Cloning and expression of a cDNA encoding human sterol carrier protein 2

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ABSTRACT We report the cloning and expression of a cDNA encoding human sterol carrier protein 2 (SCP₂). The 1.3-kilobase (kb) cDNA contains an open reading frame which encompasses a 143-amino acid sequence which is 89% identical to the rat SCP₂ amino acid sequence. The deduced amino acid sequence of the polypeptide reveals a 20-residue amino-terminal leader sequence in front of the mature polypeptide, which contains a carboxyl-terminal tripeptide (Ala-Lys-Leu) related to the peroxisome targeting sequence. The expressed cDNA in COS-7 cells yields a 15.3-kDa polypeptide and increased amounts of a 13.2-kDa polypeptide, both reacting with a specific rabbit antiserum to rat liver SCP₂. The cDNA insert hybridizes with 3.2- and 1.8-kb mRNA species in human liver poly(A)⁺ RNA. In human fibroblasts and placenta the 1.8-kb mRNA was most abundant. Southern blot analysis suggests either that there are multiple copies of the SCP₂ gene in the human genome or that the SCP₂ gene is very large. Coexpression of the SCP₂ cDNA with expression vectors for cholesterol side-chain cleavage enzyme and adrenodoxin resulted in a 2.5-fold enhancement of progestin synthesis over that obtained with expression of the steroidogenic enzyme system alone. These findings are concordant with the notion that SCP₂ plays a role in regulating steroidogenesis, among other possible functions.

Sterol carrier protein 2 (SCP₂), also known as nonspecific lipid transport protein, is a 13.2-kDa basic polypeptide which is believed to facilitate the movement of sterols and phospholipids within cells (1–3). SCP₂ has been purified to homogeneity from rat, bovine, and human liver (4–6). The amino acid sequences of the rat and bovine liver polypeptides have been determined, revealing homology (>90% identity) (4–7). The amino acid composition of the human liver polypeptide is very similar to that of the rat and cow, suggesting a high degree of conservation across species (8). Antibodies raised against rat liver SCP₂ recognize the 13.2-kDa polypeptide as well as 40- and 58-kDa proteins, which are presumably related (8). The 58-kDa protein localizes to peroxisomes, a finding concordant with the presence of a tripeptide sequence (Ala-Lys-Leu) related to the peroxisomal targeting sequence (Ser-Lys-Leu) in the carboxyl terminus of the SCP₂ molecule (9). It is noteworthy that SCP₂ is present in low levels in subjects with Zellweger syndrome (cerebro-hepatic-renal syndrome), whose cells are deficient in peroxisomes and who have an associated impairment in plasmalogen and bile acid synthesis and catabolism of phytanic acid and very-long-chain fatty acids (8).

In steroid hormone producing cells SCP₂ is thought to facilitate the transport of cholesterol to mitochondria, where the first committed step in steroidogenesis takes place (1–3). The evidence supporting this line of thinking is primarily based on the analysis of the effects of purified SCP₂ on isolated organelles (e.g., mitochondria) and the use of antibodies to neutralize SCP₂ activity in cytosolic preparations (10–12).

cDNAs encoding rat liver SCP₂ have recently been cloned and sequenced. These suggest that the 13.2-kDa polypeptide is derived from a 15.3-kDa precursor (13, 14). Moreover, the cDNA sequences suggest that mRNAs encoding proteins of 55 kDa, or greater, and 30 kDa may also exist.

We have used the rat SCP₂ cDNA which we recently reported (13) to isolate a homologous human liver SCP₂ cDNA. Here we report the sequence of this cDNA‡ and its use as a probe in Northern analysis of human RNA and genomic Southern analysis of human DNA. The cDNA was also expressed in COS African green monkey kidney cells to examine its functional activity.

MATERIALS AND METHODS

Isolation of Human SCP₂ cDNAs. A human liver cDNA library in λGT11 (Clontech) was screened with an 850-base-pair (bp) fragment of a rat liver SCP₂ cDNA (13) produced by cleaving the 1.4-kilobase (kb) insert with *Sac* I. This cDNA fragment contained the coding sequence for rat SCP₂. Eight positive clones were isolated from the screening of 100,000 plaques. After secondary and tertiary screening, cDNA inserts 1.2 and 1.3 kb in size were selected for sequence analysis. These two inserts were subcloned in pGEM-4 (Promega Biotec) and subsequently were found to have identical nucleotide sequences.

The sequences of the cDNAs were determined by using the dideoxy chain termination method of Sanger *et al.* (15), employing the Klenow fragment of DNA polymerase and reverse transcriptase. A series of deletions of the cDNA inserts were prepared by digestion with exonuclease III and mung bean nuclease as described by Henikoff (16). The ends were treated with Klenow fragment before recircularizing as previously described (17).

Northern Blot Analysis. Human liver poly(A)⁺ RNA purchased from Clontech and poly(A)⁺ RNA prepared from normal human fibroblasts, term placenta, and JEG-3 choriocarcinoma cells were electrophoresed in 1% agarose/formaldehyde gels, transferred to nylon membranes, and probed with ³²P-labeled nick-translated 1.3-kb human SCP₂ cDNA as previously described (13). For comparison, rat liver poly(A)⁺ RNA was similarly treated and probed with the 1.4-kb rat SCP₂ cDNA as previously described.

Genomic Southern Analysis. Human and rat genomic DNA was digested with *Eco*RI, *Bam*HI, *Hind*III, *Pst* I, or *Bgl* II and 10 μg was electrophoresed in 0.7% agarose gels and then transferred to nylon membranes. These filters were probed

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        10        20        30        40        50        60        70        80
GAAAG AGGCA AGTTC CTGGT GCAAA GGTGG CTCTG CAGCA TAATT TAGGC ATTGG AGGAG CTGTG GTTGT AACAC TCTAC AAG ATG
                                                                                                    Met>

        90       100       110       120       130       140       150       160
GGT TTT CCG GAA GCC GCC AGT TCT TTT AGA ACT CAT CAA ATT GAA GCT GTT CCA ACC AGC TCT GCA AGT GAT GGA TTT
Gly Phe Pro Glu Ala Ala Ser Ser Phe Arg Thr His Gln Ile Glu Ala Val Pro Thr Ser Ser Ala Ser Asp Gly Phe>

        170       180       190       200       210       220       230       240
AAG GCA AAT CTT GTT TTT AAG GAG ATT GAG AAG AAA CTT GAA GAG GAA GGG GAA CAG TTT GTG AAG AAA ATC GGT GGT
Lys Ala Asn Leu Val Phe Lys Glu Ile Glu Lys Lys Leu Glu Glu Glu Gly Glu Gln Phe Val Lys Lys Ile Gly Gly>

        250       260       270       280       290       300       310       320
ATT TTT GCC TTC AAG GTG AAA GAT GGC CCT GGG GGT AAA GAG GAC ACC TGG GTG GTG GAT GTG AAG AAT GGC CAA GGA
Ile Phe Ala Phe Lys Val Lys Asp Gly Pro Gly Gly Lys Glu Asp Thr Trp Val Val Asp Val Lys Asn Gly Gln Gly>

        330       340       350       360       370       380       390
TCA GTG CTT CCT AAC TCA GAT AAG AAG GCT GAC TGC ACA ATC ACA ATG GCT GCC TCA GAC TTC CTG GCT TTA ATG ACT
Ser Val Leu Pro Asn Ser Asp Lys Lys Ala Asp Cys Thr Ile Thr Met Ala Ala Ser Asp Phe Leu Ala Leu Met Thr>

400       410       420       430       440       450       460       470
GGT AAA ATG AAT CCT CAG TCG GCC TTC TTT CAA GGC CCA TTG AAA ATC ACT GGC AAC ATG GGT CTC GCT ATG AAG TTA
Gly Lys Met Asn Pro Gln Ser Ala Phe Phe Gln Gly Pro Leu Lys Ile Thr Gly Asn Met Gly Leu Ala Met Lys Leu>

480       490       500       510       520       530       540       550
CAA AAT CTT CAG CTT CAG CCA GGC AAC GCT AAG CTC TGA AGAAC TCCCT TTGGC TACTT TTGAA AATCA AGATG AGATA
Gln Asn Leu Gln Leu Gln Pro Gly Asn Ala Lys Leu End>

560       570       580       590       600       610       620       630       640
TATAG ATATA TATCC ATACA TTTTA TTGTC AGAAT TTAGA CTGAA ACTAC ACATC GGCAA ATAGC GTGGG ATAGA TTTGT TTCTT

        650       660       670       680       690       700       710       720
AATGG GTGTG ACCAA TCCTG TTTTT CCTAA GCTGC GGGTG AATAG AGCCT GACTG GTATA CTACT GCTTT GCGAA TTGCA TACAA

730       740       750       760       770       780       790       800       810
CTGTG CATTA CAAAG TTAAT ATGGT AATTA TGGTC TGGGG TAAAA TTGAG TTTCA GAATA AAATT AGGAA CAGTA AAATC CAAAG

820       830       840       850       860       870       880       890
AACTA TGTAA ACAAA AAAGC TTTTG TTTTG CTTAC AAAGT ATATT TAAGG ATTAT TCTGC TGAAG ATTCA GTTTA AGAGT TTTCC

900       910       920       930       940       950       960       970       980
TTGGG AGAAC TAAGT AAGAA ACACA ATGCC AACAG CTGGC CAGTA ATTAG TGTTG TGCAC TTCAT GTCAT TAATC AATTT CTCAA

        990      1000      1010      1020      1030      1040      1050      1060
TAGTT CTTAA AATTA GTGAG ATTAA AAATC TAAAA ATTTT GCATT TCATG CTATC AGAAA CAGTA TTTTC TTCCC AAATC AAAAT

1070      1080      1090      1100      1110      1120      1130      1140      1150
AAAAG AAATA TGATC AGAGC TTGAA CACAG GCTTA TTTTT AAAAT AAAAA TATTT TTAAC ATGGG TTTCC TTATT GAAAA ATCAG

        1160      1170      1180      1190      1200      1210      1220
TGTAT TAGTC ATAAA ACACC ATCAT TAAGA ATAAT TGAAC AATAA AGTTT GCTTT CAGAT GCAGT TTTCA AAAAA AAAA
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FIG. 1.   Nucleotide sequence and deduced amino acid sequence of the human liver SCP_2 cDNA. Polyadenylylation signals (AATAAA) are underlined.

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with the human 1.3-kb and rat 1.4-kb SCP$_2$ cDNAs, respectively, as previously described (13).

Expression of the Human SCP$_2$ cDNA. The 1.3-kb human SCP$_2$ cDNA was cloned in pCMV-5 in the correct-reading and reverse orientations. pCMV-5 was generously provided by David Russell (University of Texas Health Science Center, Dallas, TX). This expression system has been extremely useful when introduced into COS cells due to the amplification of the plasmid, which contains an intact simian virus 40 origin of replication, driven by the simian virus 40 large tumor antigen, which is expressed in COS cells (18). These expression vectors were then transfected into COS-7 cells by the calcium phosphate coprecipitation method used in our laboratory (19). In some experiments, the SCP$_2$ expression constructs were cotransfected with pCD vectors harboring full-length cDNA inserts for bovine cholesterol side-chain cleavage enzyme (cytochrome P450 scc) and adrenodoxin (20). These constructs were generously provided by Michael Waterman (University of Texas Southwestern Medical Center, Dallas, TX). Briefly, COS-7 cells were cultured to 60% confluence in 100-mm plastic dishes in Dulbecco's minimal essential medium supplemented with 10% fetal bovine serum. The medium was changed 4 hr prior to application of DNA/calcium phosphate coprecipitates (20 μg of DNA per dish). The cells were exposed to the DNA/calcium phosphate precipitates for 4 hr and then placed in regular growth medium. After 60 hr the cells were scraped from the dishes into a buffer consisting of 50 mM Tris chloride containing 150 mM NaCl, 0.5% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, and 1 mM phenylmethylsulfonyl fluoride. Protein was assayed by the method of Bradford (21) and 200-μg aliquots of cell lysate were then subjected to SDS/PAGE in

18% polyacrylamide gels in some experiments. After electrophoresis, the separated proteins were transferred to nitrocellulose paper. Western blotting was carried out by the procedure of Towbin (22), using a 1:50 dilution of a rabbit antiserum to rat liver SCP$_2$ and a peroxidase-linked goat antibody to rabbit IgG as the second antibody (13).

Analytical Methods. Pregnenolone was quantitated by RIA (23), using an antiserum generously provided by Charles Strott (National Institutes of Health, Bethesda, MD). Progesterone was measured by RIA using commercial reagents (Diagnostic Products, Los Angeles).

RESULTS AND DISCUSSION

The sequence of the 1.3-kb cDNA (1229 nucleotides) clone revealed an open reading frame beginning at nucleotide 84 within the context of nucleotides appropriate for the initiation of translation by eukaryotic ribosomes. The encoded polypeptide consists of 143 amino acids and has an amino acid sequence which is 89% identical (13) to rat liver SCP$_2$ (Fig. 1). In contrast to the 1.4-kb rat cDNA we previously reported (13), the human cDNA has a long 3' untranslated region with four potential polyadenylylation signals.

The deduced amino acid sequence suggests a 20 amino acid leader sequence which, when cleaved, would yield the mature 13.2-kDa polypeptide whose amino acid composition is similar to that reported for SCP$_2$ isolated from human liver (8). The presence of a 20-residue amino-terminal leader sequence is consistent with the recent studies of Fujiki *et al.* (24) indicating processing of newly synthesized SCP$_2$ from a 15-kDa to a 13-kDa molecule. The cDNA sequence confirms the absence of arginine, histidine, and tyrosine residues from
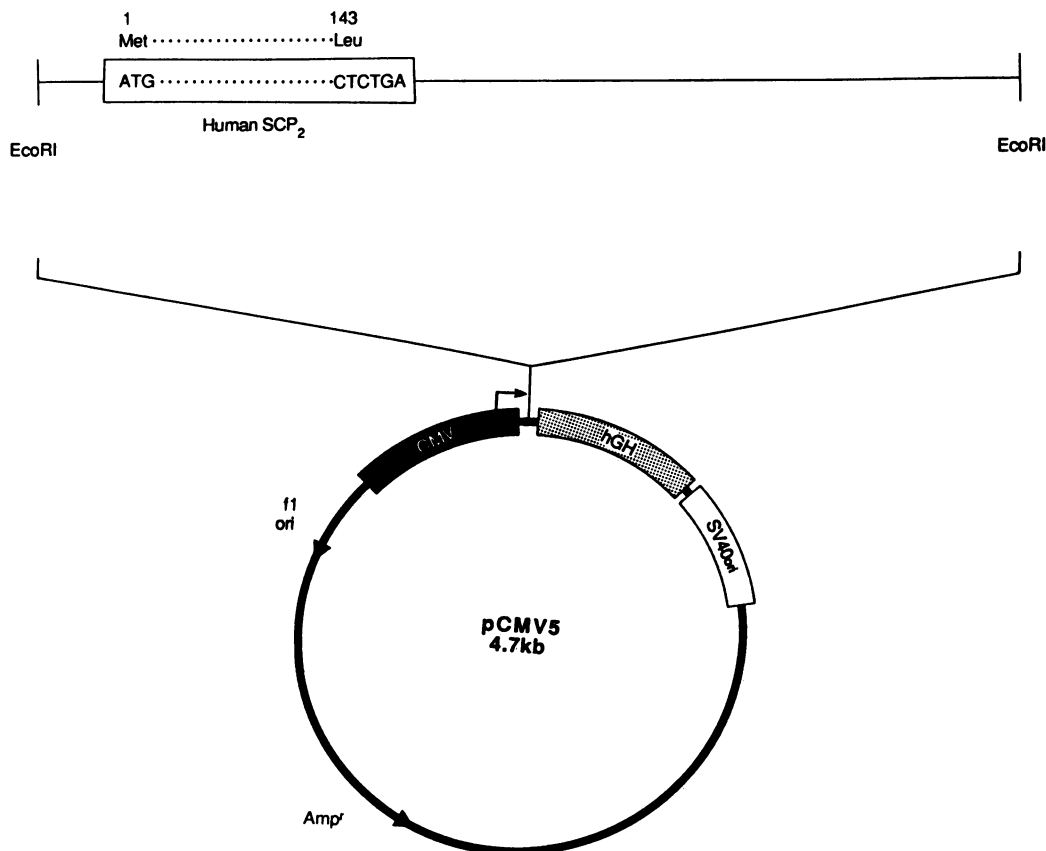
FIG. 2. SCP$_2$ expression vector based on pCMV-5. CMV, nucleotides -760 to $+3$ of human cytomegalovirus promoter of the major immediate early gene; f1 ori, phage f1 origin of DNA replication; Amp^r, ampicillin resistance; SV40ori, simian virus 40 origin of replication and early region promoter enhancer; hGH, human growth hormone transcription termination and polyadenylylation signal representing nucleotides $+1533$ to $+2157$ of the gene.
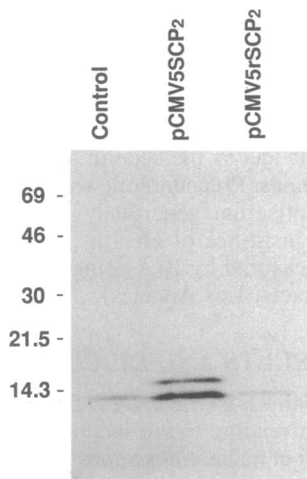
FIG. 3. Transient expression of the human liver SCP$_2$ cDNA in COS-7 cells. COS-7 cells were transfected with pCMV-5 vector harboring the SCP$_2$ cDNA in the correct and reverse orientation, using the calcium phosphate coprecipitation method. SCP$_2$ immunoreactive proteins in cell lysates were detected with a specific rabbit antiserum to rat liver SCP$_2$ by Western blotting. Lane 1, untransfected COS-7 cells; lane 2, cells transfected with pCMV-5 containing the SCP$_2$ cDNA in proper orientation; lane 3, cells transfected with pCMV-5 containing the SCP$_2$ cDNA in reverse orientation. Protein sizes are given in kDa.

the mature SCP$_2$ molecule and the presence of a carboxyl-terminal Ala-Lys-Leu peroxisome targeting sequence.

Expression of the 1.3-kb cDNA using the pCMV-5 vector (Fig. 2) in COS-7 cells promoted the appearance of a 15.3-kDa protein not readily detected in mock-transfected cells or cells transfected with the SCP$_2$ insert in pCMV-5 in the reverse orientation, as well as increased levels of the 13.2-kDa polypeptide reacting with a specific polyclonal antiserum against rat liver SCP$_2$ (Fig. 3). These findings are consistent with the production of a 15.3-kDa precursor which can be cleaved to the mature 13.2-kDa polypeptide. The antiserum reacted weakly with 30- and 58-kDa proteins in the COS-7 cells in addition to the 15.3- and 13.2-kDa polypeptides.

Northern blot analysis of human liver poly(A)$^+$ RNA revealed two mRNA species, one of 3.2 kb and one of 1.8 kb (Fig. 4). Analysis of rat liver poly(A)$^+$ RNA revealed predominant 1.4- and 3.2-kb mRNAs with a less abundant 2.1-kb mRNA. Human fibroblasts, placenta, and JEG-3 choriocar-
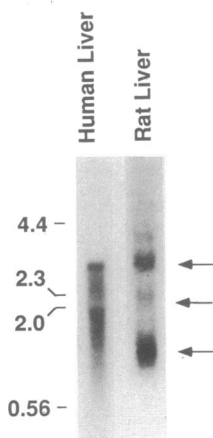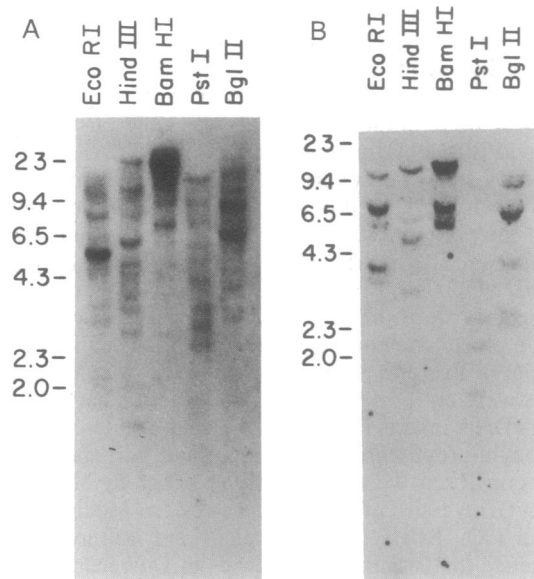

FIG. 5. Genomic Southern blot analysis of human (*A*) and rat (*B*) DNA. Genomic DNA was digested with the indicated restriction enzymes and 10 μg of each digest was electrophoresed and transferred to nylon filters. The blotted human and rat DNAs were hybridized with the respective cDNAs as described in the text. DNA lengths are given in kb.

cinoma cells contained a predominant 1.8-kb SCP$_2$ mRNA (data not shown).

Genomic Southern analysis suggested that the human genome contains multiple SCP$_2$-like gene copies or that the gene is quite large (Fig. 5). This is similar to our findings on genomic Southern analysis of rat DNA.

To begin to explore the functional activity of the expressed SCP$_2$ cDNA, we examined the effects of coexpression of SCP$_2$ with the cholesterol side-chain cleavage enzyme in COS cells. We selected this approach because of existing data indicating that SCP$_2$ plays a role in steroid hormone synthesis by enhancing transport of cholesterol to mitochondria and possibly enhancing the access of mitochondrial cholesterol to the side-chain cleavage enzyme. Untransfected COS cells or cells transfected with the pCMV-5 vector harboring the SCP$_2$ cDNA insert in either the correct or reverse orientation did not secrete detectable amounts (<0.1 ng per dish) of pregnenolone or progesterone (Table 1). Cells transfected with the pCD expression vectors for the cholesterol side-chain cleavage system secreted significant quanti-

FIG. 4. Northern blot hybridization of human liver poly(A)$^+$ RNA (10 μg) and rat liver poly(A)$^+$ RNA (10 μg) with ^{32}P-labeled nick-translated human and rat SCP$_2$ cDNAs, respectively. RNA lengths are given in kb.

Table 1. Steroid synthesis in COS-7 cells transfected with pCMV5SCP$_2$

Treatment group	Steroid synthesis, ng/dish	
	Pregnenolone	Progesterone
Control	ND	ND
pCMV5rSCP$_2$	ND	ND
pCMV5SCP$_2$	ND	ND
pCD	95.18 ± 20.92[a]	18.01 ± 3.88[a]
pCD + pCMV5rSCP$_2$	107.06 ± 20.21[a]	22.73 ± 7.25[a]
pCD + pCMV5SCP$_2$	293.97 ± 58.45[b]	39.70 ± 7.25[b]

COS cells were used untreated (control) or transfected with the indicated constructs. The pCD vectors encode adrenodoxin and cholesterol side-chain cleavage enzyme. Sixty hours after completion of transfection media were collected for assay of pregnenolone and progesterone. Values presented are means ± SEM for seven separate transfection experiments. Means with the same superscript are not statistically different, whereas means with different superscripts are significantly different by the paired t test at the $P \leq 0.025$ level. ND, not detected (<0.01 ng per dish).

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ties of progestin, with pregnenolone being the predominant product. Pregnenolone was expected to be the major steroid produced by these cells, although they apparently possess endogenous 3β-hydroxysteroid dehydrogenase activity capable of converting pregnenolone to progesterone. Coexpression of the SCP_2 cDNA with the side-chain cleavage system resulted in a more than 2.5-fold enhancement of progestin production, which was not seen when cotransfection was carried out with the pCMV-5 vector carrying the SCP_2 cDNA insert in the reverse orientation.

SCP_2 is detectable in COS cells (ref. 20 and this study) as well as a variety of other cells. In steroidogenic tissues, tropic hormones increase SCP_2 levels (13, 25). The increase in progestin synthesis found in the present experiments when SCP_2 levels were increased presumably reflects the impact of accumulation of SCP_2 above a limiting level of the polypeptide. Although we demonstrate a significant effect of SCP_2 expression on steroidogenesis in COS cells engineered to metabolize cholesterol into hormones, the exact mechanism(s) underlying this response remain to be elucidated.

In summary, we report here the cloning and sequence of a cDNA for human SCP_2. The polypeptide encoded by this cDNA is very similar in sequence to rat and bovine liver SCP_2 and the expressed protein reacts with specific rabbit antiserum to rat liver SCP_2, revealing conservation of the primary structure of the molecule across species. The cDNA probe hybridizes to mRNAs of different sizes, one (1.8 kb) which may encode the mature 13.2-kDa polypeptide and its leader sequence, and another (3.2 kb) which is of an appropriate size to encode a 58-kDa polypeptide. It remains to be determined whether these mRNAs are derived from a single or different genes.

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