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Human liver mitochondrial carnitine palmitoyltransferase I: Characterization of its cDNA and chromosomal localization and partial analysis of the gene

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ABSTRACT **Using the cDNA for rat liver mitochondrial carnitine palmitoyltransferase I (CPT I; EC 2.3.1.21) as a probe, we isolated its counterpart as three overlapping clones from a human liver cDNA library. Both the nucleotide sequence of the human cDNA and the predicted primary structure of the protein (773 aa) proved to be very similar to those of the rat enzyme (82% and 88% identity, respectively). The CPT I mRNA size was also found to be the same (≈4.7 kb) in both species. Screening of a human genomic library with the newly obtained cDNA yielded a positive clone of ≈6.5 kb which, upon partial analysis, was found to contain at least two complete exons linked by a 2.3-kb intron. Oligonucleotide primers specific to upstream and downstream regions of one of the exon/intron junctions were tested in PCRs with DNA from a panel of somatic cell hybrids, each containing a single human chromosome. The results allowed unambiguous assignment of the human liver CPT I gene to the q (long) arm of chromosome 11. Additional experiments established that liver and fibroblasts express the same isoform of mitochondrial CPT I, legitimizing the use of fibroblast assays in the differential diagnosis of the "muscle" and "hepatic" forms of CPT deficiency. The data provide insights into the structure of a human CPT I isoform and its corresponding gene and establish unequivocally that CPT I and CPT II are distinct gene products. Availability of the human CPT I cDNA should open the way to an understanding of the genetic basis of inherited CPT I deficiency syndromes, how the liver CPT I gene is regulated, and which tissues other than liver express this particular variant of the enzyme.**

The mitochondrial oxidation of long-chain fatty acids is initiated by the sequential action of carnitine palmitoyltransferase I (CPT; palmitoyl-CoA: L-carnitine *O*-palmitoyltransferase; EC 2.3.1.21) (outer membrane and detergent labile) and II (inner membrane and detergent stable), together with a carnitine–acylcarnitine translocase (1, 2). Major control over the process is exerted at the level of CPT I by virtue of the unique inhibitability of this enzyme by malonyl-CoA. This principle was first recognized in the context of hepatic ketogenesis and its regulation (3) but has since emerged as a central component of fuel "cross talk" in a variety of other tissues such as heart (4), skeletal muscle (5), and, most recently, pancreatic β cells (6, 7). Because of its pivotal role in lipid metabolism, CPT I has attracted attention as a potential site for pharmacological intervention in diabetes mellitus, where fatty acid oxidation is excessive and interferes with glucose homeostasis (8, 9). In addition, inherited defects in CPT I or CPT II form the basis of serious, and sometimes fatal, disturbances in fatty acid oxidation, although precisely which enzyme and which tissues are affected often remain unsettled (10–13).

For all of these reasons, understanding of the mitochondrial CPT system in terms of its structure/function/regulatory properties has become a matter of intense biochemical, clinical, and genetic interest. Although progress in this area has been hampered by the inherent complexity of the transport mechanism (2) and disagreement over its component parts (2, 14, 15), recent molecular studies have produced a much clearer picture. In 1990 isolation of the first cDNA encoding a mitochondrial form of CPT (rat liver CPT II) was reported (16). Soon thereafter, the same goal was achieved for human liver CPT II (17, 18). This was followed by localization of the human CPT II gene to region p32 of chromosome 1 (19), definition of mutations in its coding region (13), and identification of regulatory elements in its promoter (20). Available evidence indicates that in both rats and humans, CPT II (≈71 kDa) is expressed as the same protein in all body tissues (13, 18).

The situation with the regulated enzyme, CPT I, is far more complicated. In contrast to CPT II, CPT I in the rat appears to exist as two (and possibly more) isoforms, the liver (≈88 kDa) and skeletal muscle (≈82 kDa) variants having very different kinetic properties and sensitivity to malonyl-CoA (21–23). Interestingly, rat cardiac myocytes express both liver and muscle forms of the enzyme (24, 25), and in studies to be reported separately we have found that this also applies to human heart tissue. The only cDNA for a CPT I protein that has yet been isolated and characterized is that corresponding to the rat liver enzyme (26). Here we report the complete nucleotide sequence** of the cDNA for human liver CPT I, the predicted primary structure of the protein, a partial analysis of the CPT I gene, and its chromosomal localization. Also established is the identity of human liver and fibroblast CPT I.

In the sections that follow the use of the terms CPT I and CPT II will refer exclusively to the mitochondrial forms of these enzymes, although it is recognized that similar activities are present in other organelles such as microsomes and peroxisomes (27).

MATERIALS AND METHODS

Standard molecular biological techniques were employed throughout (28).

Screening of a Human Liver cDNA Library. Because of the strong similarity in nucleotide sequence between the cDNAs for rat and human liver CPT II (17, 18), it seemed reasonable to expect that the same would be true in the case of CPT I from

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Abbreviation: CPT, carnitine palmitoyltransferase.

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**The sequence reported in this paper has been deposited in the GenBank data base (accession no. L39211).

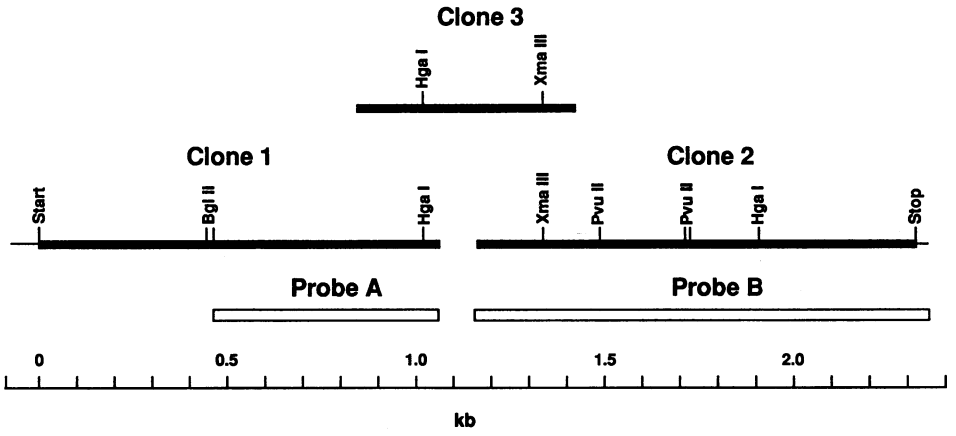

FIG. 1. Restriction maps of human liver cDNA clones. Solid bars and adjacent lines represent coding and noncoding regions, respectively. Open bars denote the positions of probes used in other experiments.

the two sources. Accordingly, rat liver CPT I cDNA was digested with the restriction enzymes *Bam*HI and *Sal* I, yielding a 1.0-kb and a 0.7-kb fragment (nt −96 to 892 and 1291 to 2005, respectively, in ref. 26). Both were used to make double-stranded, hexamer-primed ^{32}P-labeled probes to screen a human liver cDNA library in the expression vector λgt11. Filters were hybridized at 42°C in 25% formamide/5× standard saline citrate (SSC)/7× Denhardt's reagent/1% SDS/50 mM sodium phosphate, pH 6.8, containing denatured salmon sperm DNA (100 μg/ml) and labeled probe (0.3×10^6 cpm/ml). They were then washed in 2× SSC/0.5% SDS at 55°C for 1–2 hr. One positive clone was isolated with each probe and both were PCR amplified with primers specific to the λgt11 vector. After digestion of the products with *Eco*RI, the released inserts were subcloned into bacteriophage M13 vectors and both strands were sequenced by the dideoxy chain-termination method using the Sequenase T7 DNA polymerase kit (United States Biochemical). Clones 1 and 2 did not overlap (Fig. 1).

To isolate a third clone containing the presumed missing portion of the CPT I cDNA, the library was rescreened with a probe made from the 3′ fragment of a *Bgl* II digestion of clone 1 (probe A, Fig. 1). Eleven positive clones were then screened with a probe made from the entire clone 2 (probe B, Fig. 1). Four plaques gave positive signals with both probes, and one of these (clone 3, Fig. 1) was amplified, subcloned, and sequenced. All PCRs and sequencing of their products were performed at least twice.

Screening of a Human Genomic Library. A human genomic library in the cloning vector λgt10 (obtained from David Chuang of this institution) was screened with probe A by the procedure outlined in Fig. 2. Filters were hybridized at 42°C in a solution containing 50% formamide and washed as described above. One positive plaque was detected and this clone was grown in solution and its DNA was isolated. The 6.5-kb insert was released from the vector arms by digestion with *Hin*dIII. After digestion of the insert with *Pst* I, two fragments (≈850 and ≈2100 bp) were detected on a Southern blot with probe A (Fig. 2). These were subcloned into bacteriophage M13 and partially sequenced.

Mapping of the CPT I Gene. PCR primers derived from the genomic clone depicted in Fig. 2 (forward, 5′TGAGCGACTGGTGGGAGGAG-3′; reverse, 5′-GCATGAGCCAATCCCCAGAG-3′, represented by Y and Z, respectively) were tested on total human genomic DNA and were found to generate a product of 200 bp in size, as predicted from the genomic sequence. This was readily distinguished from products obtained by using mouse or hamster DNA (see below). The same primers were employed to analyze DNA from the somatic cell hybrid mapping panel 2 (NIGMS Human Genetic Mutant Cell Repository, Camden, NJ). Control and hybrid DNAs were PCR amplified in 10-μl reaction mixtures in a Perkin–Elmer GeneAmp 9600 thermal cycler using standard conditions recommended by the manufacturer (including 200 ng of template DNA, 1 μM each primer, and 1.5 mM $MgCl_2$) for 35 cycles, each consisting of 30 sec at 96°C, 30 sec at 60°C, and 30 sec at 72°C. Similar PCRs were performed with DNA from somatic cell hybrids 212A9-1 and X31 (29, 30). Mixtures were analyzed for the presence of the human-specific 200-bp product by agarose gel electrophoresis followed by ethidium bromide staining.

Northern Blot Analysis. Total RNA was isolated from human liver by a single-step method (31). Northern blot analysis was performed as described (26). The blot was hybridized in 50% formamide buffer, as above, at 42°C with a ^{32}P-labeled random hexamer-primed probe (3×10^6 cpm/ml)

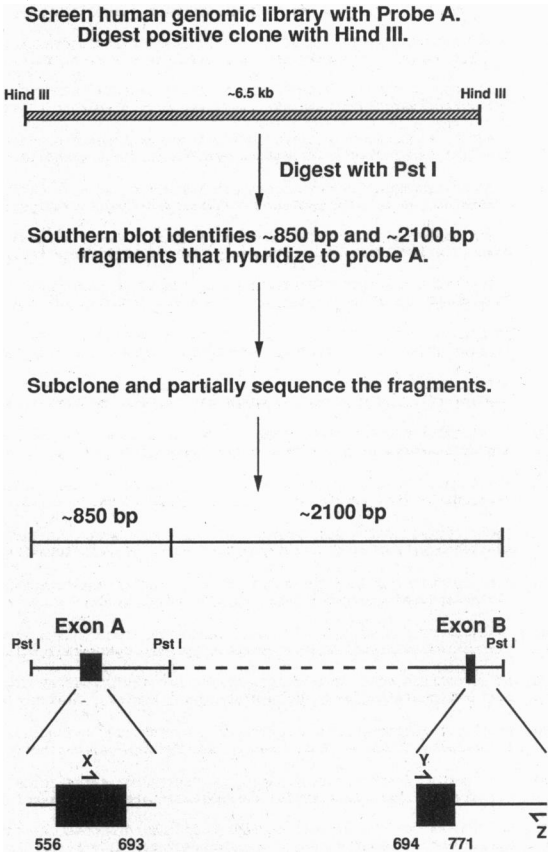

FIG. 2. Partial mapping of the human liver CPT I gene. Exons are denoted by filled boxes. Sequenced and nonsequenced regions of introns are represented by solid and dashed lines, respectively. Numbers under exons A and B correspond to the nucleotide positions in the cDNA (Fig. 3). X, Y, and Z refer to primers used in PCR experiments.

made from a PCR-generated fragment of nt 1004–2325 of the cDNA. The filter was washed at 55°C for 1 hr.

Software. Alignment of nucleotide and amino acid sequences was performed with MICROGENIE (Beckman) and the University of Wisconsin Genetics Computer Group programs.

Materials. Sources have been given in refs. 16 and 26.

RESULTS AND DISCUSSION

Molecular Cloning of a Human Liver CPT I cDNA. Screening of a human cDNA library with the 1.0-kb and 0.7-kb fragments of rat liver CPT I cDNA yielded two positive clones of 1134 and 1196 bp, respectively (clones 1 and 2 in Fig. 1). Because these did not overlap, the library was rescreened with probes A and B (Fig. 1), with the result that a third clone (clone 3, Fig. 1) was isolated. Together, the three stretches of DNA were found to encode an open reading frame of 2319 bp with a 5′ untranslated region of 76 bp (containing an in-frame stop codon 24 bp upstream of the presumed translation start site) and 35 bp of 3′ untranslated material (Fig. 3). The predicted protein contains 773 aa, exactly the same number as for the rat liver enzyme (26). As in the case of CPT II (17, 18), both the nucleotide sequence of the human CPT I cDNA and the predicted structure of the protein turned out to be very similar to those of the rat enzyme, the percent identity at each level being 82% and 86.4%, respectively. There is 94% similarity between the two amino acid sequences when allowance is made for conservative changes (accordingly, the computer-calculated molecular weights of the two proteins are very similar: rat, 88,150; human, 88,439). In addition, the mRNA for the human CPT I had the same size (≈4.7 kb; Fig. 4) as its rat equivalent (26). The fact that both the rat and human proteins contain a stretch of 20 uncharged amino acids at positions 103–122, flanked on either side by a positively charged residue, strengthens the notion that this represents a membrane-spanning domain through which CPT I is anchored to the

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-76                          CTCCACCGCCGCCGCCGCCGCCGCCGCTGCCGCTGCCGCTGCCGCACCTCCGTAGCTGACTCGGTACTCTCTGAAG
                                                                                ***

1     ATGGCAGAAGCTCACCAAGCTGTGGCCTTTCAGTTCACGGTCACTCCGGACGGGATTGACCTGCGGCTGAGCCATGAAGCTCTTAGACAA
      MetAlaGluAlaHisGlnAlaValAlaPheGlnPheThrValThrProAspGlyIleAspLeuArgLeuSerHisGluAlaLeuArgGln    30

91    ATCTATCTCTCTGGACTTCATTCCTGGAAAAAGAAGTTCATCAGATTCAAGAACGGCATCATCACTGGCGTGTACCCGGCAAGCCCCTCC
      IleTyrLeuSerGlyLeuHisSerTrpLysLysLysPheIleArgPheLysAsnGlyIleIleThrGlyValTyrProAlaSerProSer    60

181   AGTTGGCTTATCGTGGTGGTGGGCGTGATGACAACGATGTACGCCAAGATCGACCCCTCGTTAGGAATAATTGCAAAAATCAATCGGACT
      SerTrpLeuIleValValValGlyValMetThrThrMetTyrAlaLysIleAspProSerLeuGlyIleIleAlaLysIleAsnArgThr    90

271   CTGGAAACGGCCAACTGCATGTCCAGCCAGACGAAGAACGTGGTCAGCGGCGTGCTGTTTGGCACCGGCCTGTGGGTGGCCCTCATCGTC
      LeuGluThrAlaAsnCysMetSerSerGlnThrLysAsnValValSerGlyValLeuPheGlyThrGlyLeuTrpValAlaLeuIleVal    120

361   ACCATGCGCTACTCCCTGAAAGTGCTGCTCTCCTACCACGGGTGGATGTTCACTGAGCACGGCAAGATGAGTCGTGCCACCAAGATCTGG
      ThrMetArgTyrSerLeuLysValLeuLeuSerTyrHisGlyTrpMetPheThrGluHisGlyLysMetSerArgAlaThrLysIleTrp    150

451   ATGGGTATGGTCAAGATCTTTTCAGGCCGAAAACCCATGTTGTACAGCTTCCAGACATCGCTGCCTCGCCTGCCGGTCCCGGCTGTCAAA
      MetGlyMetValLysIlePheSerGlyArgLysProMetLeuTyrSerPheGlnThrSerLeuProArgLeuProValProAlaValLys    180
               ▼
541   GACACTGTGAACAGGTATCTACAGTCGGTGAGGCCTCTTATGAAGGAAGAAGACTTCAAACGGATGACAGCACTTGCTCAAGATTTTGCT
      AspThrValAsnArgTyrLeuGlnSerValArgProLeuMetLysGluGluAspPheLysArgMetThrAlaLeuAlaGlnAspPheAla    210
                                                                          ▼
631   GTCGGTCTTGGACCAAGATTACAGTGGTATTTGAAGTTAAAATCCTGGTGGGCTACAAATTACGTGAGCGACTGGTGGGAGGAGTACATC
      ValGlyLeuGlyProArgLeuGlnTrpTyrLeuLysLeuLysSerTrpTrpAlaThrAsnTyrValSerAspTrpTrpGluGluTyrIle    240
                                                              ▼
721   TACCTCCGAGGACGAGGGCCGCTCATGGTGAACAGCAACTATTATGCCATGGATCTGCTGTATATCCTTCCAACTCACATTCAGGCAGCA
      TyrLeuArgGlyArgGlyProLeuMetValAsnSerAsnTyrTyrAlaMetAspLeuLeuTyrIleLeuProThrHisIleGlnAlaAla    270

811   AGAGCCGGCAACGCCATCCATGCCATCCTGCTTTACAGGCGCAAACTGGACCGGGAGGAAATCAAACCAATTCGTCTTTTGGGATCCACG
      ArgAlaGlyAsnAlaIleHisAlaIleLeuLeuTyrArgArgLysLeuAspArgGluGluIleLysProIleArgLeuLeuGlySerThr    300

901   ATTCCACTCTGCTCCGCTCAGTGGGAGCGGATGTTTAATACTTCCCGGATCCCAGGAGAGGAGACAGACACCATCCAGCACATGAGAGAC
      IleProLeuCysSerAlaGlnTrpGluArgMetPheAsnThrSerArgIleProGlyGluGluThrAspThrIleGlnHisMetArgAsp    330

991   AGCAAGCACATCGTCGTGTACCATCGAGGACGCTACTTCAAGGTCTGGCTCTACCATGATGGGCGGCTGCTGAAGCCCCGGGAGATGGAG
      SerLysHisIleValValTyrHisArgGlyArgTyrPheLysValTrpLeuTyrHisAspGlyArgLeuLeuLysProArgGluMetGlu    360

1081  CAGCAGATGCAGAGGATCCTGGACAATACCTCGGAGCCTCAGCCCGGGGAGGCCAGGCTGGCAGCCCTCACCGCAGGAGACAGAGTTCCC
      GlnGlnMetGlnArgIleLeuAspAsnThrSerGluProGlnProGlyGluAlaArgLeuAlaAlaLeuThrAlaGlyAspArgValPro    390

1171  TGGGCCAGGTGTCGTCAGGCCTATTTTGGACGTGGAAAAATAAGCAGTCTCTTGATGCTGTGGAGAAAGCAGCGTTCTTCGTGACGTTA
      TrpAlaArgCysArgGlnAlaTyrPheGlyArgGlyLysAsnLysGlnSerLeuAspAlaValGluLysAlaAlaPhePheValThrLeu    420

1261  GATGAAACTGAAGAAGGATACAGAAGTGAAGACCCGGATACGTCAATGGACAGCTACGCCAAATCTCTACTACACGGCCGATGTTACGAC
      AspGluThrGluGluGlyTyrArgSerGluAspProAspThrSerMetAspSerTyrAlaLysSerLeuLeuHisGlyArgCysTyrAsp    450

1351  AGGTGGTTTGACAAGTCGTTCACGTTTGTTGTCTTCAAAAACGGAAGATGGGCCTCAACGCTGAACACTCCTGGGCAGATGCGCAGATC
      ArgTrpPheAspLysSerPheThrPheValValPheLysAsnGlyLysMetGlyLeuAsnAlaGluHisSerTrpAlaAspAlaGlnIle    480

1441  GTGGCCCACCTTTGGGAGTACGTCATGTCCATTGACAGCCTCCAGCTGGGCTATGCGGAGGATGGGCACTGCAAAGGCGACATCAATCCG
      ValAlaHisLeuTrpGluTyrValMetSerIleAspSerLeuGlnLeuGlyTyrAlaGluAspGlyHisCysLysGlyAspIleAsnPro    510

1531  AACATTCCGTACCCCACCAGGCTGCAGTGGGACATCCCGGGGGAATGTCAAGAGGTTATAGAGACCTCCCTGAACACCGCAAATCTTCTG
      AsnIleProTyrProThrArgLeuGlnTrpAspIleProGlyGluCysGlnGluValIleGluThrSerLeuAsnThrAlaAsnLeuLeu    540

1621  GCAAACGACGTGGATTTCCATTCCTTCCCATTCGTAGCCTTTGGTAAAGGAATCATCAAGAAATGTCGCAGGAGCCCAGACACCTTTGTG
      AlaAsnAspValAspPheHisSerPheProPheValAlaPheGlyLysGlyIleIleLysLysCysArgThrSerProAspThrPheVal    570

1711  CAGCTGGCCCTCCAGCTGGCGCACTACAAGGACATGGGCAAGTTTTGCCTCACATACGAGGCCTCCATGACCCGGCTCTTCCGAGAGGGG
      GlnLeuAlaLeuGlnLeuAlaHisTyrLysAspMetGlyLysPheCysLeuThrTyrGluAlaSerMetThrArgLeuPheArgGluGly    600

1801  AGGACGGAGACCGTGCGCTCCTGCACCACTGAGTCATGCGACTTCGTGCGGGCCATGGTGGACCCGGCCCAGACGGTGGAACAGAGGCTG
      ArgThrGluThrValArgSerCysThrThrGluSerCysAspPheValArgAlaMetValAspProAlaGlnThrValGluGlnArgLeu    630

1891  AAGTTGTTCAAGTTGGCGTCTGAGAAGCATCAGCATATGTATCGCCTCGCCATGACCGGCTCTGGGATCGATCGTCACCTCTTCTGCCTT
      LysLeuPheLysLeuAlaSerGluLysHisGlnHisMetTyrArgLeuAlaMetThrGlySerGlyIleAspArgHisLeuPheCysLeu    660

1981  TACGTGGTGTCTAAATATCTCGCTGTGGAGTCCCCTTTCCTTAAGGAAGTTTTATCTGAGCCTTGGAGATTATCAACAAGCCAGACCCCT
      TyrValValSerLysTyrLeuAlaValGluSerProPheLeuLysGluValLeuSerGluProTrpArgLeuSerThrSerGlnThrPro    690

2071  CAGCAGCAAGTGGAGCTGTTTGACTTGGAGAATAACCCAGAGTACGTGTCCAGCGGAGGGGGCTTTGGACCGGTTGCTGATGACGGCTAT
      GlnGlnGlnValGluLeuPheAspLeuGluAsnAsnProGluTyrValSerSerGlyGlyGlyPheGlyProValAlaAspAspGlyTyr    720

2161  GGTGTGTCGTACATCCTTGTGGGAGAGAACCTCATCAATTTCCACATTTCTTCCAAGTTCTCTTGCCCTGAGACGGATTCTCATCGCTTT
      GlyValSerTyrIleLeuValGlyGluAsnLeuIleAsnPheHisIleSerSerLysPheSerCysProGluThrAspSerHisArgPhe    750

2251  GGAAGGCACCTGAAAGAAGCAATGACTGACATCATCACTTTGTTTGGTCTCAGTTCTAATTCCAAAAAGTAATTCCACTGGAGCTGCTGG
      GlyArgHisLeuLysGluAlaMetThrAspIleIleThrLeuPheGlyLeuSerSerAsnSerLysLys***                      773

2341  GAAGGAAAACGAGG
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FIG. 3. cDNA and predicted amino acid sequences of human liver CPT I. Nucleotides are numbered on the left, amino acids on the right. Stars denote stop codons. Arrowheads denote the positions of three introns located to date. Boldface type indicates amino acid identity between the rat and human enzymes. Conservatively changed residues are shown in lightface type. Underlined residues are nonconservative changes.

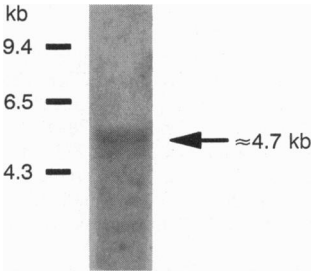

FIG. 4. Northern blot analysis of total RNA (15 μg) from human liver, using a 1.3-kb fragment of human liver CPT I cDNA as probe.

outer mitochondrial membrane (26). The same features apply to the more amino-terminal region of the two molecules bounded by residues 48 and 75, but whether this also spans the membrane is uncertain because of the presence in both sequences of a Pro-Ala-Asn (or Ser)-Pro-Ser-Ser motif which might be expected to have a high turn propensity (26). In any event, this portion of the molecule does not seem to be needed for catalytic activity but could be involved in malonyl-CoA binding to the enzyme (14). From Kyte–Doolittle analysis, the human CPT I protein appears to be quite hydrophilic between residue 122 and its carboxyl terminus, as is true for the rat enzyme (26).

Molecular Cloning of a Genomic Fragment of the Human Liver CPT I Gene. By screening a human genomic library with probe A of Fig. 1, a 6.5-kb genomic clone was obtained. Partial analysis of the material according to the scheme shown in Fig. 2 revealed the presence of at least two complete exons (A and B, Fig. 2) represented by nt 556–693 and 694–771, respectively, in the cDNA. The adjacent introns were found to contain the expected consensus sequences for intron/exon boundaries at their 5′ and 3′ termini (32). PCR analysis of the original 6.5-kb genomic fragment with primers X (forward; 5′GGGAATTCGTCGGTGAGGCCTCTTATG-3′) and Z (described in *Materials and Methods*) (Fig. 2) yielded an ≈2.6-kb product which, upon treatment with *Pst* I, was cleaved into two pieces of ≈550 and ≈2050 bp. This established two points: (*i*) the 850-bp and 2100-bp segments derived from *Pst* I digestion of the genomic clone must have been contiguous and (*ii*) exons A and B are separated by an intron of ≈2.3 kb.

Chromosomal Localization of the Human Liver CPT I Gene. The above information allowed the design of PCR primers Y and Z (Fig. 2), specific to exon B and its adjacent downstream intron. (The intron region was included here to maximize specificity of the Z primer for the human CPT I gene in DNA from somatic cell hybrids.) When these were tested on DNA extracted from a panel of human–mouse and human–hamster somatic cell hybrids, each containing a single human chromosome, only the hybrid containing human chromosome 11 yielded the predicted 200-bp product (Fig. 5). PCRs with the same primers gave positive results (not shown) with an additional hybrid, 212A9-1, which retains only the q arm of chromosome 11. Similar reactions using somatic cell hybrid X31, which contains only a small portion of chromosome 11 (11q22–23), were negative. These findings, coupled with results of the fluorescence *in situ* hybridization procedure using the 6.5-kb genomic clone as a probe (not shown), establish that the human liver CPT I gene resides on the q arm of chromosome 11 in a region outside of bands 22 and 23.

Identity of Liver and Fibroblast CPT I. Available evidence indicates that mitochondrial CPT deficiency in humans manifests itself as two distinct phenotypes, the "muscle" and "hepatic" forms of the disease (10–13). Fibroblasts from patients with the "muscle" presentation express normal levels of CPT I but are variably deficient in CPT II (10, 13). Conversely, individuals suspected to lack CPT I in liver also exhibit this defect in their fibroblasts, which express a normal

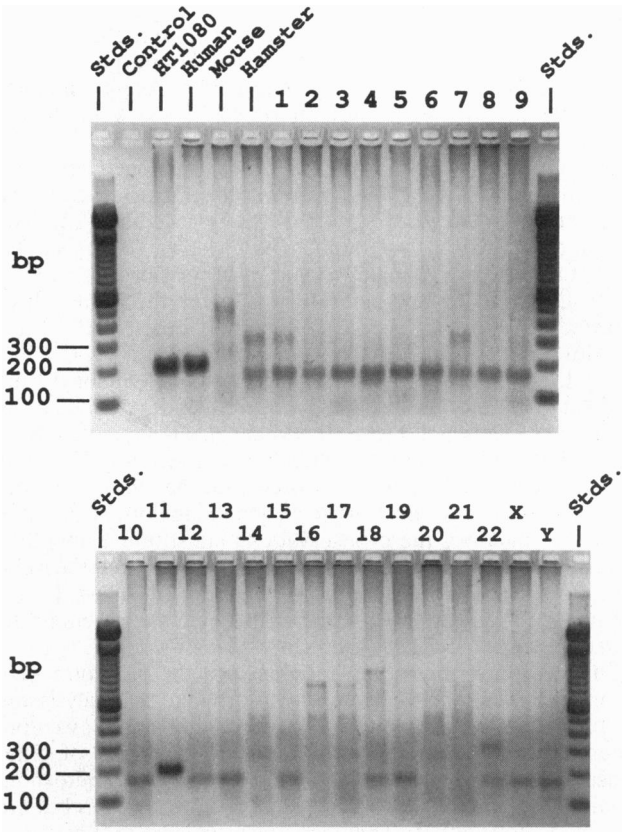

FIG. 5. PCR amplification of DNA from somatic cell hybrids. Reaction mixtures contained the following templates: Control, no template; HT1080, control human genomic DNA; human, mouse, and hamster, genomic DNA of each species from the repository; 1–22, X, and Y, genomic DNA from somatic cell hybrids containing the indicated human chromosome; Stds., DNA size markers.

complement of CPT II (10, 12); yet the same patients show no abnormality of CPT I in skeletal muscle (11). A reasonable interpretation of these findings is that, since CPT II appears to be the same protein body-wide (13, 18), its deficiency in fibroblasts reflects the same problem in other tissues including muscle. However, because there are at least two isoforms of CPT I (the liver and muscle variants, as noted in the Introduction), a low level of CPT I in liver, with attendant hepatic symptoms, coexistent with a normal muscle enzyme is not unexpected (10–12). A corollary of this argument is that liver and fibroblasts express the same CPT I protein (10–12). Because this has been an assumption, and in light of the increasing reliance on fibroblast assays for the differential diagnosis of "hepatic" and "muscle" forms of CPT deficiency, we felt it important to establish the point with certainty. To this end, the following additional experiments were carried out.

(*i*) Total RNA from human fibroblasts was subjected to Northern blot analysis using the ^{32}P-labeled human liver CPT I cDNA as probe. The major species of mRNA detected had the same size (≈4.7 kb) as the liver CPT I message shown in Fig. 4.

(*ii*) First-strand cDNA generated from fibroblast total RNA was used as a template in PCR reactions with primers based on the liver CPT I cDNA sequence. Two overlapping products were subcloned and sequenced as described above. The combined nucleotide sequence was found to be 99.8% identical to that of the entire coding region of the liver CPT I cDNA. Of the five mismatches detected, the possibility exists that one or more of these represent true polymorphisms or, alternatively, PCR errors.

(*iii*) Homogenates of normal human fibroblasts, prepared in such a way that the mitochondria remained intact, were

exposed to 3 μM [^{3}H]etomoxir [a covalent ligand for CPT I (24)] together with ATP and CoASH for 30 min. This resulted in major labeling of only one membrane-bound protein that comigrated in SDS gels with rat liver CPT I. As noted above, the latter has the same monomeric size (≈88 kDa) as the human liver enzyme.

(*iv*) Other aliquots of the fibroblast homogenates were assayed for CPT I activity over a wide range of carnitine concentration using our standard assay procedure (22). The K_m for carnitine was found to be ≈40 μM, a value very similar to that reported by Demaugre *et al.* (10) for fibroblast CPT I (≈38 μM) and by our laboratory (22) for the human liver enzyme (≈39 μM). The K_m of human muscle CPT I for carnitine is an order of magnitude higher (22).

(*v*) In similar studies, the concentration of malonyl-CoA needed to inhibit fibroblast CPT I by 50% under defined assay conditions (22) was 1.3 μM. This value also is similar to those found earlier (10, 22) for CPT I in human liver and fibroblasts (1.2–1.6 μM) and is to be contrasted with the almost 100-fold greater sensitivity of the muscle enzyme to malonyl-CoA (22).

Taken together, these observations constitute compelling evidence that liver and fibroblast CPT I are one and the same enzyme. Accordingly, the practice of using fibroblast CPT I assays as an indication of whether this enzyme is genetically defective in liver receives more formal justification.

In addition to providing insights into the structure of a human CPT I isoform, together with preliminary analysis and chromosomal localization of its corresponding gene, we consider the present findings important for a number of other reasons. First, they demonstrate unequivocally that mitochondrial CPT I and CPT II not only are distinct proteins but are the products of entirely separate genes, an issue that has been the subject of much debate (2, 14, 15, 19). Second, availability of the cDNA for human liver CPT I will allow the determination of whether this particular form of the enzyme is expressed in nonhepatic tissues other than heart and fibroblasts, such as kidney [as seems likely (21)], pancreatic β cells (as we suspect), etc. Third, the data should pave the way for a more complete analysis of the human liver CPT I gene and identification of its associated regulatory elements, which undoubtedly exist, as judged from recent studies in rat (33, 34). They should also lead to an understanding of the genetic basis of "hepatic CPT I deficiency" and whether this adversely affects cellular function in tissues other than liver. Fourth, further refinement of the position of the liver CPT I gene on chromosome 11q will provide a new genetic marker and could be useful in elucidating the cause of other diseases that might segregate with the CPT I locus. Finally, the possibility of obtaining large quantities of recombinant human liver CPT I, particularly if this can be done with retention of enzyme activity, should be helpful for the purposes of structural studies and the design of inhibitors with potential clinical use.

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