Human Sterol Carrier Protein x/Sterol Carrier Protein 2 Gene Has Two Promoters[†,‡]

Takashi Ohba,[§] John A. Holt,[§] Jeffrey T. Billheimer,[∥] and Jerome F. Strauss, III*[,§]

Department of Obstetrics and Gynecology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104, and Experimental Station, DuPont-Merck Pharmaceutical Company, Wilmington, Delaware 19880

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ABSTRACT: The human sterol carrier protein x (SCPx)/sterol carrier protein 2 (SCP2) gene gives rise to two mRNAs: a 2.8 kb mRNA encoding SCPx, a peroxisome-associated thiolase, and a 1.5 kb mRNA encoding SCP2, which is thought to be an intracellular lipid transfer protein. The SCPx/SCP2 gene is highly expressed in organs involved in lipid metabolism, but the relative abundance of SCPx and SCP2 mRNAs varies. Here we report that the two transcripts are produced under the direction of two independent promoters. We determined the DNA sequence of 3.4 kb of the proximal promoter governing the transcription of SCPx sequences. The promoter governing the transcription of SCP2 sequences was identified 45 kb downstream from the SCPx promoter in intron XI. This promoter initiates transcription within exon XII. Both the SCPx and SCP2 promoters lack TATA boxes and initiate transcription at multiple sites. They share features that are found in the promoters of genes encoding other peroxisomal proteins. The basal activities of the two promoters were tested as fusion gene constructs in selected host cells, including BeWo choriocarcinoma cells, HepG2 hepatoblastoma cells, murine Y1 adrenocortical tumor cells, and Balb 3T3 fibroblasts. Cell host-specific patterns of promoter activity were observed. In addition, 8-Br-cAMP and phorbol myristate acetate were found to increase SCPx promoter activity in a host cell-specific manner. The SCP2 promoter was not significantly influenced by these agents. Our findings reveal the basis for the generation of the two transcripts from the human SCPx/SCP2 gene, document the independent activities of the SCPx and SCP2 promoters, and demonstrate that the activities of these promoters are influenced by the host cell.

Sterol carrier protein x (SCPx) and sterol carrier protein 2 (SCP2) are structurally related proteins. SCP2 was first described as a lipid transfer protein that is thought to facilitate membrane-bound enzymatic reactions involved in cholesterol synthesis and metabolism (Scallen et al., 1975; Noland et al., 1980). Because SCP2 is able to promote the transfer of other lipids *in vitro*, including phospholipids, it has also been called a nonspecific lipid transfer protein (Wirtz & Gadella, 1990). Immunochemical studies and cDNA cloning revealed the existence of a related protein encompassing SCP2 in its carboxyl terminal sequences (He et al., 1991; Mori et al., 1991; Ossendorp et al., 1991; Seedorf & Assmann, 1991; Seedorf et al., 1993). This protein, named SCPx, has homologies to thiolases in its amino terminus (Baker et al., 1991; Mori et al., 1991) and has recently been shown to have 2-oxoacyl-CoA thiolase as well as lipid transfer activity (Seedorf et al., 1994). The carboxy termini of SCPx and SCP2 end in a peroxisome-targeting sequence, Ala-Lys-Leu (Yamamoto et al., 1990; He et al., 1991). Immunocytochemistry at the ultrastructure level and subcellular fraction studies indicate that SCPx and SCP2 are localized to peroxisomes (Keller et al., 1989; Van Heusden et al., 1990). SCP2 is also associated with other organelles. The presence of SCPx and SCP2 in peroxisomes, which are organelles that participate in fatty acid oxidation, cholesterol synthesis, and bile acid catabolism (Van den Bosch et al., 1992), is consistent with the postulated roles of these proteins in lipid metabolism. It is notable that the structures of SCPx and SCP2 are highly conserved across species (Baker et al., 1991; Pfiefer et al., 1993).

Human SCPx is encoded by a 2.8 kb mRNA, whereas SCP2 is encoded by a 1.5 kb transcript (Ohba et al., 1994). Northern hybridization analyses of RNA extracted from animal tissues revealed that these mRNAs are widely expressed, but are most abundant in tissues involved in lipid metabolism (Seedorf & Assmann, 1991; Seedorf et al., 1993). The relative abundances of the SCPx and SCP2 mRNAs in tissues are developmentally regulated (Baum et al., 1993; Pfeifer et al., 1993), vary among organs (Seedorf & Assmann, 1991; Seedorf et al., 1993), and are modulated differentially by hormonal stimulation and hypolipidemic drugs (Trzeciak et al., 1987; Rennert et al., 1991; Mori et al., 1991; Matsuo & Strauss, 1994).

We recently determined the organization of the human SCPx/SCP2 gene and suggested that both the SCPx and SCP2 mRNAs are derived from a single structural gene (Ohba et al., 1994). In order to understand the basis for the generation of two distinct transcripts, we undertook an analysis of the promoters governing the expression of these mRNAs. Here we report the localization and DNA sequence of two promoters in the SCPx/SCP2 gene and the characterization of their activities in selected host cells.

MATERIALS AND METHODS

Isolation of Genomic Clones. A human genomic library in bacteriophage λ-fix II and an arrayed human genomic

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* Address all correspondence to Jerome F. Strauss, III, M.D., Ph.D., Department of Obstetrics and Gynecology, 778 Clinical Research Building, 415 Curie Boulevard, Philadelphia, PA 19104; Telephone (215) 898-0147, FAX (215) 573-5408.

[§] University of Pennsylvania School of Medicine.

[∥] DuPont-Merck Pharmaceutical Company.

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library in the P1 cloning system were screened as described as previously (Ohba et al., 1994). The λ and P1 clones were analyzed by restriction endonuclease digestion and Southern blotting with fragments of the SCPx cDNA or with specific oligonucleotide sequences. Genomic fragments (3.4 kb for SCPx and 2.1 kb for SCP2) surrounding the putative start sites of transcription were isolated and subcloned into pBluescript (Stratagene) for manual (Sequenase version 2.0) or automated (Applied Biosystems Inc.) sequence analysis, as previously described (Ohba et al., 1994).

Determination of the Transcription Start Site of SCP2. We employed a modified rapid amplification of cDNA ends (RACE) method to identify the transcriptional start sites based on anchored PCR using uncloned single-strand human placenta cDNAs with an anchor attached to the 3′-end (5′-RACE-Ready cDNA, Clontech) as templates. PCR was carried with an anchor primer (5′-CTGGTTCGGCCCAC-CTCTGAAGGTTCCAGAATCGATAG-3′), an SCP2-specific first primer (5′-TGAGTCAGCCATTGTGATTGTGT-GCAGTCAGC-3′), and a second nested SCP2 primer (5′-CACTGATCCTTTGCCATTCTTCACATCCAC-3′), as described by Apte and Siebert (1993). The nested primer ended 242 nt downstream from the SCP2 translation start site. The anchor primer consisted of 48 nt. Thus, the number of nucleotides from the putative transcription start site to the translation start site can be determined from the following equation: nt of anchored PCR product − (48 + 242). PCR products were separated in agarose gels, subcloned into the PCR^{II} vector (Invitrogen), and sequenced with another SCP2-specific primer (5′-GCCATCTTTCACCT-TGAAGGC-3′).

Plasmid Constructs. A 3.4 kb *Sac*I fragment spanning nt −3427 to +49 of the SCPx/SCP2 gene was cloned into the plasmid vector pGL_2 (Promega), which contains the firefly luciferase as a reporter gene. 5′-Deleted constructs in pGL_2 were prepared by subcloning of PCR products generated by using various forward SCPx promoter-specific primers with attached linkers and a single reverse primer spanning from +73 to +83 with a *Hin*dIII linker (5′-AGAGATCTAAGCT-TACTGCACCAGAGCTCCCTGAAG-3′). The forward primer used to generate the "0.6 kb" SCPx promoter fragment spanned from nt −507 to −484 with linker for *Bgl*II (5′-TGAGCTCGAGATCTCGGTCTCCAATAAACAT-GGAAGGC-3′); the forward primer for the "0.5 kb" SCPx promoter fragment spanned from nt −394 to −374 with linker for *Bgl*II (5′-TGAGCTCGAGATCTGCTGGTGACG-GAGCCTGGGTT-3′); and the forward primer for the "0.2 kb" SCPx promoter fragment spanned from nt −95 to −71 with linker for *Bgl*II (5′-TGAGCTCGAGATCTCCCATGC-CCAAGTCCGCAAATTAG-3′). The sequences of all of these PCR-generated promoter fragments were confirmed to be identical to the corresponding SCPx promoter sequences.

*Sal*II−*Eco*RI (2.1 and 1.2 kb) genomic fragments containing exon XII and sequences in the preceding intron XI were subcloned into pBluescript. The putative SCP2 promoter region, spanning from −1593 to +73 ("1.7 kb") and from −809 to +73 ("0.9 kb") from the major transcription start of SCP2, were cut from 2.1 and 1.2 kb fragments and subcloned into pGL_2 with *Xho*I and *Hin*dIII linkers. Other 5′-deleted constructs were obtained by subcloning PCR products generated with various forward primers and a single reverse primer spanning from +72 to +50 with a linker for *Hin*dIII (5′-AGAGATCTAAGCTTACAACCA-

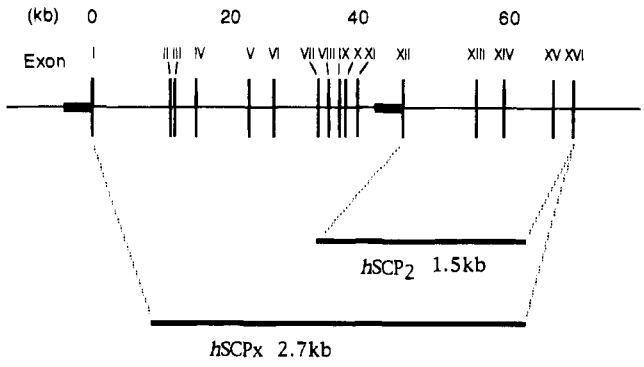

FIGURE 1: Structure of the human SCPx/SCP2 gene. Exons are indicated by vertical lines. Promoter regions are indicated by thick horizontal lines.

CAGCTCCTCCAATG-3′). To generate the "0.6 kb" SCP2 promoter fragment, the forward primer spanned from −529 to −506 with a linker for *Bgl*II (5′-TGAGCTC-GAGATCTGGGGACAGAATGTTATCCTTTTGG-3′); to generate the "0.1 kb" SCP2 promoter fragment, the forward primer spanned from −101 to −83 with a linker for *Bgl*II (5′-TGAGCTCGAGATCTGGCTTGAGAAAGCTGGTGG-3′). The sequences of the PCR-generated truncations were verified to be identical to the SCP2 promoter sequence. Other plasmids used in these experiments included the pGL_2 basic vector, which contains no promoter sequences; pGL_2 control, which places the luciferase gene under the control of the SV40 promoter and enhancer; and pCH110, a plasmid in which the Lac Z gene is under the control of the early SV40 promoter (Pharmacia).

To confirm the transcription start site of the SCP2 promoter−luciferase gene constructs, total RNA was isolated from HepG2 cells transfected with the 1.7 kb pGL_2−SCP2 construct and used for primer extension with the reverse transcription reaction containing a 30-mer primer (5′-ACAGTACCGGAATGCCAAGCTTACAACCAC-3′) projected to give an extension product of around 65-75 nt, as described by Sambrook et al. (1989).

Cell Culture and Transfection. HepG2 human hepatoblastoma cells, BeWo human choriocarcinoma cells, murine Y1 adrenal tumor cells, and mouse Balb 3T3 fibroblasts were grown in 35 mm plastic dishes in a culture medium consisting of Dulbecco's minimal essential medium supplemented with 10% fetal calf serum and 50 μg/mL gentamycin. Plasmids used for transfection were purified by using the Wizard Maxiprep (Promega) and eluted with 10 mM Tris Cl (pH 8.1). Cell cultures at 40-60% confluence were washed twice with serum-free medium before adding 1 mL of serum-free medium containing 2 μg of pGL_2 plasmid constructs and 1 μg of pCH110 plasmid with 10 μg of Lipofectamine (Gibco/BRL). After 5 h of incubation, the medium was replaced with 0.9 mL of medium with 10% serum. Cells were havested after 48 h of culture. In some experiments, 8-Br-cAMP (1 mM) or phorbol myristate acetate (100 ng/mL) in dimethyl sulfoxide as vehicle (final concentration 0.01%) or the dimethyl sulfoxide vehicle was added to the medium for the final 24 h of culture.

Luciferase and β-Galactosidase Assays. Cells were harvested 48 h after transfection, and extracts were made by three freeze−thaw cycles (Gorman, 1985). One aliquot (10 μL out of 50 μL total extract volume) was used for luciferase assays (Brasier et al., 1989), and another 10 μL was taken for β-galactosidase assay (An et al., 1982). The "blank"

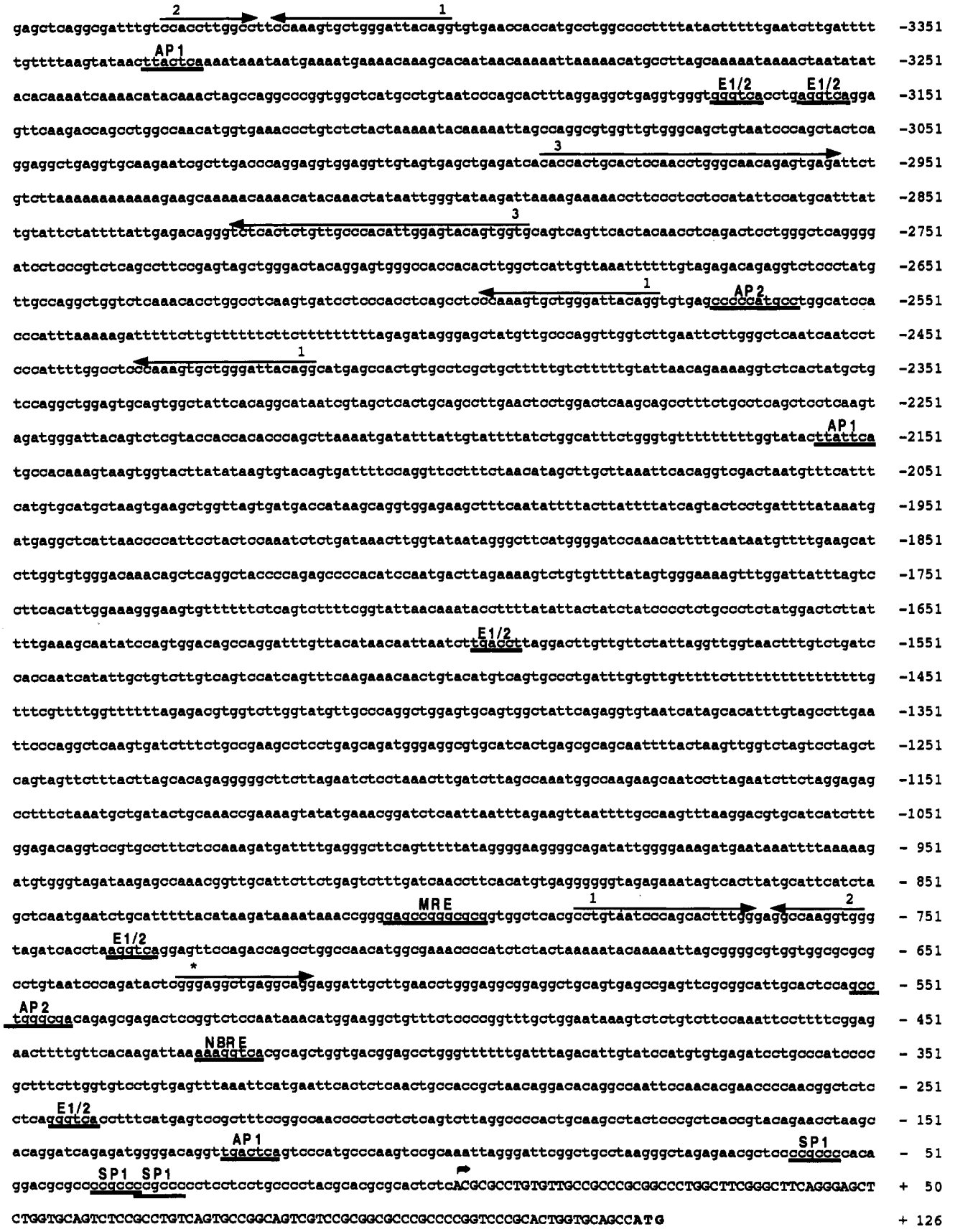

FIGURE 2: Nucleotide sequence of the human SCPx promoter. Nucleotide position 1 is assigned to one of the potential transcription start sites (curved arrow) (Ohba et al., 1994), and negative numbers refer to 5′-flanking sequences. The initiator methionine codon is boldfaced. Putative AP1, AP2, MRE, and NBRE motifs are indicated. E1/2 denotes putative ERE half-sites. Direct and inverted repeats are indicated by numbered arrows. The arrow marked with asterisk indicates a sequence similar to that found in the SCP2 promoter.

luciferase value measured in untransfected cell extracts was subtracted from the luciferase readings of transfected cell extracts. The luciferase assay results were normalized to β-galactosidase activity to compensate for variations in transfection efficiency. In each experiment, the activity of the pGL_2 control vector was defined as 100%. Each

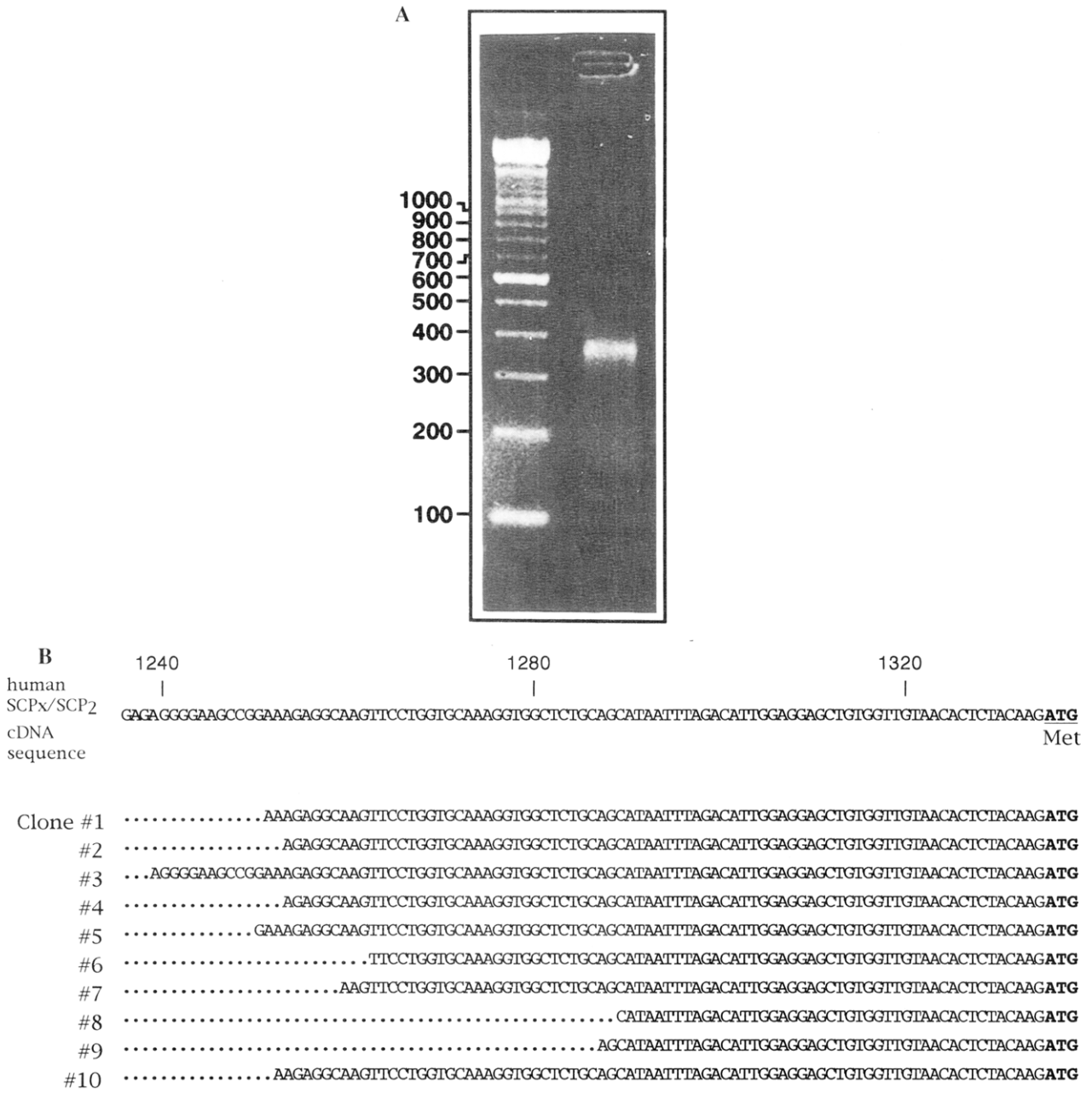

FIGURE 3: Identification of the 5′-ends of SCP2 mRNAs: (A) size of anchored PCR products generated in the RACE procedure using human placental cDNAs; (B) sequences of anchored PCR clones. The nucleotide sequence of the SCPx/SCP2 cDNA is shown above the sequences of 10 clones derived from the anchored PCR products.

treatment group contained triplicate cultures, and each experiment was repeated two to four times with different plasmid preparations. In some instances, when more than three separate experiments were carried out, means were tested for significant differences by Student's *t*-test.

RESULTS

Sequence of the SCPx Promoter. We recently described the organization of the human SCPx/SCP2 gene, which spans 80 kb and consists of 16 exons interrupted by 15 introns (Figure 1) (Ohba et al., 1994). The sequence of the first 500 nt of the SCPx promoter was also determined. The structure of this promoter resembles that of other genes encoding peroxisomal enzymes, in that it lacks a TATA box, has G+C-rich stretches, and initiates transcription from multiple sites. This segment of the promoter DNA contains several putative SP1 binding sites, a putative AP1 site, a putative estrogen response element (ERE) half-site, and a putative NGFI-B response element (NGFI-B response element or NBRE) (Wilson et al., 1991). In the present study, we determined the DNA sequence of an additional 2.8 kb of the SCPx promoter (Figure 2). Several consensus sequences for cis elements were identified in this upstream segment of DNA, including putative AP1, AP2, ERE half-sites, including an upstream pair of these sequences potentially representing a thyroid response element, and a consensus metal response element (MRE) sequence (Carter et al., 1984). Like other genes encoding peroxisomal proteins, a number of direct and inverted repeat sequences were found in the upstream SCPx promoter sequence (Ishii et al., 1987; Osumi et al., 1987; Bout et al., 1991; Varanasi et al., 1994). These observations strengthen the structural similarities between the SCPx promoter and those of genes coding for peroxisomal proteins.

Identification and Sequence of the SCP2 Promoter. In our initial characterization of the structure of the human SCPx/SCP2 gene, we were unable to localize a promoter

controlling the transcription of SCP2 sequences. Hence, we could not rule out the possibility that the SCP2 mRNA was derived through alternative splicing.

We previously reported the sequence of the 5′-end of a cDNA clone isolated from a human liver cDNA library (Yamamoto et al., 1990). These cDNA sequences ended in exon XII of the SCPx/SCP2 gene. Moreover, the 5′-ends of SCP2 cDNAs isolated from a rat liver cDNA library terminated in this region (Seedorf & Assmann, 1991), suggesting the possibility that the transcription of SCP2 sequences is initiated within exon XII. We confirmed these findings by using a modified RACE method. A prominent 370 nt band was detected by anchored PCR using human placental cDNAs (Figure 3A). The anchored PCR products were also radiolabeled using T4 polynucleotide kinase and separated by electrophoresis in a 6%/7 M urea gel along with a sequence reaction of M13 DNA to determine the sizes of the products. Autoradiography revealed bands at 375, 371, 358, and 346 nt (data not shown), which are consistent with the estimated sizes of the products by agarose gel electrophoresis. By subtracting the length of the anchor (48 nt) and the nucleotides contained in the nested SCP2-specific primer through the translation start site (242 nt), we estimate that the SCP2 mRNA ends about approximately 80 nt upstream from the translation start site of SCP2, which would place the 5′-ends within exon XII sequences. DNA sequence analysis of the 5′-ends of 20 randomly picked clones derived from ligation of the 370 nt anchored PCR product into the PCR^{II} vector gave 10 different 5′-ends, all within exon XII sequences (Figure 3B). The 5′-ends of the sequences of the cloned anchored PCR products had a range from 11 nt more than to 12 nt less than the 5′-end of the SCP2 cDNA sequence we originaliy reported (Yamamoto et al., 1990). Thus, both the determination of cDNA 5′-ends and the analysis of RACE products suggested that major transcription start sites for SCP2 are located within exon XII approximately 90 nt upstream from the SCP2 translation start site.

Nucleotide Sequence of the SCP2 Promoter. Approximately 1.7 kb of DNA from intron XI, representing the putative SCP2 promoter, and a portion of exon XII were sequenced (Figure 4). This DNA segment also lacked a TATA box. In contrast to the SCPx promoter, a G+C-rich region was not adjacent to the transcriptional start sites. However, putative AP1 and AP2 sites, an MRE sequence, two putative NFκB sites, and three putative ERE half-sites were present upstream from the transcription start sites. The 5′-most putative ERE (nt −1591 to −1578) is in the context of a DR2 suggestive of a retinoic acid response element. A 16 nt motif appeared twice in the SCP2 promoter (nt −1449 to −1434 and nt −932 to −917) and once in the SCPx promoter (nt −618 to −603). In addition, long palindromic repeats were found in the SCP2 promoter.

Functional Activities of the SCPx and SCP2 Promoters. To examine the functional activities of the two promoters, we cloned various lengths of SCPx and SCP2 promoter DNAs into the pGL$_2$ reporter plasmid. These plasmids were transfected into four different host cells: BeWo human choriocarcinoma cells, steroidogenic trophoblast-derived cell lines; HepG2 human hepatoblastoma cells, which display differentiated functions of hepatocytes; murine Y1 adrenocortical tumor cells, a steroidogenic cell line; and murine Balb 3T3 fibroblasts. All of these cells express the SCPx and SCP2 mRNAs as assessed by Northern blotting (data not shown).

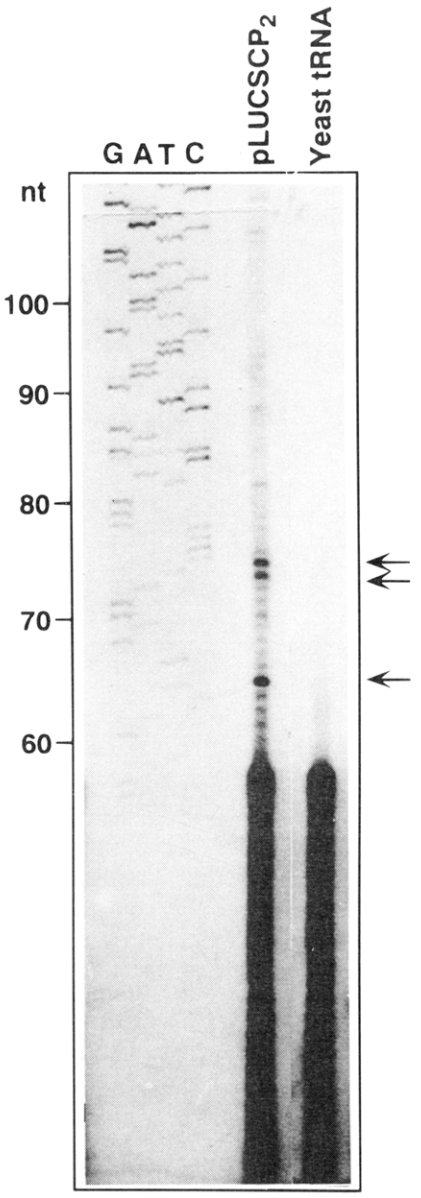

FIGURE 4: Identification of transcription start sites of the 1.7 kb SCP2 promoter luciferase fusion gene by primer extension analysis. Total RNA was extracted from HepG2 cells transfected with the 1.7 kb SCP2 promoter pGL$_2$ vector. Primer extension analysis was carried out with the HepG2 RNA and yeast tRNA as a control, as described in the text. M-13 DNA was subjected to DNA sequence analysis to determine the length of the extended products. Arrows mark prominent bands at 65, 73, and 74 nt, representing the expected sizes of extension products and reflecting transcription initiation from the SCP2 transcription start sites identified by sequence analysis of cDNA 5′-ends. These products were not found when yeast tRNA was employed as the template for primer extension.

Because of the unusual location of the SCP2 promoter and its initiation of transcription within exon XII, we confirmed by primer extension analysis that the 1.7 kb SCP2 promoter fragment initiates transcription of the luciferase fusion gene at the appropriate start sites (Figure 5). With the primer utilized, products 65−74 nt in size are expected if transcription of the fusion gene is initiated at the same positions as the SCP2 sequences. Labeled bands of this size were found when total RNA extracted from HepG2 cells transfected with the 1.7 kb SCP2 promoter pGL$_2$ construct was used as template, but not when yeast tRNA served as the template for primer extension.

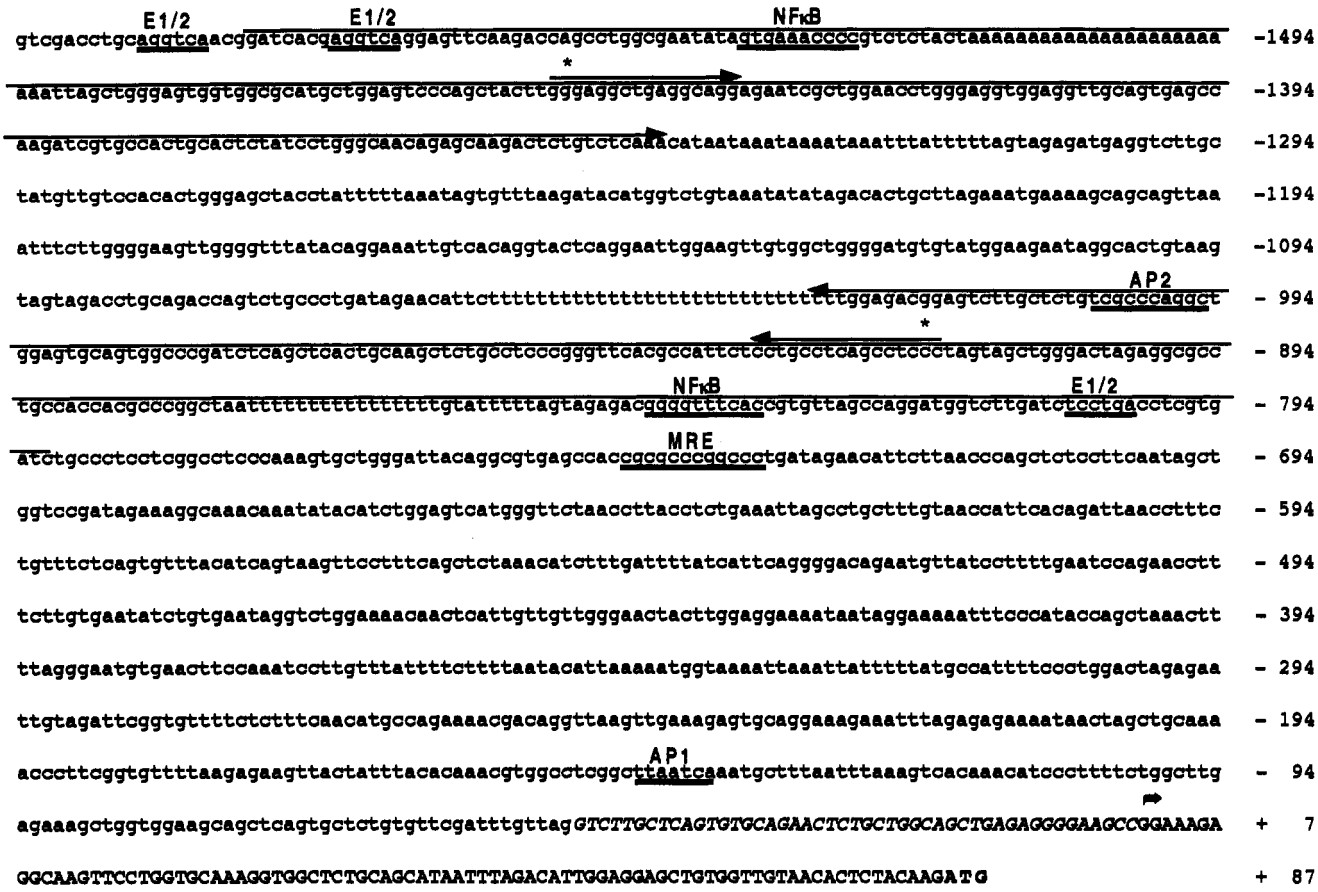

FIGURE 5: Nucleotide sequence of the SCP2 promoter region. Nucleotide position 1 is assigned to one of the potential transcription start sites (curved arrow), and negative numbers refer to 5′-flanking sequences. The initiator methionine codon is boldfaced. Putative AP1, AP2, MRE, and NFκB motifs are indicated. E1/2 denotes putative ERE half-sites. Long direct and inverted repeats are indicated by arrows. Short arrows marked with an asterisk indicate sequences similar to those found in the SCPx promoter.

The basal activities of the two largest promoter fragments relative to a control plasmid in which the luciferase reporter gene is under the control of an SV40 promoter were determined. In all host cells, the 1.7 kb SCP2 promoter displayed higher activity than the 3.4 kb SCPx promoter fragment (Figure 6), but the relative patterns of activity varied among the host cells. The ratio of SCP2 to SCPx promoter activity was substantially greater in Y1 cells than in BeWo, HepG2, and Balb 3T3 cells.

We used deletion analysis to map the basal activities of the two promoters. The patterns of SCPx promoter activity varied among the four host cell types studied (Figure 7). The first 200 nt of SCPx upstream promoter, which contains three SP1 sites, directed maximal or near-maximal expression of the luciferase reporter gene. It is remarkable that, in Y1 cells, the first 200 nt of promoter sequence drove reporter gene expression 40 times more than the largest promoter fragment. This observation raises the possibility that a strong suppressor active in Y1 cells is present just beyond the first 500 nt of promoter sequence. The large increase in basal promoter activity coincides with the removal of a consensus NBRE site, which binds orphan transcription factors including Nur77 and steroidogenic factor 1 (Wilson et al., 1993).

The 1.7 kb SCP2 promoter segment directed the greatest expression of the reporter gene in all cell hosts (Figure 8). Transcriptional activity was retained in the 900 nt from the transcription start sites. However, deletion of downstream sequences reduced promoter activity to various degrees in these four host cells.

The cAMP analog 8-Br-cAMP and the phorbol ester, phorbol myristate acetate, stimulated SCPx promoter activity by approximately 3-fold ($p < 0.01$) and 2-fold ($p < 0.01$), respectively, over basal activity in BeWo cells (Figure 9). In HepG2 cells, the cAMP analog increased promoter activity 4-fold, while the phorbol ester increased it 2-fold. A dose–response study carried out with HepG2 cells (data not shown) revealed responses to 8-Br-cAMP (0.001−1 mM) at concentrations of 1 μM, and near-maximal responses were achieved with 10 μM analog. The phorbol ester (0.01−1000 ng/mL) stimulated promoter activity at 1 ng/mL, and responses were maximal at 100 ng/mL. Both agents increased promoter activity slightly in Y1 cells. In Balb 3T3 cells, 8-Br-cAMP had a modest effect on SCPx promoter activity and the phorbol ester had no effect. The 1.7 kb SCP2 promoter displayed low and variable responses to the phorbol ester and the cAMP analog.

DISCUSSION

The SCPx/SCP2 structural gene spans some 80 kb and has been mapped to chromosome 1p32 (Ohba et al., 1994; Vesa et al., 1994). In this report, we have established that the gene contains two promoters: a 5′-promoter directing transcription of the SCPx mRNA and a promoter 45 kb downstream in intron 11, which directs transcription of the SCP2 mRNA.

We previously suggested the possibility that SCPx arose from the fusion of a thiolase gene and the gene encoding SCP2 (Baker et al., 1991). This notion recently received

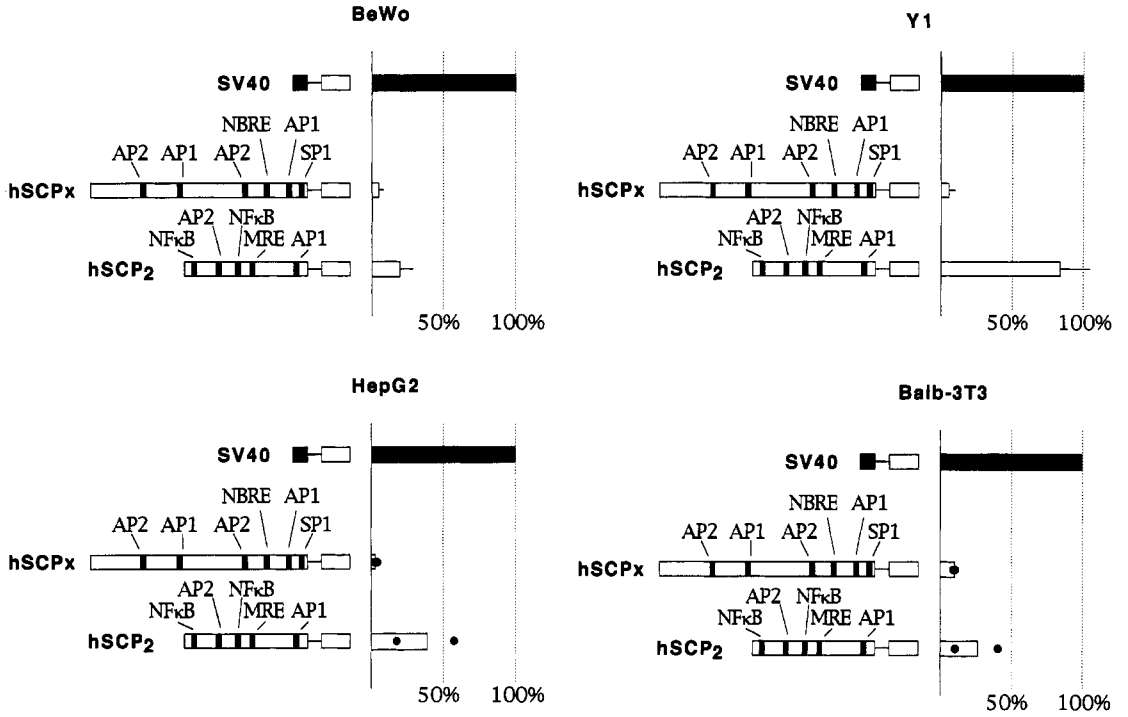

FIGURE 6: Basal activities of the 3.4 SCPx and 1.7 kb SCP2 promoter sequences in various host cells. Putative cis elements are indicated as landmarks. Promoter activities are expressed as a percentage of the activity of the pGL_2 control plasmid in which the SV40 promoter directs luciferase expression. Dots represent the mean activities from triplicate cultures of duplicate experiments. Bars represent the SE of results from four separate experiments.

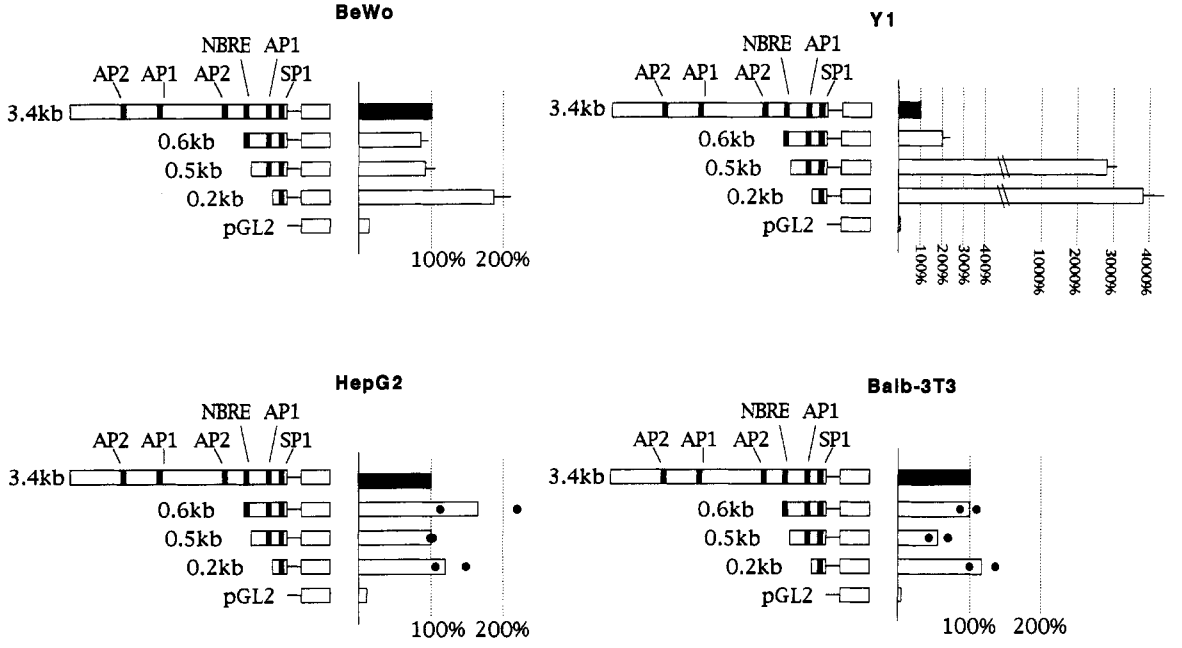

FIGURE 7: Basal SCPx promoter activity in various host cells. The indicated lengths of SCPx promoter DNA were cloned into the pGL_2 vector and transfected into the indicated host cells. Putative cis elements are indicated as landmarks. Promoter activities are expressed as a percentage of the largest promoter fragment. pGL_2 is the promoterless vector. Dots represent the mean values of triplicate cultures from two different experiments. Bars represent the SE from three or four separate experiments.

support from the discovery that an 80 kDa porcine steroid dehydrogenase, which has been localized in peroxisome-like structures of endometrium, has a carboxy terminal sequence highly homologous to SCP2 (Leenders et al., 1994). A newly discovered 100-120 kDa lipid exchange protein isolated from rabbit small intestine brush border membranes may also have a carboxy terminus similar to that of SCP2 (Lipka et al., 1995). These findings suggest that an ancestral SCP2 cassette may have been incorporated into several different genes. The presence of two widely separated promoters in the human SCPx/SCP2 gene is consistent with the idea of gene fusion.

A number of partial cDNA clones containing SCPx/SCP2 sequences have been described (Billheimer et al., 1990; Ossendorp et al., 1990; He et al., 1991; Moncecchi et al., 1991; Mori et al., 1991). Some of the 5′-ends of these cDNAs contained sequences that are not present in the SCPx cDNA, raising the possibility that there are additional transcription start sites that give rise to transcripts with unique 5′-untranslated sequences. However, there is no common

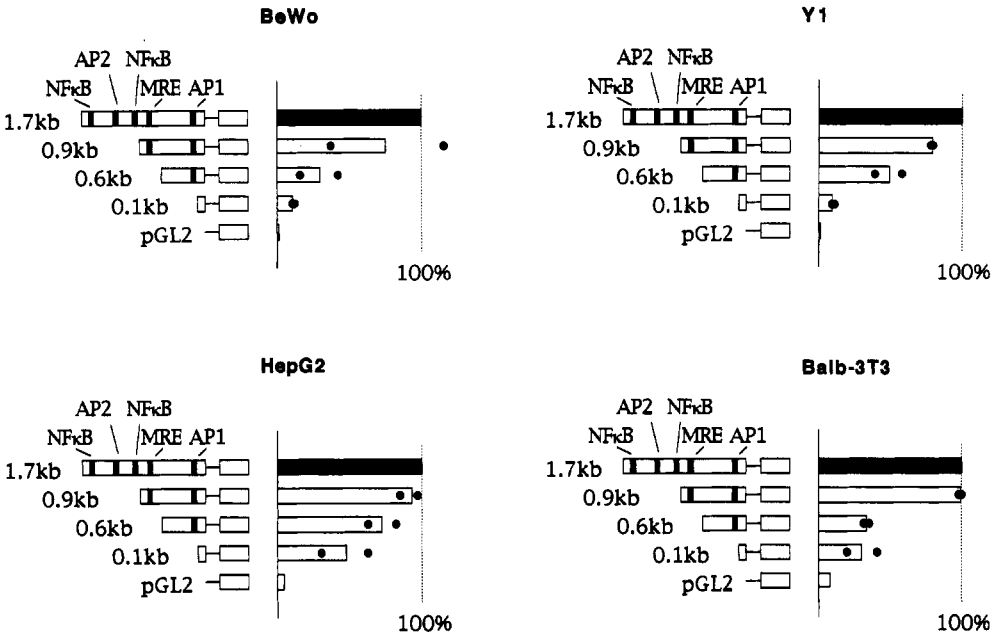

FIGURE 8: Basal SCP2 promoter activity in various host cells. The indicated lengths of SCP2 promoter DNA were cloned into the pGL_2 vector and transfected into the indicated host cells. Putative cis elements are indicated as landmarks. Promoter activities are expressed as a percentage of the largest promoter fragment. pGL_2 is the promoterless vector. Dots represent the mean values of triplicate cultures from two different experiments. Bars represent the SE from three or four separate experiments.

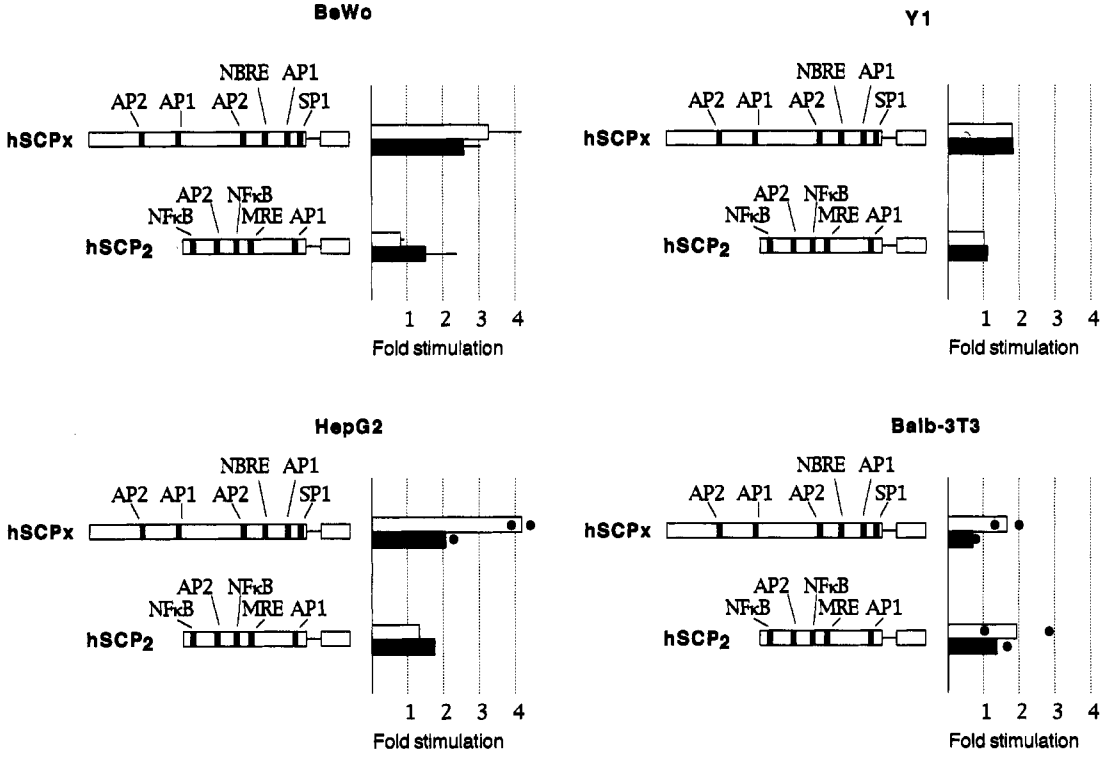

FIGURE 9: Effects of 8-Br-cAMP (open bars) and phorbol myristate acetate (solid bars) on SCPx and SCP2 promoter activities in various host cells. The 3.4 kb SCPx promoter fusion gene construct and the 1.7 kb SCP2 promoter fusion gene construct were transfected into the indicated host cells. Putative cis elements are indicated as landmarks. Transfected cells were treated either with vehicle, 8-Br-cAMP (1 mM), or phorbol myristate acetate (100 ng/mL) in dimethyl sulfoxide for the last 24 h of culture. Promoter activity is expressed relative to basal activities. Dots represent the means of triplicate cultures from two different experiments. Bars represents the SE of three or four separate experiments.

pattern among these cDNA ends. We searched extensively for human genomic sequences corresponding to the reported divergent human cDNA sequence and were unable to identify previously unrecognized exons in our genomic clones. Thus, these divergent cDNA 5′-ends could be the result of a cloning artifact.

The SCPx and SCP2 promoters we have described share several features, including the absence of TATA boxes, the initiation of transcription from multiple sites, and the presence of elements similar to those identified in the promoters of genes encoding other peroxisomal proteins, including palindromic repeats.

Although the SCPx and SCP2 promoters have several structural similarities, they appear to function independently and their activities are highly influenced by the host cell context. The pattern of basal activity of the SCPx promoter

differed markedly in different host cells. Deletion studies suggested the possibility of a strong suppressor element in the upstream sequences of the SCPx promoter that is active in Y1 cells. The removal of sequences containing a putative NBRE resulted in a 40-fold increase in promoter activity in these cells. This was not seen in other host cells, although truncation of the promoter tended to increase activity in BeWo and HepG2 cells.

The abundance of SCPx and SCP2 mRNAs has been shown to be regulated in various tissues. Peroxisome proliferators increase SCPx but not SCP2 mRNA in rat liver (Mori et al., 1991). Estrogen increases SCPx and SCP2 mRNAs in avian liver (Pfeifer et al., 1993). Retinoids increase SCP2 but not SCPx mRNA in choriocarcinoma cells (Matsuo & Strauss, 1994). Trophic stimulation of rodent adrenal cells by ACTH (Trzeciak et al., 1987) and of ovarian tissue by gonadotropins and cells by cyclic AMP analogs (Rennert et al., 1991) increases the steady state levels of the SCP2 and SCPx mRNAs. A number of putative cis elements that could account for the observed hormonal regulation of SCPx and SCP2 mRNA levels were identified in the SCPx and SCP2 promoters, including ERE half-sites, a putative RARE, and putative AP1 and AP2 sites. However, it is not yet known whether the preceding treatments alter SCPx and SCP2 mRNA levels though transcriptional mechanisms.

In the present study, we have shown that the SCPx promoter is responsive to 8-Br-cAMP and phorbol ester stimulation in certain host cells. Hence, transcriptional regulation could account, in part, for some of these observations relating to SCPx mRNA. In contrast, the first 1.7 kb of the SCP2 promoter did not show striking responses to these agents. This could mean that SCP2 mRNA is controlled by posttranscriptional mechanisms or that cis elements lying elsewhere in the gene mediate transcriptional responses. Nonetheless, these findings suggest differential regulation of the two promoters. The presence of two independent promoters and their differential activities in various host cells provide an explanation for the generation of two mRNAs from a single structural gene and for the different patterns of expression of the two mRNAs in various organs.

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