Structure and Localization of the Human Gene Encoding SR-BI/CLA-1

EVIDENCE FOR TRANSCRIPTIONAL CONTROL BY STEROIDOGENIC FACTOR 1*

(Received for publication, August 27, 1997, and in revised form, October 5, 1997)

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The scavenger receptor, class B, type 1 receptor (SR-BI) mediates the selective transport of lipids from high density lipoprotein to cells. We describe the structure and subchromosomal location of human SR-BI and provide evidence that it is regulated by the transcription factor, steroidalogenic factor 1 (SF-1). SR-BI resides on chromosome 12q24.2-qter, spans ~75 kilobase pairs, and contains 13 exons. RNA blot analysis of human tissues reveals an expression pattern similar to that described previously for rodents with the highest levels of mRNA in the adrenal gland, ovary, and liver. Unlike rodents, human SR-BI was expressed at high levels in the placenta. The transcription start site for SR-BI was mapped, and DNA sequence analysis revealed a binding site for SF-1 in the proximal 5'-flanking sequence. SF-1, an orphan member of the nuclear hormone receptor gene family, plays a key role in the regulation of steroidogenesis and is expressed at high levels in steroidogenic tissues. SF-1 binds to the SR-BI promoter in a sequence-specific manner, and efficient transcription from this promoter in adrenocortical Y1 cells is dependent on an intact SF-1 site. These data extend our understanding of SF-1 function within steroidogenic tissues and suggest that SR-BI, which serves to supply selected tissues with lipoprotein-derived lipids, is part of the repertoire of SF-1-responsive genes involved in steroidogenesis.

The scavenger receptor gene family is comprised of a series of unlinked genes that encode membrane proteins that bind multiple ligands (1). A subgroup of this family, the class B receptors, includes two plasma membrane proteins, CD36 (2) and CLA-1 (3), and a major lysosomal integral membrane protein of unknown function termed LIMPII (4, 5). A Drosophila gene called epithelial membrane protein (emp), which is expressed early in development, is also a member of this gene family (6). The gene encoding CLA-1 (gene CD36LI) resides on chromosome 12, whereas the genes for CD36 and LIMPII (CD36L2) are on chromosomes 7 and 4, respectively (7, 8). The CLA-1, CD36, and LIMPII proteins share ~20% amino acid sequence identity and are all predicted to have two transmembrane segments. The most conserved region of the class B scavenger receptors is a large extracellular domain (or in the case of LIMPII, the luminal domain), that contains similarly spaced cysteine residues and multiple sites for N-linked sugar attachments.

CD36 is the best characterized of the class B scavenger receptors (for review, see Ref. 2). It is an 88-kDa palmitoylated glycoprotein with two membrane-spanning domains flanked by short cytoplasmic sequences (9). CD36 is expressed at high levels in megakaryocytes/platelets, monocytes/macrophages, mammary epithelial cells, and adipocytes. It is expressed in the capillary endothelial cells of adipose tissue and cardiac and muscle tissue and is present in trace amounts in the vascular endothelium of the brain, lung, and kidneys (10). CD36 has been implicated in signal transduction since it is physically associated with at least three protein-tyrosine kinases of the src gene family in platelets (11). CD36 binds thrombospondin (12), type I collagen (13), apopotic cells (14), acetylated LDL, oxidized LDL (15–17), long chain fatty acids (18), and malaria-infected erythrocytes (19). It is not known which of these ligands are physiologically important.

The cDNA for CLA-1, which stands for CD36 and LIMP II Analogous-I, was cloned based on sequence similarity with CD36 and LIMPII (3). The cDNA is 2566 nucleotides in length and is predicted to encode a protein of 509 amino acids. A physiological role for CLA-1 was suggested when the hamster cDNA homolog was identified as a scavenger receptor-class B, type I receptor (SR-BI). The hamster cDNA was independently isolated based on its ability to encode a receptor for acetylated low density lipoproteins (LDL) (15). The hamster SR-BI shares 85% sequence identity with human CLA-1. Throughout this paper we refer to the proteins encoded by the CLA-1/SR-BI cDNA as SR-BI and the human gene encoding this protein as SR-BI (previously referred to as CD36LI). SR-BI, like CD36, is palmitoylated and resides in caveolae, which are specialized membrane domains on the cell surface (20, 21). Both SR-BI and CD36 bind acetylated LDL, oxidized LDL, and maleyloxidated bovine serum albumin, but unlike members of the class A scavenger receptor family, the binding of these ligands is not inhibited by fucoidan or dextran sulfate (15). CD36 and SR-BI bind anionic phospholipids (22) and may play a role in the clearance of apoptotic cells (23, 24), but only SR-BI binds native LDL and high density lipoproteins (LDL) with high affinity (15, 25).

SR-BI delivers cholesteryl esters to cells by a very different mechanism than that used by the well characterized LDL re-
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ceptron (26). After the LDL receptor binds its ligand, the receptor and ligand enter the cell by receptor-mediated endocytosis. SR-BI binds HDL at the cell surface and selectively transports lipids to the cell by a yet to be characterized mechanism (25). The selective transport of lipids, but not proteins, from lipoprotein particles is a well studied phenomenon in rodents and is the major process utilized to provide cholesterol for steroidogenesis (26). SR-BI is expressed at high levels in the adrenal gland, ovary, and liver of rats and mice, where it is implicated in the delivery of cholesterol esters from HDL to cells within these tissues (25–28). Overexpression of the SR-BI protein in mouse liver is associated with a reduction in plasma levels of HDL cholesterol, implicating a possible role for this receptor in the transport of cholesterol from peripheral tissues to the liver (29).

In this paper, we report the isolation and characterization of the human SR-BI gene and provide the first insights into the mechanism that regulates the expression of this key mediator of cholesterol transport into steroidogenic cells.

**EXPERIMENTAL PROCEDURES**

**Isolation of Human SR-BI**—A human PAC library (Genome Systems Inc., St. Louis, MO) was screened using the polymerase chain reaction (PCR) and two oppositely oriented oligonucleotides from the 5′ region of the CLA-1 cDNA (3) (3a, 5′-CTTGGCGGATCCCTCTGGCCGAGC-3′; and 3b, 5′-CTTAAAGAGCCTGCTGTGATTAGAC-3′) that correspond to nucleotides 1–25 and 171–195 to amplify the intervening 195 bp. Three clones were identified. The largest one, pSR-B1a, has an insert of ~120 kb and contains sequences from both the 5′ and the 3′ ends of the SR-BI cDNA.

**Exon/intron Junctions of SR-BI**—The exon/intron junctions of exons 1–12 were either sequenced directly from pSR-B1a or sequenced from PCR-derived fragments using oligonucleotides with sequence identity to the SR-BI cDNA (Table I). All introns greater than 2.4 kb in length, except intron 1, were amplified in a DNA thermal cycler (Robocycler, Beckman, Fullerton, CA) as a 16×1 mixture of RienToq (AB peptides, St. Louis, MO) and FruToq- Polymerase (Stratagene) with the following program: 30 cycles of 99 °C for 30 s, 67 °C for 30 s, and 68 °C for 10 min. Introns shorter than 2.4 kb in length were amplified using 1 unit of AmpliTaq (Perkin Elmer, Branchburg, NJ) with the following program: 30 cycles of 99 °C for 30 s, 60 °C for 30 s, and 72 °C for 3 min. The amplification products were gel-purified using the GeneClean II kit (Bio 101, Inc., Vista, CA). Sequencing was performed using an automated sequencer (Applied Biosystems model 373A DNA, Perkin Elmer, Foster City, CA).

**Restriction Map of SR-BI**—The lengths of introns 2–11 were determined by PCR amplification of the introns using oligonucleotides from adjacent exons (Table II). The PAC clone was digested with either EcoR1 or HindIII digestion buffer. The samples were size-fractionated on a 1.0% (w/v) horizontal agarose gel or a 1.0% (w/v) pulsed-field gel (GeneLine II, Beckman, Fullerton, CA), transferred to Biotrans membranes (ICN, Costa Mesa, CA), and hybridized with end-labeled oligonucleotides from different regions of the SR-BI cDNA or with internal fragments that were labeled with [32P]dCTP using the Megaprime DNA labeling system (Amersham Corp.) in Rapid-Hyb buffer (Amer- sham Corp.) according to the manufacturer’s instructions. The length of intron 1 was deduced from restriction mapping.

**Subchromosomal Localization of SR-BI/CLA-1**—Fluorescence in situ hybridization studies (FISH) were performed using human peripheral blood lymphocytes as described (30). Briefly, the insert from pSR-B1a was labeled by nick-translation (BioNick, Life Technologies, Inc.), preannealed in 50% (v/v) formamide, 10% (w/v) dextran sulfate, 1 M NaCl, 33 mM sodium citrate, 0.1% (w/v) Tween 20 with a 25:1 ratio of human Cot-1 DNA (Life Technologies, Inc.) for 35 min and then allowed to hybridize overnight. The slides were washed in 2 × SSC at 72 °C for 5 min and blocked with 3% (w/v) bovine serum albumin (BSA) in buffer A (buffer A contains 4 × SSC, 0.1% (w/v) Tween 20) for 20 min at 37 °C. For detection, the slides were incubated with 50 μg/ml of biotinylated D (Vector Laboratories, Inc.) in 1% (w/v) BSA in buffer A for 1 h at 37 °C. The signal was amplified by incubating the slides successively with biotinylated goat anti-avidin D (5 μg/ml) (Vector Laboratories, Inc.) and then fluorescein avidin for 1 h at 37 °C in 1% (w/v) BSA in buffer A. Each incubation was followed by three washes in buffer A at 42 °C.

Chromosome band location was facilitated by performing fluorescent G-band analysis on metaphase chromosomes using BrdU incorporation into late replicating regions (31). The slides were first blocked with 10% goat serum in phosphate-buffered saline (PBS) for 20 min followed by three washes with PBS. Slides were incubated with monoclonal anti-BrdU antibody (Sigma) for 40 min and Texas Red-conjugated affinity purified anti-mouse IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) for 20 min; both antibodies were applied at a 1:100 dilution in 2% BSA in PBS. Propidium iodide counterstain (0.1 μg/ml) in antifade solution (5.5 mM p-phenyleneediamine dihydrochloride, 80% (v/v) glycerol, 20 mM Tris-Cl at pH 8.0) was added before the slides were viewed on a Zeiss Axioplan fluorescence microscope equipped with a Bioinformed and MRC600 confocal system.

**Primer Extension to Define the Transcription Start Site of SR-BI**—Primer extension was performed using two primers in exon 1 (primer 1, 5′-CTTGGCGGATCCCTCTGGCCGAGC-3′; and primer 2, 5′-CTTAAAGAGCCTGCTGTGATTAGAC-3′) and the Primer Extension System (Promega, Madison, WI). The primers were end-labeled with T4 polynucleotide kinase and [γ-32P]ATP at 37 °C for 10 min and then the kinase was heat-inactivated by incubating the mixture at 90 °C for 2 min. Total RNA was isolated from a human adrenal cell line (H295R) (kindly provided by Dr. William Rainey, University of Texas Southwestern, Dallas) by RNA STAT-60 (Tel-Test “B,” Inc., Friendswood, TX) and 10 μg of total RNA was mixed with 0.1 pmol of the labeled primer in primer extension buffer, incubated at 60 °C for 20 min, and then slowly cooled to room temperature. The reaction was stopped by the addition of 20 μl formamide loading dye. The samples were heated to 90 °C for 10 min and then size-fractionated on a 6% polyacrylamide gel. The gel was dried and exposed to Kodak Scientific Imaging Film.

**rnase Protection Assay—**rnase protection assays were performed using HycleSpeed™ RPA system (Ambion, Austin, TX). To generate the RNA probe, a fragment of 328 nucleotides was amplified from pSR-B1a using PCR and primers 2 (sequence provided above) and 3 (5′-CTTGGC- GGATCCCTCTGGCCGAGC-3′) and cloned into pCR™II vector (Invitrogen). The plasmid was linearized, and an in vitro transcription reaction was performed in the presence of [γ-32P]UTP and T7 RNA polymerase (Promega) for 1 h at 37 °C to produce the antisense probe. The DNA template (0.5 μg) was digested by incubating the reaction products in 1 unit of RQ DNase (Promega) for 15 min at 37 °C. The reaction was then diluted in Rnase-free water to 50 μl, extracted once with an equal volume of phenol, and then purified using a G-50 spin column (5′ to 3′, Boulder, CO). Approximately 107 cpm of the probe was mixed with 10 μg of total RNA from H295R cells. The RNA was precipitated using ethanol, and then resuspended in 10 μl of Hybridization Buffer, which had been preheated to 95 °C. The mixture was incubated at 95 °C for 3 min and then incubated at 68 °C for 10 min. The Rnase A and Rnase T1 mix was diluted 20-fold in HycleSpeed RNase Digestion Buffer and then 100 μl was added to the RNA sample and incubated at 37 °C for 30 min. Then 150 μl of HycleSpeed Inactivation/Precipitation mix (Ambion) were added. The RNA was precipitated and resuspended in hybridization buffer. The sample was heated to 95 °C for 5 min and then size-fractionated on a 6% polyacrylamide gel. The gel was dried and exposed to Kodak Imaging Film.

**RNA Blot Analysis—**Filters with poly(A)⁺ RNA from multiple human tissues were purchased from CLONTECH (Palo Alto, CA). A 195-bp fragment was amplified from exon 1 of pSR-B1a using oligonucleotides 3a and 3b (see above). An aliquot (20 ng) of the purified fragment was radiolabeled using the Megaprime DNA Labeling System (Amersham Corp.). Blots were prehybridized in Rapid-hyb buffer (Amersham Corp.) for 15 min prior to addition of 10⁶ cpm/m I (~10⁶ cpm/μg DNA) probe. After 2 h at 65 °C, blots were washed for 20 min in 2 x SSC, 0.1% (w/v) SDS at 22 °C, and then washed twice for 15 min with 0.1 x SSC, 0.1% SDS at 65 °C. Blots were subjected to autoradiography for 16 h, and the size of the mRNA was estimated by comparison to size standards.

**Immunoblot Analysis of Human Tissues—**Tissues were obtained from routine post-mortem human autopsy specimens. Human fetal adrenal gland and liver were obtained from second trimester fetal tissues kindly provided by William Rainey (University of Texas Southwestern Medical Center, Dallas). The tissues were immediately placed in liquid N2 and then membrane fractions were prepared from pulverized tissues exactly as described (25). Immunoblot analysis was performed using a polyclonal rabbit anti-peptide antibody (IgG-Q820) raised against the C-terminal 14 amino acids of the human SR-BI exactly as previously reported (25).

**Expression in Cultured Cells—**A series of constructs were made that included 1055, 791, 182, and 65 bp of 5′-flanking sequence from the
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**SR-BI gene (constructs 1 through 4, respectively) placed immediately 5’ of the firefly luciferase gene in pGL2-Basic Vector (Promega, Madison WI). A total of 1.0 μg of plasmid DNA was used to transfect Y1 mouse adrenocortical cells (kindly provided by Dr. Bernard Schimmer, University of Toronto, Canada) and human embryonal kidney (293) cells using the MBS transfection kit (Stratagene). The cells were co-transfected with 30 ng of a plasmid encoding the absence of an anti-SF-1 antiserum (35) in 20 mM HEPES, 60 mM KCl, using the MBS transfection kit (Stratagene). The cells were co-

**Site-directed Mutagenesis and Expression in Y1 Mouse Adrenocortical Tumor Cells—** The SF-1 consensus sequence was altered from CCAAGGCT to ACTCGAGC according to the method of Kunkel (32) using an in vitro mutagenesis kit (Bio-Rad). Plasmids containing either the wild-type 182-bp fragment or this fragment containing the mutated sequence were placed upstream of the firefly luciferase gene. A total of 1.0 μg of plasmid plus 30 ng of the cytomegalovirus promoter/β-galactosidase under the transcriptional control sites (36). The incubations were carried out at room temperature for 20 min, and the luciferase and β-galactosidase activities were measured using Dual-Light™ Chemiluminescent Report Gene Assay System (Tropix, Bedford, MA).

**Electrophoretic Mobility Shift Assays—** Twenty-one base oligonucleotides from the human SR-BI/CLA-1 promoter (huSRB1, 5’-TGAAGCCCAAGGCTGCCCGGG-3’) and its complementary sequence (and two 28-base oligonucleotides from the promoter of the aldosterone synthase gene (−310) (32)) were synthesized with GATC overhangs at the 5’ ends. Each pair of complementary oligonucleotides was mixed, heated to 95 °C for 10 min, and then allowed to cool to room temperature. A total of 200 ng of each oligonucleotide was labeled by Klenow fragment (Stratagene) in the presence of [α-32P]dATP. Glyceraldehyde 3-phosphate dehydrogenase was added to the reaction mixture, and the reaction was loaded on a 12% nonde-

**RESULTS**

A human P1 artificial chromosome (PAC) library (Genome Systems) was screened by PCR using two oppositely oriented CCAAGGCT to ACTCGAGC according to the method of Kunkel (32) using an in vitro mutagenesis kit (Bio-Rad). Plasmids containing either the wild-type 182-bp fragment or this fragment containing the mutated sequence were placed upstream of the firefly luciferase gene. A total of 1.0 μg of plasmid plus 30 ng of the cytomegalovirus promoter/β-galactosidase under the transcriptional control sites (36). The incubations were carried out at room temperature for 20 min, and the luciferase and β-galactosidase activities were measured using Dual-Light™ Chemiluminescent Report Gene Assay System (Tropix, Bedford, MA).

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**TABLE I**

**Oligonucleotides used to define exon/intron junctions of SR-BI**

| Intron | Oligonucleotide sequence (5’ to 3’) | Nucleotide position in cDNA† |
|--------|-----------------------------------|---------------------------|
| 1      | CGTCCGGCCTCCGCTCCGGCAGG          | (−69) (−44)               |
|        | CTGCCGGTGGTGGCTAGCTGCAAAAGA      | 204–231                   |
| 2      | TTTGAGTCCTGAGACCCCGAGG          | 297–321                   |
|        | CTGCCGGTGGTGGCTAGCTGCAAAAGA      | 352–376                   |
| 3      | GTCCCAAGGCTGCTGAGGAGGCTACTAGCAGTGCAGAAGG | 376–412                  |
|        | CAAAGGCTGAGGAGGCTACTAGCAGTGCAGAAGG | 447–483                  |
| 4      | TACCTTTCCAGGCTCTCTGCCAAAGACAGTTC | 580–616                   |
|        | TGCTGATGTCCGATCTGCCAGGCTGTCGGAGG | 653–689                   |
| 5      | CCAGAACACTGACACGAGCAGTGCAGTGCAGAAGG | 696–732                  |
|        | TTCCATTGAATGCTAGCTGCTGAGGCTGCAAGA | 733–769                   |
| 6      | GGGCAAATGGGCCCCCTCTGAGT          | 775–799                   |
|        | GCGGATATGGGGCTGGCTGAGGAGG       | 864–900                   |
| 7      | CTTGCTGAGGCTCCAAACCCCTGTT        | 900–924                   |
|        | TCCTGGTCTGAGGCTCCAGCAGTC        | 1074–1098                 |
| 8      | GCCGAGCCGGCTTCTGCGAGAAGG       | 1047–1071                 |
|        | GAGACATTTCCATGAGGAGGCTAGC        | 1167–1191                 |
| 9      | AGCGGATCCCAACTGAGGCTGCT          | 1132–1156                 |
|        | TGCAAGAACGACGGCGAGCGACACAGGCGCTAATT | 1215–1251               |
| 10     | TGGACCTGCTGCTGAGGCTGCTCTCT      | 1218–1243                 |
|        | AGTGTGAATGGTGGTGAAGGTCTC         | 1274–1299                 |
| 11     | GATGCCATTTGGCAGTGAGCTGCT        | 1321–1345                 |
|        | CCTCGAGAACCTCAACTTTGTGCTT       | 1516–1540                 |
| 12     | GATAGGAGGCGCCCTTGGAGCCCTAT      | 1516–1539                 |
|        | CTGCGCAAGGCGGCCCTGAGTGTTAGTTGCGAGA | 1765–1800               |

† Number 1+ is at translation start ATG.
region of the SR-BI cDNA (primer 1 and primer 2) in the presence or absence of total RNA isolated from the H295R human adrenal cell line (Fig. 3A). Extension products with both oligonucleotides were detected only when adrenal RNA was included in the reaction (lanes 2 and 4). Primer 1 produced a predominant extension fragment of 159 nucleotides. Primer 2 produced a 214-nucleotide extension product. When considered with the locations of the two primers within exon 1, the data are consistent with a major start site of transcription located 140 bp upstream of the translation start site (Fig. 3C).

To confirm the primer extension results, an RNase protection assay was performed using a 32P-nucleotide radiolabeled RNA fragment that contained sequences extending from primer 2 to primer 3 (Fig. 3B). The probe was hybridized with total RNA extracted from H295R cells, and the hybridization mixture was treated with RNase A and T1. In the presence of yeast carrier RNA and no RNase, the full-length probe was seen (lane 2); addition of RNase to the reaction mixture in the absence of cellular RNA led to complete digestion of the probe (lane 3). When both probe and adrenal cell RNA were present, a 217-bp protected fragment was detected (lane 4). The same protected fragment was obtained when poly(A) + RNA from HepG2 cells was used (data not shown), suggesting that the same transcription initiation site is used in hepatocytes. Based on these results, the start site of transcription is approximately 143 bp upstream of the translation start site (shown with an asterisk in Fig. 3C). Thus, the RNase protection assay and the primer extension assays predicted a very similar location for the start site of transcription from the SR-BI gene.

A comparison of the intron-exon structure of the gene to the predicted amino acid sequences of human SR-BI and CD36 is shown in Fig. 4. The relative positions of the introns are remarkably similar between the genes with two exceptions. 1) Two additional exons and introns (solid triangles, Fig. 4) are present at the 5′ end of the human CD36 gene. The second exon is alternatively spliced (37). 2) Intron 8 of CD36 is not present in SR-BI (solid triangle, Fig. 4). Alternate splicing takes place in the last intron of CD36 (38). Recently it was reported that SR-BI is also alternatively spliced in this region, producing two transcripts of similar size (SR-BI.1 and SR-BI.2) that differ in their coding sequence at the 3′ end (39). Both alternatively spliced transcripts are expressed in human cultured cells derived from liver (HepG2), monocytes/macrophages (THP1), and a choriocarcinoma (HeLa) (39).

CD36 and SR-BI have different tissue distributions. SR-BI is expressed at high levels in the adrenal, ovary, testes, and liver of mice and rats (25, 28) and in only trace amounts in rodent placenta. To examine the SR-BI expression pattern in humans, RNA blot analysis was performed using total mRNA from various tissues (Fig. 5A). Because the probe used for this experiment hybridizes with the sequences in exon 1, and thus detects both of the ~2.8-kb alternatively spliced forms of SR-BI (SR-BI.1 and SR-BI.2), the relative amounts of these two transcripts cannot be assessed by mRNA blotting using this probe. A single major band of the expected size (2.8 kb) was detected in multiple tissues. The largest amount of total SR-BI transcripts was present in the ovary, whereas lower levels were detected in the placenta, liver, prostate, testes, and small intestine. A prominent signal was also detected in the adrenal gland (data not shown). Low levels were detected in the spleen, thymus, colon, and leukocytes. Thus, the tissue distribution of the human SR-BI mRNA is similar to that of rodents with the highest mRNA levels present in the liver and steroidogenic tissues. However, unlike rodents, the SR-BI mRNA is readily detectable in human placenta (Fig. 5).

The distribution of SR-BI protein in human tissues is shown in Fig. 5B. A total of 50 μg of membrane proteins from each tissue (except for the human fetal adrenal where 5 μg was used) was subjected to immunoblot analysis using an anti-SRBI specific antibody. The antibody used in this experiment is directed against the COOH terminus of the protein, so will only detect the protein generated from SR-BI.1, which is the major expressed form of SR-BI in most murine tissues (39). A prominent ~80-kDa band was seen in a number of adult tissues including the adrenal, placenta, liver, kidney, and ovary. An

![Table II](image)

**Table II**

Exon/intron organization of human SR-BI

| Exon number | Exon size | Sequence at exon/intron junction | Amino acid(s) interrupted | Intronsize
|-------------|-----------|---------------------------------|--------------------------|-------------
| 1           | 266       | TAAAGtgggggtga...            | cctcgagAAGC             | 33071       |
| 2           | 158       | ACAGGtgggggtga...            | cccacagGAGG             | -50,000     |
| 3           | 142       | CTTGtgggggtga...            | cctcgagGTTG             | 725         |
| 4           | 204       | TGGGtgggggtga...            | gttcttagCTGA             | 2,300       |
| 5           | 96        | CAAAGtgggggtga...            | cccacagGGTG             | 1,750       |
| 6           | 116       | GCAGGtgggggtga...            | cctcgagATCC             | 2,350       |
| 7           | 167       | TGGGtgggggtga...            | cttcttagGTCG             | 3,000       |
| 8           | 119       | CCCGtgggggtga...            | gttcttagGTC             | 5,100       |
| 9           | 74        | TTGGGtgggggtga...            | atttcagACAA             | 7,500       |
| 10          | 52        | AGGGGtgggggtga...            | gttcttagAGCG             | 800         |
| 11          | 147       | CGAGGtgggggtga...            | gttcttagGAGA             | 3,700       |
| 12          | 129       | GGGGtgggggtga...            | gttcttagGTC             | 500         |
| 13          | ~1 kb     |                                  |                          |             |

![Fig. 2](image)

**Fig. 2. Localization of human SR-BI/CLA-1 gene to chromosome band 12q24.2-qter by FISH.** The hybridization signals were confined to chromosome 12 (data not shown), and a high magnification of chromosome 12 showing the site of probe hybridization is shown (yellow).
The shaded arrows indicate introns present in huCD36 not found in huSR-BI/CLA-1. Both SR-BI and CD36 undergo alternative splicing at their 3’ end (3, 39).

In general, the relative amounts of expression of SR-BI protein tissues mirrored that of the SR-BI mRNA in the adult human tissues (Fig. 5B). The highest concentrations of SR-BI in the adult tissues was in the adrenal gland, placenta, liver, and ovary. A proportionally greater amount of immunodetectable protein was detected in the human kidney relative to the amount of mRNA (upper panel). This may reflect contamination of the kidney sample with adrenal cortical tissue. Relative to other tissues, the amount of ovarian SR-BI mRNA was much greater than the amount of SR-BI protein. It is possible that SR-BI.2 is the predominant form of SR-BI in the human ovary. Alternatively, the relative differences in the amount of SR-BI in the mRNA blot and the immunoblot may reflect differences in the hormonal status of the women sampled. The age and hormonal status of the woman whose ovary was used for the immunoblot was from a 20-year-old, presumably menstruating Caucasian woman. Immunoblot analysis of another sample from a post-menopausal woman disclosed no detectable SR-BI protein (data not shown). Analysis of the SR-BI mRNA and
protein from additional ovarian samples at different stages of the hormonal cycle will be required to explain the apparent discrepancy between the relative amounts of SR-BI mRNA and protein in the ovarian tissues sampled. The highest overall level of SR-BI was in the fetal adrenal tissue. Only one-tenth of the amount of fetal adrenal membrane proteins as compared with the other tissues was loaded on the gel. The level of SR-BI in the human fetal adrenal is >50-fold greater than that in the adult adrenal gland. The human fetal adrenal is distinguished from all nonprimate species by having a so-called fetal zone which synthesizes large amounts of dehydroepiandrosterone, which is converted to estradiol in the human placenta (40). Immunohistochemical studies show intense cell surface staining of SR-BI in the fetal zone of the adrenal gland (data not shown), which is consistent with SR-BI playing an important role in the synthesis of adrenal steroids in the fetus.

The DNA sequence of the 5'-flanking region of SR-BI is shown in Fig. 6. This region is very GC-rich; within the first 1200 nucleotides, 82% of the sequence contains nucleotides having a GC content of 60% or higher. One such sequence is shown in Fig. 6. This sequence is 121 bp in length and has 95% GC content. It is preceded by a 9-bp sequence (TCAGGTGAT) that contains an E box, a potential TATA box-like sequence is underlined twice, and a consensus site of known transcription factors. This search of the sequence of the SF-1 motif in construct 3, which contains 182 bp of 5'-flanking sequence, essentially abolishes promoter activity in both cell lines. As a first step in determining the sequences required for transcription of SR-BI, a series of plasmids were produced containing 1055, 791, 182, and 65 bp of 5'-flanking sequence of the SR-BI gene placed upstream of a luciferase reporter gene (Fig. 7, constructs 1–4). Each construct was co-transfected with a constitutively expressed β-galactosidase gene into Y1 cells, a line of mouse adrenocortical tumor cells, and 293 cells, a line of human embryonal kidney cells. Relative transcriptional activities of each construct were normalized to the activity of the β-galactosidase control plasmid to correct for differences in transfection efficiency. Transcription from constructs 1–3, which contain the putative SF-1 binding site, was 40 to 80 times higher in Y1 cells than in 293 cells (Fig. 7A), indicating these 5'-flanking sequences confer cell-selective expression to the reporter gene. Reduction in the length of the 5' sequence caused a progressive reduction in the level of transcription, suggesting that multiple sequences in the SR-BI promoter are required for maximum expression of this gene. Finally, deletion to position −65, which removes the SF-1 site, essentially abolishes promoter activity in both cell lines. To determine more directly the role of the SF-1 site in the promoter activity, we used site-directed mutagenesis to scramble the sequence of the SF-1 motif in construct 3, which contains 182 bp of 5'-flanking sequence. Mutating this sequence
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reduced expression by over 50% in Y1 adrenocortical cells (Fig. 7B). This level of reduction of expression is similar to that observed in other SF-1-dependent genes with mutated SF-1 binding sites (36). Taken together these results are consistent with high level SR-BI expression requiring at least 182 bp of 5’-flanking sequence and that SF-1 participates in the transcriptional regulation of SR-BI in adrenal cells.

To determine if SF-1 binds the DNA sequence present in SR-BI, we performed gel mobility shift assays with a 20-bp fragment from the human gene (Fig. 8A, left). A known SF-1 binding site from the aldosterone synthase gene promoter (Fig. 8A, lanes 1 and 2) was used as a positive control. Incubation of either the radiolabeled control or SR-BI oligonucleotide with nuclear extracts from Y1 adrenocortical cells resulted in the production of slower migrating protein-DNA complexes (lanes 2 and 4). These shifted bands were eliminated when a 200-fold molar excess of unlabeled control oligonucleotide was included in the reaction with the huSR-BI oligonucleotide (Fig. 8A, lane 5) as well as the control oligonucleotide (data not shown). Preincubation of the nuclear extract with an antibody to the DNA-binding domain of SF-1 eliminated the predominant complex (Fig. 8A, lane 7). These data demonstrate that SF-1 binds to the sequence at –75 in the promoter region of SR-BI.

To validate further that SF-1 binds this sequence, the human SR-BI oligonucleotide was incubated with a partially purified GST-SF-1 fusion protein. As shown in Fig. 8B, a major shift was detected (Fig. 8B, lane 4); the complex migrated at the same position as did the complex formed by GST-SF-1 and the aldosterone synthase –310 probe (Fig. 8A, lane 2). The migration of this complex was abolished by adding antisera specific for SF-1 (lane 6), and the complex was specifically competed by adding unlabeled oligonucleotides containing a known SF-1 binding site (lane 8) but not by mutated SF-1 sites (lane 9). Thus, these experiments demonstrate that SF-1 can interact directly with the –72 element shown above to regulate SR-BI promoter activity. Collectively, our data strongly suggest that SF-1 is an important regulator of SR-BI expression within steroidogenic cells.

**DISCUSSION**

We have characterized the human gene encoding the scavenger receptor, SR-BI. The gene is –75 kb in length, contains 13 exons and 12 introns, and is located on the long arm of chromosome 12 (12q24.2-qter). The intron-exon boundaries of SR-BI closely resemble CD36, another member of the class B scavenger receptor gene family. Only a single SR-BI transcription start site was identified in cultured human adrenal cells using primer extension and RNase protection assays. A consensus binding site for the transcription factor SF-1 is present at position 13. This SR-BI gene sequence binds SF-1 in a specific manner and enhances transcription in Y1 adrenocortical tumor cells. These data strongly suggest that SF-1 regulates SR-BI in steroidogenic cells, a model supported by our finding that SR-BI mRNA is abundant in human steroidogenic tissues, including the adrenal and ovary.

The SR-BI/CLA-1 cDNA was originally isolated from an amplified HL60 library and was 300 bp shorter than a subsequently isolated and more abundant cDNA. The initial cDNA clone was thought to represent a minor, alternatively spliced mRNA species (8). Our analysis of the SR-BI/CLA-1 gene structure is consistent with this model, since the missing 300 bp correspond exactly to the sequences encoded by exons 2 and 3. This truncated message would be predicted to encode a protein with a deletion of 100 amino acids in the extracellular domain adjacent to the first membrane spanning region. Whether this alternatively spliced form of SR-BI is expressed in tissues is not known and will require further studies. The absence of any transcript other than the 2.8-kb mRNA on RNA blot analysis, or any immunoreactive proteins of other sizes on immunoblot analysis, suggests that if a truncated, alternatively spliced form of SR-BI is expressed, it is a quantitatively minor species in tissues so far examined. Interestingly, a splice variant involving the corresponding region of the CD36 gene was isolated from an erythroblast cell line (HEL cells) (45), although there is no evidence that this form of CD36 mRNA is expressed in tissues.

Recently Webb and colleagues (39) identified an alternatively spliced form of SR-BI at the 3’ end of the murine SR-BI gene. The alternative splicing occurs at the splice donor site of intron 11. In most tissues, the major splice form is SR-B1, where exon 11 splices directly to the 5’ end of exon 12. The less abundant form (SR-BL2) uses the same 5’ splice site, but skips exon 12 and splices to exon 13. The predicted sequences of the two forms of SR-BI differ in sequence starting at the fourth residues downstream of the putative membrane spanning region. Thus, the alternative splicing results in the production of
another form of SR-BI that contains both membrane-spanning domains, the entire extracellular domain, and yet a different COOH terminus, which may significantly affect the cellular localization and trafficking of this receptor. In rodents, SR-BI.2 is the major transcript in testes and adipose tissue and accounts for ~40% of total hepatic SR-BI mRNA (39). On our mRNA blot we use a probe that hybridizes with exon 1, detecting both forms of SR-BI mRNA. Because the two species of mRNA are predicted to be similar in size, we cannot estimate the relative abundance of these two forms in human tissues from our data. It is important to note that the antibody we employed to analyze the relative amounts of SR-BI protein in human tissues is directed against the COOH terminus of SR-BI.1 so it only detects the SR-BI.1 isof orm. The correlation between the levels of total SR-BI mRNA and immunodetectable SR-BI.1 protein in most human tissues suggests that the SR-BI.2 form is expressed at low levels or at levels proportional to SR-BI.1. A possible exception is human testes where there were easily detectable levels of SR-BI mRNA in the absence of any detectable SR-BI protein. SR-BI.2 appears to be the predominant mRNA species expressed in the mouse testes (39); experiments are in progress to determine if this is also true in humans.

The overall pattern of SR-BI expression in human tissues is similar to rodents with the highest levels of mRNA detected in the adrenal and ovary (24, 25, 28). SR-BI protein is abundantly expressed in the primary steroidogenic tissues of the rat including the zona fasciculata and zona reticularis of the adrenal, the corpus luteum of the ovary, and the interstitial (Leydig) cells of the testes (28). The most dramatic difference in the tissue distribution of SR-BI in humans and rodents is in the placenta. In mice and rats, only trace amounts of SR-BI mRNA and protein are found in the placenta (25, 28), whereas the mRNA is readily detected in the human placenta.

The highest levels of SR-BI protein were in the human fetal adrenal gland where the levels were estimated to be over 50-fold greater than in the adult adrenal gland. It has been estimated that the rate of steroidogenesis in the human fetal adrenal is up to 5 times higher than in the adult adrenal (40). The major steroid produced by the human adrenal is dehydroisoandrosterone sulfate, which is made in the so-called fetal zone, a specialized region in the cortical adrenal gland present in fetal primates but not lower organisms. We have performed immunocytochemical studies on fetal adrenal tissues and found that SR-BI is highly expressed in the fetal zone.2 Freshly isolated fetal adrenocortical cells have a high affinity, prionase-resistant binding site for 125I-HDL, which is likely to be SR-BI (46). The fetal adrenal gland also expresses very high levels of the LDLR (47), and cultured fetal adrenal cells actively take up and degrade LDL (48). The relative role of SR-BI and the LDLR in providing the cells with sufficient amounts of cholesterol during human fetal development is not known. Individuals with homozygous familial hypercholesterolemia have no functional LDLR, yet develop normally; this may be due to the high levels of SR-BI in the fetal adrenocortical cells. LDL, rather than HDL, appears to be the major source of cholesterol for fetal adrenal steroidogenesis. Since SR-BI binds LDL as well as HDL (15, 24), it may mediate the selective uptake of sterol from LDL in the fetal adrenal in the absence of the LDLR.

The expression of SR-BI in rodents is hormonally regulated. The level of SR-BI in the adrenal gland and testes increases dramatically with the administration of ACTH or human chorionic gonadotropin (28, 49). These increases are associated with enhanced delivery of HDL-associated lipid to the respec-

tive tissues (28). Thus, we have proposed that SR-BI mediates the selective uptake of lipids from HDL to provide steroidogenic tissues with cholesterol substrate (25, 28). Our finding that SR-BI is regulated by SF-1, which activates the expression of many components of the steroidogenic complex, and that SF-1 binds to this DNA in vitro, supports the idea that this receptor plays an essential role in steroidogenesis.

SF-1 is a member of the nuclear receptor gene family, which includes receptors for steroid hormones, thyroid hormone, vitamin D, retinoic acid, and other yet to be identified ligands (33, 43). The gene encoding SF-1 (Ftz-F1) is constitutively expressed in adrenal cortical cells, the Leydig cells of the testes, and the granulosa, thecal, and corpus luteal cells of the ovary (33). Thus, SF-1 and SR-BI are expressed in many of the same tissues and cell types. SF-1 activates the expression of many components of the steroidogenic complex including P450 side chain cleavage enzyme (50), P450 17α-hydroxylase (51), 21-hydroxylase (52), 11β-hydroxylase (53), and the steroidogenic acute regulatory protein (54). Our finding that SR-BI is also regulated by SF-1 further supports the concept that SR-BI plays an important role in delivering lipids for steroidogenesis. Recent studies showed that SF-1 transactivation in cell transfection studies can be enhanced by oxysterols, suggesting that cholesterol-derived compounds may act as ligands for this orphan nuclear receptor (55). To the extent that the precursor for these activating oxysterols may be delivered to steroidogenic cells by SR-BI, the potential exists for an autoregulatory loop in which SF-1 activates SR-BI expression, which in turn supplies larger amounts of the SF-1 ligand to steroidogenic cells.

SF-1 is also expressed in a number of nonsteroidogenic tissues and cell types including the hypothalamus, Sertoli cells of the testes, and pituitary gonadotropes of the anterior pituitary (56). At the present time, it is not known if there is coordinate expression of SR-BI in these locations. We are in the process of performing in situ mRNA hybridization studies to examine the ontogeny of SR-BI expression in relation to SF-1. Preliminary results indicated that the patterns of SR-BI and SF-1 mRNA expression are similar during early development, at least in the mouse.

Although our studies establish SF-1 as an important regulator of SR-BI promoter activity in cultured adrenocortical cells, the step-wise decrease in the 5’ deletion studies (Fig. 8) strongly suggests that other transcription factors participate in the regulation of this gene, even in steroidogenic tissues. Moreover, SF-1 is not expressed in a number of tissues that express SR-BI including the liver, mammary gland, and human placenta, indicating that other transcription factors must regulate SR-BI expression in these tissues. SR-BI may thus resemble the P450 side chain cleavage enzyme, which is regulated by SF-1 in steroidogenic tissues, but not in the brain, placenta, and fetal gut (57, 58), which are all tissues that do not express SF-1.

The sequences that regulate expression of SR-BI in non-steroidogenic tissues remain to be determined. Of particular interest are the factors that regulate SR-BI expression in the liver, where it may play an important role in the reverse cholesterol transport pathway (59). No sites for liver-specific transcription factors were detected within 1055 base pairs of the transcription start site of the SR-BI gene. RNase protection assays using poly(A)+ RNA from HepG2 cells, a human hepatoma cell line, showed the same pattern of protected bands as was detected in cultured adrenal cells (data not shown). This result suggests that a different combination of transcription factors in the liver may direct expression from the same start site. How-

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2 K. L. Wyne and H. H. Hobbs, unpublished observations.

3 G. Cao and H. H. Hobbs, unpublished observations.
ever, this does not rule out the possibility that SR-BI, like CD36, has alternative splicing at its 5’ end and that a different promoter sequence regulates SR-BI gene expression in the liver as well as other nonsteroidogenic tissues. Alternatively, the important sequences that regulate gene expression may lie further upstream or may be localized in another region of the gene. Further studies in nonsteroidogenic tissues will be required to define the regulation of SR-BI.

Acknowledgments—We thank Tommy Hyatt, Melissa Christiansen, and Jeana Meade for excellent technical assistance and Dr. Thomas Weber for preparing the GST-SF-1 protein. We also thank David W. Russell, Kathleen Caron, and Timothy Osborne for helpful discussions. We are grateful to Laura Fuller for assistance in the preparation of the manuscript.

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