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Human Hydroxysteroid Sulfotransferase SULT2B1: Two Enzymes Encoded by a Single Chromosome 19 Gene

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We have cloned and characterized cDNAs that encode two human hydroxysteroid sulfotransferase (SULT) enzymes, SULT2B1a and SULT2B1b, as well as the single gene that encodes both of these enzymes. The two cDNAs differed at their 5′-termini and had 1050- and 1095-bp open reading frames that encoded 350 and 365 amino acids, respectively. The amino acid sequences encoded by these cDNAs included "signature sequences" that are conserved in all known cytosolic SULTs. Both cDNAs appeared, on the basis of amino acid sequence analysis, to be members of the hydroxysteroid SULT "family," SULT2, but they were only 48% identical in amino acid sequence with the single known member of that family in humans, SULT2A1 (also referred to as DHEA ST). Northern blot analysis demonstrated the presence of SULT2B1 mRNA species approximately 1.4 kb in length in human placenta, prostate, and trachea and—faintly—in small intestine and lung. Expression of the two human SULT2B1 cDNAs in COS-1 cells showed that both of the encoded proteins catalyzed sulfation of the prototypic hydroxysteroid SULT substrate, dehydroepiandrosterone, but both failed to catalyze the sulfate conjugation of 4-nitrophenol or 17β-estradiol, prototypic substrates for the phenol and estrogen SULT subfamilies. Both of these cDNAs were encoded by a single gene, *SULT2B1.* The locations of most exon–intron splice junctions in *SULT2B1* were identical to those of the only other known human hydroxysteroid SULT gene *SULT2A1* (previously *STD*). The divergence in 5′-terminal sequences of the two SULT2B1 cDNAs resulted from alternative transcription initiation prior to different 5′ exons, combined with alternative splicing. *SULT2B1* mapped to human chromosome band 19q13.3, approximately 500 kb telomeric to the location of *SULT2A1.*

INTRODUCTION

Sulfate conjugation is an important reaction in the biotransformation of steroid hormones, neurotransmitters, drugs, and other xenobiotic compounds (Weinshilboum and Otterness, 1994; Falany, 1997). The cytosolic sulfotransferase enzymes that catalyze these reactions were previously referred to as "STs" (Weinshilboum and Otterness, 1994). However, a recent International Sulfotransferase Nomenclature Workshop suggested that the abbreviation "SULT" be used to refer to these enzymes and their genes. At least seven SULTs are presently known to be expressed in human tissues. Three are phenol SULTs; one is an estrogen SULT; one, SULT1B1, is capable of catalyzing the sulfation of thyroid hormones; another, SULT1C1 may represent an orthologue of a rat enzyme that can catalyze the sulfation of *N*-hydroxy-2-acetylaminofluorene; and the final currently known isoform in humans is a hydroxysteroid SULT, SULT2A1, referred to previously as "DHEA ST" (Weinshilboum and Otterness, 1994; Weinshilboum *et al.,* 1997; Falany, 1997; Her *et al.,* 1997; Wang *et al.,* 1998). cDNAs have been cloned for all of these enzymes, and chromosomal localizations have been reported for six of their genes. The three human phenol SULT genes have been mapped to a gene cluster located between bands 16p12.1 and 16p11.2 on the short arm of chromosome 16 (Dooley *et al.,* 1993; Aksoy *et al.,* 1994a; Her *et al.,* 1996; Raftogianis *et al.,* 1996), the *SULT1C1* gene was localized to chromosome 2q11.1–11.2 (Her *et al.,* 1997), the human estrogen SULT gene has been mapped to chromosome band 4q13.1 (Her *et al.,* 1995), and the *SULT2A1* gene (previously *STD*) was localized to chromosome band 19q13.3 (Otterness *et al.,* 1995a). All of

Sequence data from this article have been deposited with the GenBank Data Library under Accession Nos. U92314 (SULT2B1a), U92315 (SULT2B1b), and U92316–U92322 (*SULT2B1*).

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TABLE 1

Human Cytosolic SULT Enzymes

(A) Human cytosolic SULT enzymes and genes

Original enzyme nomenclature	Original gene nomenclature	Proposed gene nomenclature	Gene Chromosomal localization
TS PST1	*STP1*	*SULT1A1*	16p11.2–p12.1
TS PST2	*STP2*	*SULT1A2*	16p11.2–p12.1
TL PST	*STM*	*SULT1A3*	16p11.2
ST1B2	—	*SULT1B1*	—
EST	*STE*	*SULT1E1*	4q13.1
SULT1C1	—	*SULT1C1*	2q11.1–q11.2
DHEA ST	*STD*	*SULT2A1*	19q13.3
SULT2B1a	—	*SULT2B1*	19q13.3
SULT2B1b	—		

(B) Human SULT enzymes % amino acid sequence identity

	1A1	1A2	1A3	1B1	1C1	1E1	2A1
1A1	—						
1A2	95	—					
1A3	93	90	—				
1B1	54	54	53	—			
1C1	52	53	52	53	—		
1E1	51	51	50	56	48	—	
2A1	37	38	38	39	36	37	—
2B1a	41	38	42	38	37	35	48
2B1b	39	37	40	38	37	35	48

Note. (**A**) Selected examples of the previous nomenclature for these proteins, both the previous and a proposed gene nomenclature, and chromosomal localizations of the genes when that information is known. A comprehensive list of SULT nomenclatures can be found in Weinshilboum *et al.,* 1997. (**B**) Comparisons of the percentage amino acid sequence identity of human SULTs. These comparisons were made with the Bestfit program (Devereux *et al.,* 1984). Sources of sequence data and chromosomal localizations included TS PST1 or SULT1A1 (Wilborn *et al.,* 1993; Dooley *et al.,* 1993, 1994; Raftogianis *et al.,* 1996), TS PST2 or SULT1A2 (Ozawa *et al.,* 1995; Her *et al.,* 1996), TL PST or SULT1A3 (Wood *et al.,* 1994; Aksoy *et al.,* 1994b), SULT1B1 (Fujita *et al.,* 1997; Wang *et al.,* 1998), SULT1C1 (Her *et al.,* 1997), SULT1E1 (Aksoy *et al.,* 1994a; Her *et al.,* 1995), and DHEA ST or SULT2A1 (Otterness *et al.,* 1992, 1995b).

these enzymes as well as selected examples of their previous trivial abbreviations, the recently proposed SULT nomenclature, and the chromosomal locations of their genes are listed in Table 1A.

In the present study, we report the identification of two new human hydroxysteroid SULTs encoded by a single gene, *SULT2B1,* which maps to the long arm of human chromosome 19. On the basis of Northern blot analysis, *SULT2B1* was most highly expressed in the human placenta, prostate, and trachea. Sulfate-conjugated hydroxysteroids are known to play an important functional role during pregnancy in the human fetoplacental unit (Hobkirk, 1993), and the prostate is a steroid-hormone-dependent organ (Sandberg, 1980). The only other known human hydroxysteroid SULT—SULT2A1 or DHEA ST (see Table 1)—is highly expressed in the liver, small intestine, and adrenal cortex, but not in the placenta or prostate (Otterness *et al.,* 1992; Comer *et al.,* 1993; Otterness and Weinshilboum, 1994). The discovery of two novel human hydroxysteroid SULTs that are expressed in the placenta and prostate opens the way for studies of the possible functional significance of hydroxysteroid sulfation in those organs.

MATERIALS AND METHODS

Human placental SULT2B1 cDNA cloning. SULT enzymes share at least four areas of high amino acid sequence homology (Weinshilboum and Otterness, 1994; Marsolais and Varin, 1995; Weinshilboum *et al.,* 1997). We took advantage of the existence of these signature sequences to search for novel human cytosolic SULTs in the expressed sequence tag database (Boguski and Schuler, 1995). That search involved the highly conserved amino acid sequence motif "RKGxxGDWKNxFT" (Weinshilboum and Otterness, 1994; Weinshilboum *et al.,* 1997) in which "x" represented any amino acid. That search resulted in the identification of an expressed sequence tag (GenBank Accession R73584) located at the 3′-end of a clone isolated from a human placental cDNA library by the IMAGE Consortium (IMAGE clone ID 141495; Lennon *et al.,* 1996). Unfortunately, the American Type Culture Collection (ATCC) was unable to retrieve this clone; so we used 5′- and 3′-rapid amplification of cDNA ends (RACE) (Frohman *et al.,* 1988) to obtain the 5′- and 3′-ends of the cDNA with both human placental and prostate Marathon-Ready cDNA as template (Clontech, Palo Alto, CA). The placental mRNA used to generate the Marathon-Ready cDNA had been isolated from a 16-year-old Caucasian/Japanese woman, and the prostate mRNA had been isolated from a "pool" obtained from 24 Caucasian subjects.

The antisense primers used to perform these initial 5′-RACE experiments were R273 during the initial amplification and R203 during the second, nested reaction. Both of these primers had been designed on the basis of the sequence of IMAGE clone ID 141495 (expressed sequence tag R72969). Sequences of these and all other primers used in the course of the experiments described subsequently are listed in Table 2. The 5′-RACE studies demonstrated the presence of two different 5′ coding sequences, both of which shared a common 3′-terminus, resulting in the identification of two cDNAs, SULT2B1a and SULT2B1b. The sense primers used to perform the 3′-RACE experiments were F839 during the initial amplification and F917 during the second, nested reaction. Both F839 and F917 were designed on the basis of the sequence of expressed sequence tag R73584, the tag that included the signature sequence that initially resulted in our selection of this clone for study. The "anchor" primers used to perform the 5′- and 3′-RACE experiments were those provided by the manufacturer of the Marathon-Ready cDNA (Clontech). The common central portion of the cDNA was amplified with the same Marathon-Ready cDNA as template and with primers F442 and R797, primers designed on the basis of the nucleotide sequences of expressed sequence tags R72969 and R73584, respectively. Confirmatory 5′- and 3′-RACE experiments were then performed with cDNA that had been reverse transcribed from pooled human placental or pooled human prostate poly(A)$^+$ RNA (Clontech). First-strand cDNA for those experiments was generated with Superscript II reverse transcriptase (Gibco BRL, Gaithersburg, MD) and, for 5′-RACE, primer R536, located within the "common" region of the open reading frames (ORFs) of the two cDNAs. A poly(G) sequence was added to these first-strand cDNAs with terminal transferase (Boehringer Mannheim, Indianapolis, IN). The PCR for these 5′-RACE experiments was performed with the poly(G) "tailed" cDNAs as template and with primers R479 and d(C)$_{15}$. Amplification products from all RACE experiments were subcloned into pCR2.1 (Invitrogen, San Diego, CA) prior to DNA sequencing. A total of 26 5′-RACE subclones for placenta and 37 subclones for prostate were sequenced and analyzed. The two primers used to perform the 3′-RACE experiment were F839 and F917. A total of 6 3′-RACE subclones were analyzed for the placenta and 12 for the prostate.

TABLE 2

Sequences of Primers Used either to Perform PCR Amplifications or as Probes for Southern Blot Analysis

Primer designation	Primer location	Primer sequence
5′-RACE		
R203	Exon II	TCCGGGATGGATCCCCTTCCTTCA
R273	Exon II	TCCGGGAGGCTGAAAGCACCCACAAT
R479	Exon III	CTTCGCCTTTGAGGAAGTCCCTCAGGAACT
R536	Exon IV	CTTTGCCCTTCATCCGAAGCCAGCCCTTAA
3′-RACE		
F839	Exon VI	ATGCCGACCTTCCCCTGGGATGAA
F917	Exon VI	AAGCCCAGCCTTGAGCCCAACACC
Central cDNA		
F422	Exon III	ACCCGACCAGTTCCTGAGGGACTT
R797	Exon VI	TCTGGGCCACCGTGAAGTGGTTCT
Complete cDNA		
BF(−121)	Exon IB	TGCCGCCTGCTCCCTGTTGGTCCTC
AF(−79)	Exon IA	GTCCGAGTGTCGCCACCCTGAGAACTC
R1047	Exon VI	AGACAGAATCGACGTGTTTATTATGAG
R1047L	Exon VI	AGACAGAATCGACGTGTTTATTATGAGGGTCGTGGGTG-CGGGGTCTCACAGGCCTGGCCGGGGCTGGGGCTGGGGC
Chromosomal localization		
IN1AF	Intron I	GCCCTCCCACACCCAATTAATCTG
R273	Exon II	TCCGGGAGGCTGAAAGCACCCACAAT
Southern analysis		
BF(−144)	Exon IB	TCGCTGCGCACACCTGGCCTCTGT
AR(−95)	Exon IA	CCTCCTCAGAGCCTCAGTCCCTCTTCTGTA
AF(−25)	Exon IA	TGGCGTCTCCCCCACCTTTCCACAGCCAGAAGTT
IN1AR	Intron IA	TCGCCCAGGCTGGAGTGCAATGGCGCGATCTCGGCTCACT
R203	Exon II	TCCGGGATGGATCCCCTTCCTTCA
R479	Exon III	CTTCGCCTTTGAGGAAGTCCCTCAGGAACT
R536	Exon IV	CTTTGCCCTTCATCCGAAGCCAGCCCTTAA
R683	Exon V	TGGCCTTCATGGCGCTGAAGGTTGAGTGTG
F917	Exon VI	AAGCCCAGCCTTGAGCCCAACACC
Intron length determination		
BF(−144)	Exon IB	TCGCTGCGCACACCTGGCCTCTGT
R52	Exon IA	GACGGGGAAGGGGACGCCCTTGTA
AF(−29)	Exon IA	CTCATGGCGTCTCCCCCACCTTTC
R203	Exon II	TCCGGGATGGATCCCCTTCCTTCA
F221	Exon II	TGGGAGCGGGCACCCTGGTGTGAG
R479	Exon III	CTTCGCCTTTGAGGAAGTCCCTCAGGAACT
F442	Exon III	ACCCGACCAGTTCCTGAGGGACTT
R536	Exon IV	CTTTGCCCTTCATCCGAAGCCAGCCCTTAA
F520	Exon IV	TCGGATGAAGGGCAAAGACAACTTCCTATT
R683	Exon V	TGGCCTTCATGGCGCTGAAGGTTGAGTGTG
R664	Exon V	CTTCAGCGCCATGAAGGCCAACACCATGTC
R1047	Exon VI	AGACAGAATCGACGTGTTTATTATGAG

Note. The numbering scheme for the primers is based on the cDNA sequence, in which +1 is the first nucleotide within exon IA that is common to the sequences of the two SULT2B1 cDNAs (see Fig. 1). Nucleotides 5′ of this location have been assigned negative numbers, while those located in the 3′ direction have been assigned positive numbers. Abbreviations: F, forward; R, reverse; IN, intron. Bracketed primer pairs were used to determine intron lengths.

Each of the two different cDNAs identified by performing RACE was amplified by performing the PCR with ExTaq DNA polymerase (PanVera, Madison, WI) and with 3′ primer R1047, located within the 3′ untranslated region (3′-UTR) of both cDNAs, paired with two different 5′ primers, AF(−79) and BF(−121) (Table 2). The templates used to perform these amplifications included Marathon-Ready cDNA from both human placenta and prostate and first-strand cDNA that had been reverse transcribed from both human placental and prostate poly(A)$^+$ RNA (Clontech). Once again, two different cDNA sequences were amplified that diverged in sequence at exactly the point predicted by the 5′-RACE experiments. The cDNA with the shorter ORF will be referred to subsequently as SULT2B1a, while that with the longer ORF will be referred to as SULT2B1b (Fig. 1).

Northern blot analysis. Human Multiple Tissue Northern Blots (Clontech) were used to perform Northern blot analyses. Each lane of the Northern blots contained approximately 2 μg of poly(A)$^+$ RNA. The probes were the human SULT2B1a cDNA ORF that had been radioactively labeled with [α-^{32}P]dCTP by random priming with the Oligolabeling Kit (Pharmacia, Piscataway, NJ) and radioactively labeled human ß-actin cDNA.

COS-1 cell expression. The ORFs of SULT2B1a and SULT2B1b were PCR amplified with primer AF(−79), designed on the basis of the 5′-UTR sequence of SULT2B1a, or primer BF(−121), designed on the basis of the 5′-UTR sequence of SULT2B1b, both paired with reverse primer R1047L (Table 2). The template was human placental Marathon-Ready cDNA. ExTaq DNA polymerase was used to perform these reactions. Both amplification products were ligated into the eukaryotic expression vector pCR3.1 (Invitrogen), and the inserts were sequenced completely on both strands to ensure that no variant sequence had been introduced during the amplifications. These expression constructs were used to transfect COS-1 African green

monkey SV-40-transformed kidney cells with the DEAE dextran method as described elsewhere (Wood *et al.*, 1996). Transfected cells were harvested after 48 h in culture, and 100,000*g* cytosol preparations were prepared and stored at −80°C.

SULT and protein assays. SULT activities were assayed with the method of Foldes and Meek (1973) as modified to measure activity under optimal conditions for the prototypic substrates DHEA, 4-nitrophenol, 17ß-estradiol, and dopamine (Campbell *et al.*, 1987; Hernández *et al.*, 1992). This assay utilizes 0.4 μM [^{35}S]3′-phosphoadenosine-5′-phosphosulfate (PAPS) as a sulfate donor. Blanks were samples that did not contain a sulfate acceptor substrate. Protein concentrations were measured by the dye-binding method of Bradford (1976) with bovine serum albumin as a standard.

SULT2B1 gene cloning. The cloning of *SULT2B1* began with a determination of the chromosomal localization of the gene to make it possible to screen a chromosome-specific genomic DNA library. As a first step, partial sequence for one intron was determined by using the anchored PCR technique "rapid amplification of genomic DNA ends" (RAGE) (Mizobuchi and Frohman, 1993). Determination of this partial intron sequence made it possible, as described subsequently, to use NIGMS Human/Rodent Hybrid Cell DNA Mapping Panels 1 and 2 (Coriell Institute for Medical Research, Camden, NJ) to determine that the gene was located on human chromosome 19. The next step involved using the human SULT2B1a cDNA ORF as a probe to screen—unsuccessfully—a human chromosome 19-specific cosmid contig library (deJong *et al.*, 1989), followed by use of the cDNA to screen a total human genomic DNA bacterial artificial chromosome (BAC) library (Shizuya *et al.*, 1992). The cDNA probe had been radioactively labeled with [α-^{32}P]dCTP by random priming. A single positive clone, BAC45957, was isolated from the BAC library, and the presence of *SULT2B1* in this clone was confirmed by performing the PCR with gene-specific primers IN1AF and R273. The clone was then digested with a series of restriction enzymes, and the restriction fragments were ligated to pBluescript (pBSK) DNA that had been digested with the same restriction enzymes to create a series of BAC45957 DNA RAGE panels. The PCR was then performed either with pairs of cDNA-specific primers and with BAC45957 DNA as template or with pairs of pBSK-specific and cDNA-specific primers with BAC45957 RAGE panel DNA as template to obtain intron, 5′-flanking region, or 3′-flanking region sequences.

The length of each intron, except that of intron IA, was determined by performing the long PCR with ExTaq DNA polymerase using primer pairs located on contiguous exons (Table 2). Each of these amplification products was sequenced to confirm exon–intron splice junctions. Southern analysis of the BAC45957 clone was also performed to confirm intron lengths. Specifically, 10 μg of BAC45957 DNA was digested with each of 13 restriction enzymes, followed by electrophoresis on a 0.8% SeaKem Gold agarose gel (FMC BioProducts, Rockland, ME). After electrophoresis, DNA fragments were transferred to a nylon membrane that was probed with radioactively labeled oligonucleotide probes specific for each of the *SULT2B1* exons (Table 2).

SULT2B1 chromosomal localization. The chromosomal localization of *SULT2B1* was initially determined by performing the PCR with template DNA from NIGMS Human/Rodent Somatic Cell Hybrid Mapping Panels 1 and 2. Intron-based forward primer IN1AF and exon-based reverse primer R273 were used to perform these reactions. After this approach had localized the gene to chromosome 19, sublocalization was achieved by performing the PCR with the same primers and with template DNA from a human chromosome 19 regional mapping panel (Bachinski *et al.*, 1993). The sublocalization of *SULT2B1* was confirmed by performing FISH analysis of human metaphase chromosomes with BAC45957 DNA as a probe (Tasken *et al.*, 1996). The sublocalization was also confirmed by use of the same high-density arrayed human chromosome 19 cosmid contig library that had originally been screened, unsuccessfully, with the SULT2B1 cDNA probe. In this case, the library was screened with probes generated from BAC45957 DNA by the use of long-range *Alu* PCR (Parrish *et al.*, 1995). That approach resulted in the identification of two positive nonoverlapping chromosome 19 clones, cosmid 1438 and 1229, that overlapped the BAC clone, information that allowed BAC45957 to be localized within the chromosome 19 cosmid contigs.

DNA sequencing and data analysis. DNA sequencing was performed in the Mayo Clinic Molecular Biology Core Facility with Applied Biosystems Model 373A and 377A sequencers (Perkin–Elmer). Sequencing reactions were performed with the ABI Prism Dye Terminator Cycle Sequencing Ready Reaction Kit using AmpliTaq FS DNA polymerase (Perkin–Elmer). The University of Wisconsin Genetics Computer Group software package, versions 8.0 and 9.0 (Devereux *et al.*, 1984), was used to analyze nucleotide and peptide sequences, and the transcription factor database TFSITES, release 5.0 (Ghosh, 1990), was used to identify putative promoter or transcription-modifying DNA sequence motifs. Apparent K_m values for transiently expressed proteins were determined by the method of Wilkinson (1961) with a computer program written by Cleland (1963).

RESULTS

SULT2B1 cDNA cloning. The human placental SULT2B1 cDNA was identified by performing a search of the expressed sequence tag database for possible cytosolic SULT cDNAs. The first step involved a search of the database for a highly conserved amino acid sequence motif, RKGxxGDWKNxFT, which is present in all known cytosolic SULTs (Weinshilboum and Otterness, 1994; Weinshilboum *et al.*, 1997). That search resulted in the identification of an expressed sequence tag that represented the 3′-terminus of a clone from a human placental cDNA library (GenBank Accession No. R73584, IMAGE clone ID 141495; Lennon *et al.*, 1996). As described subsequently, amino acid sequence alignment analysis also showed that the 5′-end of that clone (GenBank Accession No. R72969) encoded an amino acid sequence motif that is highly conserved among hydroxysteroid SULTs. Unfortunately, the ATCC was unable to retrieve the clone that contained these two tag sequences. Therefore, we used the anchored PCR technique RACE (Frohman *et al.*, 1988) with primers designed on the basis of the expressed tag sequences (GenBank Accession Nos. R72969 and R73584) and with human placental and prostate cDNA (Clontech) as template to obtain the 5′- and 3′-ends of what proved to be two cDNAs. Analysis of subcloned 3′-RACE amplification products resulted in the identification of 20 additional nucleotides that extended beyond the 3′-terminus of the expressed sequence tag and ended in a poly(A)$^+$ tract. A polyadenylation signal (AATAAA) was located 18 nucleotides 5′ upstream of the poly(A) tract (Fig. 1). The polyadenylation signal overlapped the translation termination codon by 2 nucleotides (Fig. 1). However, two different 5′-termini were amplified by performing 5′-RACE with human placental and prostate Marathon-Ready cDNAs as template with primers designed on the basis of the 5′ expressed tag sequence. Specifically, a total of 26 5′-RACE subclones were analyzed for the placenta and 37 for prostate. The longest of these two different groups of 5′-RACE products were 423 and 408 bp in length,

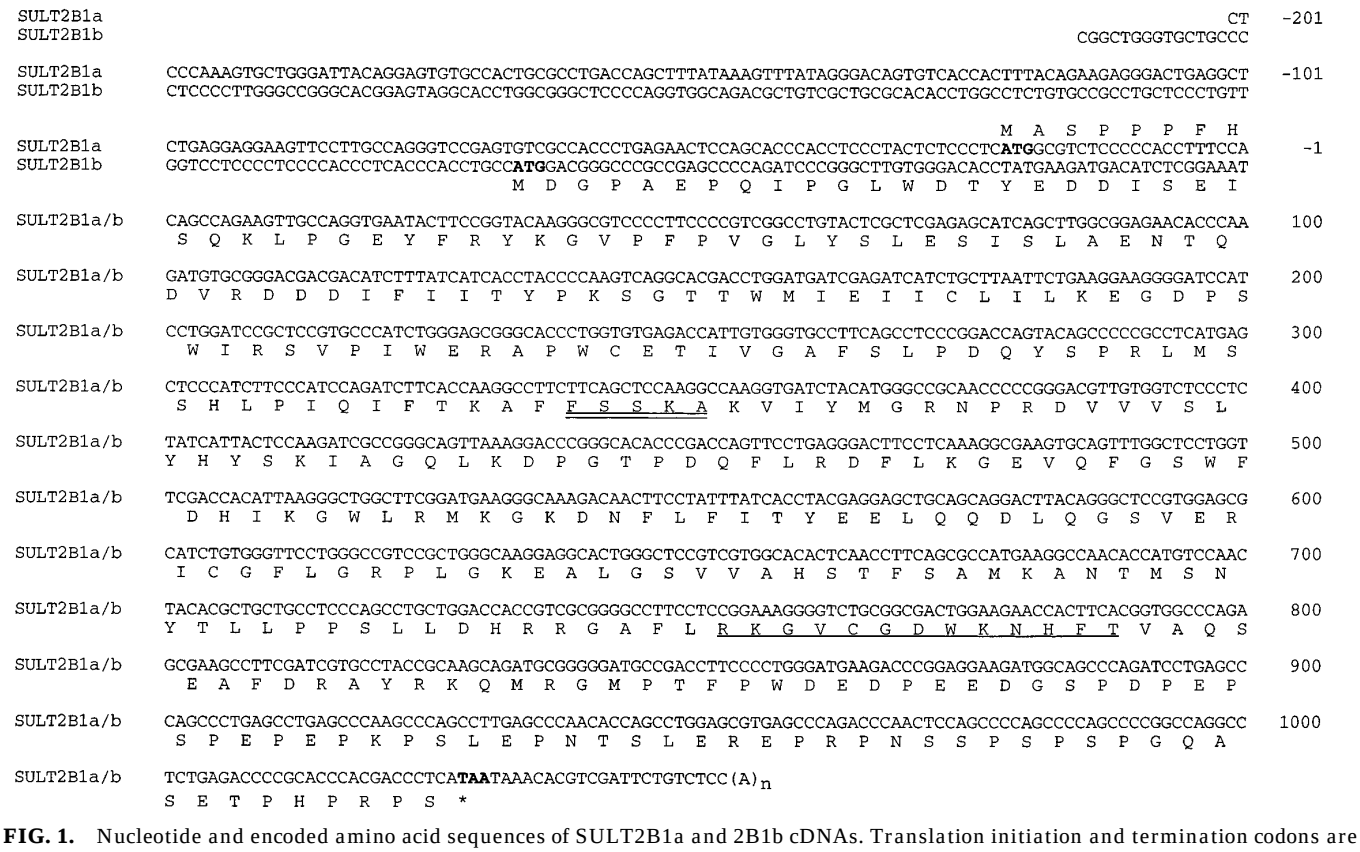

FIG. 1. Nucleotide and encoded amino acid sequences of SULT2B1a and 2B1b cDNAs. Translation initiation and termination codons are shown in bold. The numbering scheme for nucleotides begins with the first common nucleotide for the two cDNAs. The underlined amino acid sequence is the SULT signature sequence that was used to screen the expressed sequence tag database. The double-underlined sequence is a hydroxysteroid SULT signature sequence. The GenBank accession numbers for the SULT2B1a and 2B1b cDNA sequences are U92314 and U92315, respectively.

respectively—although, obviously, the exact length of the 5′-UTRs remains unclear. Analysis of the sequences of amplification products of each of the two "types" observed showed that they both included an identical 203-bp sequence at their 3′-ends that overlapped that of the tag sequence by 57 nucleotides. However, the sequences of the two types of amplification products differed 5′ of those 203 identical nucleotides.

The next step involved an attempt to amplify complete cDNAs with primers designed on the basis of the two different 5′-terminal sequences, but using a common 3′ primer. Two separate cDNAs were amplified that differed only in the nucleotide sequences present at their 5′-ends—with sequence divergence at exactly the point that the sequences of the 5′-RACE subclones had diverged. The cDNA with the shorter ORF, the one that we have designated SULT2B1a, was 1282 bp in total length with a 179-bp 5′-UTR, while SULT2B1b was 1297 bp in length with a 149-bp 5′-UTR (Fig. 1). SULT2B1a and 2B1b had 1050- and 1095-bp ORFs that encoded either 350 or 365 amino acids, respectively. The GenBank accession numbers for the SULT2B1a and 2B1b cDNAs are U92314 and U92315, respectively. Although both SULT2B1a and 2B1b sequences were found to be expressed in both tissues during the 5′-RACE studies, SULT2B1b was observed more frequently than was the 2B1a sequence, with a ratio of 21 to 5 in placenta and 32 to 5 in prostate for the 2B1b and 2B1a sequences, respectively.

SULT2B1a and 2B1b appeared to be members of the hydroxysteroid SULT family on the basis of amino acid sequence analysis, with the highest sequence identity, 48%, to that of human SULT2A1 (DHEA ST, Table 1B) when comparisons were made within comparable portions of the encoded proteins. Furthermore, both of the SULT2B1 cDNAs contained a sequence motif, FSSKA, that is found only among members of the hydroxysteroid SULT family (Fig. 2, double underlined in Fig. 1). At that same location, there are also highly conserved subfamily-specific amino acid sequence motifs, LDQKV and WEKxC, in the phenol and estrogen SULT subfamilies, respectively (Fig. 2). When the 2 SULT2B1 proteins were compared with the 10 other currently known hydroxysteroid SULTs, 9 cloned from nonhuman species (Fig. 2), they were longer at both the N- and the C-termini than were any of those other enzymes. Of particular interest was the fact that the final 54 residues at the C-terminus of the proteins encoded by *SULT2B1* were proline-rich and contained several "xP" tandem repeats, in which x could represent any amino acid (Fig. 1). The possible functional significance of this repetitive amino acid sequence is unknown. In summary, we cloned two separate

	SPECIES	SEQUENCE	AA LOCATION	REFERENCE
HYDROXYSTEROID SULTs	Human (2B1a)	FSSKA	121-125	----------
	Human (2B1b)	FSSKA	136-140	----------
	Rat	FSSKA	107-111	Chatterjee et al., 1987
	Human (2A1)	FSSKA	110-114	Otterness et al., 1992
	Mouse	FSSKA	110-114	Kong and Fei, 1994
	Guinea Pig	FSSKA	110-114	Lee et al., 1994
	Monkey	FSSKA	110-114	Ogura et al., 1996
	Mouse	FSSKA	110-114	Kong et al., 1993
	Rat	FSSKA	109-113	Ogura et al., 1989
	Guinea Pig	FSSKA	110-114	Luu et al., 1995
	Rat	FSSKA	109-113	Ogura et al., 1990
	Rat	FSSKA	110-115	Watabe et al., 1994
	Phenol SULTs	LDQKV	----------	----------
	Estrogen SULTs	WEKxC	----------	----------

FIG. 2. Hydroxysteroid SULT "subfamily-specific" amino acid signature sequences. The species from which each hydroxysteroid SULT was cloned is listed. The numbers refer to amino acid positions within each of the SULTs listed. Subfamily-specific sequences for phenol and estrogen SULTs at comparable locations within their respective proteins are also shown.

SULT2B1 cDNAs, which differed at their 5′-termini, including a portion of the ORF. Possible explanations for these observations would involve alternative sites of transcription initiation, alternative splicing, or a combination of the two. To evaluate those possibilities, it was necessary to clone and characterize structurally the *SULT2B1* gene. However, before doing that, we performed Northern blots to determine the tissues in which these cDNAs were expressed, as well as transient expression experiments to help characterize the enzymes encoded by the cDNAs.

Northern blot analyses. Northern blot analysis was performed with 23 different human tissue preparations (Fig. 3). Human Multiple Tissue Northern blots (Clontech) were probed with the human SULT2B1a cDNA ORF. This probe would hybridize to both SULT2B1a and SULT2B1b mRNA. The major transcript detected was approximately 1.4 kb in length and was present in human placenta, prostate, and trachea, with a fainter signal also detected in the small intestine and lung (Fig. 3). Virtually identical results were obtained when the Northern analysis was repeated with a separate set of Multiple Tissue Northern blots. The only other known human hydroxysteroid SULT, SULT2A1 (DHEA ST), is highly expressed, on the basis of Northern blot analysis or Western blot analysis in the liver, intestine, and adrenal cortex, but not in the placenta, prostate, or trachea (Comer and Falany, 1992; Comer *et al.*, 1993; Otterness and Weinshilboum, 1994; Otterness *et al.*, 1995b).

COS-1 cell expression. SULT2B1a and SULT2B1b cDNA ORFs were subcloned into the eukaryotic expression vector pCR3.1, and COS-1 cells were transfected with both expression constructs. Cytosol preparations from the transfected cells were then used to study the substrate specificities of each expressed protein. Specifically, SULT enzyme activities were tested with DHEA, 4-nitrophenol, dopamine, and 17ß-estradiol—prototypic substrates for human hydroxysteroid SULTs, phenol SULTs, and estrogen SULTs (Weinshilboum and Otterness, 1994). Because these enzymes display profound substrate inhibition (Weinshilboum and Otterness, 1994), all experiments were performed in two stages. During the first stage, six substrate concentrations that varied over four orders of magnitude, from 10^{-7} to 5×10^{-3} M, were studied. If activity was detected, a narrower range of concentrations was studied to determine apparent K_m values. The proteins encoded by the SULT2B1a and 2B1b cDNAs were capable of catalyzing the sulfation of the hydroxysteroid DHEA, with apparent K_m values of 8.8 and 11.6 μM, respectively. Double inverse plots of the data used to calculate these K_m values are shown in Fig. 4A. Apparent K_m values of the proteins encoded by SULT2B1a and 2B1b for PAPS, the "sulfate donor" cosubstrate for the reaction, were 0.033 and 0.056 μM, respectively. Double inverse plots of the data used to calculate these values are shown in Fig. 4B. However, neither protein catalyzed the sulfation of 4-nitrophenol, dopamine, or 17ß-estradiol, even though six different concentrations of each compound that extended over four orders of magnitude were tested. Thus, biochemical studies of the proteins encoded by these cDNAs after transient expression as well as amino acid sequence analysis were compatible with the conclusion that SULT2B1a and 2B1b encoded members of the hydroxysteroid SULT family, SULT2.

SULT2B1 gene cloning. The fact that the two SULT2B1 cDNAs differed at only their 5′-termini

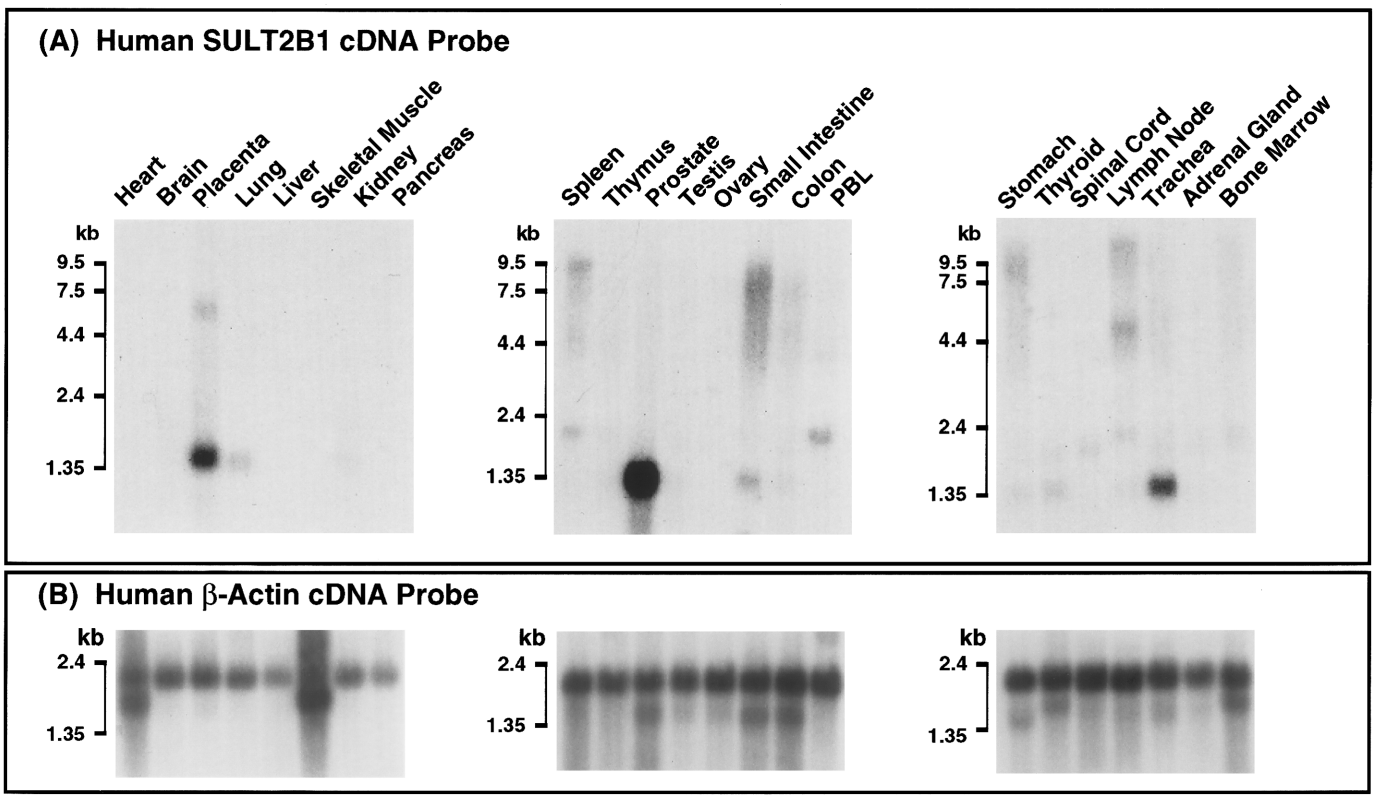

FIG. 3. Northern blot analysis of human tissues. Each lane contained approximately 2 μg of poly(A)$^+$ RNA (Clontech). (**A**) SULT2B1 Northern blot analysis performed with SULT2B1a cDNA as a probe. (**B**) The same blots were probed with human ß-actin cDNA.

made it most likely that they were encoded by a single gene. To determine whether that was the case, we cloned the gene for *SULT2B1*. The first step involved the identification of partial intron sequence by use of the anchored PCR technique RAGE (Mizobuchi and Frohman, 1993). That partial intron sequence was then used to design primers to perform the PCR with human/rodent hybrid cell DNA mapping panels that localized the gene to chromosome 19. We then screened, without success, a human chromosome 19-specific cosmid contig library (deJong *et al.*, 1989). However, we were successful in isolating a BAC clone, BAC45957, from a total genomic DNA library (Shizuya *et al.*, 1992) with SULT2B1a cDNA as a probe.

The next step involved digestion of BAC45957 DNA with 13 different restriction enzymes and use of this DNA to create a series of RAGE panels after ligation to pBSK DNA as an anchor. The PCR was then performed with either BAC45957 DNA or BAC45957 RAGE panel DNA as template to obtain intron, 5′-flanking region, or 3′-flanking region sequences for the gene. Analysis of these data showed that *SULT2B1* encoded both SULT2B1a and 2B1b cDNAs (Fig. 5). The SULT2B1a cDNA was encoded by exons IA to VI. Exon IA con-

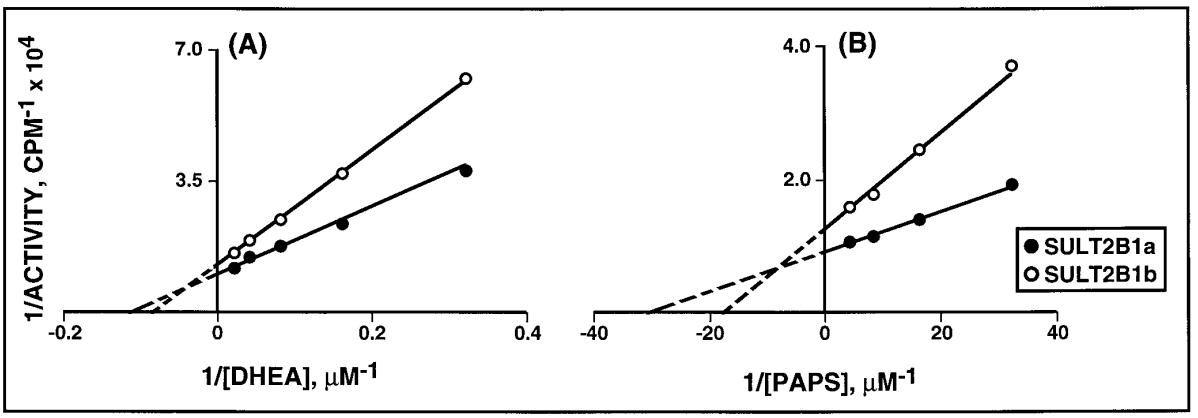

FIG. 4. Recombinant human SULT2B1 substrate kinetics in COS-1 cells. Double inverse plots of the relationship between enzyme activity and substrate concentration for recombinant SULT2B1a and SULT2B1b with (**A**) DHEA as the varied substrate or with (**B**) PAPS as the varied substrate with DHEA as the sulfate acceptor substrate. Each point represents the average of three determinations.

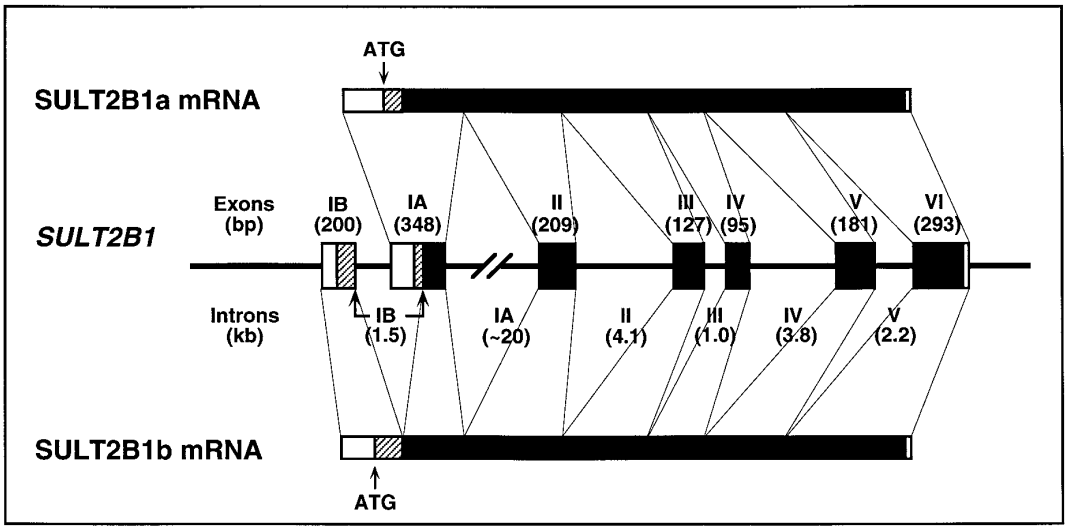

FIG. 5. Human *SULT2B1* gene structure as well as structures of the two mRNAs encoded by this gene. Black and cross-hatched rectangles represent portions of exons that encode mRNA ORF sequences. Open rectangles represent 5′- and 3′-UTR sequences. Exon lengths in bp and intron lengths in kb are also indicated. The GenBank accession numbers for the *SULT2B1* gene are U92316 through U92322, respectively.

tained 179 nucleotides of 5′-UTR and the first 169 bp of the coding sequence of SULT2B1a (Fig. 5). The SULT2B1b cDNA was encoded by *SULT2B1* exon IB, the final 143 nucleotides of exon IA plus exons II to VI (Fig. 5). Exon IB contained the entire 5′-UTR and the first 71 bp of the SULT2B1b ORF. No TATA box sequence was located near either site of transcription initiation. A canonical TATA box has been identified in only one human SULT gene, *SULT1E1* (Her *et al.*, 1995). However, an "initiator" sequence, PyPyCAPyPyPyPyPy (Smale and Baltimore, 1989), was located at the 5′-terminus of the longest SULT2B1a 5′-RACE product. Approximate lengths of *SULT2B1* introns were determined by performing Southern analysis of BAC45957 and were confirmed by performing the PCR with primers designed on the basis of the sequences of adjacent exons (Fig. 5). This PCR-based approach was successful for all introns except IA, which, on the basis of the Southern analysis, was at least 20 kb in length. All *SULT2B1* exon–intron splice junction sequences, including that located "within" exon IA, conformed to the GT–AG rule (Mount, 1982). Finally, after the exon–intron structure of the gene had been defined, intron-specific and gene-flanking sequence-specific primers were used to amplify each of the seven *SULT2B1* exons shown in Fig. 5 with BAC45957 DNA as template. Sequences of the exons amplified from the BAC DNA could then be compared with those of the SULT2B1 cDNAs that had been amplified with human placental and prostate cDNA as template. The *SULT2B1* gene sequence has been submitted to GenBank under Accession Nos. U92316, U92317, U92318, U92319, U92320, U92321, and U92322.

The human *SULT2B1* gene showed a high degree of structural homology with other human SULT genes (Fig. 6), particularly with the human *SULT2A1* gene (previously *STD*, see Table 1)—the only other hydroxysteroid SULT currently known to be expressed in human tissue. The locations of most exon–intron splice junctions as well as the lengths of internal coding exons for *SULT2B1* were identical to those for *SULT2A1* (Otterness *et al.*, 1995b). *SULT2B1*, like *SULT2A1*, contained a 209-bp exon II, a structural feature found, thus far, only in genes for members of the hydroxysteroid SULT family (Fig. 6) (Weinshilboum *et al.*, 1997). Finally, there was striking identity among amino acids encoded by *SULT2B1* and *SULT2A1* codons that were interrupted by splice junctions (data not shown). Since the gene for SULT2A1 had been localized to human chromosome band 19q13.3 (Otterness *et al.*, 1995a), and since we had also mapped *SULT2B1* to chromosome 19, the next step in our analysis involved an attempt to determine the sublocalization of *SULT2B1* on chromosome 19.

SULT2B1 chromosomal localization. *SULT2B1* was initially mapped to human chromosome 19 by performing the PCR with DNA from NIGMS Human/Rodent Somatic Cell Hybrid DNA Mapping Panels 1 and 2. When the PCR was performed with primers IN1AF and R273, an amplification product of the anticipated length, 171 bp, was obtained with Panel 1 DNA from hybrid cell lines GM/NA09925, GM/NA09926, GM/NA09927, GM/NA09928, GM/NA09933, and GM/NA09936, as well as with DNA from the parental human cell line NAIMR91. Those results were compatible with a chromosome 19 localization, with concordance and discordance percentages for that chromosome of 83 and 17%, respectively. PCR data obtained with Mapping Panel 2 DNA confirmed that *SULT2B1* was located on human chromosome 19.

The mapping of *SULT2B1* was completed by the use of a series of complementary techniques. As a first step, the same pair of primers used in the experiments per-

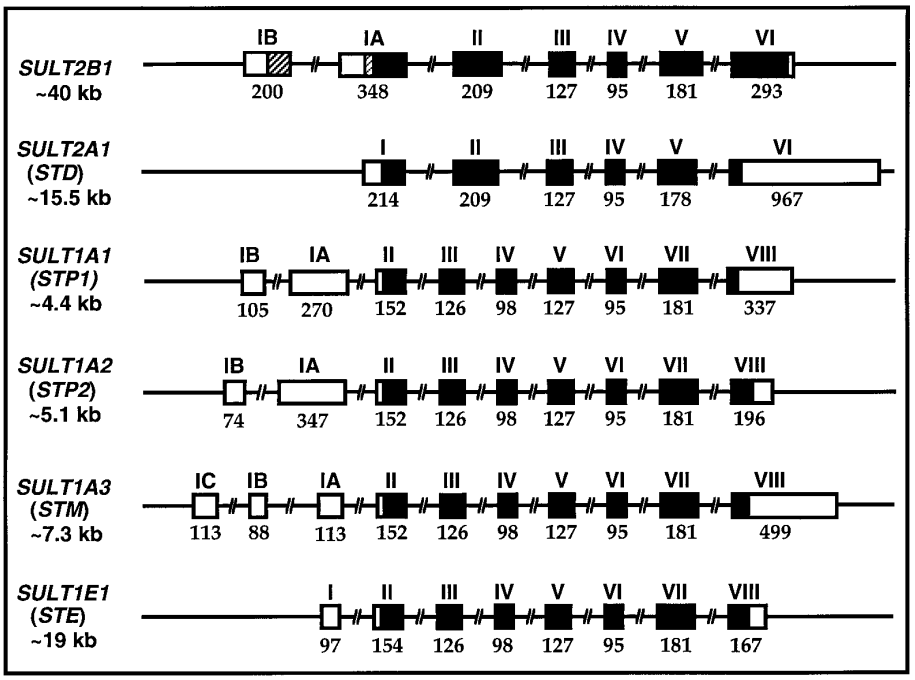

FIG. 6. Human SULT gene structures. Black and cross-hatched rectangles represent portions of exons that encode mRNA ORF sequences. Open rectangles represent 5′- and 3′-UTR sequences. These gene structures were reported by Otterness *et al.* (1995b) for *SULT2A1,* Raftogianis *et al.* (1996) for *SULT1A1,* Her *et al.* (1996) for *SULT1A2,* Aksoy and Weinshilboum (1995) for *SULT1A3,* and Her *et al.* (1995) for *SULT1E1.*

formed with the NIGMS mapping panels was used to perform the PCR with DNA from a human chromosome 19/hamster somatic cell hybrid regional mapping panel (Bachinski *et al.,* 1993; Tasken *et al.,* 1996). That analysis made it possible to assign the gene to a region on the terminal portion of the long arm of chromosome 19 between band 19q13.3 and 19qter (Fig. 7, right). In parallel studies, fluorescence *in situ* hybridization (FISH) was performed with human metaphase chromosomes and with BAC45957 DNA as a probe. The FISH analysis mapped *SULT2B1* to 19q13.3 (Fig. 7, left), the same region of the chromosome that con-

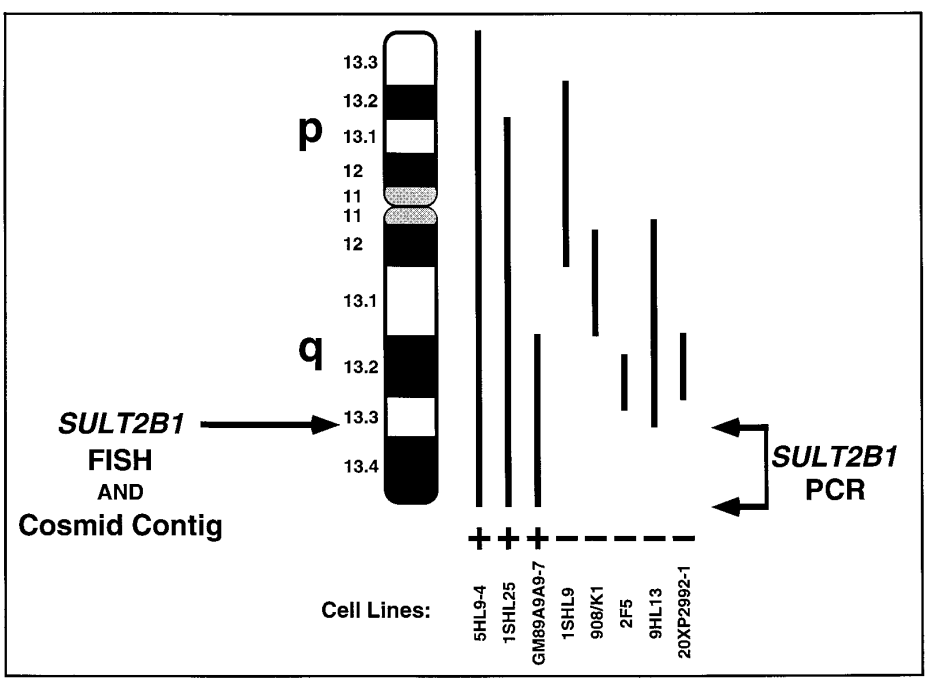

FIG. 7. *SULT2B1* localization on human chromosome 19. The **right** depicts schematically results of the PCR-based hybrid cell DNA panel mapping of *SULT2B1* to 19q13.3–qter and the **left,** the fluorescence *in situ* hybridization mapping of *SULT2B1* to band 19q13.3. The arrayed human chromosome 19 cosmid contig-based sublocalization of *SULT2B1* is also indicated on the **left**.

tained the gene for *SULT2A1* (*STD*), the other known human hydroxysteroid SULT (Otterness *et al.*, 1995a). Specifically, FISH analysis showed that both chromatids of all 20 chromosomes analyzed hybridized to the biotinylated probe at band 19q13.3. Finally, use of the same high-density arrayed human chromosome 19 cosmid contig library that we had originally screened without success made it possible to determine the relative locations of *SULT2B1* and *SULT2A1* on chromosome 19. When this library was screened with probes generated from BAC45957 DNA by the use of long-range *Alu* PCR (Parrish *et al.*, 1995), multiple clones in contigs represented by cosmids 18567 and 29764 were identified. Since the locations of those cosmid clones were known (Gordon *et al.*, 1995), it was possible to map *SULT2B1* to a position approximately 500 kb telomeric to that of *SULT2A1*. These observations made it possible to identify and close a "hole" in the cosmid contig library. In summary, three different complementary approaches all indicated that *SULT2B1* mapped to the long arm of chromosome 19 within band 19q13.3.

DISCUSSION

We have cloned and characterized cDNAs for two novel human hydroxysteroid SULTs, SULT2B1a and SULT2B1b. These two cDNAs differed only at their 5′-termini. That observation also led us to clone the *SULT2B1* gene, a gene that mapped to human chromosome band 19q13.3 in a location telomeric to that of the gene for SULT2A1, the only other known human hydroxysteroid SULT (Otterness *et al.*, 1995a). Sequence and structural characterization of *SULT2B1* demonstrated that it encoded both of the SULT2B1 cDNAs. The two different 5′-terminal sequences for these cDNAs were encoded by different 5′ exons, presumably as a result of a combination of the initiation of transcription at alternative locations plus alternative splicing (Fig. 5). Alternative sites of transcription initiation have also been described for the three human phenol SULT genes that are located in a cluster on the short arm of chromosome 16 (Aksoy and Weinshilboum, 1995; Her *et al.*, 1996; Raftogianis *et al.*, 1996; Weinshilboum *et al.*, 1997). Amino acid sequence alignment performed with all currently known cytosolic SULTs indicated that these two new cDNAs appeared to be members of the hydroxysteroid SULT family. That conclusion was supported both on the basis of the gene structure (Fig. 7) and by functional characterization of the proteins encoded by the cDNAs (Fig. 4). Northern blot analysis demonstrated that these cDNAs were most highly expressed in the placenta and prostate (Fig. 3), organs in which steroid hormones play important biological roles.

SULT2A1 (DHEA ST) was the only hydroxysteroid SULT previously known to be expressed in human tissues (Otterness *et al.*, 1992; Comer *et al.*, 1993; Otterness and Weinshilboum, 1994). Although the proteins encoded by the two SULT2B1 cDNAs are 48% identical in amino acid sequence with that of human SULT2A1, and even though both recombinant SULT2B1s have apparent K_m values for DHEA comparable to that of SULT2A1 (Wood *et al.*, 1996), there are significant differences between these enzymes. SULT2A1 is highly expressed in the human adrenal cortex, liver, and small intestine, but not in the placenta and prostate, where SULT2B1 is expressed (Comer and Falany, 1992; Comer *et al.*, 1993; Otterness and Weinshilboum, 1994; Otterness *et al.*, 1995b). The expression of an enzyme capable of catalyzing the sulfate conjugation of hydroxysteroids in steroid-hormone-dependent organs such as the placenta and prostate raises the possibility of a functional role in the fetoplacental unit during pregnancy or in the pathophysiology of diseases such as carcinoma of the prostate. That possibility can now be studied systematically. The two SULT2B1 proteins also contain amino acid sequences at both their N- and C-termini that are not found in any other known hydroxysteroid SULT, with very high proline content at the C-terminus (Fig. 1). Most other proline-rich proteins that have been described in mammalian tissues either have a structural role or are thought to perform a "binding" function (Williamson, 1994). Obviously, the possible functional implications of the additional amino acid sequence at the N- and C-terminii of SULT2B1 will have to be explored in future studies.

In summary, we have cloned and characterized two new human hydroxysteroid SULT cDNAs. Northern blot analysis indicated that the single gene encoding these cDNAs is expressed primarily in placenta, prostate, and trachea of the adult tissues studied (Fig. 3). We have also determined the structure of the gene that encodes these two enzymes and have mapped that gene to the long arm of chromosome 19, near the location of the gene for the only other hydroxysteroid SULT known to be expressed in human tissues. It will now be possible to study the function of SULT2B1a and SULT2B1b to determine their possible role in the regulation of steroid hormone function in the human placenta and prostate.

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