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Human SULT1A Genes: Cloning and Activity Assays of the SULT1A Promoters

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Abstract

The three human SULT1A sulfotransferase enzymes are closely related in amino acid sequence (>90%), yet differ in their substrate preference and tissue distribution. SULT1A1 has a broad tissue distribution and metabolizes a range of xenobiotics as well as endogenous substrates such as estrogens and iodothyronines. While the localization of SULT1A2 is poorly understood, it has been shown to metabolize a number of aromatic amines. SULT1A3 is the major catecholamine sulfonating form, which is consistent with it being expressed principally in the gastrointestinal tract. SULT1A proteins are encoded by three separate genes, located in close proximity to each other on chromosome 16. The presence of differential 50 ‐ untranslated regions identified upon cloning of the SULT1A cDNAs suggested the utilization of differential transcriptional start sites and/or differential splicing. This chapter describes the methods utilized by our laboratory to clone and assay the activity of the promoters flanking these different untranslated regions found on SULT1A genes. These techniques will assist investigators in further elucidating the differential mechanisms that control regulation of the human SULT1A genes. They will also help reveal how different cellular environments and polymorphisms affect the activity of SULT1A gene promoters.

Introduction

The human SULT1A subfamily of cytosolic sulfotransferases is unique as it contains more than one isoform (SULT1A1, SULT1A2, and SULT1A3), compared to a solitary SULT1A1 member identified in all other species to date. The human SULT1A enzymes were first classified as the aryl/phenol sulfotransferases according to their substrate preference for small phenolic compounds (Hart et al., 1979). Originally, two isoforms were differentiated based on their substrate preference and thermostability (Veronese et al., 1994). The more thermostable (TS ‐ PST) and phenolpreferring (P ‐ ST) form is now referred to as SULT1A1 and the thermolabile (TL ‐ PST) and monoamine ‐ preferring (M ‐ ST) isoform as SULT1A3 (Blanchar d et al., 2004 ; Wilborn et al., 1993; Zhu et al., 1993a,b). Later, a second thermostable isoforms was cloned and named SULT1A2 (Ozawa et al., 1995; Zhu et al., 1996). The three SULT1A enzymes exhibit relative molecular masses on SDS–PAGE between 32 and 34 kDa and each form is composed of 295 amino acids, which share >90% sequence identity (Fig. 1). Although the three SULT1A enzymes share high amino acid sequence identity, their tissue ‐ specific exp ression and substr ate pr ofiles vary. Not surpri singly, the greatest variab ility in amino acid sequence is found in the areas of substrate binding. Site ‐ directed mutag enesis studies and eluci dation of the human SULT1A1 and SULT 1A3 protei n crystal struc tures have given insight into the mecha nisms determ ining substrate specifi city diff erences betw een these two enzym es (Bar nett et al., 2004; Bidwe ll et al ., 1999; Brix et al., 1999; Dajan i et al ., 1999 ; Gamage et al., 2003). The neg atively charged glutamic acid resid ue at position 146 has be en sh own to play an impo rtant role in the bindi ng of positive ly charged biogen ic ami nes such as dopa mine by SULT 1A3 (Bar nett et al., 2004 ; Brix et al ., 1999). SUL T1A1, which lacks a charge at this pos ition, has less affini ty for these substrates (Brix et al ., 1999). How ever, compar ed to SUL T1A3, the hy drophob ic substrate ‐ bindin g pocket of SULT 1A1 has great er affinity for small phenolic compounds such as the model sub strate p ‐ nitrop henol and the drugs acetamin ophen and minoxidi l (Brix et al., 1999; Fa lany, 1997; Gam age et al., 200 3). SUL T1A1 also ha s a ctivity in sulfonatin g estrogen s an d thyroid hormone, and crystallogr aphic studi es have shown that the hydrophobic ‐

binding poc ket is large eno ugh to accommodat e two sub strate molecul es, explaini ng the su bstrate inhi bition kinet ic pr ofile observed at high substr ate con centrati ons (Gam age et al., 2003).

Studie s investigati ng the mecha nisms unde rlying the different ial tissue expression profi les of the SUL T1A enzym es ha ve only recent ly be en undertake n. One of the most strikin g differenc es in express ion occurs in the adult liver, wher e SULT 1A1 is express ed in high amoun ts, yet SULT1A3 at negligi ble levels (Richard et al ., 2001; Windm ill et al ., 1998). Our laboratory has report ed on a different ial mechan ism of transcrip tional regulatio n between SULT1A 1 and SUL T1A3 genes, which is a first step in eluci dating the tissue - specific pa tterns of exp ression of these tw o clos ely related enzym es (Hemp el et al ., 2004). This chap ter de scribes the met hods employed by our labora tory to inves tigate the pro perties of the human SULT1A g enes, with specifi c emphas is on the app roach use d to clone and study the properties of the SULT1A promoters in mammalian and Drosophila S2 cell lines.

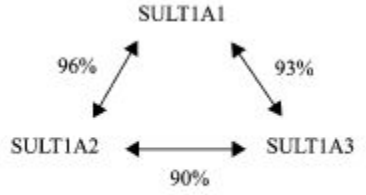

FIG. 1. Human SULT1A isoform amino acid homology.

Properties of Human SULT1A Genes

Members of the human SULT1A subfamily are encoded by three separate genes, which are found in close proximity to each other on chromosome 16 (16p11.2–12.1). Their relative position on chromosome 16 of the Homo sapiens genome can be attained using the NCBI Entrez Map View browser (htt p://www.n cbi.nlm .nih.gov/m apview /) with the searc h terms ''SULT1A1,'' ''SULT1A2,'' or ''SULT1A3.'' A schematic representation with links to sequence information can be gained by clicking the ''Map Element'' Link. The gene symbol leads to a summary data page with information on the gene, including links to references and NCBI curated nucleotide and protein sequence records. For reference, the curated nucleotide records for SULT1A1, SULT1A2, and SULT1A3 are NM_001055, NM_001054, and NM_003166, respectively. Within the Map Viewer page the area spanning between the three SULT1A genes can be displayed by entering the two genes of interest (e.g., SULT1A1 and SULT1A3) in the search field ''Region shown'' on the left - hand side of the page. With current data from the annotated genome sequence, SULT1A2 is positioned the most telomeric of the three SULT1A genes. The SULT1A2 and SULT1A1 genes are positioned head to tail, approximately 9.75 kb apart from the stop codon of SULT1A1 to the ATG codon of SULT1A2. These two genes share more than 93% sequence identity and no other gene has been positioned between the two (Aksoy andWeinshilboum, 1995; Bernier et al., 1996; Her et al., 1996). Their close proximity and high homology suggest that these genes have arisen recently in evolution due to a gene duplication event. SULT1A3 is located most centromeric and at a distance of approximately 1.58 Mb from the position of SULT1A1 and SULT1A2, with several other genes occupying the sequence between them. SULT1A3 is currently positioned in the opposite direction to the other SULT1A genes. The SULT1A3 gene differs mainly in its 5o promoter sequence and shares about 70% sequence identity with the other two SULT1A genes (Aksoy and Weinshilboum, 1995; Bernier et al., 1994a; Dooley et al., 1994).

Structurally the three SULT1A genes are similar. All contain seven coding exons and have been shown to have alternatively transcribed 5o - untranslated regions (5oUTRs). Figure 2 shows the 5oUTRs thus far identified in the SULT1A cDNA species isolated to date. Why these alternate 5oUTRs are transcribed is currently unknown. Due to their isolation from different tissue libraries, it was suggested that transcription of these may represent a tissue - specific mechanism (Zhu et al., 1993a,b). The endogenous consequences for this phenomenon are unclear, as all SULT1A cDNAs contain the same open reading frame. It has not been investigated whether this compromises mRNA translation or stability as a way to control SULT1A expression posttranscriptionally. Alternate splicing and/or the utilization of alternate promoters in front of these sequences has been proposed as the mechanism for the occurrence of these 5oUTRs. Our laboratory and others have shown that sequences on the SULT1A genes flanking these alternate 5oUTRs have different promoter activities.

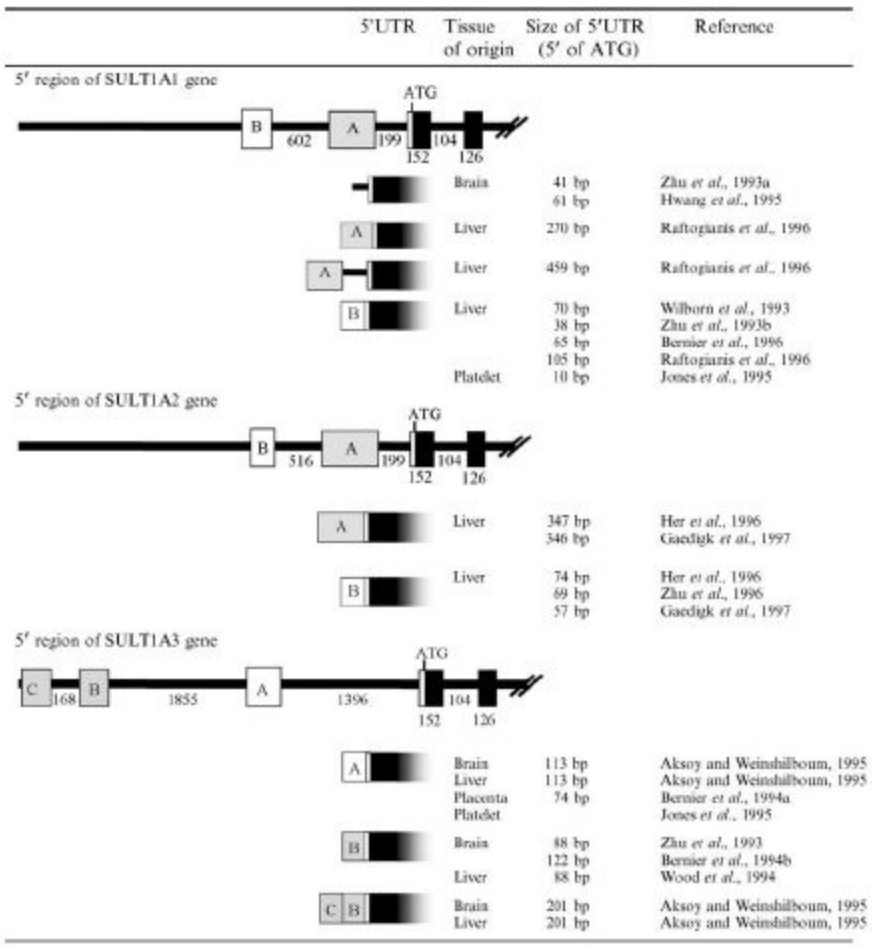

FIG. 2. 5′-Untranslated regions of SULT1A cDNAs. Black boxes represent coding exons; white and gray boxes represent alternate 5′UTRs. Sizes of introns are given below on the 5′ region of the genes (Aksoy and Weinshilboum, 1995; Her *et al.*, 1996; Raftogianis *et al.*, 1996).

Cloning of Human SULT1A Promoters

The SULT1A promoters can be amplified easily from human genomic DNA. Primers were originally designed by our laboratory based on the SULT1A3 gene sequence submitted to GenBank prior to the release of human genome data (Accession No.: U20499; Aksoy and Weinshilboum, 1995). The sense primer 5′-ACGCGTGCTAGCGAGCTGTGAGGAAGTTCAGGTC-3′, containing MluI and NheI restriction sites (underlined), and the antisense primer 5′-AGATCTCTCGAGGATCAGCTCCATGTTCCTGCATC-3′, containing BglII and XhoI restriction sites (underlined), are located 4023 bp upstream and 12 bp downstream of the ATG start codon of SULT1A3, respectively.

The TaqPlus Long polymerase system by Stratagene, containing a mixture of Taq2000 and the proofreading polymerase Pfu, allows for efficient and more accurate amplification of long polymerase chain reaction (PCR) products. To a 300-ng genomic DNA template the following components are added in a final volume of 50 μl: 1 x low-salt TaqPlus Long polymerase buffer, 800 μM dNTP mix, 1 μM of each sense and antisense primer, and 2 U of TaqPlus Long polymerase. The PCR cycling parameters are as follows: an initial 2-min denaturing step at 94° is followed by 30 cycles of denaturing at 92° for 30 s, annealing of primers at 66° for 1 min, and extension at 72° for 5 min. This is followed by an additional 5 cycles, with an increased extension time of 15 min. The PCR product is electrophoresed on a 1% agarose gel containing ethidium bromide, excised, and purified. Approximately 150 ng of the PCR product is used for cloning into the TOPO TA pCR2.1 vector (Invitrogen) according to the manufacturer's instructions, followed by transformation into TOP10 chemically competent cells (Invitrogen).

Sequences are subcloned into the pGL3Basic luciferase reporter gene vector using restriction sites MluI and BglII at the 3′ and 5′ ends of the insert, respectively. The pGL3Basic reporter gene vector encodes the firefly (Photinus pyralis) luciferase protein when a functional promoter is cloned upstream of its transcriptional start site. This vector has no enhancer or intrinsic promoters, and therefore will only transcribe the luciferase gene based on the activity of the cloned promoter. The high sequence similarity of the three SULT1A genes in areas near the ATG start codon and 4 kb upstream means that the sequences of all three SULT1A genes are amplified in the aforementioned PCR. The restriction enzymes NsiI, ClaI, and KpnI, which specifically digest the SULT1A1, SULT1A2, and SULT1A3 sequences, respectively, can be used to differentiate between the amplified sequences. Figure 3 shows the restriction digest of three plasmid preparations containing the SULT1A1, SULT1A2, or SULT1A3 5′ regions cloned into the 4818‐bp pGL3Basic vector.

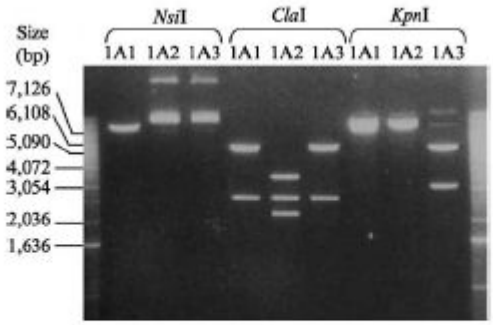

FIG. 3. Differential restriction digestion of 4-kb upstream *SULT1A* gene sequences cloned into pGL3Basic. Four kilobases of the *SULT1A* sequences flanking the ATG start codon was isolated from human genomic DNA by PCR and cloned into the pGL3Basic vector as described. The constructs (1 μg) were digested with 2 U of *Nsi*I, *Cla*I, or *Kpn*I restriction enzymes for 4 h at 37°. Digested DNA was electrophoresed on a 1% agarose gel containing ethidium bromide.

To amplify the 6‐kb sequence flanking the 5′UTR C of SULT1A3 the sense primer 5′‐GTGAGAGACCTGGCAGGAACAGG‐3′ and the antisense primer 5′‐AGATCACATGGGCCCTTAGC‐3′, which are 9689 and 3691 bp upstream of the SULT1A3 ATG start codon, respectively, are used. The following components are added to 300 ng of human genomic DNA in a final volume of 50 μl: 1 x Expand Long PCR buffer 1 (Roche), 1.75 mM $MgCl_2$, 4 mM dNTP mix, 300 ng of each primer, and 0.75 μl of Expand Long PCR enzyme mix (Roche). The cycling parameters are as follows: initial denaturing at 94° for 5 min, followed by 10 cycles of 30 sec denaturing at 94°, 30 s annealing at 62°, and 7 min extension at 68°. This is followed by 25 cycles of the same temperature parameters, where the extension time is increased in length by 20 s at each cycle. A final extension at 68° for 10 min concludes the PCR. The product is electrophoresed, gel extracted, and cloned into the TOPO TA pCR2.1 vector. For subcloning into the pGL3Basic vector the sequence is excised from pCR2.1 using XhoI and HindIII restriction sites of the vector. A 2564‐bp deletion construct of the SULT1A3 promoter flanking 5′UTR C can be created using the restriction enzyme NheI.

The cloned SULT1A 5′ gene sequences are used as templates for amplification of the different promoters flanking the alternate 5′UTRs and promoter deletion constructs using the primers listed in Table I. To allow for subcloning of sequences into pGL3Basic, sense primers are designed to incorporate 5′ MluI or NheI restriction enzyme recognition sites and antisense primers 5′ BglII or XhoI sites. The PCR is carried out with 2.5 U Taq DNA polymerase, 200 ng of each primer, 50 ng of template DNA, 1.5 mM $MgCl_2$, and polymerase buffer in a final volume of 50 μl. Cycling is carried out under the following conditions: an initial denaturing step at 94° for 2 min, followed by 25 cycles at 94° for 30 s, annealing at 64° for 30 s, and extension at 72° for 1 min per kilobase PCR product length. The cycling is concluded with a final extension at 72° for 5 min. The PCR products are electrophoresed on 1–2% agarose gels and purified. After initial TA cloning into pCR2.1 the sequences are subcloned into the pGL3Basic vector.

TABLE I
OLIGONUCLEOTIDE PRIMERS FOR *SULT1A* PROMOTER PCR

Name	Location	S/AS	Sequence[a]
SULT1A promoter deletions			
Promoter flanking first coding exon 1			
1A1/2/3 RPExI	−1 bp of ATG	AS	5′-AGATCTCTCGAGTGTTCCTGCGTCAGGGGCCAGAGC-3′
Promoter flanking 5′UTR A 1A1/1A2			
1A1/2 FP1A	5′UTR B	S	5′-ACGCGTTCACCCTGCTCAGCTTGTGGCTC-3′
1A1/2/3 RP1	5′UTR A 1A1/2	AS	5′-AGATCTCTCGAGTGTCTCACCATTTCCTGCTGG-3′
Promoter flanking 5′UTR B 1A1/1A2 and 5′UTR A 1A3			
	Location from transcriptional start site		
1A1/2/3 RP2	5′UTR B 1A1/2 5′UTR A 1A3	AS	5′-AGATCTCTCGAGACCTGAGCTCTTGGGAACCTG-3′
1A1/2 FP3	−2325 1A1 −2258 1A2	S	5′-ACGCGTGCTAGCGGTAGCTGTGAGGCGTCACTGCTTTGG-3′
1A2 FP4	−1663	S	5′-ACGCGTGCTAGCGGCTCTTGGCACCTTAGCCAGA-3′
1A3 FP4	−1554	S	5′-ACGCGTGCTAGCGCCTCTGAGCTCATGCAATTCTTGG-3′
1A1/2 FP5	−1217 1A1 −1156 1A2	S	5′-ACGCGTGCTAGCCACATTCTCGCCTCTTTTCTGTGTCA-3′
1A3 FP5	−1074	S	ACGCGTGCTAGCCCGCCATCATGCCCAGCTAA
1A1/2 FP6	−542 1A1 −488 1A2	S	5′-ACGCGTGCTAGCGGCCTTGTGGTCAGAGCCTGGA-3′
1A3 FP6	−560	S	ACGCGTGCTAGCCATGGCAAAACCCCGTCTCTACTAAA
1A1/2 FP7	−232 1A1 −221 1A2	S	5′-ACGCGTGCTAGCCCTTTCCCCTTTCATTCTTCTGTTTTC-3′
1A3 FP7	−265	S	5′-ACGCGTGCTAGCCCAAATACCAATGTTGGCCCCTTTT-3′
1A1/2/3 FP8	−144 1A1 −132 1A2 −157 1A3	S	5′-ACGCGTTAAGGAGGGTAATGGAGAAGCT-3′
1A1/3 FP9	−112 1A1 −125 1A3	S	5′-ACGCGTCAACCCCACCCCTTCCTTCC-3′
1A2 FP9	−100	S	5′-ACGCGTCAACCCTACCCCTTCCTTCC-3′
1A1 FP9b	−89	S	5′-GGACGCGTAGCAAATCTAAGTCCAGCCCCG-3′
1A1/2 FP10	−68 1A1 −56 1A2	S	5′-ACGCGTGGCTCCAGATCCCTCCCACA-3′
1A3 FP10	−89	S	5′-ACGCGTGACTTTAGATCCCTCCCACACTG-3′
1A1 FP11	−1	S	5′-ACGCGTCACAGCACCCACAATCAGCCACT-3′
1A2 FP11	+10	S	5′-ACGCGTCACAACACCCACACTCAGCCACT-3′
1A3 FP11	−22	S	5′-ACGCGTCACCACACCCATACTCAGCCCCT-3′
Promoter flanking 5′UTR B 1A3			
1A3 utrB RP	5′UTR B	AS	5′-CTCGAGAGATCTCAGTGTCCATCTGGCACAGCCA-3′

[a] Underlined bases represent restriction enzyme recognition sites.

Cell Based Transfection of SULT1A Promoter Constructs

A range of cell lines are useful tools in studying the activity of the human SULT1A promoters. In the past we have utilized mammalian cell lines such as the human hepatocarcinoma HepG2 and Hep3B cells as representatives of a liver‐like environment, human Caco2 cells as a representative of the gastrointestinal tract, and the human breast cancer cell line MCF‐7 as a representative of mammary tissue. Further, Schneider's Drosophila melanogaster 2 cells (S2) lack a large variety of mammalian transcription factors (Galvagni et al., 2001) and are a useful system to study the regulation of promoters by ubiquitous or highly expressed mammalian transcription factors using cotransfection. We have used this method to show an induction of the human SULT1A1 promoter by Ets and Sp1 transcription factors (Hempel et al., 2004).

Mammalian cells are grown in MEM containing 10% fetal bovine serum and penicillin (50 U/ml)/streptomycin (50 μg/ml) at 37° in a humidified incubator with 5% CO_2. For transfection of luciferase reporter plasmids, cells are seeded into 24‐well plates at approximately $5x10^4$ cells per well in 500 μl medium. Transfection is carried out at 70–80% confluence using a modified protocol of the calcium phosphate transfection

method of the CellPhect transfection kit (Amersham). Each SULT1A promoter luciferase reporter construct or the empty pGL3 - Basic vector (0.1 μg/well) is transfected together with the transfection control reporter vector pRL - SV40 (0.05 μg/well). For comparison of different promoter constructs that vary significantly in length, the DNA amounts are adjusted to bring samples to equimolar concentrations of plasmid. In cotransfection experiments, mammalian expression vectors containing transcription factor cDNAs (0.1–1 μg/well) can be included in the transfection mix. Empty vectors are used to bring transfection samples to equal DNA concentrations between wells. Each sample is transfected in triplicate. For transfection into 3 wells the DNA is mixed with MilliQ H_2O to a final volume of 37.5 μl, to which an equal volume of buffer A (CellPhect kit) is added. After brief vortexing and incubation at room temperature for 10 min, 75 μl of buffer B is added, followed by immediate vortexing and incubation at room temperature for 15 min. The amount of DNA and the volumes of buffers A and B are scaled up or down according to the number of wells transfected. The DNA mix (50 μl per well) is added to the cells and the medium is replaced 24 h posttransfection. After an additional 24 h in culture the medium is aspirated and the cells are washed once with phosphate - buffered saline, followed by the addition of 1x passive lysis buffer (100 μl/well) of the dual luciferase assay (Promega). Cells are lysed by gentle rocking at room temperature.

S2 cells are grown in the dark in Schneider's Drosophila medium (Invitrogen), supplemented with 10% heat - inactivated fetal bovine serum and penicillin/streptomycin at room temperature. Cells are transferred to 12 - well plates at a cell density of $0.5x10_6$ cells per well in 1 ml media and transfected after 24 h in culture using the calcium phosphate method. Each well is transfected with 2 μg SULT1A promoter reporter constructs and 2–5 _g of the desired transcription factor, cloned into a Drosophila expression vector such as pAC5.1 (Invitrogen). The DNA is mixed with $2MCaCl_2$ and the sample is brought to a final volume of 105 μl with sterile $MilliQH_2O$. An equal volume of 2 x HEPES - buffered saline (50 mM HEPES, 1.5 mM Na_2HPO_4, 280 mMNaCl; pH 7) is added drop wise while mixing the sample gently. The mix is incubated at room temperature for 30 min and 200 μl is added to each well containing the S2 cells while swirling the plates gently. The medium is changed 24 h posttransfection by collecting the cells using centrifugation at 100g for 10 min in 1.7 - ml tubes, resuspending them in 1 ml fresh medium, and returning cells to the wells. After an additional 24 h in culture, cells are collected by centrifugation at 1000g for 10 min in 1.7 - ml tubes. The medium is aspirated, 100 μl of 1x passive lysis buffer is added, and the cells are shaken vigorously for 10 min. After aiding lysis by freeze/ thaw at -80°, the cells are centrifuged at 3000g for 10 min.

SULT1A Promoter Luciferase Activity Assays

The luciferase reporter assay is a quantitative gene reporter system used to assess the ability of sequences to act as promoters by driving transcription of the firefly luciferase enzyme. The transfection standard vector pRLSV - 40 encodes a second Renilla reniformis luciferase enzyme. The activities of both enzymes are assessed by a sequential chemiluminescence assay (dual luciferase assay system, Promega), according to the manufacturer's specifications. Briefly, after lysis of cells with passive lysis buffer, 50 μl of the firefly luciferase enzyme substrate (luciferase assay reagent II) is added to 2–10 μl of total lysed mammalian cells or 20 μl cleared lysate of the S2 Drosophila cells. Chemiluminescence is measured for 10 s after an initial 2 - s premeasurement delay on either a single sample or a 96 - multiwell injection luminometer. The reaction is quenched by the addition of 50 μl Renilla luciferase substrate (Stop & Glo) and Renilla chemiluminescence is measured for 10 s. Firefly luciferase activity is generally normalized against Renilla luciferase activity. Alternatively, in S2 cells, firefly luciferase activity is normalized against the protein concentration of the cleared lysate.

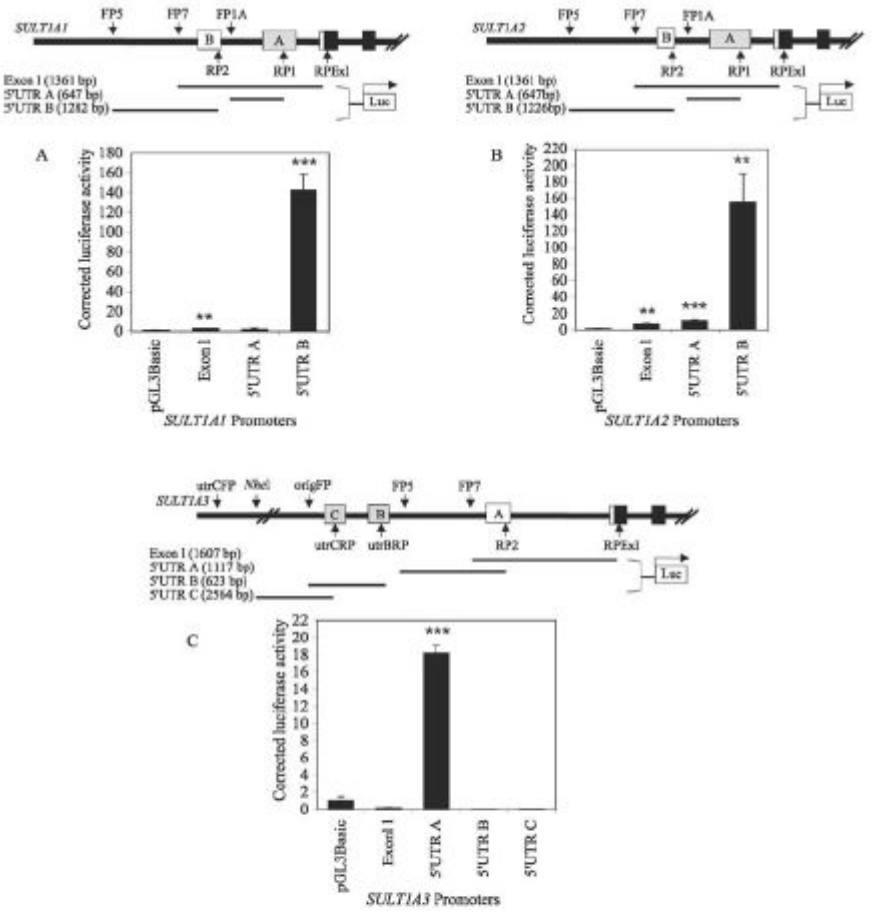

FIG. 4. Promoter activities of sequences flanking the 5′UTRs of *SULT1A* genes. Sequences flanking the different 5′UTRs of the *SULT1A1* (A), *SULT1A2* (B), and *SULT1A3* (C) genes were created by PCR and cloned into the pGL3Basic vector. The location and size of the constructs and the position of primers (arrows) used to amplify these are displayed schematically. Constructs were transfected into HepG2 cells and lysed cells were assayed for luciferase activity as described. Results represent firefly luciferase activity corrected for *Renilla* luciferase activity of the transfection control and are expressed relative to the activity of the empty pGL3Basic vector. Each bar represents the mean result from three transfected wells of cells ± SD. Asterisks indicate significant differences to the activity of the empty pGL3Basic basic vector (**$p < 0.01$; ***$p < 0.001$, Student t test). (Adapted with permission from Hempel *et al.*, 2004.)

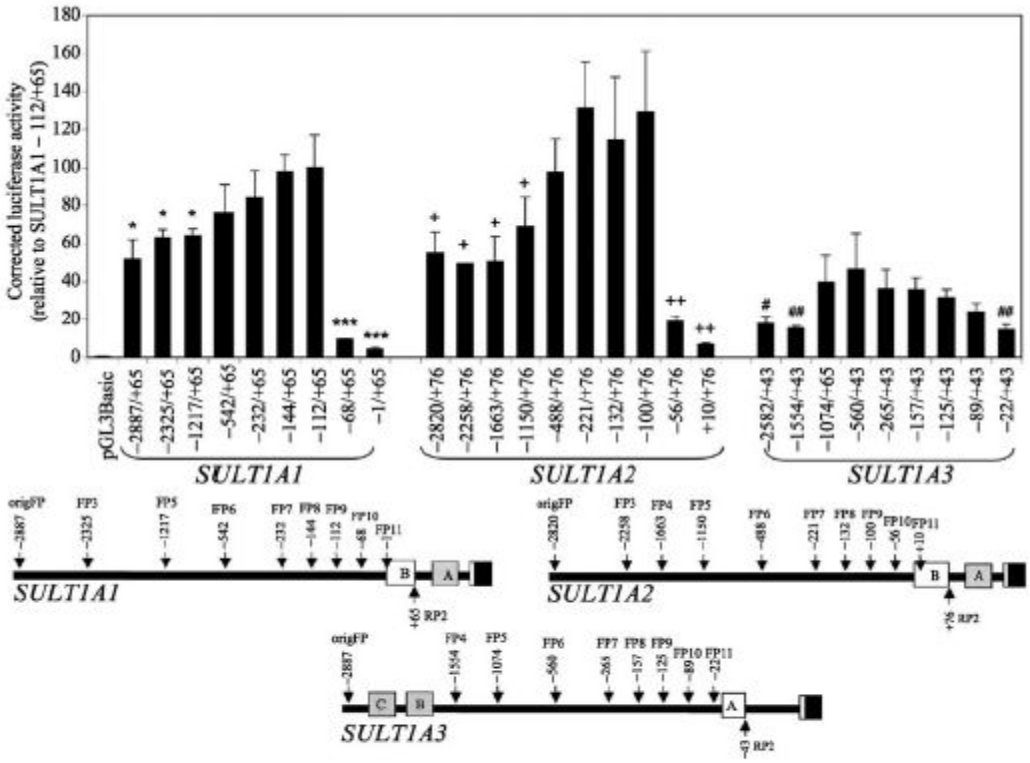

FIG. 5. *SULT1A* promoter deletion construct activities in HepG2 cells. Promoter deletion constructs of the sequences flanking 5′UTR B of *SULT1A1* and *SULT1A2* and 5′UTR A of *SULT1A3* were created by PCR and cloned into the pGL3Basic vector. Positions of the sense primers used to create these are represented schematically by arrows above the gene and are numbered according to their position relative to the transcriptional start site (+1). The antisense primer is marked below the gene by an arrow. Constructs were transfected into HepG2 cells and lysed cells were assayed for luciferase activity as described. Results represent firefly luciferase activity corrected for *Renilla* luciferase activity of the transfection standard and are expressed relative to the activity of the *SULT1A1* −112/+65 construct. Each bar represents the mean result from three transfected wells of cells ± SD. Asterisks indicate significant differences to the activity of the *SULT1A1* −112/+65 construct (*$p<0.05$; ***$p<0.001$, Student *t* test); plus signs indicate significant differences to the activity of the *SULT1A2* −100/+76 construct ($^{+}p < 0.05$; $^{++}p < 0.01$, Student *t* test); and hash marks indicate significant differences to the activity of the *SULT1A3* −125/+43 construct ($^{\#}p < 0.05$; $^{\#\#}p < 0.01$, Student *t* test). (Adapted with permission from Hempel *et al.*, 2004.)

Activity of SULT1A Promoters

Using the aforementioned methods, we cloned and assessed the activity of sequences flanking the different 5′UTRs of the SULT1A genes in HepG2 cells (Hempel et al., 2004). Sequences flanking the first coding exon of SULT1A1 and SULT1A2 increase luciferase expression by 2 - and 6 - fold, respectively, compared to the empty vector (exon I; Fig. 4A and B). This is not observed for the sequence flanking exon I of SULT1A3 (Fig. 4C). The SULT1A1 sequence flanking 5′UTR A, which is a 5′UTR so far only observed in SULT1A1 and SULT1A2 cDNA species, has no statistically significant promoter activity (Fig. 4A). However, the highly homologous SULT1A2 sequence displays a statistically significant 11 - fold higher luciferase activity than the empty pGL3Basic vector (Fig. 4B). Of the regions flanking the different SULT1A 5′UTRs identified in the literature, sequences upstream of SULT1A1 and SULT1A2 5′UTR B have the highest promoter activity (Fig. 4; Hempel et al., 2004). Similarly, the homologous sequence upstream of SULT1A3 5′UTR A also efficiently drives luciferase transcription.

Figure 5 shows the activity of promoter deletion constructs of the SULT1A promoters flanking 5o UTR B of SULT1A1 and SULT1A2 and the homologous SULT1A3 5o UTR A. Upon deletion of 44 bp from construct -112/þ65 and -100/þ76 of the SULT1A1 and SULT1A2 promoters, respectively, a significant decrease in promoter activity is observed, indicating the presence of a crucial regulatory element (Fig. 5; Hempel et al., 2004). Our laboratory has reported on the importance of the Ets and Sp1 transcription factor response elements in this region and their role in regulating the SULT1A1 promoter (Hempel et al., 2004). In HepG2 cells the SULT1A3 promoter constructs display approximately 70% less activity than the SULT1A1 and SULT1A2 promoters. The promoter activities of SULT1A3 constructs -2582/þ43 and -1554/þ43 are approximately half that observed for the -1074/þ43 construct, which may indicate the presence of an inhibitory transcription factor ‐ binding site. This is particularly apparent when Caco2 cells are used as the experimental model (Fig. 6).

The relative activities of the SULT1A promoters in different cellular environments are shown in Fig. 6, where constructs are transfected into another hepatocarcinoma cell line, Hep3B, the colon carcinoma cell line Caco2, and the human breast cancer cell line MCF ‐ 7. Although no direct comparison among cell lines can be made due to differential transfection efficiencies, differences in activities can be assessed by comparison with the highly active SULT1A1 -112/þ65 promoter construct (Fig. 6). The general pattern of the promoter activities in the different cell lines tested is similar to that observed in HepG2 cells. In all cell lines the sequence flanking SULT1A1 and SULT1A2 5o UTR B is the most highly active promoter (Fig. 6). Similarly, the promoter flanking 5o UTR A of SULT1A3 has the highest activity of the SULT1A3 constructs (Fig. 6). As observed for the transfection in HepG2 cells, the deletion of 44 bp from -122 to -68 of the SULT1A1 promoter and -100 to -56 of the SULT1A2 promoter results in a greater than 90% drop in activity, suggesting that the regulatory element present in this region controls the activity of these two promoters in all the cells tested. One interesting observation is the activity of the SULT1A3 promoter in Caco2 cells. Relative to the SULT1A1 and SULT1A2 promoters, SULT1A3 promoter activity in the colon carcinoma cell line is higher than in the other cell lines tested. It cannot be concluded whether this indicates that SULT1A1 and SULT1A2 promoters display a decreased activity level in Caco2 cells or whether there is an increase in SULT1A3 promoter activity in this cell line. However, tissue distribution profiles match this difference in observed promoter activity (Windmill et al., 1998). Similar to its promoter activity, SULT1A3 protein levels are lower in the liver and higher in the gastrointestinal tract (Windmill et al.,
1998).

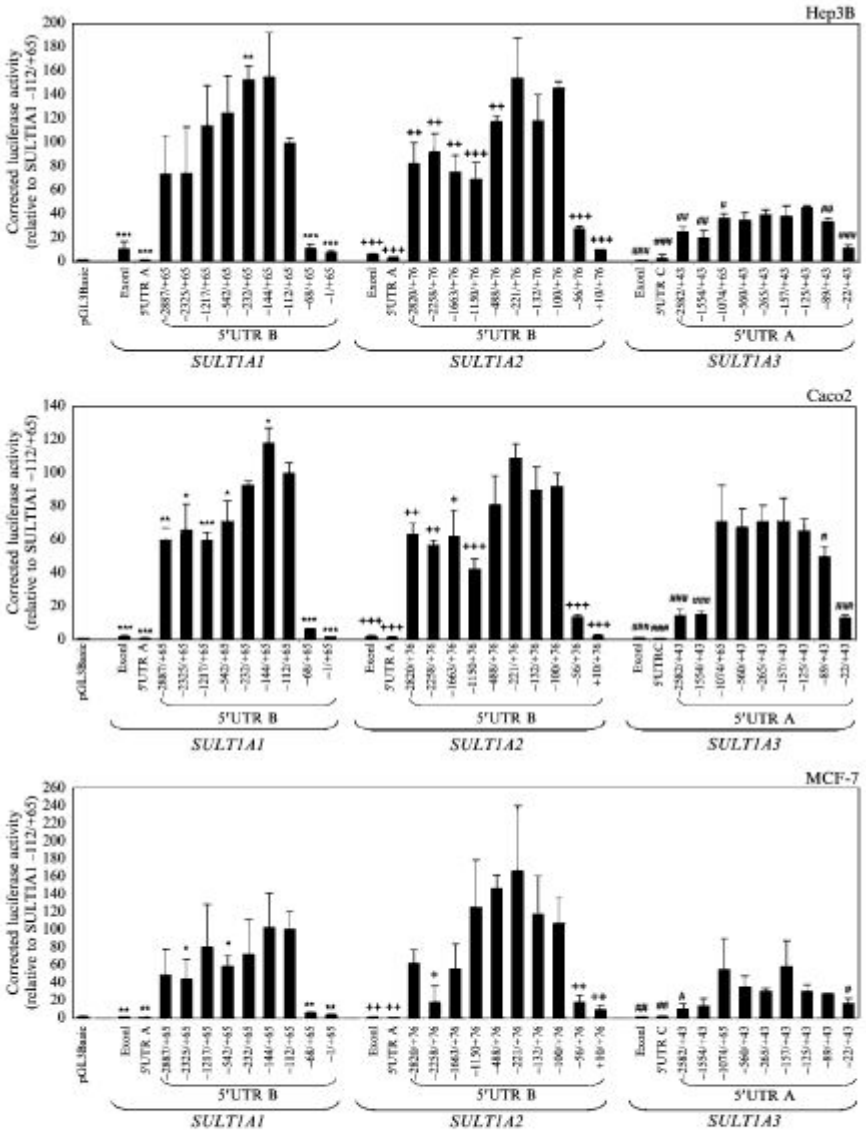

FIG. 6. *SULT1A* promoter constructs activities in different cell lines. *SULT1A* promoter deletion constructs were created by PCR and cloned into the pGL3 Basic vector. Promoter constructs were transfected into Hep3B, Caco2, and MCF-7 cells and lysed cells were assayed for luciferase activity as described. Results represent firefly luciferase activity corrected for *Renilla* luciferase activity of the transfection standard and are expressed relative to the activity of the *SULT1A1* promoter deletion construct −112/+65. Each bar represents the mean result from three transfected wells of cells ± SD. Asterisks indicate significant differences to the

Conclusion

Using the aforementioned met hods, we have studied the mechanisms of the SULT 1A promot ers and have identified a crucial regulatory region in the proximal SULT 1A1 pro moter con taining Ets and Sp1 trans cripti on factor ‐ binding sites (Hemp el et al ., 2004). Further , we were able to sho w that a lack of the full Ets ‐ binding site in the SULT 1A3 promoter compromised its activity in hepatocarcinoma cells due to an inability of Ets and Sp1 transcription factors to act in synergy in a ctivating this promoter (Hem pel et al ., 2004). The described techniques in this chapter will allow further investigations into the promoter propert ies of the human SULT 1A genes, inclu ding the influence of polym rphisms on the activity of the promoters. Interindividual differences in SULT 1A express ion levels have been reported in numerous studies (Aben haim et al., 1981; Iida et al ., 2001) and thus far a

mecha nism controll ing this variati on has not been fully explained. Singl e nucleot ide polymorp hisms (SNPs) ha ve been de scribed for all three SUL T1A ge nes. One study of a Japan ese popul ation report ed 13 and 7 SNPs in the 3 ‐ kb region upstream of the ATG start codon of the SULT1A1 and SULT1A2 genes, respectively (Iida et al., 2001). Additionally, SNPs on the SUL T1A genes can be visua lized using the NC BI SNP site (htt p://www.n cbi.nlm .nih.gov/ SNP/). Whether these nucleotide changes influence promoter activities of the genes by altering transcription factor recognition sequences has not been investigated.

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