# Cloning, Sequencing, and Regulation of Rat Liver Carnitine Octanoyltransferase: Transcriptional Stimulation of the Enzyme during Peroxisome Proliferation[†,‡]

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ABSTRACT: Several complementary DNAs for the peroxisomal enzyme carnitine octanoyltransferase (COT), cloned in the expression vector λgt11, have been isolated. Together, these clones cover 2143 bp of the COT cDNA sequence with an open reading frame for 523 amino acids. Northern analysis showed the mRNA size for this enzyme to be 3.5 kilobases. The 523 residue long amino acid sequence amounts to a molecular mass of 60 269 daltons, indicating that the cloned cDNAs contain most or all of the coding sequence for COT ($M_r$ ~62 000). Hybridization studies showed that the increased COT activity in the liver of rats, fed the potent peroxisome-proliferating drug Wy-14,643, is associated with a more than 40-fold rise in the steady-state level of the COT mRNA.

Three separate carnitine acyltransferase activities, catalyzing the reversible reaction L-(−)-carnitine + acyl-CoA ⇌ acylcarnitine + CoASH, have been identified in the rodent and bovine livers (Solberg, 1972; Markwell et al., 1973; Miyazawa et al., 1983; Farrell et al., 1983, 1984). These transferases are structurally and immunologically distinct from each other and show different substrate specificities. Carnitine acetyltransferase (CAT) is specific for short-chain acyl derivatives ($C_2$–$C_4$), and carnitine palmitoyltransferase (CPT) is most active toward medium- and long-chain molecules ($C_{10}$–$C_{18}$). Carnitine octanoyltransferase (COT) on the other hand has a preferential substrate specificity toward hexanoyl-CoAs. COT is localized mostly in peroxisomes (Farrell et al., 1984); CAT activity can be detected both in mitochondria and in peroxisomes, while the activity for CPT is confined in the mitochondria only.

Fatty acid β-oxidation in the peroxisome is carnitine independent, and the physiological role of COT in this organelle is thus not clear. It has been suggested however that COT plays a role in the transport of medium-chain acyl-coenzyme A's from peroxisomes to mitochondria (Farrell & Bieber, 1983; Farrell et al., 1984). Because of specificities of the peroxisomal β-oxidative enzymes toward long-chain fatty acid substrates, oxidation of fatty acyl-CoAs in peroxisomes terminates at the medium-chain length ($C_6$–$C_8$). Formation of acylcarnitines may then allow export of these molecules to the mitochondria for further metabolism. Likewise, peroxisomal CAT may aid in the removal of accumulated acetyl-CoAs. CPT on the other hand catalyzes the transfer of cytosolic long-chain fatty acyl-CoAs through the mitochondrial inner membrane to the site of β-oxidation.

Several peroxisome proliferators such as hypolipidemic drugs and phthalate plasticizers induce both peroxisomal and mitochondrial carnitine acyltransferases (Farrell et al., 1984; Lazarow & Fujiki, 1985; Hashimoto, 1987). Such induction has facilitated purification and characterization of these enzymes. Carnitine octanoyltransferase (COT) shows a much lower $K_M$ toward octanoyl-CoA as opposed to that toward palmitoyl-CoA and acetyl-CoA, respectively, thus demonstrating its specificity toward medium-chain acyl coenzyme A's. Furthermore, high $K_M$ values of COT for acylcarnitines suggest that under physiological conditions COT catalyzes the forward reaction to produce acylcarnitines and is likely to be involved in the export of incompletely oxidized fatty acyl-CoAs of peroxisomes to the mitochondria.

Delineation of the structure–function relation of these transferase enzymes and a study of their regulation under a variety of physiological, endocrine, and nutritional conditions require availability of the corresponding complementary DNA clones. In this paper we report the isolation of rat liver carnitine octanoyltransferase cDNA clones and determination of the COT cDNA sequence. The cloned cDNA for COT has been utilized as the hybridization probe to demonstrate that peroxisome proliferation, mediated by [[4-chloro-6-(2,3-xylidino)-2-pyrimidinyl]thio]acetic acid (Wy-14,643), a hypolipidemic drug, is associated with a marked induction in the hepatic level of the COT mRNA.

## Materials and Methods

*Isolation of Rat Liver Poly(A+) RNAs and Construction of a cDNA Library in λgt11.* Total nucleic acid was extracted from the liver with phenol–SDS (Rosenfeld et al., 1972), and mRNAs were purified by oligo(dT)–cellulose chromatography (Aviv & Leder, 1972). The λgt11 liver cDNA library was constructed from mRNAs of adult male rats (Fischer 344) fed for 4 weeks with Purina Chow 5012 mixed with 0.1% (w/w) of Wy-14,643, a product of Wyeth Laboratories, Radnor, PA (a gift from Dr. A. K. Hajra). The experimental conditions for construction of the cDNA library were described in details earlier (Chatterjee et al., 1987b).

*Purification of Rat Liver COT.* Carnitine octanoyltransferase was purified to apparent homogeneity from the rat liver homogenate according to the purification scheme of Miyazawa et al. (1983). Prior to the sacrifice of animals, livers were induced for the COT activity through dietary administration of dioctyl phthalate (2% w/w, Aldrich Chemical Co.) for 4 weeks. The COT activity at each step of the purification was monitored by assaying for production of coenzyme A from octanoyl-CoA in the presence of L-(−)-carnitine. The release of coenzyme A was monitored through disulfide exchanges between 5,5′-dithiobis(2-nitrobenzoic acid) and coenzyme A,

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resulting in an increase in light absorbance at 412 nm (Farrell et al., 1984).

*Antibody and Oligonucleotide Screening of the Rat Liver λgt11 cDNA Library.* The polyclonal rabbit antiserum to mouse liver carnitine octanoyltransferase (Farrell et al., 1984; courtesy of Dr. J. K. Reddy) was used to screen the λgt11 library for immunopositive plaques in the presence of isopropyl β-D-thiogalactopyranoside as described by Young and Davis (1983) with minor modifications (Chatterjee et al., 1987b). Positive recombinant phage were plaque purified through an additional three rounds of screening.

In order to isolate cDNA clones encompassing terminal 5′ and 3′ sequences of the COT mRNA, we prepared 5′- and 3′-specific oligonucleotide probes from the known sequence of the longest COT cDNA insert. The recombinant phage DNA containing this cDNA insert is referred to as λ-2A$_x$COT. The oligonucleotide probes, labeled at the 5′ end with $^{32}$P by use of [γ-$^{32}$P]ATP and the kinase reaction, were used to screen the λgt11 library for isolation of additional COT-specific cDNA clones.

*Isolation of Phage DNA, Purification of cDNA Inserts, and Construction of DNA Restriction Map.* DNA from positive recombinants were grown on a large scale according to established procedures (Maniatis et al., 1982). Phage particles were recovered by centrifugation through cesium chloride step gradients, and the DNA was isolated as described by Maniatis et al. (1982). The recombinant phage DNA was digested with *Eco*RI, and the cDNA insert was purified from a 0.7% agarose gel by electroelution.

For restriction map analysis, the nonrecombinant and recombinant phage DNAs were digested with the restriction enzyme *Bam*H1 following the conditions specified by the supplier (Bethesda Research Laboratories, Gaithersburg, MD). DNA fragments were separated by agarose gel electrophoresis and visualized by ethidium bromide staining.

*Hybrid Selection and mRNA Translation.* The purified cDNA insert (1 μg) of λ-2A$_x$COT was immobilized on nitrocellulose filters under the conditions of Maniatis et al. (1982). Prehybridization and hybridization of the filter disks were carried out under the conditions described before (Chatterjee et al., 1987a). The hybridization solution contained rat liver mRNAs (500 μg/mL) derived from animals fed a diet containing 0.1% (w/w) Wy-14,643 for 4 weeks. The bound RNA was eluted with boiling water, and in vitro translation of hybrid-selected mRNAs was carried out in the rabbit reticulocyte lysate (Bethesda Research Laboratories, Gaithersburg, MD) in the presence of [$^{35}$S]methionine (>1000 Ci/mmol, NEN, Boston, MA).

For immunoprecipitation, the translation products (30 μL) were incubated for 2 h with 10 μL of rabbit antiserum in the presence of 0.14 M NaCl at 25 °C, and the antigen–antibody complex was precipitated by *Staphylococcus aureus* protein A. Washing of the immunoprecipitate and electrophoretic analysis of the precipitated antigen on an SDS–polyacrylamide slab gel were done as described previously (Chatterjee & Roy, 1980).

*Dideoxy Sequencing of the Cloned cDNA.* A library of M13 clones was produced at the *Sma*1 site of the mp19 vector by random sonication of the self-ligated insert DNA (Deininger, 1983), and sequencing was performed by the dideoxy chain termination procedure (Sanger et al., 1977). Recombinant M13 phage growth, DNA preparation, and DNA chain termination sequencing with [α-$^{35}$S]dATPαS were performed according to the protocols of Bankier and Barrell (1983). Sequencing reactions were carried out in 96-well microtiter trays, and electrophoresis was done through a buffer gradient sequencing gel (Biggin et al., 1983). The sequences were determined from both strands, and each nucleotide was read an average of four times in both directions. Sequence data was assembled by DB SYSTEM programs (Staden, 1982).

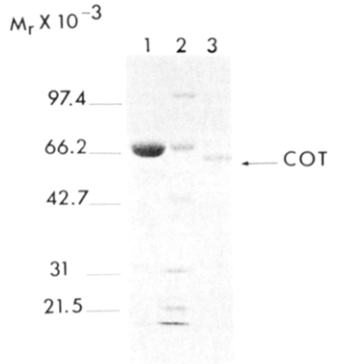


FIGURE 1: Estimated molecular weight of rat liver COT as analyzed by SDS–polyacrylamide gel electrophoresis. Lane 1, bovine serum albumin; lane 2, molecular weight markers; lane 3, purified COT after Blue Dextran–Sepharose 4B column chromatography.

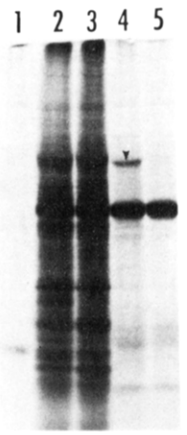


FIGURE 2: Clone identification by hybrid-selected mRNA translation. The figure shows the autoradiogram of an SDS–polyacrylamide slab gel. Lane 1, $^{14}$C-labeled protein markers; lanes 2 and 3, in vitro translation products of hepatic mRNAs from control (lane 2) and Wy-14,643-treated rats (lane 3); lane 4, translation products of the mRNAs hybrid selected by the insert of the recombinant phage DNA λ-2A$_x$COT. The 62 000-dalton translation product is shown by an arrow. Lane 5, translation products of mRNAs hybrid selected by the nonrecombinant λgt11 DNA (negative control). The endogenous band from the reticulocyte lysate is evident in both lanes 4 and 5.

*Electrophoresis and Blot Hybridization of Nucleic Acids.* For Northern analysis, 6 μg of poly(A+) RNAs was electrophoresed on an agarose gel (1.7%) containing 2.2 M formaldehyde (Maniatis et al., 1982). Prehybridization and hybridization conditions were same as before (Chatterjee et al., 1987a). $^{32}$P labeling of the cDNA insert was carried out by random priming (Feinberg & Vogelstein, 1983). Messenger RNA levels were analyzed by the dot blot procedure. Serially diluted RNA samples (starting with 1 μg of RNA and serial dilution of 2-fold thereafter) in the presence of 7.4% formaldehyde were spotted on the nitrocellulose filter. RNAs were fixed by baking the filter at 80 °C in vacuo. Filters were prehybridized and hybridized as in the Northern analysis and then exposed to Kodak XAR-5 film.

## RESULTS

*Purification of Carnitine Octanoyltransferase (COT) and Estimation of Its Molecular Weight by SDS–PAGE Analysis.* As shown in Figure 1, lane 3, COT has been purified to

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           10          20          30          40          50
           *           *           *           *           *
GGG AGT CGT GGT TCC CAT GGT ACA TAA TGG ATA CGG CTT TTT CTA CCA CAT CAG AGA

  60          70          80          90          100         110
  *           *           *           *           *           *
TGA CAG GTT TGT GGT GAC ATG TTC ATC CTG GAG GTC ATG TCT TGA GAC TGA TGC AGA

     120         130         140         150         160         170
      *           *           *           *           *           *
AAA GTT AGT GGA GAT GAT TTT TCA TGC TTT CCA CGA TAT GAT ACA TCT GAT GAA CAC

         180         190         200         210         220
          *           *           *           *           *
GGC TCA TCT TTA GAG ACT CAG AGA CAT ACA GGT CAC AGA AAC TGG GTA CAG AGA ATG

230         240         250         260         270         280
 *           *           *           *           *           *
GGA TGG TGA TAC GAC ATG GAA GGA ATG TTG ACT TAA AGG AAA CCT GTT AAT GCA GGG

    290         300         310         320         330         340
     *           *           *           *           *           *
ATT AGA GAG GGA TGC ACT CTA GAT TTA TTC TAC CTT AAA GCC TTC TGT TGC AAC AGC

        350         360         370         380         390
         *           *           *           *           *
AAT GCA AAC TCA GAC ATA GTG AAT AGA ACT ATG CAA TGT CTT AAG CCT CAA CAA TGC
                                    Met Gln Cys Leu Lys Pro Gln Gln Cys

400         410         420         430         440         450
 *           *           *           *           *           *
ACA TCT GTA TAT TTT AAC AAT ACA AAT CCT ACT CTA ATG TTA AAA TAT TTT TGT TGG
Thr Ser Val Tyr Phe Asn Asn Thr Asn Pro Thr Leu Met Leu Lys Tyr Phe Cys Trp

   460         470         480         490         500         510
    *           *           *           *           *           *
CAC ATG TGT AGG TTG CAA GTC CTC TGT GAC TAT ACC ATG GAA AAT CAA TTG GCT AAG
His Met Cys Arg Leu Gln Val Leu Cys Asp Tyr Thr Met Glu Asn Gln Leu Ala Lys

       520         530         540         550         560         570
        *           *           *           *           *           *
TCA ATT GAA GAA CGA ACA TTC CAG TAC CAG GAC TCT CTT CCG CCC TTG CCC GTT CCT
Ser Ile Glu Glu Arg Thr Phe Gln Tyr Gln Asp Ser Leu Pro Pro Leu Pro Val Pro

           580         590         600         610         620
            *           *           *           *           *
TCG CTT GAA GAA TCA CTG AAG AAG TAC CTT GAG TCA GTG AAG CCA TTT GCA AAT GAA
Ser Leu Glu Glu Ser Leu Lys Lys Tyr Leu Glu Ser Val Lys Pro Phe Ala Asn Glu

 630         640         650         660         670         680
  *           *           *           *           *           *
GAC GAA TAC AAG AAA ACT GAA GAA ATA GTT CAA AAG TTT CAA GAT GGA GTT GGC AAG
Asp Glu Tyr Lys Lys Thr Glu Glu Ile Val Gln Lys Phe Gln Asp Gly Val Gly Lys

     690         700         710         720         730         740
      *           *           *           *           *           *
ACA TTG CAT CAG AAG TTA CTT GAA AGG GCT AAA GGA AAA AGA AAC TGG CTG GAA GAG
Thr Leu His Gln Lys Leu Leu Glu Arg Ala Lys Gly Lys Arg Asn Trp Leu Glu Glu

         750         760         770         780         790
          *           *           *           *           *
TGG TGG CTC AAT GTC GCC TAC TTG GAT GTG CGT ATT CCA TCA CAA CTG AAC GTG AAC
Trp Trp Leu Asn Val Ala Tyr Leu Asp Val Arg Ile Pro Ser Gln Leu Asn Val Asn

 800         810         820         830         840         850
  *           *           *           *           *           *
TTT GTG GGT CCG TCT CCC CAC TTT GAA CAC TAC TGG CCT GCA AGG GAA GGC ACT CAG
Phe Val Gly Pro Ser Pro His Phe Glu His Tyr Trp Pro Ala Arg Glu Gly Thr Gln
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     860          870          880          890          900          910
      *            *            *            *            *            *
TTG GAA AGA GGA AGC ATA CTA CTG TGG CAC AAC TTG AAC TAC TGG CAG CTG CTA AGA
Leu Glu Arg Gly Ser Ile Leu Leu Trp His Asn Leu Asn Tyr Trp Gln Leu Leu Arg

         920          930          940          950          960
          *            *            *            *            *
AGA GAA AAA TTG CCT GTA CAT AAA TCT GGA AAT ACT CCT CTA GAC ATG AAC CAA TTC
Arg Glu Lys Leu Pro Val His Lys Ser Gly Asn Thr Pro Leu Asp Met Asn Gln Phe

970          980          990          1000         1010         1020
 *            *            *            *            *            *
CGG ATG CTG TTT TCT ACC TGC AAG GTT CCG GGA ATC ACT AGA GAT TCG ATT ATG AAT
Arg Met Leu Phe Ser Thr Cys Lys Val Pro Gly Ile Thr Arg Asp Ser Ile Met Asn

   1030         1040         1050         1060         1070         1080
    *            *            *            *            *            *
TAT TTT AAG ACT GAG AGC GAG GGG CAT TGT CCG ACC CAC ATT GCC GTG CTG TGT CGA
Tyr Phe Lys Thr Glu Ser Glu Gly His Cys Pro Thr His Ile Ala Val Leu Cys Arg

      1090         1100         1110         1120         1130         1140
       *            *            *            *            *            *
GGC AGA GCG TTT GTC TTC GAT GTC CTC CAT GAC GGT TGT TTG ATC ACC CCA CCA GAA
Gly Arg Ala Phe Val Phe Asp Val Leu His Asp Gly Cys Leu Ile Thr Pro Pro Glu

          1150         1160         1170         1180         1190
           *            *            *            *            *
CTT CTC AGA CAA CTG ACA TAC ATC TAC CAG AAA TGC TGG AAT GAA CCT GTT GGG CCC
Leu Leu Arg Gln Leu Thr Tyr Ile Tyr Gln Lys Cys Trp Asn Glu Pro Val Gly Pro

 1200         1210         1220         1230         1240         1250
  *            *            *            *            *            *
AGT ATA GCG GCA TTA ACC AGT GAG GAG CGA ACT CGG TGG GCG AAG GCA AGA GAA TAT
Ser Ile Ala Ala Leu Thr Ser Glu Glu Arg Thr Arg Trp Ala Lys Ala Arg Glu Tyr

    1260         1270         1280         1290         1300         1310
     *            *            *            *            *            *
CTG ATT GGT CTT GAT CCA GAG AAC TTG ACT TTA TTA GAA AAA ATT CAA TCC AGT TTA
Leu Ile Gly Leu Asp Pro Glu Asn Leu Thr Leu Leu Glu Lys Ile Gln Ser Ser Leu

        1320         1330         1340         1350         1360
         *            *            *            *            *
TTT GTG TAT TCC ATA GAA GAC ACC AGT CCA CAT GCA ACC CCA GAA AAT TTT TCT CAG
Phe Val Tyr Ser Ile Glu Asp Thr Ser Pro His Ala Thr Pro Glu Asn Phe Ser Gln

1370         1380         1390         1400         1410         1420
 *            *            *            *            *            *
GTC TTT GAA ATG CTT CTT GGT GGA GAT CCA GCA GTG CGC TGG GGT GAC AAG TCC TAT
Val Phe Glu Met Leu Leu Gly Gly Asp Pro Ala Val Arg Trp Gly Asp Lys Ser Tyr

   1430         1440         1450         1460         1470         1480
    *            *            *            *            *            *
AAT CTG ATT TCC TTT GCT AAC GGA ATA TTT GGC TGT AGC TGT GAT CAT GCT CCT TAT
Asn Leu Ile Ser Phe Ala Asn Gly Ile Phe Gly Cys Ser Cys Asp His Ala Pro Tyr

       1490         1500         1510         1520         1530
        *            *            *            *            *
GAT GCA ATG TTT ATG GTG AAC ATT GCT CAC TAT GTT GAT GAG AAG CTC CTA GAG ACG
Asp Ala Met Phe Met Val Asn Ile Ala His Tyr Val Asp Glu Lys Leu Leu Glu Thr

1540         1550         1560         1570         1580         1590
 *            *            *            *            *            *
GAA GGG AGA TGG AAG GGT TCA GAA AAA GTC CGG GAT ATA CCG TTG CCA GAG GAG CTG
Glu Gly Arg Trp Lys Gly Ser Glu Lys Val Arg Asp Ile Pro Leu Pro Glu Glu Leu

  1600         1610         1620         1630         1640         1650
   *            *            *            *            *            *
GCT TTC ACT GTG GAT GAG AAG ATA CTG AAT GAC GTC TAC CAA GCC AAA GCC CAA CAC
Ala Phe Thr Val Asp Glu Lys Ile Leu Asn Asp Val Tyr Gln Ala Lys Ala Gln His

      1660         1670         1680         1690         1700         1710
       *            *            *            *            *            *
CTC AAA GCA GCA TCT GAT TTA CAG ATA GCA GCA TCT ACC TTC ACA TCT TTT GGC AAA
Leu Lys Ala Ala Ser Asp Leu Gln Ile Ala Ala Ser Thr Phe Thr Ser Phe Gly Lys
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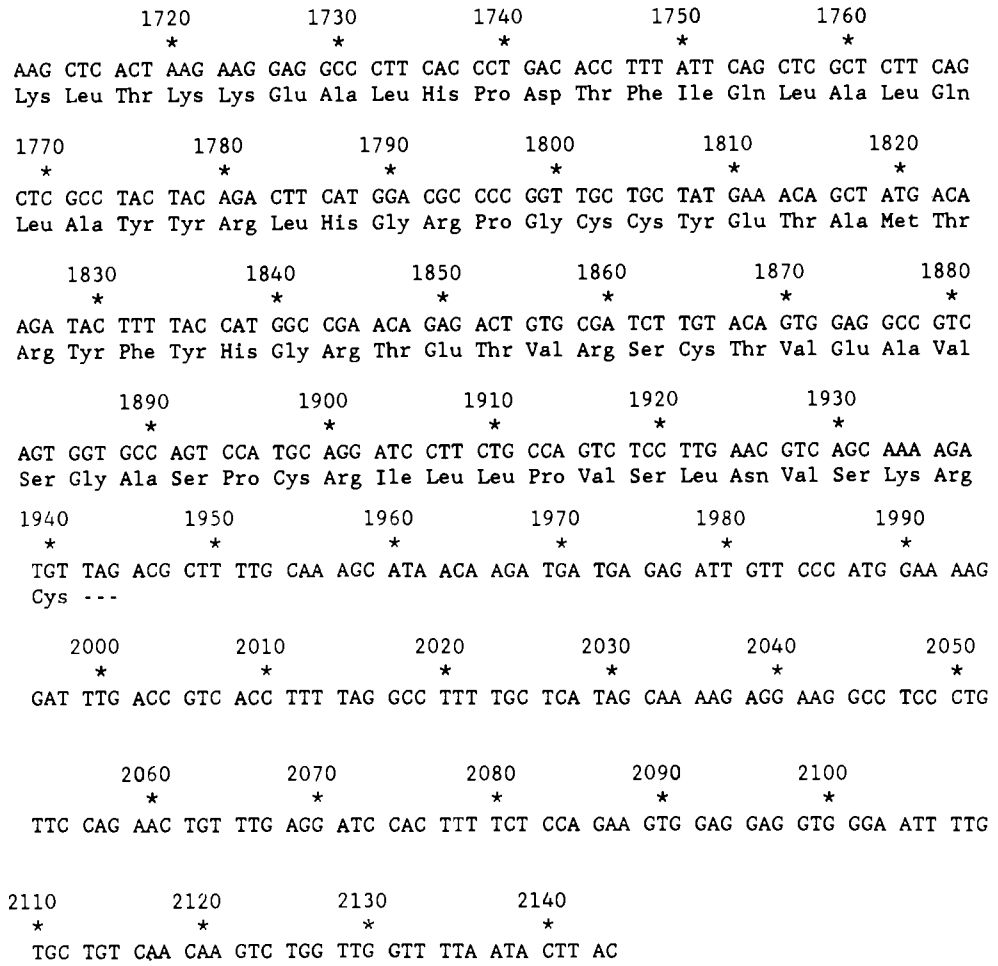


FIGURE 3: Composite nucleotide sequence and the translated amino acid sequence of the open reading frame for 2143 base pairs of COT cDNA.

electrophoretic homogeneity after the final purification step, i.e., after elution from the Blue Dextran–Sepharose 4B column. This fraction yields a single band upon staining with Coomassie blue. The stained band corresponds to a protein of 62 000 daltons as judged from the standard molecular mass markers (Figure 1, lane 2). The stained band in lane 1 of Figure 1 is for bovine serum albumin with an $M_r$ of about 66 000. Our estimated molecular weight of 62 000 for COT is similar to that reported by Miyazawa et al. (1983).

*Isolation and Characterization of Complementary DNAs (cDNAs) to Carnitine Octanoyltransferase.* The λgt11 cDNA library constructed from 1 μg of rat liver poly(A+) RNAs yielded $0.8 \times 10^5$ independent recombinant clones (Chatterjee et al., 1987b). Screening of the amplified library with the antibody to carnitine octanoyltransferase (anti-COT) gave eight immunopositive plaques. One of the recombinant plaques, λ-2A$_x$COT, was chosen for further analysis as it contained a large cDNA insert (1642 base pairs). The cDNA hybrid selected an mRNA species from rat liver mRNAs, which upon in vitro translation produced a 62-kdalton polypeptide (Figure 2, lane 4), a size the same as that of purified COT. The 62-kdalton protein synthesized in vitro by the hybrid-selected mRNA is also immunoprecipitable with anti-COT (not shown in the figure). Lanes 2 and 3 of Figure 2 represent electrophoretically separated in vitro translation products of total liver mRNAs from control (lane 2) and drug-treated (lane 3) rats. The presence of the predominant albumin band masks the enriched COT band in the drug-treated sample.

*Sequence Analysis of COT cDNA.* The cloned cDNA in λ-2A$_x$COT was further characterized by nucleotide sequence analysis. The sequence data established the insert size to be 1642 base pairs, including the *Eco*RI recognition sequence at the two termini. Out of the six possible reading frames of the sequenced DNA, four were ruled out by consideration of the fixed reading frame of the lac Z gene in λgt11 DNA. Digestion of the recombinant phage λ-2A$_x$COT with *Bam*H1 generated six DNA fragments of size 14.2, 11.2, 7.7, 6.5, 5.5, and 0.17 kilobase pairs. The comparative restriction cleavage patterns of the phage DNAs from λgt11 and λ-2A$_x$COT established the polarity of this cDNA insert.

The known nucleotide sequences at the 5′ and 3′ ends of the cDNA insert of λ-2A$_x$COT were used to synthesize 5′- and 3′-specific oligonucleotide probes (5′-specific, ATTGAAGA-ACGAACATTCCAGT; 3′-specific, TGCTGTCAA-CAAGTCT). The $^{32}$P-labeled probes were used to screen the λgt11 library to isolate additional cDNA clones for COT. The overlapping clones together span 2143 bp of COT cDNA, and the composite nucleotide sequence is given in Figure 3. The cDNA sequence corresponds to an open reading frame for 523 amino acids with a calculated $M_r$ of 60 269 (Figure 3). No poly(A) tail sequence, the AATAAA homology for the polyadenylation signal (Birnstiel et al., 1985) or processing sequence (Proudfoot & Brownlee, 1976), was found in any of the isolated cDNA clones. Thus the 3′ noncoding sequence of the COT mRNA is not represented in the composite nucleotide sequence given in Figure 3. The 523 amino acid long open reading frame ($M_r$ 60 269) suggests that the isolated cDNA corresponds to the major portion of the coding region of the COT mRNA.

*Transcriptional Induction of COT mRNA by Wy-14,643.* Northern analysis (Figure 4a) with the $^{32}$P-labeled cDNA

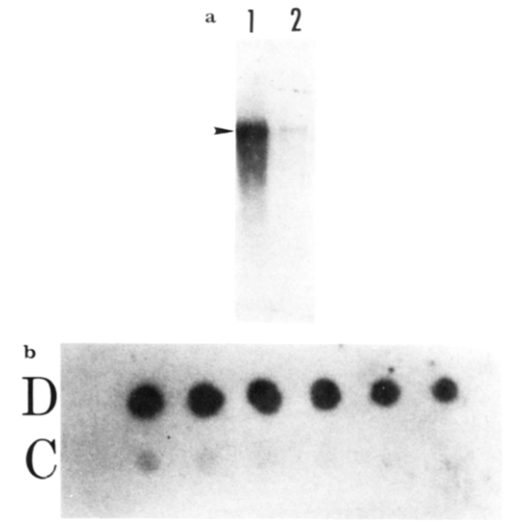


FIGURE 4: Induction of the hepatic COT mRNA in the presence of the hypolipidemic drug Wy-14,643. Drug-treated animals represent rats which were fed a diet containing 0.1% (w/w) Wy-14,643 for 4 weeks. (a) Northern blot analysis of rat liver mRNAs. Lane 1, drug-treated rats; lane 2, control rats. The arrowhead shows the position of the hybridizable COT mRNA. Its size (3.5 kb) has been estimated from the sizes of 28S (4.73 kb) and 18S (1.87 kb) ribosomal RNAs. (b) Dot blot analysis of the COT mRNA. D, Drug-treated; C, control rats. Hybridization of Northern blots with the $^{32}$P-labeled albumin cDNA probe revealed no differences in the levels of albumin mRNAs for the livers of drug-treated and control rats (Chatterjee et al., 1987b).

insert of λ-2A$_x$COT shows that the mRNA size for COT is approximately 3500 base pairs. The size of the hybridizable mRNA species was estimated by comparing its electrophoretic mobility with that of 28S (4.72 kbp) and 18S (1.87 kbp) ribosomal RNAs which were visualized by staining the nitrocellulose filter with methylene blue (Maniatis et al., 1982). The liver mRNA isolated from Wy-14,643-fed rats (lane 1, Figure 4a) showed a marked increase in the level of the COT mRNA as compared to that from nontreated control animals (lane 2, Figure 4a). The degree of induction of the hepatic content of the COT mRNA was quantitated by the dot blot analysis (Figure 4b). From densitometric scanning of the dot blot autoradiogram, it is evident that the lipid-lowering drug Wy-14,643 causes more than a 40-fold induction in the COT mRNA. The same RNA preparations (i.e., control and drug treated) when assayed for albumin mRNA with a cloned cDNA probe did not show any significant alteration in the level of albumin mRNA (Chatterjee et al., 1987b). Thus the increased carnitine octanoyltransferase activity during peroxisome proliferation reflects a corresponding rise in the steady-state level of its mRNA.

## Discussion

The present study describes cloning and sequencing of the complementary DNA (cDNA) to rat liver carnitine octanoyltransferase (COT). The identity of the COT cDNA clone is established by the following criteria: (1) immunoreactivity of the λgt11 recombinant protein product with the authentic COT antiserum [this antiserum has been extensively characterized by Farrell et al. (1984)]; (2) close correlation between the molecular weights of the hybrid-selected mRNA translation product and purified COT of the rat liver; (3) more than a 40-fold increase, by a potent peroxisome proliferator, in the level of the specific mRNA which hybridizes with the cloned cDNA insert. The cloned cDNA for COT hybridizes with a 3.5-kilobase mRNA species, although overlapping cDNA clones together so far have spanned 2143 bases of the mRNA. The sequence data revealed an open reading frame (ORF) for 523 amino acids with a calculated $M_r$ of 60 269. The AUG initiator codon of this ORF is preceded by the base "A" at position −3 and by "C" at position −2 and thus is likely to be the true initiator codon in accordance with the Kozak consensus sequence of $CC^{A}_{G}CCAUG$ for the eukaryotic initiation site (Kozak, 1984). Repeated attempts to screen the λgt11 cDNA library with the 3′-specific oligonucleotide probe failed to extend the λ-2A$_x$COT cDNA clone toward the 3′ end. The cDNA library used for isolation of clones probably does not represent the 3′ end of the COT mRNA. A specific secondary structure of the mRNA in this region may account for the absence of such sequences. The close correspondence between the calculated and estimated $M_r$'s of COT suggests that isolated cDNA clones represent most of the coding sequence of the COT mRNA. The relatively large (3.5 kilobases) size of the COT mRNA points to the possibility for a long untranslated region. It is interesting to note that long 3′ noncoding sequences have also been found in the mRNAs of other rat liver peroxisomal enzymes, i.e., acyl-CoA oxidase with ~1800 bases and enoyl-CoA-hydratase:3-hydroxyacyl-CoA dehydrogenase and catalase with 700–800 bases (Lazarow & Fujiki, 1985; Osumi et al., 1984; Furuta et al., 1986). However, the mRNAs for yeast peroxisomal enzymes usually have shorter noncoding sequences (Lazarow & Fujiki, 1985).

A wide variety of hypolipidemic compounds with diverse chemical structures induce peroxisome biogenesis and β-oxidation activity of this organelle. Results reported here also show that the increased COT enzymatic activity during peroxisome proliferation is due to a rise in the steady-state level of the corresponding mRNA. This finding is similar to what has been reported for other inducible peroxisomal enzymes (Chatterjee et al., 1983, 1987b; Osumi et al., 1984; Lazarow & Fujiki, 1985; Rachubinski et al., 1985; Fijiki et al., 1986; Reddy et al., 1986; Furuta et al., 1986). Earlier we also showed that in the primary rat hepatocytes about 10 h of time lag exists for the transcriptional activation of the gene for the bifunctional enzyme hydratase–dehydrogenase following exposure of the cells to Wy-14,643 (Chatterjee et al., 1987b). The delayed drug effect may indicate a cascade mechanism for the drug-mediated induction in peroxisomal enzymes. However, in whole animal studies the drug was reported to induce the mRNA rather rapidly (within 1 h) (Reddy et al., 1986). At present we cannot explain the discordant results of in vitro and in vivo studies. A study of the kinetics of induction of hepatic COT and quantification of the rate of transcription of this gene before and after drug-mediated peroxisome proliferation will further delineate the relationship between this enzyme and the β-oxidation activity in this organelle.

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**Registry No.** COT, 39369-19-2; COT cDNA, 117183-16-1; Wy-14,643, 50892-23-4; rat liver COT, 117183-21-8.

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# Human Ethanol-Inducible P450IIE1: Complete Gene Sequence, Promoter Characterization, Chromosome Mapping, and cDNA-Directed Expression[†,‡]

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ABSTRACT: The human P450IIE1 gene, coding for an ethanol-inducible nitrosamine-metabolizing P-450, was isolated from a λEMBL3 genomic library and completely sequenced. The human gene spanned 11 413 base pairs and contained nine exons and a typical TATA box. Upstream and downstream DNAs of 2788 and 559 base pairs were also sequenced and compared to the rat gene. Significant areas of sequence similarity were observed within 140 base pairs upstream of the transcription start site in the rat and human genes. Human DNA 539 base pairs upstream of the transcription start site was inserted into the expression vector pSVOALΔ5′, and luciferase activity was detected when the constructs were introduced into a rat hepatoma cell line. The activity was over 100-fold lower than that of pRSVL, a Rous sarcoma virus LTR-driven luciferase gene. By use of panels of rodent–human cell hybrids, the gene was mapped to chromosome 10 (CYP2E locus). A full-length cDNA, constructed with the first exon of the genomic clone and a partial cDNA clone, was expressed in COS cells and found to code for *N*-nitrosodimethylamine demethylase activity.

The cytochrome P-450 gene superfamily consists of at least ten families of enzymes that share several common features among which is the presence of a noncovalently bound heme associated with a conserved cysteine-containing peptide near the carboxy terminus of the enzyme. The P-450s all contain about 500 amino acid residues and a noncleaved signal sequence and are intrinsically bound to endoplasmic reticulum membranes. These enzymes carry out a myriad of diverse biotransformations including both anabolic and catabolic reactions. The enzymology (Waxman, 1986; Black & Coon,

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