acid sequence domains among mammalian CK/EKs (Fig. 1A). Incidentally, the classical phosphotransferase (Brenner's) consensus sequence present in domain-6 was shown to be derived from the nucleotide sequence in between exons VI and VII.

Comparison of the entire amino acid sequences of mouse CK/EK-α and CK/EK-β to those from other eukaryotic CK or EK origins

A search of the DNA database resulted in the possible existence of 17 different origins of eukaryotic CK and/or EK including mouse CK/EK-α and CK/EK-β, all of which presumably contained the sequences to encode the entire amino acids. Then, we compared the similarity of the putative amino acid sequences of various CK/EK origins to

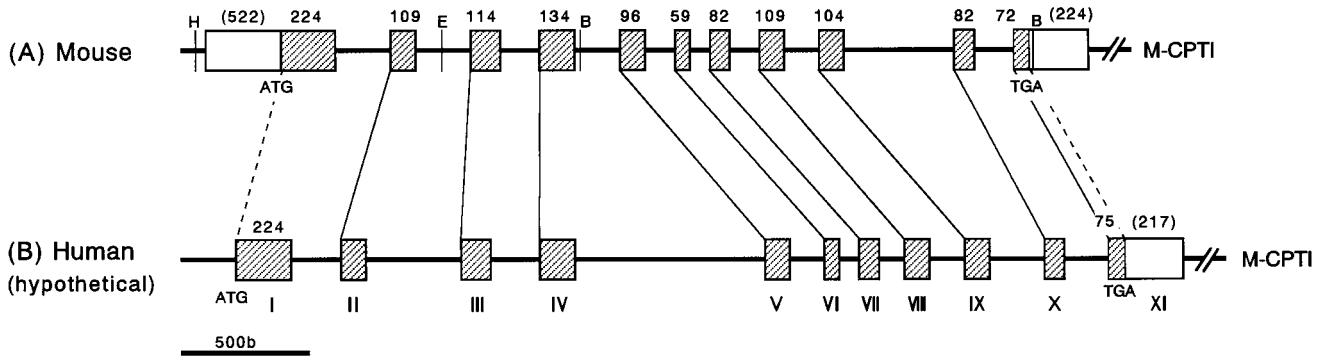

Fig. 4. Structure of the mouse gene for CK/EK-β (A) and its comparison to the hypothetical human CK/EK-β gene structure (B). The ddy mouse genome clones MG3 and MG5, both of which had originally been isolated by screening with a M-CPTI cDNA probe as described in Experimental Procedures, were shown to contain also the entire gene for CK/EK-β and its 5′-flanking region. The homologous human gene has recently been submitted in databases (Accession No. U62317 in EMBL/GenBank/DDBJ) by the Genome Project and its computer-assisted exon/intron structure proposed. We found that the human sequence reported in the database had an error in its putative exon I which resulted in a frameshift to cause a longer coding exon. We corrected its sequencing error (Accession No. 029885), then arranged the hypothetical human CK/EK-β gene structure as shown in (B). The boxes (open, non-coding; hatched, coding) and thick lines indicate exons and introns, respectively. The nucleotide size of each coding exon is indicated above the box. The sites of restriction enzymes (B, BamHI; E, EcoRI; H, HindIII) are also indicated.

the mouse CK/EK-α and CK/EK-β, respectively (**Table 3**). The amino acid sequences for the human CK/EK-β, all four isoforms of *C. elegans* CK and *Arabidopsis* CK were those hypothesized through the computer-assisted exon organization for their genomic DNA sequences. The results showed that the homology scores of all nonmammalian CK/EKs against mouse CK/EKs were relatively low ranging from 25 to 40%, when compared to those between any of the mammalian CK/EK-α and CK/EK-β isozymes (see above). Interestingly, when these 17 sequences were positioned in an evolutionary tree (data not shown), three soy bean CK isoforms were all shown to be in closer relationship to the mammalian enzymes than any other nonmammalian CK/EKs including the *C. elegans* isoforms. Both *D. melanogaster* EK, which was reported to have no CK activity at all (30), and one (U58734) of *C. elegans* CK isoforms were located at the most distal positions toward the mammalian CK/EKs.

TABLE 2. Comparison of the nucleotide sequences in the corresponding exons between CK/EK-α and CK/EK-β genes

Exon No.	α(nt)	β(nt)	Homology	Conserved Amino Acid Domains
			%	
I	338	224	51.1	d-1
II	112	109	71.3	d-2, d-3
II′	(54)	—	—	
III	114	114	69.0	d-4
IV	134	134	68.2	d-5
V	105	96	52.9	
VI	59	59	65.5	d-6
VII	88	82	63.6	d-6, d-7
VIII	109	109	74.5	d-7
IX	107	104	55.3	
X	82	82	68.8	d-8
XI	60	72	72.0	
I~XI	α1:1308nt (α2:1362nt)	1185nt	62.2	
	α1:435aa α2:(453aa)	394aa	59.7	

The coding exon sequences were compared for their similarity between the every corresponding exon of CK/EK-α and CK/EK-β by GENETYX MAC. The positions of the conserved amino acid domains (d-1 to d-8) are indicated in Fig. 1.

Discovery of the two novel CK/EK consensus amino acid sequences in addition to the classic phosphotransferase consensus sequence

Finally, GENETYX MAC multialignment was performed for the putative amino acid sequences of 17 different origins of eukaryotic CK and/or EK which have been reported in the EMBL/GenBank/DDBJ databases. Each alignment was done for every 2 or 3 exons of the mouse CK/EK genes. The overall results led to an identification of three highly homologous domains in which several key amino acids were completely conserved among all eukaryotic CK/EK proteins. One of which was so-called Brenner's (20) phosphotransferase consensus sequence which was found in domain-6 derived from exons VI and VII of the mammalian CK/EK genes (**Fig. 6A**, see also Fig. 1 and Table 2). In the CK/EK consensus sequence, it could be ameliorated to **h**x**F**x**HND**xxxx**Nhh**.... ..**D**. The second CK/EK consensus sequence, **h**x**hhDhE**xxxx**N**xxx**hDh**xx**Hh**x**E**, was found to be located in domain-7 derived from exons VII and VIII, the latter of which has been shown to have the highest sequence homology between the mouse CK/EK-α and CK/EK-β genes (Table 2). It contained six completely conserved amino acids, four of which are acidic and the other two are polar, suggesting that the alignment of these amino acids is most likely to be critical for interacting with the positive charge of the substrate choline and/or ethanolamine. The third consensus sequence was found in the more N-terminal region corresponding to the C-terminal half of domain-4 (Fig. 6B), which could be derived from exon III of the mammalian CK/EK genes. The role of this highly conserved sequence, GPx**L**hGx**F**xx**GR**x**h**Ex**hh**, for the expression of CK/EK activity is presently unknown.

Fig. 5. Nucleotide sequence in the 5′-flanking promoter (proximal) region of mouse CK/EK-β gene. The putative transcription start sites estimated by 5′-RACE (mouse kidney) and Cap Site cDNA™ (mouse brain) analyses are indicated by closed and open arrows, respectively. A nucleotide of the most 5′-site is tentatively numbered as +1 and the preceding nucleotides are negatively numbered as indicated in the right margin. Capital letters indicate the sequence from the cDNA (13) and the putative translation start codon in exon I is boxed. The consensus sequences of well-characterized transcription factor-binding elements by TFSEARCH are also boxed (CCAAT, with reverse orientation) or indicated with underlines.

Other completely conserved amino acid residues among all eukaryotic CK/EK proteins identified from the multi-alignment data were: ^{142}R(^{104}R), ^{214}H(^{175}H), ^{222}K(^{183}K), ^{360}Y(^{316}Y) and ^{398}W(^{353}W) with respect to the mouse CK/EK-α1 (CK/EK-β) primary amino acid sequence.

DISCUSSION

In this study, we have cloned mouse genes for CK/EK-α and CK/EK-β and determined their entire structures for the first time. The results demonstrated clearly that both CK/EK-α and -β genes are composed of 11 major exons split by 10 introns. While the sizes of coding exons as well as their organization were shown to be quite similar to each other, there was a big difference in their entire gene sizes: it was not less than 40 kb for CK/EK-α, whereas it was only 3.5 kb for CK/EK-β. Furthermore, the CK/EK-α gene had an extra 54 b coding exon (exon II′) which could be alternatively spliced to yield two isoforms, α1 and α2. The meaning of the presence of two CK/EK-α isoforms in mammalian cells as well as the difference in their catalytic nature is presently unknown. The physiological significance of the existence of two CK/EK isozymes, α

and β, is also unknown. Our previous finding indicated that the expression of either of the CK/EK isozymes occurred ubiquitously in rat tissues with the highest expression in testis. No significant tissue specificity has yet been observed in their mRNA abundance between the two isozymes. Thus, understanding of the exact meaning for the presence of more than one form of CK/EK in mammalian tissues must await future studies. It has now become possible from the present study to construct a knockout mouse for each CK/EK isozyme to observe whether or not its phenotype may have a change in PC/PE metabolism. Analysis of the possible *trans*-acting factors that regulate their gene expressions would provide further insight into the function of each isozyme and this kind of investigation has now become available for both CK/EK-α and -β isozymes. In this respect, the possible function of two XREs discovered in the 5′-flanking promoter region of CK/EK-α gene would be of particular interest because the critical role of CK/EK in mitogenic signal transduction as well as in carcinogenesis has recently been proposed (for review, see refs. 16, 17) and that major PAHs as well as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) that bind to XRE via the aryl hydrocarbon receptor (Ah receptor) have been known to be highly mitogenic and carcinogenic.

Although both CK/EK-α and -β have been shown to have significant EK activities, choline is much more predominant than ethanolamine as their substrate. Thus, it has been postulated that another enzyme that is specific for ethanolamine or prefers ethanolamine to choline as its substrate should exist also [such an enzyme actually exists in lower eukaryotes including yeast (31) and *Drosophila* (30)] in mammals. Recently, two reports have described the possible existence of relatively EK-specific kinases in human (32) and rat (33) tissues. No molecular information for these enzymes, however, could be obtained at the present.

Another important result from the present investigation is an identification of the two novel CK/EK consensus sequences, in addition to the classical Brenner's (20) phosphotransferase consensus, which have been shown to be completely conserved among all of the eukaryotic CK/EK putative amino acid sequences. One was located shortly downstream of the C-terminal region of Brenner's consensus sequence and it contained four acidic and two polar amino acids aligned in every 2 to 5 amino acid intervals. The characteristic nature of the region suggests that this amino acid alignment may be required for the interaction with a positive charge of choline and/or ethanolamine substrate. The other CK/EK consensus sequence was found to be located in the more N-terminal region which corresponded to the domain-4, one of the most highly conserved amino acid sequence domains among all mammalian CK/EK proteins. The role of several key amino acids of this region in catalytic or regulatory function of CK/EK activity needs to be evaluated in further analysis.

From the genomic point of view, we are very much concerned about the fact that CK/EK-β gene was found to be localized in close proximity to the gene for mammalian M-CPTI (18, 19). Particularly in the human genome, the 3′-end of CK/EK-β gene was shown to be only 300 b upstream of the first exon (exon la) of M-CPTI gene (18), indicating that there could be some 5′-flanking regulatory region of the M-CPTI gene expanding into the CK/EK-β gene. The tissue-specific expression of M-CPTI gene has been well characterized (34) and occurred only in heart, skeletal muscle, and both brown and white adipose tissues but not in many other mammalian tissues. Although the exact mechanism underlying its tissue-specific expression as well as the functional *cis*-elements have not yet been clarified with mammalian M-CPTI gene, it would be quite fascinating to know how the expression of these two genes located in close proximity can be regulated. At present, however, the expression of CK/EK-β gene has not been investigated in detail with any of these mammalian tissues where M-CPTI is expressed in high levels. Incidentally, the chromosomal mapping of M-CPTI gene, and thereby of CK/EK-β gene, has been reported to reside on 22q13 for the human (35), 15E3 for the mouse (19, 36) and 7q34 for the rat (19). There is no information at present about the chromosomal mapping of CK/EK-α gene in any mammalian species.

An earlier study from our laboratory demonstrated that administration of several PAHs (27) and carbon tetrachloride (CCl_4) (37) caused induction of CK/EK activities in rat liver, and that this induction was most likely through

TABLE 3. Amino acid sequence similarity of mouse CK/EK-α and CK/EK-β to other CKs or EKs reported in the literature or hypothesized from the genomic sequence in the DNA data bank

Origin	Description (Accession No. in GenBank/EBI)	Similarity	
		Mouse α[a]	Mouse β
		%	
Mouse	α1(50 kDa CK), α2(52 kDa CK)	—	59.7
	β(42 kDa CK/EK)	59.7	—
Rat	α1(CKR1), α2(CKR2)	97.9	59.7
	β42 kDa CK/EK)	59.7	95.9
Human	α2(52 kDa CK)	87.5	56.2
	β(AB029885/6)	62.5	86.0
S. cerevisiae	Cki1p	30.3	31.8
	Eki1p(Z50046)	29.5	26.1
C. elegans	Z34533	24.5	28.7
	Z34533/Z30973	25.7	28.6
	U64598	37.7	40.7
	U58734	29.7	30.9
Arabidopsis	AC005168	33.8	33.4
Soy bean	GmCK1	31.6	35.2
	GmCK2	32.2	34.3
	GmCK3	33.2	33.3
D. melanogaster	EK	28.5	31.3

The previous descriptions for CK/EK-α1, -α2, and CK/EK-β are indicated in parentheses. The origins of sequences are as follows: mouse α1, α2, and β(13); rat α1(9), rat α2(10), rat β(12); human α2(11), Cki1p(39), Eki1p(31), GmCK1~3(40), and *D. melanogaster* EK(30). Other sequences including human β were derived from their genomic DNA sequences reported in the EMBL/GenBank/DDBJ Data Bank.

[a] For the CK/EK-α isozyme, α1 sequence was used for all similarity comparisons except for that against the human α2 where mouse α2 sequence was used.

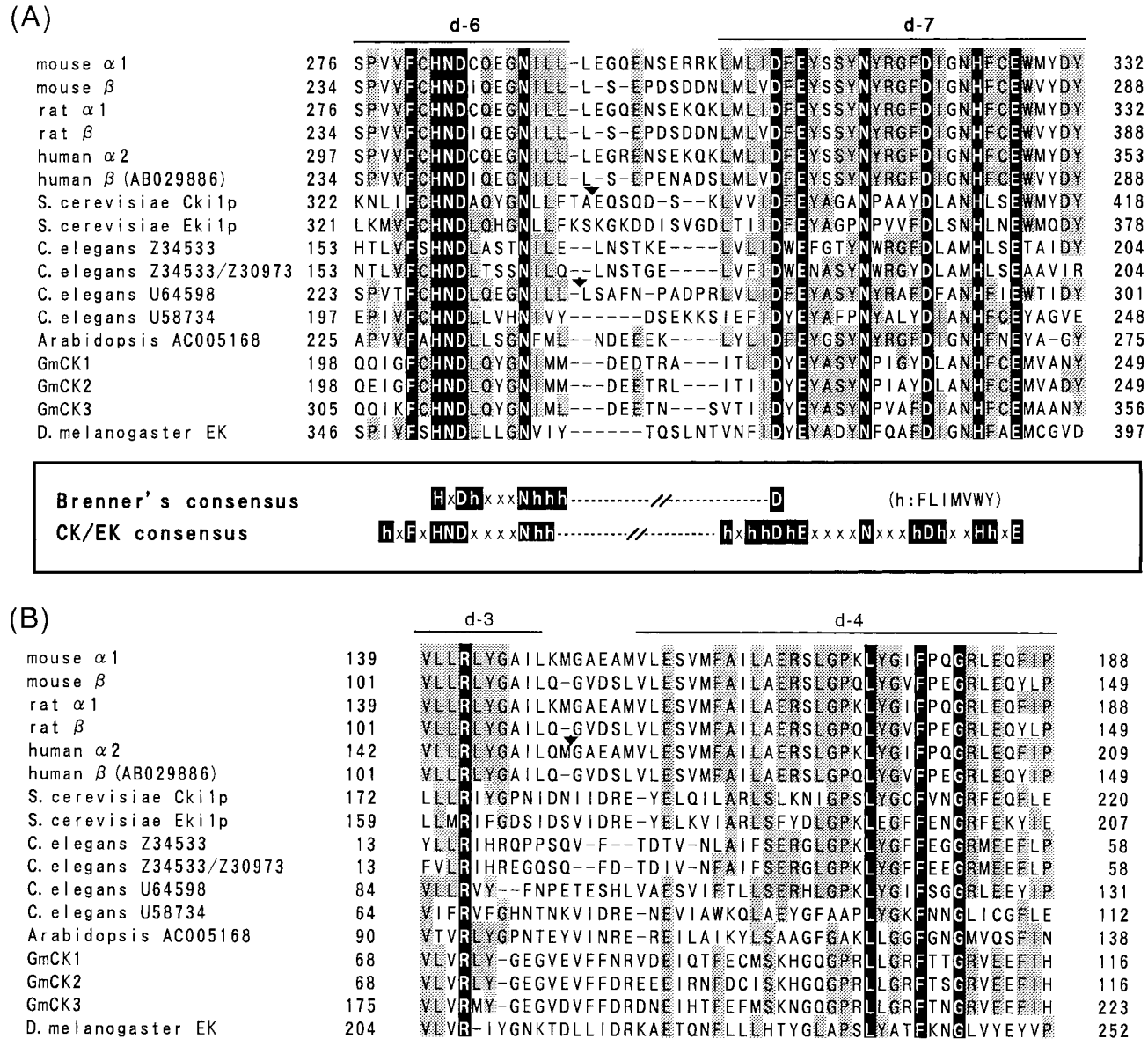

Fig. 6. Identification of the two novel CK/EK consensus amino acid sequences in addition to the classical phosphotransferase (Brenner's) consensus. GENETYX MAC multialignment was performed for the putative amino acid sequences of 17 different origins of eukaryotic CK and/or EK which have been reported in the EMBL/GenBank/DDBJ databases (see Table 3). Each alignment was done for every 2–3 exons of the mouse CK/EK gene. Two highly conserved regions (A and B) in addition to the Brenner's phosphotransferase consensus was discovered. One was located in the C-terminal side of the Brenner's consensus sequence (A), and the other was located in the more N-terminal region corresponding to the C-terminal half of domain-4 (B). In (A), 42 amino acids from *S. cerevisiae* Cki1p (39) and 23 amino acids from *C. elegans* U64598 were removed at the positions indicated (by arrowheads) so that maximum matching could be obtained. In (B), the arrowhead indicates the position where the reported human CK/EK-α2 sequence (11) had 18 amino acids insertion.

the synthesis of new CK/EK protein(s). Subsequently, we confirmed immunologically that the inducible form(s) of CK/EK could not be the same enzyme(s) as those constitutively expressed in rat tissues (4, 8). These observations indicated that another isozyme or isoform other than CK/EK-α (α1 and α2) and CK/EK-β could be present in the rat because both of the latter forms of CK/EK have been shown to be ubiquitously expressed enzymes. Alternatively, the inducible form of CK/EK might have been subject to conformational change in some way so that it could not cross-react with the antibody raised against the highly purified CK/EK-β protein. At present, however, there is no molecular information for the possible existence of a third isozyme or isoform of CK/EK (except for the relatively EK-specific one as described above) in any mammalian sources. On the other hand, Uchida (38) suggested by both Western blot with an antibody raised against recombinant rat CK/EK-α1 and Northern blot with a rat cDNA probe for CK/EK-α that both PAH and CCl_4 increased the protein and transcript levels of CK/EK-α in rat liver. Thus, molecular characterization of the major PAH- and/or CCl_4-inducible form of CK/EK in the liver

still remains to be clarified. The CK/EK-α gene disruption studies would provide conclusive evidence for the above discrepancies between our results (4) and those by Uchida (38).

Finally, CK/EK has been shown to be activated or induced in a number of situations where the increased activities of CK/EK resulted in a similarly increased rate of PC/PE biosynthesis (3). There have been several instances, however, where the increased activity of CK/EK did not appear to associate directly with the increased synthesis of PC/PE. In most cases, an accumulation of intracellular phosphocholine or phosphoethanolamine has been observed (16, 17). Thus, the physiological meaning of activation or induction of the enzyme in these latter situations has not long been evaluated experimentally. In recent years, both phosphocholine and phosphoethanolamine have been proposed to be the possible second messengers in mitogenic signal transduction pathway. In addition, certain chemical carcinogens mentioned above and/or ras/raf oncogene transfection have shown to cause an induction of CK activity and, at the same time, an accumulation of phosphocholine in the cell (2, 16, 17). Although the exact mechanism leading to activation or induction of CK/EK as well as an exact site of phosphocholine/phosphoethanolamine association in mitogenic signaling pathway remains to be determined, the isolation and characterization of each CK/EK gene, as presented in this study, would no doubt prove the critical role of each isozyme or isoform in not only PC/PE biosynthesis but also mitogenic signal transduction in animal cells.

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REFERENCES

1. Vance, D. E. 1996. Glycerolipid biosynthesis in eukaryotes. *In* Biochemistry of Lipids, Lipoproteins and Membranes. D. E. Vance and J. Vance, editors. Elsevier Science Publishers B. V., Amsterdam. 153–181.
2. Kent, C. 1995. Eukaryotic phospholipid biosynthesis. *Annu. Rev. Biochem.* **64:** 315–343.
3. Kent, C. 1990. Regulation of phosphatidylcholine biosynthesis. *Prog. Lipid Res.* **29:** 87–105.
4. Ishidate, K., K. Nakagomi, and Y. Nakazawa. 1984. Complete purification of choline kinase from rat kidney and preparation of rabbit antibody against rat kidney choline kinase. *J. Biol. Chem.* **259:** 14706–14710.
5. Porter, T. J., and C. Kent. 1990. Purification and characterization of choline/ethanolamine kinase from rat liver. *J. Biol. Chem.* **265:** 414–422.
6. Uchida, T., and S. Yamashita. 1990. Purification and properties of choline kinase from rat brain. *Biochim. Biophys. Acta.* **1043:** 281–288.
7. Ishidate, K., K. Iida, K. Tadokoro, and Y. Nakazawa. 1985. Evidence for the existence of multiple forms of choline (ethanolamine) kinase in rat tissues. *Biochim. Biophys. Acta.* **833:** 1–8.
8. Tadokoro, K., K. Ishidate, and Y. Nakazawa. 1985. Evidence for the existence of isozymes of choline kinase and their selective induction in 3-methylcholanthrene- or carbon tetrachloride-treated rat liver. *Biochim. Biophys. Acta.* **835:** 501–513.
9. Uchida, T., and S. Yamashita. 1992. Molecular cloning, characterization, and expression in *Escherichia coli* of a cDNA encoding mammalian choline kinase. *J. Biol. Chem.* **267:** 10156–10162.
10. Uchida, T. 1994. Regulation of choline kinase R: Analyses of alternatively spliced choline kinases and the promotor region. *J. Biochem. (Tokyo)* **116:** 508–518.
11. Hosaka, K., S. Tanaka, J. Nikawa, and S. Yamashita. 1992. Cloning of a human choline kinase cDNA by complementation of the yeast cki mutation. *FEBS Lett.* **304:** 229–232.
12. Aoyama, C., K. Nakashima, M. Matsui, and K. Ishidate. 1998. Complementary DNA sequence for a 42kDa rat kidney choline/ethanolamine kinase. *Biochim. Biophys. Acta.* **1390:** 1–7.
13. Aoyama, C., K. Nakashima, and K. Ishidate. 1998. Molecular cloning of mouse choline kinase and choline/ethanolamine kinase: their sequence comparison to the respective rat homologs. *Biochim. Biophys. Acta.* **1393:** 179–185.
14. Ishidate, K. 1989. Choline transport and choline kinase. *In* Phosphatidylcholine Metabolism. D. E. Vance, editor. CRC Press Inc., Boca Raton, FL. 9–32.
15. Ishidate, K., and Y. Nakazawa. 1992. Choline/ethanolamine kinase from rat kidney. *Methods Enzymol.* **209:** 121–134.
16. Ishidate, K. 1997. Choline/ethanolamine kinase from mammalian tissues. *Biochim. Biophys. Acta.* **1348:** 70–78.
17. Kiss, Z. 1999. Regulation of mitogenesis by water-soluble phospholipid intermediates. *Cell. Signal.* **11:** 149–157.
18. Yamazaki, N., Y. Yamanaka, Y. Hashimoto, Y. Shinohara, A. Shima, and H. Terada. 1997. Structural features of the gene encoding human muscle type carnitine palmitoyltransferase I. *FEBS Lett.* **409:** 401–406.
19. Wang, D., W. Harrison, L. M. Buja, F. F. B. Elder, and J. B. McMillin. 1998. Genomic DNA sequence, promoter expression, and chromosomal mapping of rat muscle carnitine palmitoyltransferase I. *Genomics.* **48:** 314–323.
20. Brenner, S. 1987. Phosphotransferase sequence homology. *Nature (Lond.)* **329:** 21.
21. Green, M. R. 1991. Biochemical mechanisms of constitutive and regulated pre-mRNA splicing. *Annu. Rev. Cell Biol.* **7:** 559–599.
22. Fujisawa-Sehara, A., K. Sogawa, M. Yamane, and Y. Fujii-Kuriyama. 1987. Characterization of xenobiotic responsive elements upstream from the drug-metabolizing cytochrome P-450c gene: a similarity to glucocorticoid regulatory elements. *Nucleic Acids Res.* **15:** 4179–4191.
23. Rushmore, T. H., R. G. King, K. E. Paulson, and C. B. Picket. 1990. Regulation of glutathione S-transferase Ya subunit gene expression: Identification of a unique xenobiotic-responsive element controlling inducible expression by planar aromatic compounds. *Proc. Natl. Acad. Sci. USA.* **87:** 3826–3830.
24. Favreau, L. V., and C. B. Picket. 1991. Transcriptional regulation of the rat NAD(P)H: quinone reductase gene: Identification of regulatory elements controlling basal level expression and inducible expression by planar aromatic compounds and phenolic antioxidants. *J. Biol. Chem.* **266:** 4556–4561.
25. Asman, D. C., K. Takimoto, H. C. Pitot, T. J. Dunn, and R. Lindahl. 1993. Organization and characterization of the rat class 3 aldehyde dehydrogenase gene. *J. Biol. Chem.* **268:** 12530–12536.
26. Emi, Y., S. Ikushiro, and T. Iyanagi. 1996. Xenobiotic responsive element-mediated transcriptional activation in the UDP-glucuronosyltransferase family 1 gene complex. *J. Biol. Chem.* **271:** 3952–3958.
27. Ishidate, K., M. Tsuruoka, and Y. Nakazawa. 1980. Induction of choline kinase by polycyclic aromatic hydrocarbon carcinogens in rat liver. *Biochem. Biophys. Res. Commun.* **96:** 946–952.
28. Zwingman, T., H. Fujimoto, L-W. Lai, T. Boyer, A. Ao, J. R. D. Stalvey, S. R. Blecher, and R. P. Erickson. 1994. Transcription of circular and noncircular forms of *Sry* in mouse testis. *Mol. Reprod. Dev.* **37:** 370–381.
29. Ito, E., T. Toki, H. Ishihara, H. Ohtani, L. Gu, M. Yokoyama, J. D. Engel, and M. Yamamoto. 1993. Erythroid transcription factor GATA-1 is abundantly transcribed in mouse testis. *Nature (Lond.)* **362:** 466–468.
30. Pavlidis, P., M. A. Ramaswami, and M. A. Tanouye. 1994. The drosophila *easily* shocked gene: A mutation in a phospholipid synthetic pathway causes seizure, neuronal failure, and paralysis. *Cell.* **79:** 23–33.
31. Kim, K., K-H. Kim, M. K. Storey, D. R. Voelker, and G. M. Carman. 1999. Isolation and characterization of the *Saccharomyces cerevisiae EKI1* gene encoding ethanolamine kinase. *J. Biol. Chem.* **274:** 14857–14866.

32. Draus, E., J. Niefind, K. Vietor, and B. Havsteen. 1990. Isolation and characterization of the human liver ethanolamine kinase. *Biochim. Biophys. Acta.* **1045:** 195–204.
33. Uchida, T. 1997. A novel high-molecular mass mammalian ethanolamine kinase. *Biochim. Biophys. Acta.* **1349:** 13–24.
34. McGarry, J. D., and N. F. Brown. 1997. Mitochondrial carnitine palmitoyltransferase system: From concept to molecular analysis. *Eur. J. Biochem.* **244:** 1–14.
35. Britton, C. H., D. W. Mackey, V. Esser, D. W. Foster, D. K. Burns, D. P. Yarnall, P. Froguel, and J. D. McGarry. 1997. Fine chromosome mapping of the genes for human liver and muscle carnitine palmitoyltransferase I (CPT1A and CPT1B). *Genomics.* **40:** 209–211.
36. Cox, K. B., K. R. Johnson, and P. A. Wood. 1998. Chromosomal locations of the mouse fatty acid oxidation genes *Cpt1a*, *Cpt1b*, *Cpt2*, *Acadvl*, and metabolically related *Crat* gene. *Mamm. Genome.* **9:** 608–610.
37. Ishidate, K., S. Enosawa, and Y. Nakazawa. 1983. Actinomycin D-sensitive induction of choline kinase by carbon tetrachloride intoxication in rat liver. *Biochem. Biophys. Res. Commun.* **111:** 683–689.
38. Uchida, T. 1994. Immunologically and enzymatically distinct rat choline kinase isozymes. *J. Biochem. (Tokyo)* **116:** 1241–1250.
39. Hosaka, K., T. Kodaki, and S. Yamashita. 1989. Cloning and characterization of the yeast CKI gene encoding choline kinase and its expression in *Escherichia coli*. *J. Biol. Chem.* **264:** 2053–2059.
40. Monks, D. E., J. H. Goode, and R. E. Dewey. 1996. Characterization of soybean choline kinase cDNAs and their expression in yeast and *Escherichia coli*. *Plant Physiol.* **110:** 1197–1205.