Cloning and mRNA tissue distribution of rabbit cholesteryl ester transfer protein

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Abstract The amino acid sequence of rabbit cholesteryl ester transfer protein (CETP) has been obtained from cloned cDNA and genomic sequences. The 496 amino acid rabbit CETP has an overall sequence homology of 81% compared to the 476 amino acid human CETP, with two-thirds of the amino acid substitutions being conservative. Like human CETP, rabbit CETP is extremely hydrophobic, which is consistent with its function in the transfer of neutral lipids. The data implies extensive structural similarity between rabbit and human CETP. Rabbit CETP mRNA is estimated to be 2.2 kilobases in size, 300 nucleotides longer than the corresponding human mRNA, and contains the unusual polyadenylation signal sequence AGTAAA. In rabbit, CETP mRNA is found mainly in the liver, with small amounts also present in adrenal glands and kidney. In contrast to human spleen, rabbit spleen does not have detectable amounts of CETP mRNA. Northern blot analysis of liver poly(A)+ RNAs revealed significant amounts of CETP message in human, rhesus, and rabbit, and undetectable levels in pig, mouse, and rat, in agreement with reported plasma levels of transfer activity.—**Nagashima, M., J. W. McLean, and R. M. Lawn.** Cloning and mRNA tissue distribution of rabbit cholesteryl ester transfer protein. *J. Lipid Res.* 1988. **29:** 1643–1649.

Supplementary key words rabbit cholesteryl ester transfer protein cDNA • mRNA tissue distribution

Cholesteryl ester transfer protein (CEPT) is a plasma glycoprotein that functions to transfer neutral lipids among lipoprotein particles (1–5). Free cholesterol from peripheral tissues is transferred to high density lipoproteins (HDL) where it is esterified by lecithin:cholesterol acyl transferase (LCAT). CETP is capable of transferring cholesteryl esters in exchange for triglycerides to low and very low density lipoproteins (LDL,VLDL) which can subsequently be catabolized in the liver. In addition, CETP may be involved in the direct transfer of cholesteryl ester between plasma and cells or interstitium (6, 7).

The plasma levels of CETP activity vary greatly among species, which may account in part for their distinctive lipoprotein profiles (8). Plasma transfer activity appears to be modulated by CETP mass and by circulating inhibitors (9). It has also been observed that species with relatively high plasma CETP activity such as humans and rabbits are susceptible to diet-induced atherosclerosis, while species with low CETP activity such as rat are resistant to this disease. Thus, by altering the composition of plasma lipoproteins, CETP plays an important role in the metabolism of lipoproteins and may be involved in the development of atherosclerosis.

Presently, there is little known about the regulation of CETP metabolism. In order to enhance the applicability of rabbit as a model system in these studies, we have isolated and characterized rabbit CETP cDNA and genomic clones using human CETP cDNA as a probe, and have investigated the distribution of CETP mRNA in rabbit tissues and in the liver of several other species.

EXPERIMENTAL PROCEDURES

Materials

Restriction enzymes, Klenow fragment, T4 polynucleotide kinase, and T4 DNA ligase were purchased from Boehringer Mannheim and New England Biolabs. Reagents used for the preparation of RNA probes were from Promega Biotec. [α-^{32}P]-dATP and [α-^{32}P]-dCTP (3000 Ci/mmol) and [γ-^{32}P]ATP (>5000 Ci/mmol) were from Amersham.

Isolation of rabbit CETP cDNA and genomic clones

Both oligo(dT)-primed and randomly primed New Zealand White rabbit liver cDNA libraries in λgt10 vector (provided by David Leung and William Wood, Genentech) were screened with a ^{32}P-labeled 1.36 kb *Ava* I fragment of human CETP cDNA (10). Following the plaque hybridization at 42°C in 40% formamide, 5 × SSC, 50 mM so-

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Abbreviations: CETP, cholesteryl ester transfer protein; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; LCAT, lecithin:cholesterol acyltransferase.

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dium phosphate (pH 6.8), 5 × Denhardt's 5% dextran sulfate, 0.1% SDS, and 200 μg/ml of boiled sperm DNA, nitrocellulose filters were washed in 0.2 × SSC/0.1% SDS at 53°C and exposed to X-ray film. Insert DNAs from positive clones were isolated as described (11). Since none of the rabbit CETP clones isolated from oligo(dT)-primed or random oligonucleotide-primed cDNA libraries extended to the 5' terminus of the coding region, a rabbit genomic DNA library in λCharon 4A (provided by Ross Hardison; 12) was screened with ^{32}P-labeled full-length insert DNA from rabbit CETP cDNA clones. The regions of the genomic clone containing the 5'-terminal coding sequence were identified by hybridizing restriction enzyme digests of the insert DNA isolated from the genomic clone with a 63-base oligonucleotide 5'-AATGCCCATGCCTGCTCCAAAGG-CACCTCGCACGAGGCAGGCATCGTGTGCCGCAT-CACCAAG-3' representing the 5' end of human CETP cDNA (10). The hybridization was done in 30% formamide, and the filters were washed in 0.5 × SSC, 0.1% SDS at 50°C.

DNA sequencing strategy

Restriction digest fragments of rabbit CETP clones were subcloned into either M13 mp18/mp19 vectors or pUC118/pUC119 vectors (13) for DNA sequencing by the dideoxy chain termination method (14), using both universal and synthetic primers. For the sequencing of clones containing the GC-rich 5' end, reactions with Klenow fragment of DNA polymerase were performed at 55°C in the presence of 3% glycerol (E. Y. Chen, personal communication). Ninety percent of the sequence was derived from both DNA strands.

RNA isolation and Northern blot analysis

Total RNAs were extracted from various tissues by the guanidine thiocyanate method (15), and poly(A+) RNAs were isolated by oligo(dT)-cellulose chromatography. For Northern blot hybridization analysis, RNA samples were electrophoresed in a 1% agarose, 6% formaldehyde gel and transferred to nitrocellulose filters. Filters were hybridized with either cDNA probes at 42°C as described above, or with RNA probes at 60°C in hybridization solution containing 40% formamide and 160 μg/ml yeast RNA. Washing was performed in 0.5 × SSC plus 0.1% SDS at 55°C for DNA probes and in 0.2 × SSC plus 0.1% SDS at 70°C for RNA probes. All filters were reprobed with rat β-actin cDNA (16) to demonstrate the presence of intact mRNA in all lanes.

RESULTS AND DISCUSSION

Isolation of rabbit CETP cDNA and genomic clones

A single rabbit CETP cDNA clone was isolated by screening 5 × 10^5 phage plaques from the oligo(dT)-primed rabbit liver cDNA library probed with a human CETP cDNA clone (10). The rabbit cDNA clone contained a 1.4 kb DNA insert extending to a poly(A) tail (designated λRCETP.1) (**Fig. 1**). The sequence of this clone showed substantial homology to human CETP cDNA, but lacked the NH_2-terminal coding region. Screening of 1.8 × 10^6 plaques from the randomly primed cDNA library yielded four more clones. The longest of these clones, (λRCETP.2) extended 320 nucleotides more than λRCETP.1 into the 5' end. Since

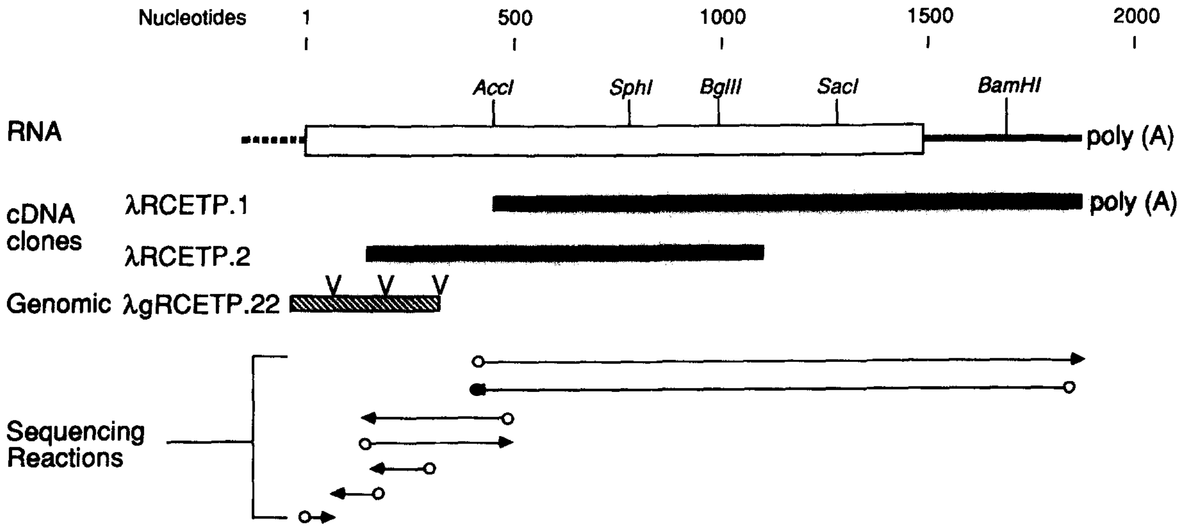

Fig. 1. Partial rabbit CETP mRNA. Rabbit CETP mRNA, as deduced from cDNA clones, is depicted beneath a size scale in nucleotides. Direction of transcription is from left to right. Features of the message are the undetermined length of 5'-untranslated and leader prepeptide coding region (dotted line), mature protein coding region (open box), and 3'-untranslated region ending in poly(A) site (line with bar). Recognition sites for several restriction enzymes are shown. Below this line, the horizontal solid bars indicate the extent of cDNA clones, and the hatched bar depicts the region of a genomic clone that was sequenced to elucidate the 5' end of mature protein coding region. The location of introns is shown by V. Below this, arrows indicate the extent and direction of cDNA sequencing reactions.

screening of two cDNA libraries failed to yield a clone containing the complete 5' end (probably due to high GC content), a rabbit genomic library constructed in λ Charon 4A was tested. One out of 10^6 plaques hybridized to a ^{32}P-labeled cDNA probe prepared from rabbit cDNA clones. In order to identify the region of this genomic clone (λgRCETP.22) coding for the NH_2-terminus, *Eco*RI/*Sal*I restriction digest fragments of the bacteriophage DNA were probed in Southern blots with a 63-base oligonucleotide that coded for the last 4 amino acids of the prepeptide and the initial 17 residues of mature human CETP. Portions of a 3.5 kb*Eco*RI/*Sal*I fragment that hybridized to the 63-base oligonucleotide were sequenced. Comparison to the human cDNA sequence implied the presence of three short exons that encoded 107 amino acids. Each exon is flanked by consensus splice site sequences (17), and the first exon was preceded by the sequence of the synthetic oligonucleotide linker used in library construction.

The cDNA sequence and the predicted amino acid sequence of rabbit CETP are shown in **Fig. 2.** By analogy to human sequence, we have designated cysteine as the first residue of the mature protein. As with human CETP, this cysteine residue is preceded by alanine, which is the most common residue found at the site of signal peptide cleavage (18). The predicted mature rabbit CETP is 496 amino acids long, and the 3' untranslated region is 376 bases long followed by a poly(A) tail. A polyadenylation signal sequence of AGTAAA (19) is situated 27 bases upstream of the poly(A) tail. The predicted polyadenylation hexapeptide is preceded by the similar sequence ATGAAA, 233 bp upstream. At the corresponding position in human CETP, G is replaced by T, resulting in polyadenylation near this site. Thus, assuming the similar sizes for the 5' untranslated regions, signal sequence and poly(A) tail, rabbit CETP mRNA would be at least 230 bp longer than human CETP mRNA, which is consistent with Northern blot hybridization results shown in Fig. 5a.

The comparison of amino acid sequences of mature rabbit and human CETP, shown in **Fig. 3**, revealed that rabbit CETP is 20 amino acids longer than human CETP. These differences consist of an additional 19 amino acids near the carboxy terminus and a threonine residue at position 317. Apart from these insertions, rabbit and human CETP share overall amino acid sequence homology of 81% and nucleotide homology of 85%. Furthermore, 60 of the 92 amino acid substitutions are of a conservative nature. This degree of structural homology implies functional similarity of the two proteins, enhancing the applicability of rabbit as a model system in the studies of CETP metabolism. The calculated molecular weight of rabbit CETP is 54,442. Since the molecular weight of rabbit CETP from plasma was estimated to be 68,000–70,000 (20, 21), this protein may be heavily glycosylated, as is human CETP. There are six potential N-linked glycosylation sites (Asn-X-Ser/Thr) distributed throughout rabbit CETP (Fig. 2), and three of these sites are found in corresponding positions in the human protein sequence. Like human CETP, rabbit CETP is extremely hydrophobic, with an overall hydropathy index of 0.08. This is more hydrophobic than all known apolipoproteins, consistent with the role of CETP in the transfer of neutral lipids between the core of lipoprotein particles. Using the algorithm of Garnier, Osguthorpe, and Robson (22), rabbit CETP has a predicted α-helical content of about 40% and residues 300 to 315 may form an α-helix with amphipathic nature. Another noteworthy feature is that while there are two extra Cys residues toward the COOH-terminus of rabbit CETP (Cys 390 and Cys 474), the positions of other seven Cys residues are conserved between rabbit and human CETP.

Tissue distribution of CETP mRNA

In humans, CETP mRNA has been detected in the liver and small intestine, the two major sources of apolipoprotein synthesis. Surprisingly, the mRNA was also detected in comparable or larger amounts in human adrenal gland and spleen (10). We made similar studies in a normal New Zealand white rabbit using Northern blot analysis (**Fig. 4**). In contrast to the result in humans, rabbit CETP appeared to be synthesized chiefly by the liver. Low amounts of CETP mRNA were detectable in adrenal glands and possibly in the kidney, while CETP mRNA in the spleen, small intestine, brain and testes (Fig. 4) as well as stomach, heart, lung, and skeletal muscle (not shown) were below the sensitivity of detection. While the contribution to the total circulating CETP mass by extrahepatic tissues would likely be insignificant, it would be possible that the local CETP production in these tissues might be important in the regulation of either efflux or influx of cholesteryl esters in these tisues. The increased efflux of cholesteryl esters from the smooth muscle cells of the arterial wall in the presence of exogenous CETP has been reported (23). Lack of CETP mRNA in rabbit spleen, in contrast to human spleen, has been confirmed in spleen samples from three humans and three rabbits. This difference may be attributed partly to the differences in the contents of macrophages, which have been shown to secrete a lipid transfer protein (24). Currently, we are investigating the possible synthesis of CETP in macrophages using *in situ* hybridization techniques.

In order to correlate the reported levels of CETP activity with synthesis, we monitored the presence of CETP mRNA in the liver of several species by Northern blot hybridization with rabbit and human CETP cDNA probes (**Fig. 5a**). While the differences in probe cross-reactivity rule out precise quantitation, the results show that large amounts of CETP mRNA are present in rabbit and rhesus monkey liver, much less in human and pig liver, and below the limit of detection in rat and mouse liver. These results are in agreement with the reported levels of plasma transfer ac-

```
 -1   1                                 10                                      20
ala cys pro lys gly ala ser tyr glu ala gly ile val cys arg ile thr lys pro ala leu leu val leu asn gln glu thr ala lys
GCC TGT CCC AAA GGC GCC TCC TAC GAG GCT GGC ATC GTG TGT CGC ATC ACC AAG CCC GCC CTC TTG GTG TTG AAC CAA GAG ACG GCC AAG

30                                      40                                      50
val val gln thr ala phe gln arg ala gly tyr pro asp val ser gly glu arg ala val met leu leu gly arg val lys tyr gly leu
GTG GTC CAG ACG GCC TTC CAG CGC GCC GGC TAT CCG GAC GTC AGC GGC GAG AGG GCC GTG ATG CTC CTC GGC CGG GTC AAG TAC GGG CTG

60                                      70                                      80
                                                                                                                 *
his asn leu gln ile ser his leu ser ile ala ser ser gln val glu leu val asp ala lys thr ile asp val ala ile gln asn val
CAC AAC CTC CAG ATC AGC CAC CTG TCC ATC GCC AGC AGC CAG GTG GAG CTG GTG GAC GCC AAG ACC ATC GAC GTC GCC ATC CAG AAC GTG

90                                      100
                                 *                                       •
ser val val phe lys gly thr leu asn tyr ser tyr thr ser ala trp gly leu gly ile asn gln ser val asp phe glu ile asp ser
TCC GTG GTC TTC AAG GGG ACC CTG AAC TAC AGC TAC ACG AGT GCC TGG GGG TTG GGC ATC AAT CAG TCT GTC GAC TTC GAG ATC GAC TCT

120                                     130                                     140
ala ile asp leu gln ile asn thr glu leu thr cys asp ala gly ser val arg thr asn ala pro asp cys tyr leu ala phe his lys
GCC ATT GAC CTC CAG ATC AAC ACA GAG CTG ACC TGC GAC GCT GGC AGT GTG CGC ACC AAT GCC CCC GAC TGC TAC CTG GCT TTC CAT AAA

150                                     160                                     170
leu leu leu his leu gln gly glu arg glu pro gly trp leu lys gln leu phe thr asn phe ile ser phe thr leu lys leu ile leu
CTG CTC CTG CAC CTC CAG GGG GAG CGC GAG CCG GGG TGG CTC AAG CAG CTC TTC ACA AAC TTC ATC TCC TTC ACC CTG AAG CTG ATT CTG

180                                     190                                     200
lys arg gln val cys asn glu ile asn thr ile ser asn ile met ala asp phe val gln thr arg ala ala ser ile leu ser asp gly
AAG CGA CAG GTC TGC AAT GAG ATC AAC ACC ATC TCC AAC ATC ATG GCT GAC TTT GTC CAG ACG AGG GCC GCC AGC ATC CTC TCA GAT GGA

210                                     220                                     230
asp ile gly val asp ile ser val thr gly ala pro val ile thr ala thr tyr leu glu ser his his lys gly his phe thr his lys
GAC ATC GGG GTG GAC ATT TCC GTG ACG GGG GCC CCT GTC ATC ACA GCC ACC TAC CTG GAG TCC CAT CAC AAG GGT CAC TTC ACG CAC AAG

                                        250                                     260
 *
asn val ser glu ala phe pro leu arg ala phe pro pro gly leu leu gly asp ser arg met leu tyr phe trp phe ser asp gln val
AAC GTC TCC GAG GCC TTC CCC CTC CGC GCC TTC CCG CCC GGT CTT CTG GGG GAC TCC CGC ATG CTC TAC TTC TGG TTC TCC GAT CAA GTG

270                                     280                                     290
leu asn ser leu ala arg ala ala phe gln glu gly arg leu val leu ser leu thr gly asp glu phe lys lys val leu glu thr gln
CTC AAC TCC CTG GCC AGG GCC GCC TTC CAG GAG GGC CGT CTC GTG CTC AGC CTG ACA GGG GAT GAG TTC AAG AAA GTG CTG GAG ACC CAG

300                                     310                                     320
gly phe asp thr asn gln glu ile phe gln glu leu ser arg gly leu pro thr gly gln ala gln val ala val his cys leu lys val
GGT TTC GAC ACC AAC CAG GAA ATC TTC CAG GAG CTT TCC AGA GGC CTT CCC ACC GGC CAG GCC CAG GTA GCC GTC CAC TGC CTT AAG GTG

330                                     340                                     350
pro lys ile ser cys gln asn arg gly val val val ser ser ser val ala val thr phe arg phe pro arg pro asp gly arg glu ala
CCC AAG ATC TCC TGC CAG AAC CGG GGT GTC GTG GTG TCT TCT TCC GTC GCC GTG ACG TTC CGC TTC CCC CGC CCA GAT GGC CGA GAA GCT

360                                     370                                     380
val ala tyr arg phe glu glu asp ile ile thr thr val gln ala ser tyr ser gln lys lys leu phe leu his leu leu asp phe gln
GTG GCC TAC AGG TTT GAG GAG GAT ATC ATC ACC ACC GTC CAG GCC TCC TAC TCC CAG AAA AAG CTC TTC CTA CAC CTC TTG GAT TTC CAG

390                                     400                                     410
                                                 *                                                               *
cys val pro ala ser gly arg ala gly ser ser ala asn leu ser val ala leu arg thr glu ala lys ala val ser asn leu thr glu
TGC GTG CCG GCC AGC GGA AGG GCA GGC AGC TCA GCA AAT CTC TCC GTG GCC CTC AGG ACT GAG GCT AAG GCT GTT TCC AAC CTG ACT GAG

420                                     430                                     440
ser arg ser glu ser leu gln ser ser leu arg ser leu ile ala thr val gly ile pro glu val met ser arg leu glu val ala phe
AGC CGC TCC GAG TCC CTG CAG AGC TCT CTC CGC TCC CTG ATC GCC ACG GTG GGC ATC CCG GAG GTC ATG TCT CGG CTC GAG GTG GCG TTC

450                                     460                                     470
thr ala leu met asn ser lys gly leu asp leu phe glu ile ile asn pro glu ile ile thr leu asp gly cys leu leu leu gln met
ACA GCC CTC ATG AAC AGC AAA GGC CTG GAC CTC TTC GAA ATC ATC AAC CCC GAG ATT ATC ACT CTC GAT GGC TGC CTG CTG CTG CAG ATG

480                                     490                     496
asp phe gly phe pro lys his leu leu val asp phe leu gln ser leu ser AM
GAC TTC GGT TTT CCC AAG CAC CTG CTG GTG GAT TTC CTG CAG AGC CTG AGC TAG CAGGGAGCTGGAGACACAAGACACGCTGACGTCTCCGCCCATCGGGGT

GGAGGTCAGGGAGTGGGTCGGAGGACGGGCGATGGCTCCCAACTCCTTCTGTCCTGAAGACCCCTAGCATGAAAGCAGCATACCCTGGGCAGGCATCTGGCTGAGCGGCTAAGCCACTGG

TCAGGACACCTGCGTCTAGGGTGCTCCGGATCCCAGCTGCCTGCTAACGTGCACCCTGGGGAGCAGCCGTGCTGGTTCCCGCCAGCCACATCGGAGACCCAGACTGAGGTCCTGGCTCCT

GGCTTTAGCCTGCCCAGTGAGTGGCAGCTAAATCTCTCTGGCTGTCTCTCTCTGCCTCTCAAGTAAACGAATATCTCAAAAACAAGAG
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Fig. 2. Sequence of rabbit CETP cDNA. The complete amino acid sequence of rabbit CETP is shown above the DNA sequence. The negative amino acid refers to the COOH terminus of the presumed leader prepeptide, whereas positive numbers refer to the mature protein. AM denotes the stop codon. The polyadenylation signal hexanucleotide is underlined, as is the location of the homologous polyadenylation hexanucleotide of human CETP (ATTAAA). Predicted N-linked glycosylation sites are marked by asterisks.

```
           +       10  +     20        30        40        50
rcetp      CPKGASYEAGIVCRITKPALLVLNQETAKVVQTAFQRAGYPDVSGERAVM
           *·**·* *****************·*****·*******·***··**·*·*
cetp       CSKGTSHEAGIVCRITKPALLVLNHETAKVIQTAFQRASYPDITGEKAMM
           +       10  +     20        30        40        50


                   60        70        80        90       100
                                                *         *
rcetp      LLGRVKYGLHNLQISHLSIASSQVELVDAKTIDVAIQNVSVVFKGTLNYS
           ***·*******·***************·**·***·************·*·
cetp       LLGQVKYGLHNIQISHLSIASSQVELVEAKSIDVSIQNVSVVFKGTLKYG
                   60        70        80       *90       100


                  110       120       130       140       150
                    *                    +           +
rcetp      YTSAWGLGINQSVDFEIDSAIDLQINTELTCDAGSVRTNAPDCYLAFHKL
           **·** ***·**·**************·****·* ***·******·****
cetp       YTTAWWLGIDQSIDFEIDSAIDLQINTQLTCDSGRVRTDAPDCYLSFHKL
                  110       120       130+      140  +    150


                  160       170       180   +   190       200
rcetp      LLHLQGEREPGWLKQLFTNFISFTLKLILKRQVCNEINTISNIMADFVQT
           ************·**************·** *·*·*** ***********
cetp       LLHLQGEREPGWIKQLFTNFISFTLKLVLKGQICKEINVISNIMADFVQT
                  160       170       180   +   190       200


                  210       220       230       240       250
                                                  *
rcetp      RAASILSDGDIGVDISVTGAPVITATYLESHHKGHFTHKNVSEAFPLRAF
           ****************·** *****·**********  ***** ·** ·*
cetp       RAASILSDGDIGVDISLTGDPVITASYLESHHKGHFIYKNVSEDLPLPTF
                                                  *
                  210       220       230       240       250


                  260       270       280       290       300
rcetp      PPGLLGDSRMLYFWFSDQVLNSLARAAFQEGRLVLSLTGDEFKKVLETQG
           ·* *************··*··***· ***·***·*** ***** **** *
cetp       SPTLLGDSRMLYFWFSERVFHSLAKVAFQDGRLMLSLMGDEFKAVLETWG
                  260       270       280       290       300


                  310       320     + 330   +   340       350
rcetp      FDTNQEIFQELSRGLPTGQAQVAVHCLKVPKISCQNRGVVVSSSVAVTFR
           *·********·  *·* ·****·*****·*******·****·*** * *
cetp       FNTNQEIFQEVVGGFP-SQAQVTVHCLKMPKISCQNKGVVVNSSVMVKFL
                  310       320     + 330   +   340 *


                  360       370       380       390       400
                                                  +
rcetp      FPRPDGREAVAYRFEEDIITTVQASYSQKKLFLHLLDFQCVPASGRAGSS
           ***** ···*** *****·********·***** *****  *
cetp       FPRPDQQHSVAYTFEEDIVTTVQASYSKKKLFLSLLDFQITP--------
                  360       370       380       390


            *     410     * 420       430       440       450
rcetp      ANLSVALRTEAKAVSNLTESRSESLQSSLRSLIATVGIPEVMSRLEVAFT
                      *·******* ***·** *·*·*··************ **
cetp       -----------KTVSNLTESSSESIQSFLQSMITAVGIPEVMSRLEVVFT
                          * 400       410       420       430


                  460       470   +   480       490
rcetp      ALMNSKGLDLFEIINPEIITLDGCLLLQMDFGFPKHLLVDFLQSLS
           *******· **·******** ** ********** ***********
cetp       ALMNSKGVSLFDIINPEIITRDGFLLLQMDFGFPEHLLVDFLQSLS
                  440       450       460       470
```

Fig. 3. Comparison of mature rabbit and human CETP. The top line presents the amino acid sequence of the mature rabbit CETP derived from cDNA sequencing, and the bottom line is the cDNA-derived sequence of human CETP (10). Asterisks between the two sequences indicate identical amino acids and dots indicate conservative substitutions. The overall amino acid homology is 81%. Cysteine residues are highlighted by + above the rabbit or below the human sequences and potential asparagine-linked glycosylation sites are highlighted by asterisks.

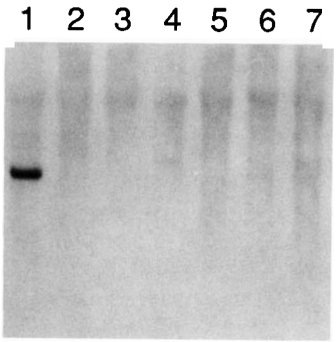

Fig. 4. Tissue distribution of CETP mRNA in rabbit. Rabbit poly(A$^+$) RNA samples were hybridized with an RNA probe derived from λRCETP.1 (*Eco*RI/*Bam*HI fragment) insert subcloned into pSP64 (30) and exposed to X-ray film. RNA sources and amounts (in parentheses) are: lane 1, liver (8 μg); lane 2, spleen (15 μg); lane 3, small intestine (7 μg); lane 4, kidney (10 μg); lane 5, brain (5 μg); lane 6, adrenal gland (3.5 μg); lane 7, testes (8 μg). Reprobing of the same filter with actin cDNA demonstrated the presence of intact RNA in all lanes (not shown).

tivity in these species (25). However, recent reports concerning a cholesteryl ester transfer inhibitor protein imply that the inhibitor masks substantial amounts of transfer activity in rats and pigs (26). It remains possible that extrahepatic synthesis of CETP is substantial in these species, or that the transfer protein of pig and rat shares comparatively little sequence homology to human and rabbit CETP.

The same mRNA samples were also hybridized with human and mouse LCAT probes (Fig. 5b). In previous studies human LCAT mRNA was only detected in liver (27). Furthermore, human and mouse LCAT cDNA sequences show 85% identity, implying substantial sequence conservation and probe cross-reactivity. (J. W. McLean, unpublished results). Liver LCAT mRNA levels are conspicuously higher in rat and mouse than in other species. Thus the ratio of CETP mRNA to LCAT mRNA is high in rabbit and rhesus monkey, intermediate in human, and very low in rat and mouse. In these latter species, most cholesteryl esters converted from cholesterol by relatively large amounts of LCAT on high density lipoproteins must be cleared from the circulation by a pathway that differs from the CETP and LDL receptor-mediated process that operates in humans. While nonhuman primates such as rhesus monkey would be an effective model for the study of CETP metabolism, a rabbit model would be more practical. Furthermore, using a cloned rabbit cDNA as a probe, possible involvement of CETP in the development of atherosclerosis may be investigated in cholesterol-fed rabbits and Watanabe heritable hyperlipidemic rabbits, in which elevated levels of plasma CETP activities have been detected (28, 29).

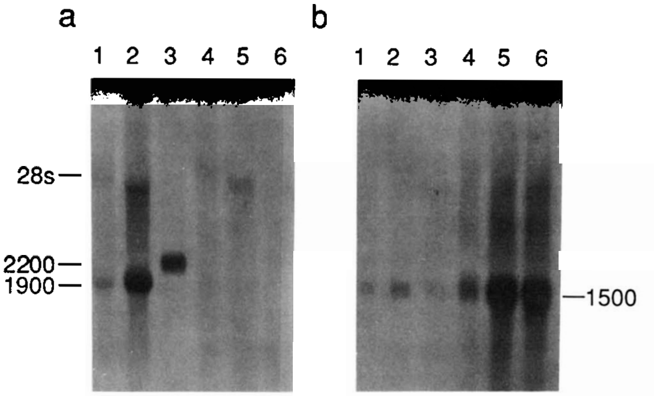

Fig. 5. Northern blot analysis of liver poly(A$^+$) RNA samples from various species hybridized with CETP and LCAT cDNA probes. Liver poly(A$^+$) RNA samples were subjected to electrophoresis as described in Methods, and hybridized with either ^{32}P-labeled rabbit and human CETP cDNA probes (panel a), or with human and mouse LCAT cDNA probes (panel b). (Rehybridization with rat actin cDNA probe is not shown.) Sources and amounts (in parentheses) of liver poly(A$^+$) RNA samples are: lane 1, human (14 μg); lane 2, rhesus monkey (10 μg); lane 3, rabbit (2 μg); lane 4, pig (14 μg); lane 5, rat (19 μg); lane 6, mouse (16 μg). The size, in nucleotides, of CETP mRNA and the location of 28s RNA is shown on the side.

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REFERENCES

1. Zilversmith, D. B., L. B. Hughes, and J. Balmer. 1975. Stimulation of cholesteryl ester exchange by lipoprotein-free rabbit plasma. *Biochim. Biophys. Acta.* **409:** 393–398.
2. Barter, P. J., Y. C. Ha, and G. D. Calvert. 1981. Studies of esterified cholesterol in subfractions of plasma high density lipoproteins. *Atherosclerosis.* **38:** 165–175.
3. DeParscau, L., and P. E. Fielding. 1984. Lecithin:cholesterol acyltransferase and cholesteryl ester transfer activity from the isolated perfused rabbit liver. *J. Lipid Res.* **25:** 721–728.
4. Albers, J. J., J. H. Tollefson, C-H. Chen, and A. Steinmetz. 1984. Isolation and characterization of human plasma lipid transfer proteins. *Arteriosclerosis.* **4:** 49–58.
5. Hesler, C. B., T. L. Swenson, and A. R. Tall. 1987. Purification and characterization of a human plasma cholesteryl ester transfer protein. *J. Biol. Chem.* **262:** 2275–2282.
6. Granot, E., I. Tabas, and A. R. Tall. 1987. Human plasma cholesteryl ester transfer protein enhances the transfer of cholesteryl ester from high density lipoproteins into cultured HepG2 cells. *J. Biol. Chem.* **262:** 3482–3487.
7. Stein, V., O. Stein, T. Olivecrona, and G. Halperin. 1985. Putative role of cholesteryl ester transfer protein in removal of cholesteryl ester from vascular interstitium studied in a model system in cell culture. *Biochim. Biophys. Acta.* **834:** 336–345.
8. Tall, A. R. 1986. Plasma lipid transfer proteins. *J. Lipid Res.* **27:** 361–367.
9. Morton, R. E., and D. B. Zilversmith. 1981. A plasma inhi-

bitor of triglyceride and cholesteryl ester transfer activities. *J. Biol. Chem.* **256:** 11992-11995.

10. Drayna, D., A. S. Jarnagin, J. McLean, W. Henzel, W. Kohr, C. Fielding, and R. Lawn. 1987. Cloning and sequencing of human cholesteryl ester transfer protein cDNA. *Nature.* **327:** 632-634.
11. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular Cloning. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
12. Maniatis, T., R. C. Hardison, E. Lacy, J. Lauer, C. O'Connell, D. Quon, G. K. Sim, and A. Efstratiadis. 1978. The isolation of structural genes from libraries of eukaryotic DNA. *Cell.* **15:** 687-701.
13. Vieira, J., and J. Messing. 1987. Production of single-stranded plasmid DNA. *Methods Enzymol.* **153:** 3-11.
14. Sanger, F., S. Nicklen, and A. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74:** 5463-5467.
15. Cathala, G., J-F. Savouret, B. Mendez, B. L. West, M. Karin, J. A. Martial and J. D. Baxter. 1983. Laboratory methods. A method for isolation of intact, translationally active ribonucleic acid. *DNA.* **2:** 329-335.
16. Nudel, U., R. Zakut, M. Shani, S. Neuman, Z. Levy, and D. Yaffe. 1983. The nucleotide sequence of the rat cytoplasmic β-actin gene. *Nucleic Acids Res.* **11:** 1759-1771.
17. Shapiro, M. B., and P. Senapathy. 1987. RNA splice junctions of different classes of eukaryotes: sequence statistics and functional implications in gene expression. *Nucleic Acids Res.* **15:** 7155-7174.
18. Perlman, D., and H. O. Halvorson. 1983. A putative signal peptidase recognition site and sequence in eukaryotic and prokaryotic signal peptides. *J. Mol. Biol.* **167:** 391-409.
19. Birnstiel, M. L., M. Busslinger, and K. Strub. 1985. Transcription termination and 3′ processing: the end is in site. *Cell.* **41:** 349-359.
20. Abbey, M., G. D. Calvert, and P. J. Barter. 1984. Changes in lipid and apolipoprotein composition of pig lipoproteins facilitated by rabbit lipid transfer protein. *Biochim. Biophys. Acta.* **793:** 471-480.
21. Ierides, M., N. Dousset, M. Potier, J. Manent, M. Carton, and L. Douste-Blazy. 1985. Cholesteryl ester transfer protein. *FEBS Lett.* **193:** 59-62.
22. Garnier, J., D. J. Osguthorpe, and B. Robson. 1978. Analysis of the accuracy and implications of simple methods for predicting the secondary structure of globular proteins. *J. Mol. Biol.* **120:** 97-120.
23. Stein, O., G. Halperin, and Y. Stein. 1986. Cholesteryl ester efflux from extracellular and cellular elements of the arterial wall. *Arteriosclerosis.* **6:** 70-78.
24. Tollefson, J. H., R. Faust, J. J. Albers, and A. Chait. 1985. Secretion of a lipid transfer protein by human monocyte-derived macrophages. *J. Biol. Chem.* **260:** 5887-5890.
25. Ha, Y. C., and P. J. Barter. 1982. Differences in plasma cholesteryl ester transfer activity in sixteen vertebrate species. *Comp. Biochem. Physiol.* **71:** 265-269.
26. Nishide, T., J. H. Tollefson, and J. J. Albers. 1987. Inhibition of lipid transfer by a specific high-density lipoprotein subclass containing an inhibitor protein. *Circulation.* **76:** IV-416 (abstr).
27. McLean, J., C. Fielding, D. Drayna, H. Dieplinger, B. Baer, W. Kohr, W. Henzel, and R. Lawn. 1986. Cloning and expression of human lecithin cholesterol acyltransferase cDNA. *Proc. Natl. Acad. Sci. USA.* **83:** 2335-2339.
28. Tall, A., E. Granot, R. Brocia, I. Tabas, C. Hesler, K. Williams, and M. Denke. 1987. Accelerated transfer of cholesteryl esters in dyslipidemic rabbit plasma. *J. Clin. Invest.* **79:** 1217-1225.
29. Son, Y. C., and D. B. Zilversmit. 1986. Increased lipid transfer activities in hyperlipidemic rabbit plasma. *Arteriosclerosis.* **6:** 345-351.
30. Melton, D. A., P. A. Krieg, M. R. Rebagliati, T. Maniatis, K. Zinn, and M. R. Green. 1984. Efficient in vitro synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage Sp6 promoter. *Nucleic Acids Res.* **12:** 7035-7056.