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The Human Sulfotransferase *SULT1A1* Gene Is Regulated in a Synergistic Manner by Sp1 and GA Binding Protein

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ABSTRACT

Human sulfotransferase SULT1A1 is an important phase II xenobiotic metabolizing enzyme that is highly expressed in the liver and mediates the sulfonation of drugs, carcinogens, and steroids. Until this study, the transcriptional regulation of the SULT1A subfamily had been largely unexplored. Preliminary experiments in primary human hepatocytes showed that SULT1A mRNA levels were not changed in response to nuclear receptor activators, such as dexamethasone and 3-methylcolanthrene, unlike other metabolizing enzymes. Using HepG2 cells, the high activity of the TATA-less *SULT1A1* promoter was shown to be dependent on the presence of Sp1 and Ets transcription factor binding sites (EBS), located within −112 nucleotides from the transcriptional start site. The homologous promoter of the closely related SULT1A3 catecholamine sulfotransferase, which is expressed at negligible levels in the adult liver, displayed 70% less activity than *SULT1A1*. This was shown to be caused by a two-base pair difference in the EBS. The Ets transcription factor GA binding protein (GABP) was shown to bind the *SULT1A1* EBS and could transactivate the *SULT1A1* promoter in *Drosophila melanogaster* S2 cells. Cotransfection of Sp1 could synergistically enhance GABP-mediated activation by 10-fold. Although Sp1 and GABP alone could induce *SULT1A3* promoter activity, the lack of the EBS on this promoter prevented a synergistic interaction between the two factors. This study reports the first insight into the transcriptional regulation of the *SULT1A1* gene and identifies a crucial difference in regulation of the closely related *SULT1A3* gene, which accounts for the two enzymes' differential expression patterns observed in the adult liver.

The human SULT1A subfamily of cytosolic sulfotransferases (SULT) belongs to a supergene family of enzymes that catalyze the transfer of a sulfonate group from 3′-phosphoadenosine 5′-phosphosulfate (PAPS) to a variety of xenobiotic and endogenous compounds such as drugs, carcinogens, steroids, and neurotransmitters (Falany, 1997). Unlike other species the human SULT1A subfamily contains more than one member (SULT1A1, SULT1A2, and SULT1A3), which share >92% identity at the amino acid level (Wilborn et al., 1993; Zhu et al., 1993a,b; Ozawa et al., 1995). The three *SULT1A* genes are found within proximity to each other on chromosome 16 (16p12.1-p11.2), suggesting their recent evolution from a gene duplication event (Dooley et al., 1994; Bernier et al., 1996; Dooley and Huang, 1996; Raftogianis et al., 1996). SULT1A1 plays a significant role in the sulfo-conjugation of xenobiotics, such as *p*-nitrophenol, *N*-hydroxy-heterocyclic and -aromatic amines, and endogenous compounds such as di-iodothyronine and estrogens (Falany, 1997; Brix et al., 1999; Richard et al., 2001). SULT1A1 is found in large abundance in the liver and in a variety of tissues, including the intestine, lung, kidney, endometrium, placenta, skin, and platelets (Butler et al., 1983; Zhu et al., 1993b; Windmill et al., 1998; Rubin et al., 1999; Dooley et al., 2000; Stanley et al., 2001). In contrast, SULT1A3, which is the major sulfotransferase involved in catecholamine sulfonation, is barely expressed in the adult liver but is present in significant amounts in the intestine, lung, platelets, and the

ABBREVIATIONS: SULT, sulfotransferase; PAPS, 3′-phosphoadenosine 5′-phosphosulfate; CAR, constitutive androstane receptor; UTR, untranslated region; GABP, GA binding protein; EBS, Ets binding sequence; DMSO, dimethyl sulfoxide; Citco, 6-(4-chlorophenyl)imidazo[2,1-*b*][1,3]thiazole-5-carbaldehyde *O*-(3,4-dichlorobenzyl)oxime; FBS, fetal bovine serum; RT-PCR, reverse transcription-polymerase chain reaction; FAM, 5-carboxyfluorescein; TAMRA, 5-carboxytetramethylrhodamine; RACE, rapid amplification of cDNA ends; OP, outer primer; PCR, polymerase chain reaction; EMSA, electrophoretic mobility shift assay; bp, base pair(s); UGT, uridine diphosphate glucuronosyltransferase.

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fetal liver (Butler et al., 1983; Windmill et al., 1998; Richard et al., 2001). Although considerable information is available on the structure-function relationships of these two enzymes, their transcriptional regulation has not been explored in great detail.

Most studies investigating the gene regulation of cytosolic SULTs have focused on the rodent isoforms and their response to nuclear receptor activators. Rat and mouse SULT1A1 and SULT2 isoforms and human SULT2A1 enzyme levels were shown to be induced by glucocorticoids (Runge-Morris et al., 1998; Wu et al., 2001; Duanmu et al., 2002). In addition, the constitutive androstane receptor (CAR) activator phenobarbital has been shown to cause a decrease in rat SULT1A1 and 2A1 and a 4-fold increase in rat SULT2A2 mRNA levels (Runge-Morris, 1998). A recent study demonstrated that dexamethasone increased SULT1A1 expression in the rat liver, but no such effect was observed in cultures of human primary hepatocytes (Duanmu et al., 2001, 2002). Although the rodent and human SULT1A1 enzymes share 77% amino acid identity, their promoter regions are distinct in sequence. These differences, together with the fact that humans possess three SULT1A members compared with a solitary isoform in rodents, make it difficult to extrapolate the mechanisms regulating human SULT1A expression from animal *SULT* gene regulation studies.

When the human SULT1A cDNA species were isolated variable 5′ untranslated regions (5′UTR) were observed, which were suggestive of either the use of alternate transcriptional start sites or alternate post-transcriptional splicing (Wilborn et al., 1993; Zhu et al., 1993a,b; Bernier et al., 1994a; Wood et al., 1994; Aksoy and Weinshilboum, 1995; Raftogianis et al., 1996). It was noted that these alternate transcripts may be tissue-specific; however, the true endogenous ramifications remain unclear, because all transcripts contain the same coding sequence. Bernier et al. (1996) showed that the human *SULT1A1* gene sequence upstream of the more distal 5′UTR had higher promoter activity than the sequence immediately upstream of the ATG start codon. The distal 5′UTR seems to be part of the most common SULT1A1 mRNA species identified by investigators and was the transcript first isolated by our laboratory from a liver cDNA library (Zhu et al., 1993a).

The objective of this study was to unravel the mechanisms of gene regulation of the human SULT1A subfamily, with particular emphasis on the *SULT1A1* promoter. In this study, we provide the first explanation as to how this gene is regulated at the transcriptional level. The region immediately upstream of the distal 5′UTR on the *SULT1A1* gene harbors a highly active promoter, lacking canonical TATA box elements but containing G/C-rich regions, which are responsible for the action of Sp1 on the promoter. Furthermore, the high activity of the human *SULT1A1* promoter is driven by a synergistic action between the Ets transcription factor GA binding protein (GABP) and Sp1, and the lower promoter activity observed for *SULT1A3* is the result of a two-base pair difference in an Ets transcription factor binding sequence (EBS) on this gene that prevents this synergistic effect.

Materials and Methods

Materials. Dexamethasone, rifampicin, 3-methylcolanthrene, phenobarbital, DMSO, and other common reagents were purchased from Sigma-Aldrich (St. Louis, MO) or local suppliers, unless otherwise stated. 6-(4-Chlorophenyl)imidazo[2,1-*b*][1,3]thiazole-5-carbaldehyde *O*-(3,4-dichlorobenzyl)oxime (Citco) was kindly provided by Dr. Steven Kliewer (University of Texas Southwestern Medical Center, Dallas, TX; Maglich et al., 2003). Cell culture media, fetal bovine serum (FBS), and trypsin/EDTA were obtained from Invitrogen (Carlsbad, CA). Oligonucleotides were purchased from Sigma Genosys (The Woodlands, TX) or Invitrogen Custom Primers. TaqMan real-time probes were synthesized by Applied Biosystems (Foster City, CA)

Treatment of Primary human Hepatocytes and Real-Time RT-PCR. Liver tissues for primary human hepatocytes cultures were obtained with the patient's consent and the approval of the University of North Carolina Hospitals Ethics Committee. All tissues were isolated by qualified medical staff from patients undergoing liver resection from metastatic tumors. From these, only hepatocytes exhibiting normal morphology were isolated and cultured in six-well plates as described previously (Wang et al., 2003). Cells were treated for 16 to 18 h with nuclear receptor activators dexamethasone (1 μM), rifampicin (10 μM), 3-methylcolanthrene (5 μM), phenobarbital (1 mM), Citco (1 μM), or vehicle DMSO. RNA was isolated from cells with TRIzol reagent (Invitrogen), and reverse transcription of 2 μg of RNA was carried out using the SuperScript Reverse transcription kit for RT-PCR (Invitrogen). The RT-PCR was carried out on an ABI Prism 7700 sequence detector (Applied Biosystems), with 2× TaqMan Universal Mastermix (Applied Biosystems); 1 μl of reverse-transcribed RNA; 20× β-actin internal standard control mix, containing the Vic fluorescent label (Applied Biosystems); 50 ng of each sense and antisense primer; and 5 pmol of probe. The reaction was carried out after an initial hold at 50°C for 2 min and a denaturing step at 95°C for 10 min, followed by 40 cycles of denaturing at 95°C for 15 s and combined annealing and extension at 60°C for 1 min. The probe and primer sets used were designed on Primer Express Software (Applied Biosystems) and are shown in Table 1.

SULT1A Immunoblotting. Recombinant human SULT1A1 and SULT1A3, cloned in pET28(a)+ (Promega, Madison, WI), were bacterially expressed in BL21(DE3) cells as described previously (Brix et al., 1999), and the histidine tag was cleaved by overnight incubation with 2 units of thrombin. Total bacterial cytosol (0.1 μg) and 75 μg of primary human hepatocyte cytosol were electrophoresed on a 14% acrylamide gel and subjected to Western blotting. The goat anti-human SULT1A1 antibody is able to cross-react with all three human SULT1A proteins (Brix et al., 1999). After secondary incubation with anti-goat horseradish peroxidase-conjugated antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA), proteins were visualized by enhanced chemiluminescence exposure (Amersham Biosciences, Piscataway, NJ) and autoradiography.

5′Rapid Amplification of cDNA Ends. SULT1A1 5′RACE was carried out using human liver Marathon Ready cDNA (0.2 ng; BD Biosciences Clontech, Palo Alto, CA), with *SULT1A1* gene-specific antisense outer primer (OP; 1 μM; 5′-GGGAATCCCTGGGGCTTTGAACTC-3′), located within the second coding exon, the cDNA adapter primer AP1 (1 μM), 200 μM dNTP mix, and the Advantage 2 Polymerase mix and buffer (BD Biosciences Clontech), using the following PCR cycling conditions: initial denaturing at 94°C for 30 s; 5 cycles of denaturing at 94°C for 5 s and a combined annealing and extension time at 72°C for 4 min; followed by 30 cycles of denaturing at 94°C for 5 s and annealing/extension at 70°C for 4 min. This was followed by a second amplification of 1/50 of the PCR product with the SULT1A1 inner primer (5′-CACTTCTCCAGGTCACCACCCTGGTA-3′), located just upstream of the OP site. A control PCR was carried out with OP and a control sense primer (5′-CGCCCGCCACTGGAGTACGTG-3′) located in the first coding exon. 5′RACE PCR products were electrophoresed, purified, cloned into the pCR2.1 vector (Invitrogen), and sequenced using BigDye Terminator Ready Reaction mix (Stratagene, La Jolla, CA).

Promoter Constructs and Expression Plasmids. The *SULT1A* promoter regions were amplified using the Long *Taq* Plus

DNA polymerase system (Stratagene) from human genomic DNA. The heterologous primer set (sense, 5′-GAGCTGTGAGGAAGTTCAGGTC-3′; antisense, 5′-GATCAGCTCCATGTTCCTGCATC-3′) was used to amplify approximately 4 kilobases of sequences upstream of the start codon of all three human *SULT1A* genes. The PCR was performed with the following cycling conditions: an initial 2-min denaturing step at 94°C was followed by 30 cycles of denaturing at 92°C for 30 s, annealing of primers at 66°C for 1 min, and extension at 72°C for 5 min, followed by an additional 5 cycles, with increased extension time to 15 min. The PCR products were cloned into the pCR2.1 vector (Invitrogen) and sequenced. Restriction enzymes NsiI, ClaI, and KpnI (New England Biolabs, Beverly, MA) were used to differentiate the three sequences by specifically cutting only *SULT1A1*, *SULT1A2*, and *SULT1A3* sequences, respectively. Promoter deletion constructs were created by PCR, using *Pfu Turbo* DNA polymerase (Stratagene) and the above-mentioned *SULT1A* promoter constructs as template. The constructs were cloned into the pGL3Basic luciferase reporter vector (Promega). The QuikChange site-directed mutagenesis kit (Stratagene) was used to incorporate site mutations as indicated in Fig. 4a.

Human GABPα (GenBank accession no. NM_002040), GABPβ1 (GenBank accession no. NM_005254), Ets-1 (GenBank accession no. NM_005238), Ets-2 (GenBank accession no. NM_005239), Elf-1 (GenBank accession no. NM_172373), Elk-1 (GenBank accession no. NM_005229), and Tel-1 (GenBank accession no. NM_001987) cDNAs were isolated from HepG2 cDNA by PCR using TaKaRa *LATaq* polymerase (Panvera, Madison, WI). Primers were based on the sequence available from the GenBank/National Center for Biotechnology Information database, and the PCR was carried out at an initial denaturing step at 94°C for 1 min, followed by 40 cycles of denaturing at 94°C for 30 s, annealing of primers at 49°C for 30 s, and extension at 72°C for 3 min, with a 15-s increase in extension time, per cycle. The PCR was concluded by a final extension at 72°C for 10 min and the purified PCR products cloned into the TOPO TA pCDNA3.1 vector (Invitrogen). Ets factors were subcloned into the pAC5.1 *Drosophila melanogaster* expression vector (Invitrogen). Sp1 and Sp3 in pPac were kindly provided by Dr. Guntram Suske (Philipps University, Marburg, Germany).

Cell Culture, Transfection, and Luciferase Assay. Human hepatocarcinoma cell lines HepG2 and Hep3B were obtained from American Type Culture Collection (Manassas, VA) and grown in minimum essential medium containing 10% FBS. Transfection of promoter constructs was carried out at ~70 to 80% confluence using the CellPhect Transfection kit (Amersham Biosciences). The *SULT1A* promoter luciferase reporter constructs or the empty pGL3-Basic vector (0.1 μg/well) were transfected together with control *Renilla reniformis* luciferase reporter vector pRL-SV40 (0.05 μg/well) in 24-well plates. Media were changed 24 h after transfection, and luciferase activities of the lysed cells were measured using the dual luciferase kit (Promega), after an additional 24 h in culture. Each result represents the mean and standard deviation of three transfections, and assays were repeated 2–3 times to confirm reproducibility. Primary human hepatocytes were seeded into 24-well biocoated plates and grown in Williams' medium E (Sigma-Aldrich), supplemented with 1% insulin/transferrin/sodium selenite supplement (Sigma-Aldrich) and 0.1 μM dexamethasone. Cells were transfected with *SULT1A* promoter constructs (0.25 μg/well) and the pRLSV40 control (0.025 μg/well) after 48 h in culture, using Effectene transfection reagent (QIAGEN, Valencia, CA). Media were changed the following day, and luciferase activities were measured as stated above, after an additional 24 h in culture. *D. melanogaster* S2 cells were cultured in Schneider's Drosophila medium (Invitrogen) supplemented with 10% FBS at 25°C. Cells were transferred to 12-well dishes at a density of 0.5 × 10^6 cells/well, and transfections were carried out the following day using the calcium phosphate method, as per protocol of the Drosophila Expression System kit (Invitrogen). Each well was transfected with 1 μg of *SULT1A* promoter reporter construct and where indicated with 1–5 μg of the following: Sp1, Sp3, GABPα, GABPβ, Ets1, Ets2, Elf, Elk, or the empty pAC5.1 or pPac-UBX vectors. Media were changed after 18 h post transfection. After 24 h in culture, cells were lysed and luciferase activity was measured (Promega). Luciferase activity was normalized against protein concentration of the cleared lysate (Bio-Rad protein assay; Bio-Rad, Hercules, CA).

Electrophoretic Mobility Shift Assay. Nuclear extracts from cell lines were isolated as described by Dignam et al. (1983). Recombinant Ets transcription factors were expressed using the in vitro rabbit TNT reticulocyte lysate transcription/translation system (Promega). Complimentary oligonucleotide probes with 5′ linkers GATC [*SULT1A1* wild type (wt), 5′CCTTCCTTCCGGAAGCAA3′; and EBS mutants (m); Fig. 4a] were annealed and end-labeled with [α-^{32}P]dATP (Amersham Biosciences). The radiolabeled probe (~15,000 cpm) was incubated with 3 to 5 μg of nuclear extract or 3 to 6 μl of rabbit reticulocyte lysate-expressed protein, in 20 mM Tris-HCl, pH 7.5, buffer, containing 1 μg of poly(dIdC), 0.1 mM dithiothreitol, 50 mM NaCl, and 10% glycerol for 45 min at room temperature. The protein-DNA complexes were electrophoresed on a 5% polyacrylamide gel in 7 mM Tris-HCl, pH 7.5, 3 mM sodium acetate and 1 mM EDTA, and visualized by autoradiography. In supershift experiments, nuclear extract was preincubated with 2 μg of antibody for 1 h on ice. Anti-mouse

TABLE 1
Primers and Probes

SULT1A1	
Sense	5′-GCAACGCAAAGGATGTGGCA-3′
Antisense	5′-TCCGTAGGACACTTCTCCGA-3′[a]
Probe	6FAM-ACATGGCCAAGGTGCACCCTGAGCC-TAMRA
SULT1A3	
Sense	5′-GGAACCCTCAGGGCTGGAG-3′
Antisense	5′-CGTCCTTTGGGTTTCGGG-3′
Probe	6FAM-GCCCCCACGGCTCATCAAGTCACACC-TAMRA
CYP2B6	
Sense	5′-AAGCGGATTTGTCTTGGTGAA-3′
Antisense	5′-TGGAGGATGGTGGTGAAGAAG-3′
Probe	6FAM-CATCGCCCCGTGCGGAATTGTTC-TAMRA
CYP3A4	
Sense	5′-TCAATAACAGTCTTTCCATTCCTCAT-3′
Antisense	5′-CTTCGAGGCGACTTTCTTTCA-3′
Probe	6FAM-TTTCCAAGAGAAGTTAC-MGBNFQ
UGT1A1[b]	
Sense	5′-GGCCCATCATGCCCAATAT-3′
Antisense	5′-TTCAAATTCCTGGGATAGTGGATT-3′
Probe	6FAM-TTTTTGTTGGTGGAATCAACTGCCTTCAC-TAMRA

[a] Dooley et al. (2000).
[b] Sugatani et al. (2001).

GABPα and β polyclonal antibodies were prepared previously (Yokomori et al., 1995) and normal rabbit Immunoglobulin G (Rb-IgG), Ets-2, and Elf-1 antibodies were purchased from Santa Cruz Biotechnology Inc. The anti-mouse GABP antibodies were shown to cross-react with the human recombinant GABP proteins (data not shown).

Results

Human SULT1A1 and 1A3 mRNA Levels Are Not Induced by Activators of the Glucocorticoid Receptor, CAR, Pregnane X Receptor, or Aryl Hydrocarbon Receptor in Primary Human Hepatocytes. Fig. 1, a–e, shows the result for real-time RT-PCR of human primary hepatocytes from one of two donors treated with various nuclear receptor activators. Phenobarbital, which elicits the well known induction pathway for the CYP2B family and the phase II enzyme UGT1A1 via CAR (Sugatani et al., 2001; Pascussi et al., 2003), did not influence SULT1A1 mRNA levels in these cells. Even the newly described human CAR activator Citco (Maglich et al., 2003) failed to change SULT1A1 mRNA levels. As reported previously, the glucocorticoid receptor agonist dexamethasone had no influence on SULT1A1 mRNA levels (Duanmu et al., 2002), contrary to the increases in CYP3A4 levels observed, a known target gene of this receptor (El-Sankary et al., 2000). Likewise, the pregnane X receptor activator rifampicin did not alter the SULT1A1 levels, but it had a significant effect on inducing CYP3A4 and UGT1A1 mRNA levels. Treatment of the aryl hydrocarbon receptor ligand 3-methylcolanthrene caused a 5-fold induction of UGT1A1 levels, but again it had no effect on SULT1A1 mRNA. Treatments of hepatocytes from the other donor resulted in similar induction profiles for the P450 and UGT1A1 mRNAs, but again they did not produce significant changes in SULT1A1 levels. In addition, no changes in SULT1A3 levels were observed with the treatments. Generally, the mRNA levels of SULT1A3 in primary human hepatocytes were lower than those of SULT1A1 (Fig. 1f), and SULT1A3 protein could not be detected in hepatocyte cytosol using immunoblotting, unlike the high abundance of SULT1A1 protein observed (Fig. 1g).

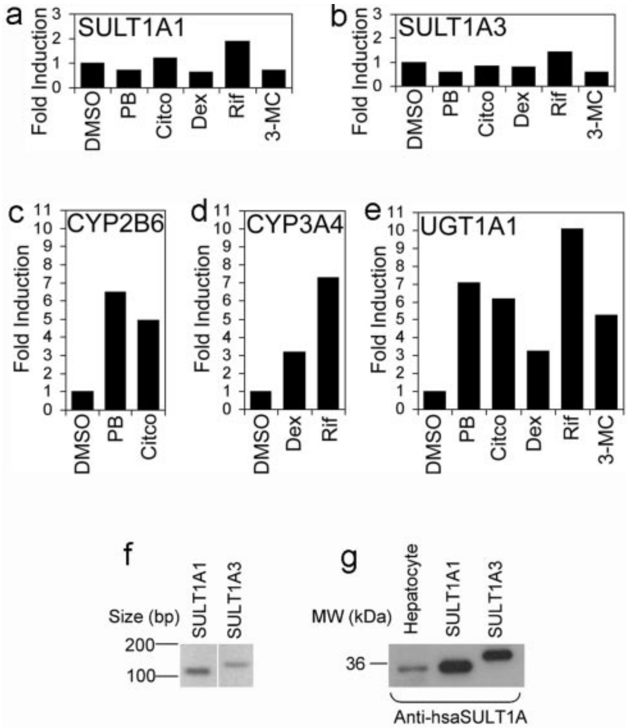

Fig. 1. a–e, real-time RT-PCR of primary human hepatocyte RNA after treatment with nuclear receptor activators. Data represent the results obtained from primary hepatocytes of one liver donor, isolated as stated under *Materials and Methods*. Hepatocytes were treated with 1 mM phenobarbital (PB), 1 μM Citco, 1 μM dexamethasone (Dex), 10 μM rifampicin (Rif), 5 μM 3-methylcolanthrene (3-MC), or vehicle DMSO, and RNA was isolated and reverse transcribed 18 h after treatment. Real-time PCR was carried out as stated under *Materials and Methods*, and results represent the fold-induction in SULT1A1 (a), SULT1A3 (b), CYP2B6 (c), CYP3A4 (d), and UGT1A1 (e) mRNA levels after treatment compared with the DMSO control (n = 2). f, SULT1A1 and SULT1A3 mRNA levels in primary human hepatocytes. RNA was isolated from untreated primary human hepatocytes and reverse transcribed as stated under *Materials and Methods*. RT-PCR was carried out with SULT1A1- and SULT1A3-specific primers. g, immunoblot of primary human hepatocyte cytosol with anti-human SULT1A antibody. Human primary hepatocyte cytosol (75 μg) and recombinant, bacterially expressed SULT1A1 and SULT1A3 proteins were electrophoresed and immunoblotted as described under *Materials and Methods*.

***SULT1A1* Promoter Properties.** Early cloning studies of human SULT1A1 cDNAs demonstrated the presence of different mRNA species, which varied only in their 5′UTRs. Figure 2a shows a schematic of the 5′region of the *SULT1A1* gene, indicating the positions of the three 5′UTRs reported in the literature; a 5′UTR immediately upstream of the ATG start codon (Exon 1, Fig. 2A; Zhu et al., 1993b), a proximal 5′UTR (5′UTR A, Fig. 2A; Raftogianis et al., 1996) and a distal 5′UTR (5′UTR B, Fig. 2A; Wilborn et al., 1993; Zhu et al., 1993a). To identify the transcriptional start site of the *SULT1A1* gene in the liver, 5′RACE was carried out, which resulted in the amplification of a single 63-bp product, located 1071 bp upstream from the ATG start codon of *SULT1A1* (data not shown). This represents the most common cDNA species described previously, containing the distal 5′UTR (5′UTR B, Fig. 2A; Wilborn et al., 1993; Zhu et al., 1993a). The furthest 5′ transcriptional start site identified in this study was denoted as bp +1, with other 5′RACE products displaying start sites at 3, 8, and 10 bp downstream of this site. This distal 5′UTR was also the major sequence amplified from HepG2 cDNA when RT-PCR with primers positioned in the alternate 5′UTRs was performed (data not shown). This hepatocarcinoma cell line was used to initially characterize the *SULT1A1* promoter.

The distal 5′UTR B was further confirmed to contain the start site of transcription when promoter activities of the sequences in front of all reported 5′UTRs were assessed in HepG2 cells. The 1371-bp sequence 5′ of the start codon (exon I) exhibited only 2.7-fold higher luciferase activity than the empty vector alone (Fig. 2b). The sequence immediately upstream of the proximal 5′UTR A did not produce a significant luciferase signal, which reflects our RT-PCR data, where an mRNA species containing this 5′UTR could not be amplified. In contrast, the sequence located upstream of the transcriptional start site identified in 5′RACE (5′UTR B) displayed considerable basal activity, with the −1217/+65 construct having 140-fold higher activity than the empty pGL3Basic vector alone (Fig. 2b).

To characterize this highly active *SULT1A1* promoter, we analyzed the properties of several deletion constructs in HepG2 cells. These displayed steadily increasing luciferase activity when deleted, with the −112/+65 construct exhibiting highest activity; a 230-fold increase from the empty vector (Fig. 2c). Further deletion of an additional 44 bp (−68/

+65) resulted in a 90% decrease in activity, indicating the presence of a crucial regulatory element in this region. The *SULT1A1* promoter constructs displayed similar activity patterns when transfected into primary human hepatocytes and Hep3B cells, and the deletion to −68/+65 resulted in an equally dramatic decrease in promoter activity, suggesting a common mechanism of regulation in these cell types (Fig. 2d).

Identification of a Crucial EBS by Comparison with the Less Active *SULT1A3* Promoter. Three different 5′UTRs were previously shown to exist in SULT1A3 cDNA species isolated from a variety of cDNA libraries (Fig. 3a; Zhu et al., 1993a; Bernier et al., 1994a; Aksoy and Weinshilboum, 1995). SULT1A3 5′RACE results from liver cDNA were inconclusive, most likely because of the limited transcription of this gene in this tissue. However, cloning of the *SULT1A3* gene sequences flanking these 5′UTRs into the pGL3Basic vector and assessing their ability to act as promoters in driving luciferase transcription revealed that only the sequence flanking 5′UTR A, which is homologous to the distal 5′UTR B of *SULT1A1*, was a functional promoter in HepG2 cells (Fig. 3b).

These two *SULT1A* promoters share >60% sequence identity in the 3 kb upstream of their transcriptional start sites. A notable 3-fold difference in activities between the *SULT1A1* and *SULT1A3* promoters was observed, which was used as an effective tool to delineate the molecular mechanisms differentially controlling the transcriptional regulation of the two *SULT1A* genes in the liver-derived cell line (Fig. 4a). The *SULT1A3* −125/+43 promoter construct displayed 70% less activity than the homologous *SULT1A1* −112/+65 construct in HepG2 cells. Both *SULT1A* genes seem to have TATA-less promoters, which are rich in GC areas. Analysis of the *SULT1A1* sequence using the Patch, Match, and Transfac database programs (http://www.gene-regulation.com) identified a high homology match to the EBS core sequence GGAA, located on the sense strand at bases −92 to −89 (EBS1) and as a tandem repeat immediately upstream of this on the antisense strand (Fig. 4b; EBS2 and 3). Flanking the EBS repeats on either side are two Sp1 sites. The *SULT1A3* sequence lacks 6 bp downstream of the EBS repeats (Fig. 4b). In addition, *SULT1A3* has a two-nucleotide mismatch in the EBS1 motif, but it has EBS2, EBS3, and the Sp1 sites conserved. To assess whether the difference in promoter activities between *SULT1A1* and *SULT1A3* is caused by a difference in sequence between bases −112 and −68, a chimeric construct was created that introduced the *SULT1A3* region into the *SULT1A1* promoter (alt1A3). This caused a 70% decrease in *SULT1A1* promoter activity and resulted in luciferase levels similar to those observed for wild-type *SULT1A3* (wt; Fig. 4c). Interestingly, this same reduction in activity could also be achieved by changing two bases in the EBS1 motif of *SULT1A1* to those found in *SULT1A3*, thereby disrupting the Ets binding core motif GGAA (1A1EBS1m; Fig. 4c). The importance of this motif

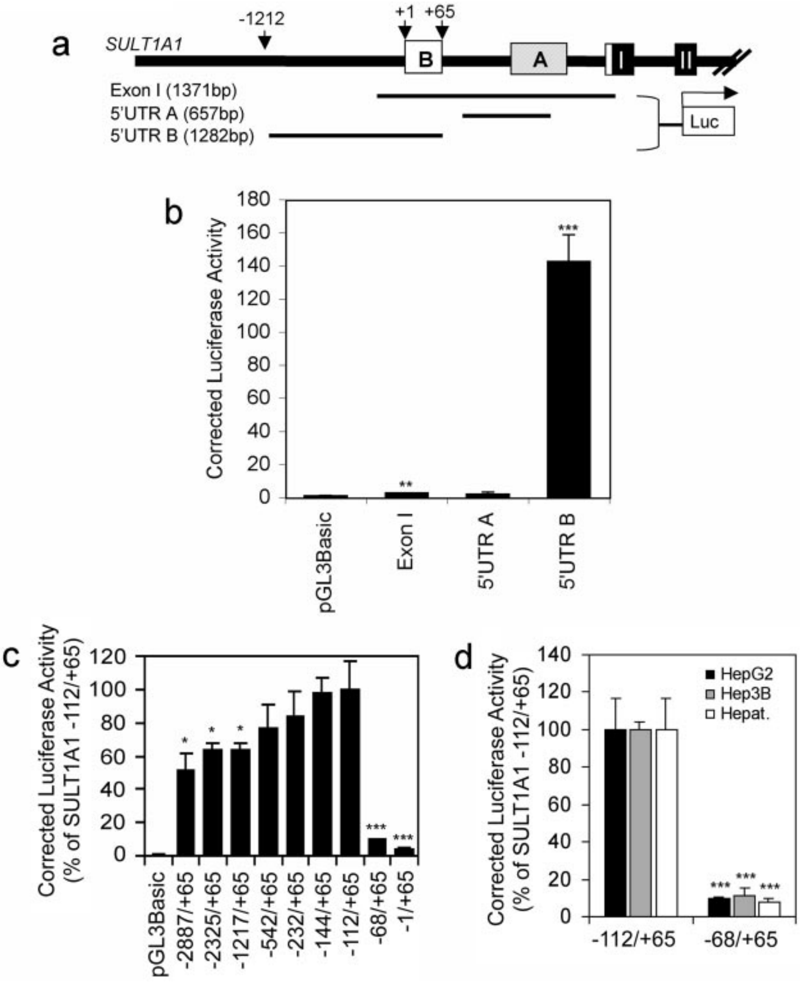

Fig. 2. a, schematic of the 5′ region of the human *SULT1A1* gene. Open boxes represent the location of the alternate 5′UTRs of SULT1A1 cDNA species identified in the literature, with proximal and distal 5′UTR labeled as A and B, respectively, and closed boxes represent the first and second coding exons of *SULT1A1*. The transcriptional start site identified by 5′RACE is labeled as +1. Black columns represent promoter constructs, and these were cloned into the pGL3Basic Luciferase reporter vector as stated under *Materials and Methods*. b, promoter activities of sequences flanking the alternate 5′UTRs of *SULT1A1* in HepG2 cells. All promoters were cloned into the pGL3Basic vector, transfected into HepG2 cells, and lysed cells assayed for luciferase activity as stated under *Materials and Methods*. Results are corrected for *R. reniformis* luciferase activity of the pRLSV40 transfection standard and represent the mean ± S.D. of three transfections. Results are expressed as fold increases in luciferase activity relative to the empty pGL3Basic vector. Asterisks indicate significant differences to the activity of the empty pGL3Basic vector (Student's *t* test; **, $p < 0.01$; ***, $p < 0.001$). c, *SULT1A1* promoter deletion construct activities in HepG2 cells. Deletion constructs of the distal promoter were cloned into the pGL3Basic vector and transfected into HepG2 cells as described above. Results are expressed as a percentage of the *SULT1A1* −112/+65 construct promoter activity, with significant differences to this construct indicated by asterisks (*, $p < 0.05$; ***, $p < 0.001$). d, promoter activities of *SULT1A1* promoter deletion constructs in HepG2, Hep3B, and primary human hepatocytes (Hepat). The −68/+65 and −112/+65 *SULT1A1* promoter constructs were cloned into the pGL3Basic vector, transfected into cell lines, and lysed cells assayed for luciferase activity as stated above. Results are expressed as a percentage of the *SULT1A1* −112/+65 construct promoter activity in each cell line, with significant differences to the activity of this construct indicated by asterisks (***, $p < 0.001$).

was further highlighted by introducing the consensus GGAA EBS1 into *SULT1A3*, which significantly increased promoter activity and effectively converted it to that observed for the wild-type *SULT1A1* promoter (Fig. 4c).

The roles of the adjacent EBS2 and EBS3 on the antisense strand were assessed by a series of mutations in the *SULT1A1* −112/+65 construct and by analysis of promoter activity in HepG2 cells (Fig. 5a). Mutations disrupting either EBS2 or EBS3 resulted in a 50% reduction in promoter activity. Mutations of all three EBS GGAA motifs resulted in a decrease to 10% of the original *SULT1A1* −112/+65 wild-type promoter activity, and resembled the activity observed with the −68/+65 deletion construct, suggesting the need for the presence of all three EBS sites for maximal promoter activity.

Identification of Ets Factors Binding the *SULT1A1* EBS Motifs. EMSA of HepG2 nuclear extract with the *SULT1A1* −102/-85 ^{32}P-labeled oligonucleotide probe, incorporating the EBS motifs, revealed the formation of several specific protein-DNA complexes (Fig. 5b, A–C). These were competed out by molar excess of unlabeled probe, but not by a probe containing mutations in the three EBS. Three bands were observed at position A with HepG2 and Hep3B nuclear extract. The band at position B was enhanced in the EMSA with Hep3B nuclear extract. Additionally there was a lower migrating doublet at C. A nonspecific (*n/s*) signal was also observed. To assess the importance of the individual EBS motifs, ^{32}P-radiolabeled *SULT1A1* −102/−85 probes containing mutations in these sites were incubated with HepG2 nuclear extract (Fig. 5c). It was observed that the bands at position A were only present if EBS3 was intact (Fig. 5c, −102/−85, EBS1m, EBS2m). Bands B and C lost intensity with a mutation in EBS1, which also represents the *SULT1A3* wild-type sequence, but seemed to increase in intensity when mutations were made in EBS3, suggesting that the protein-DNA complexes at position B and C require the presence of EBS1 and that a shift of binding can occur when one of the GGAA triplicate motifs is disrupted. All binding was abolished when nuclear extract was incubated with the probe containing mutations in all three EBS motifs.

Several Ets transcription factors could be isolated from HepG2 cDNA, which were potential candidates of the endogenous Ets factors controlling *SULT1A1* gene regulation in these cells. Indeed, using an antibody supershift EMSA, it was possible to show the presence of two Ets factors in the protein-DNA complexes formed by the *SULT1A1* EBS probe and hepatocarcinoma cell nuclear extract. Figure 6a shows the supershift EMSA performed with preincubation of HepG2 nuclear extract with antibodies against the widely expressed Ets factors Ets2, Elf1, and GABPα and β. No supershift was observed with the antibody against Ets2, which is also able to cross-react with Ets1. The protein-DNA complex at position B could be identified to contain the Ets transcription factor Elf1. The presence of Elf1 in band B was further highlighted when the EBS3 mutant probe was incubated with nuclear extract and Elf1 antibody. This probe enhanced binding of band B, which clearly disappears with addition of this antibody. Anti-mouse GABPβ was able to supershift all bands at position A (Fig. 6a). The antibody against mouse GABPα also supershifted these, except the lowest band, which may represent a variant of human GABPα that is not readily detectable by the antibody raised against the mouse isoform, yet is able to form a heterodimer with GABPβ and is hence supershifted with the GABPβ antibody. GAPBα requires its non-DNA-binding heterodimeric partner GABPβ for its transcriptional activity (LaMarco et al., 1991). It has been shown that GABPα and β can form both heterodimers and tetramers and that these have the same effect on promoter function (Genuario et al., 1993), which could explain the multiple bands observed at position A. The proteins of complex C could not be identified using antibody supershift EMSA. A probe with the mutation in EBS1, resembling the *SULT1A3* sequence displayed similar nuclear extract binding pattern to the *SULT1A1* wild-type probe, and a supershift with the GABP α and β antibodies. No binding or supershifts were observed for the probe containing mutations in all three Ets

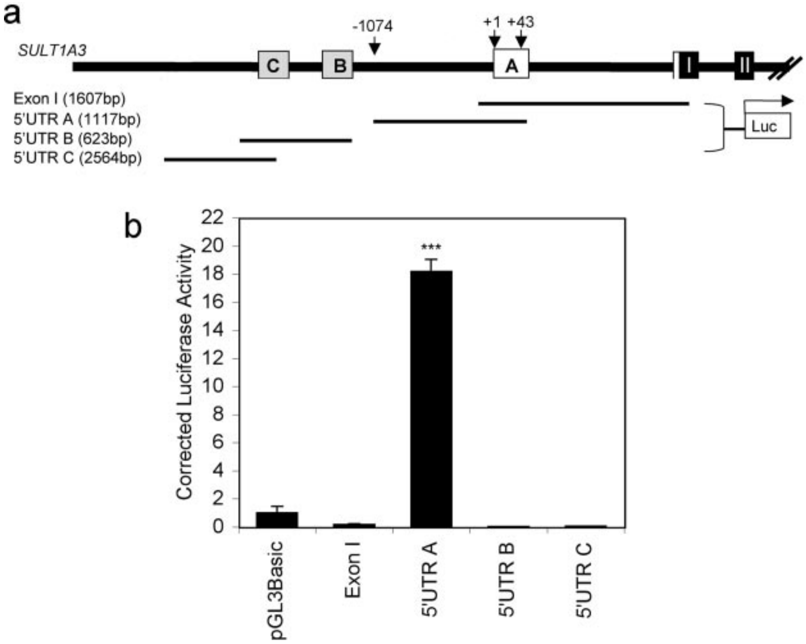

Fig. 3. a, schematic of the 5′ region of the human *SULT1A3* gene. Open boxes represent the location of the alternate 5′UTRs of SULT1A3 cDNA species identified in the literature, and closed boxes represent the first and second coding exons of *SULT1A3*. The 5′UTR A transcriptional start site identified in the literature is labeled as +1 (Bernier et al., 1994a). Black columns represent promoter constructs, and these were cloned into the pGL3Basic Luciferase reporter vector as stated under *Materials and Methods*. b, promoter activities of sequences flanking the alternate 5′UTRs of *SULT1A3* in HepG2 cells. All promoters were cloned into the pGL3Basic vector, transfected into HepG2 cells, and lysed cells were assayed for luciferase activity as stated under *Materials and Methods*. Results are corrected for *R. reniformis* luciferase activity of the pRLSV40 transfection standard and represent the mean ± S.D. of three transfections. Results are expressed as fold increases in luciferase activity relative to the empty pGL3Basic vector. Asterisks indicate significant difference to the activity of the empty pGL3Basic vector (***, $p <$ 0.001).

binding sequences. The ability of these Ets factors to bind the *SULT1A1* −102/−85 EBS probe was further highlighted when recombinant, in vitro expressed Ets proteins Elf1, Elk1, and the GABP heterodimer were shown to form DNA-protein complexes in EMSA (Fig. 6b). Ets1, Ets2, Tel, and the empty expression vector did not form visible complexes with the *SULT1A1* probe, which suggests that the EBS site of the *SULT1A1* promoter shows selectivity in the binding of Ets factors. A mutation in EBS3 of the *SULT1A1* probe did not change the binding affinity for Elf1 or Elk1, but compromised binding of the GABP heterodimer. Binding of all factors was drastically reduced with the mutation in EBS1, which represents the same sequence as wild-type *SULT1A3* (Fig. 6b). Binding was totally abolished for all factors when the three EBS sites were mutated, suggesting that the presence of the triplicate EBS repeat is required for optimal binding of a broader variety of Ets factors.

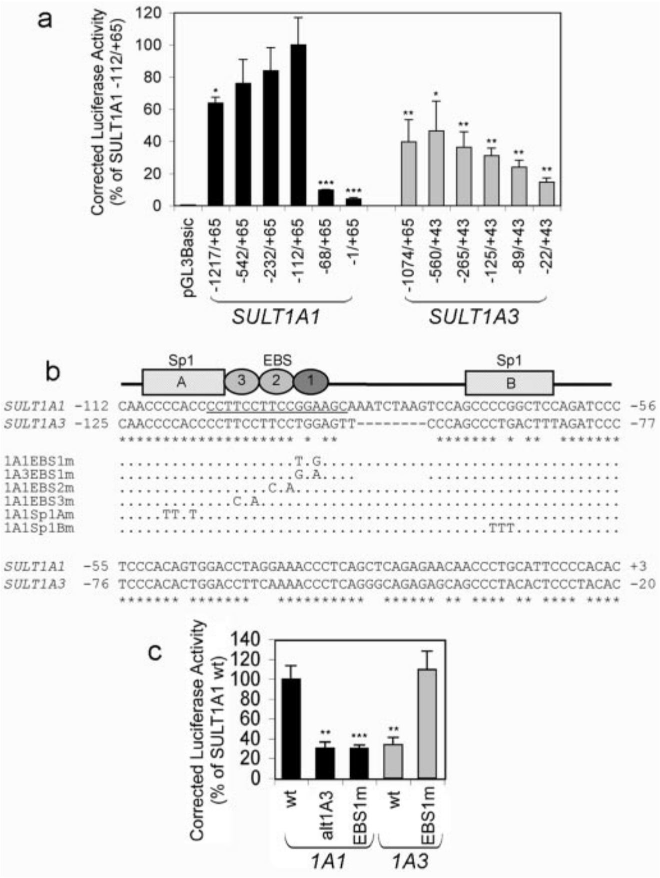

Fig. 4. a, comparison of promoter activities between *SULT1A1* and the homologous *SULT1A3* sequences. Constructs were cloned into pGL3Basic, transfected into HepG2 cells, and lysed cells were assayed for luciferase activity as stated under *Materials and Methods*. Black and gray columns represent SULT1A1 and SULT1A3 promoter activities, respectively. Results are corrected for *R. reniformis* luciferase activity of the pRLSV40 transfection standard and expressed relative to the *SULT1A1* −112/+65 promoter activity (mean ± S.D.; $n = 3$), with significant differences to this construct indicated by asterisks (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). b, alignment of the *SULT1A1* −112/+3 and *SULT1A3* −125/−20 promoter sequences, and position of Sp1 and EBS. Asterisks represent areas of sequence identity between *SULT1A1* and *SULT1A3*. Circles and rectangle schematically represent EBS and Sp1 binding sites, respectively. The locations and sequence modifications of the consensus site mutations are shown below. The transcriptional start site is marked as +1, and the bases underlined display the probe sequence used for EMSA. c, effects of nucleotide −112 to −68 exchange between the *SULT1A1* and *SULT1A3* promoters and mutation of EBS1. The *SULT1A1* fragment from −112 to −68 was excised and replaced by the homologous *SULT1A3* sequence [1A1(alt1A3)]. Using site-directed mutagenesis, EBS1 of *SULT1A1* was changed to the site found in *SULT1A3* (1A1EBS1m), and EBS1 was introduced into *SULT1A3* (1A3EBS1m). Constructs were transfected into HepG2 cells as described above. *SULT1A1* and *SULT1A3* promoter activities are represented by black and gray columns, respectively, and are expressed as a percentage of the *SULT1A1* −112/+65 promoter activity, with significant differences to this construct indicated by asterisks (**, $p < 0.01$; ***, $p < 0.001$).

Recombinant Ets Factors Influence *SULT1A* Promoter Activity in S2 Cells. To assess the ability of Ets factors to induce the *SULT1A1* promoter, we used the *D. melanogaster* cell line S2, which lacks a large variety of mammalian transcription factors. Transfection of Ets factors in HepG2 cells suggested an effect of these on *SULT1A1* promoter activity; however, the changes in activity were moderate, most probably caused by the action of endogenous Ets factors on the promoter. In contrast, the *SULT1A1* promoter had no basal activity in the S2 cells, with luciferase values close to those observed for the empty pGL3Basic vector, suggesting that no endogenous transcription factors were affecting the promoter in these cells (Fig. 7a). When the Ets transcription factors were cotransfected, an increase in *SULT1A1* promoter activity was observed with the GABP heterodimer and Elf1, but not with other Ets factors. This induction was dependent on increasing amounts of Ets factor transfected and 5 μg of either Elf1 or GABPα/β resulted in a 4-fold induction of promoter activity. The data show that transcriptional activation by GABP proteins could only be conferred in the presence of both heterodimeric partners (Fig. 7A). Even though we showed recombinant Elk1 binding, it seems that under the conditions in S2 cells, Elk1 is unable to transactivate the *SULT1A1* promoter. Tel, a known Ets repressor, which showed no obvious binding on EMSA, and no inducibility of the *SULT1A1* promoter was able to repress the promoter activity induced by the GABP heterodimer (Fig. 7a; Lopez et al., 1999). The inducibility of GABP and Elf1 on *SULT1A1* promoter activity was compromised when mutations were made in the individual EBS motifs and totally abolished in the triplicate mutant (Fig. 7b). Interestingly, inducibility of the *SULT1A3* promoter could be observed with GABP and Elf1, although the binding of the recombinantly expressed proteins was compromised in EMSA with the EBS1 mutant, which corresponds to the *SULT1A3* sequence (Fig. 6b). This suggests that the reason for the difference between *SULT1A1* and *SULT1A3* promoter activities observed in the hepatocarcinoma cell lines is more complex than the difference in binding and consequent action of Ets transcription factors at EBS1.

Sp1 Acts in Synergy with GABP to Induce the *SULT1A1* Promoter. The role of the two Sp1 motifs flanking the EBS region in the *SULT1A1* promoter were assessed using site-directed mutagenesis. Mutations in either site reduced *SULT1A1* −112/+65 promoter activity by 30% in HepG2 cells (Fig. 8a). A mutation in both sites resulted in a 70% reduction in activity. When the recombinant Sp1 transcription factor was cotransfected with the *SULT1A1* promoter and its Sp1 site mutants into S2 cells, a similar pattern of activity was seen as in HepG2 cells, suggesting that the endogenous levels of Sp1 play an important role in driving the *SULT1A1* promoter (Fig. 8b). Sp1 had strong effects on the wild-type promoter, inducing its activity 17-fold.

Previous studies have shown that Sp1 and GABP can interact to coactivate transcription of genes (Galvagni et al., 2001; Jiang et al., 2002). When cotransfecting Sp1 and GABP into S2 cells, we noted an overall 45-fold induction of *SULT1A1* promoter activity (Fig. 9). Alone GABP and Sp1 were able to induce promoter activity 4- and 17-fold, respectively, indicating that this high induction represents a synergy between the two factors, with Sp1 inducing the GABP-mediated activation approximately 10-fold. Although on its own Ets1 seemed not to induce the *SULT1A1* promoter, an enhanced effect of the Sp1 induction was observed when cotransfected with Ets1. Elf1 failed to show a synergistic effect; with cotransfection of both Elf1 and Sp1 resulting in a 13-fold additive induction.

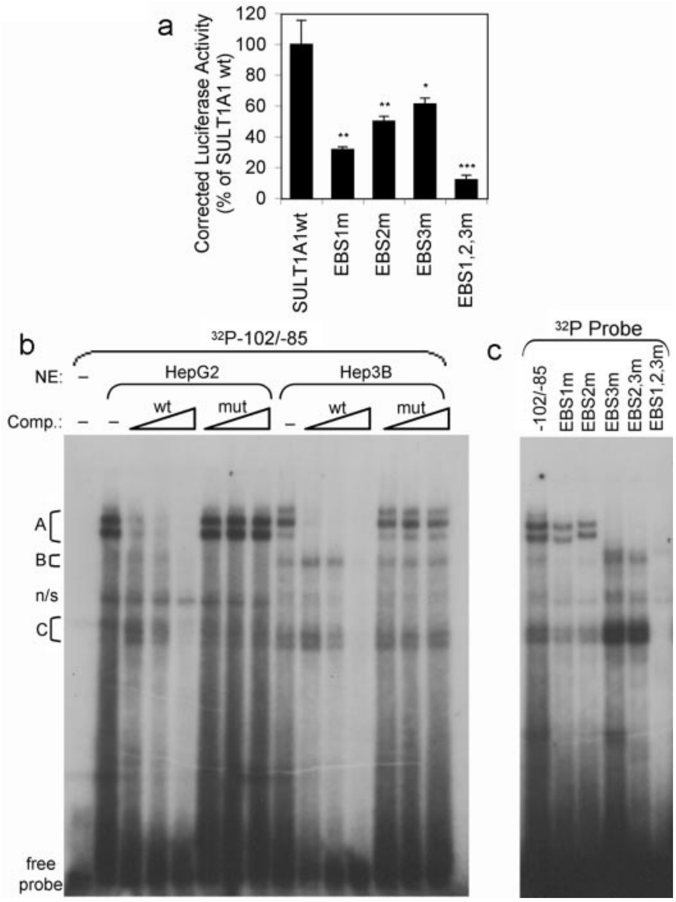

Fig. 5. a, effects of EBS motif mutations on *SULT1A1* promoter activity. EBS consensus elements were disrupted in the *SULT1A1* −112/+65 promoter construct using site-directed mutagenesis (Fig. 4a). Constructs were transfected into HepG2 cells, and lysed cells were assayed for luciferase as indicated under *Materials and Methods*. Luciferase activity was normalized against *R. reniformis* luciferase activity of the transfection control pRLSV40, and results are expressed as a percentage of the wild-type *SULT1A1* −112/+65 construct (mean ± S.D.; $n = 3$). Asterisks indicate significant difference to the activity of this construct (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). b, binding of HepG2 and Hep3B nuclear extract to the *SULT1A1* EBS motifs. EMSAs were carried out as described under *Materials and Methods*. The −102/−85 *SULT1A1* ^{32}P-radiolabeled probe containing the EBS motifs was incubated with either 4 μg of HepG2 or Hep3B nuclear extract (NE). In competition (Comp) experiments 10, 100, or 500× molar excess of unlabeled wild-type probe or the probe containing mutations in all three EBS (EBS1, 2, and 3m) was included. Specific DNA-protein complexes that are competed out by molar excess of cold competitor are labeled by letters (A–C). A nonspecific complex is labeled n/s. c, binding of HepG2 nuclear extract to ^{32}P-radiolabeled mutant EBS probes. EMSAs were carried out as described above with radiolabeled mutant and wild-type −102/−85 *SULT1A1* probes and 4 μg of HepG2 nuclear extract.

Synergistic Interaction between Sp1 and GABP Is Compromised on the *SULT1A3* Promoter Because of a Lack of EBS1. To asses the role of the Ets and Sp1 binding sites in determining the synergy between Sp1 and GABP, the ability of these two transcription factors to induce the promoter activity of the *SULT1A1* mutant constructs was assessed in S2 cells. A mutation in the first Sp1 site (Sp1A) reduced the ability of Sp1 to induce the promoter by 50%, which in turn caused a decrease in the synergistic induction produced with GABP from 45- to 20-fold. Synergy was totally abolished after mutating the second Sp1 site (Sp1B), indicating its importance in this transcriptional mechanism (Fig. 10a).

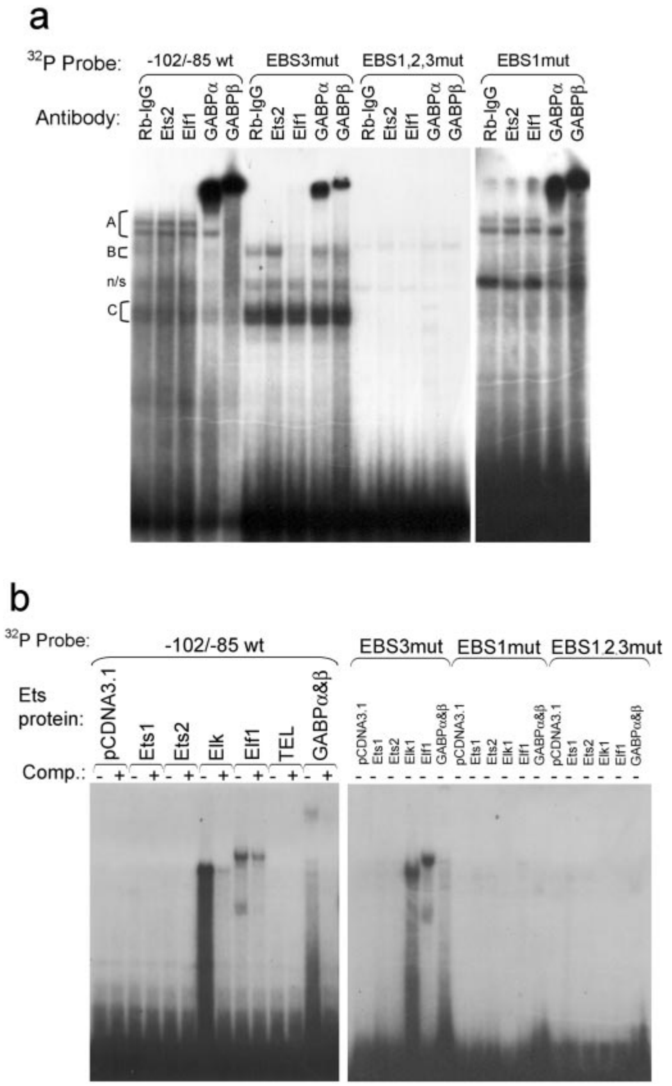

Fig. 6. a, Ets transcription factor antibody supershift EMSA. EMSA was carried out as described under *Materials and Methods*. HepG2 nuclear extract (5 μg) was incubated with 2 μg of polyclonal antibody against Ets2, Elf1, GABPα, GABPβ, or normal rabbit IgG (Rb-IgG), and either the ^{32}P-radiolabeled *SULT1A1* −102/−85 wild-type probe (SULT1A1 wt) or the radiolabeled EBS mutant probes. b, binding of recombinant Ets factors to the *SULT1A1* EBS probe. Recombinant Ets proteins were in vitro transcribed/translated in rabbit reticulocytes as described under *Materials and Methods*. ^{32}P-Radiolabeled *SULT1A1* −102/−85 wild-type probe was incubated with 2 μl of reticulocyte expressed protein and 100× molar excess of unlabeled probe was included in competition (Comp) experiments. Reticulocyte lysate containing empty expression vector pCDNA3.1 was run as control and EMSA carried out as described under *Materials and Methods*.

Furthermore, it was found that EBS1 and EBS3 are needed for the synergistic effect between GABP and Sp1 to occur. Mutations in either site abolished synergy between the two transcription factors, suggesting that it is necessary for Ets factors to have a specific conformational binding to the promoter for the interaction with Sp1 to occur, which is dependent on the presence of all three Ets binding sequences (Fig. 10b). Because EBS1 seems to be necessary for this synergy, we tested the ability of GABP and Sp1 to act in synergy in the induction of the *SULT1A3* promoter, which lacks this site. No synergy in induction could be observed with this promoter, and it seems that the activity of the *SULT1A3* promoter is solely driven by Sp1 and perhaps an additive effect of Ets factors binding at EBS2 and 3 (Fig. 10b).

Discussion

Previous studies have shown that rat and bovine SULT1A1 levels can be modulated by polycyclic aromatic hydrocarbon receptor agonists and nuclear receptor activators, such as dexamethasone and phenobarbital (Runge-Morris, 1998). In addition, levels of other phase I and II metabolic enzymes, including the P450 family, UGT, and the hydroxysteroid sulfotransferases (SULT2), are influenced by nuclear receptor activators (Runge-Morris, 1998; Sugatani et al., 2001; Pascussi et al., 2003). Unlike these enzymes, human SULT1A1 expression does not seem to be induced by any foreign chemical stimuli thus far explored, but it is constitutively expressed at high levels in hepatic and many extrahepatic tissues. In this study, the negligible effects of the glucocorticoid receptor agonist on human SULT1A1 levels in primary human hepatocytes were reconfirmed, which is in contrast to the dexamethasone-inducible expression of the rat SULT1A1 enzyme (Duanmu et al., 2001, 2002). In addition, all other nuclear receptor ligands failed to change human SULT1A1 and SULT1A3 mRNA levels in primary human hepatocytes. It seems that the human SULT1A1 enzyme exhibits a regulation profile that is unique from its rodent homologues, with high expression, wide tissue distribution, and a lack of gender-specific regulation. The high levels of human SULT1A1 reported in the liver and many extrahepatic tissues is suggestive of a more ubiquitous pattern of regulation. This study aimed to investigate the molecular mechanisms of *SULT1A1* gene regulation at the promoter level.

The isolation of different 5′UTRs of SULT1A cDNA species suggested the use of alternate transcriptional start sites on the genes (Wilborn et al., 1993; Zhu et al., 1993a,b; Bernier et al., 1994a; Wood et al., 1994; Aksoy and Weinshilboum, 1995; Raftogianis et al., 1996). In this study, the primary transcript from 5′RACE and RT-PCR of HepG2 cells contained the previously identified distal 5′UTR of SULT1A1 (Zhu et al., 1993b). When assessing the promoter activities of sequences flanking all 5′UTRs reported, this distal 5′UTR B was shown to house the most active and primary promoter in the liver-like environment of hepatocarcinoma cell lines and primary human hepatocytes. This promoter lacks a canonical TATA box element but contains G/C-rich regions near its transcriptional start site. High activity of the distal promoter was also seen in other cell lines, other than those derived from a liver origin, such as MCF-7 and Caco2 (data not shown), suggesting a ubiquitous mechanism of transcriptional control of the *SULT1A1* gene in a variety of tissues.

Although the SULT1A1 and SULT1A3 enzymes share >93% amino acid homology, their tissue-specific expression varies markedly, best demonstrated by the high abundance of SULT1A1 in the adult liver compared with very low SULT1A3 levels (Fig. 1, f and g; Windmill et al., 1998; Richard et al., 2001). The human *SULT1A1* and *SULT1A3* genes share >70% sequence identity, with highest diversity being present in the promoter and intron regions. A cDNA species containing a 5′UTR homologous to the distal 5′UTR identified for SULT1A1 was also found for SULT1A3 cDNAs isolated from a liver (Aksoy and Weinshilboum, 1995) and placental (Bernier et al., 1994b) library. Other 5′UTRs further upstream of this have also been reported (Zhu et al., 1993a; Bernier et al., 1994b; Aksoy and Weinshilboum, 1995). Bernier et al. (1994a) reported that the *SULT1A3* sequence flanking the 5′UTR homologous to the *SULT1A1* distal 5′UTR B has higher promoter activity than that flanking the other *SULT1A3* 5′UTR, which was confirmed in this study. This suggests that each gene uses a homologous region of its

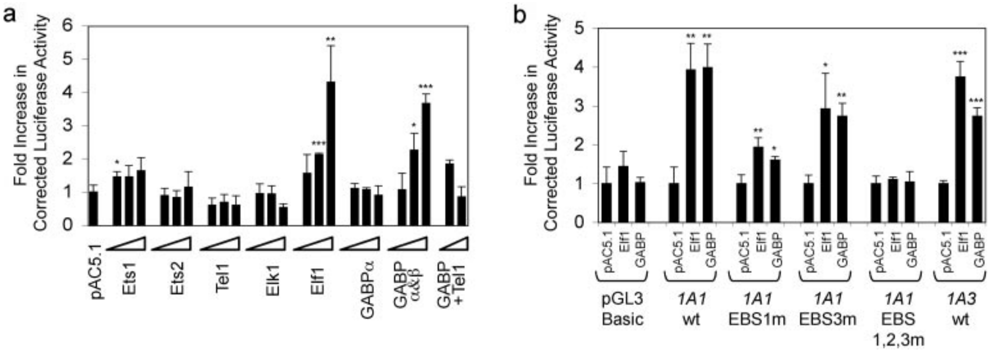

Fig. 7. a, Ets factors influence *SULT1A1* −112/+65 promoter activity in S2 cells. S2 cells were transfected as indicated under *Materials and Methods* with 1 μg of *SULT1A1* −112/+65 promoter in pGL3Basic and 1, 2.5, or 5 μg of each Ets transcription factors in pAC5.1 vector. The last two columns represent 5 μg of GABPα and β cotransfected with 2.5 and 5 μg of Tel. Luciferase activity of lysed cells was normalized against total protein, and data represent the mean ± S.D. of three transfections and are expressed relative to promoter activity cotransfected with empty pAC5.1 vector. Asterisks indicate significant differences to the activity of the promoter transfected with pAC5.1 (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). b, effects of Ets factors on *SULT1A1* −112/+65 EBS mutant and *SULT1A3* promoter constructs. S2 cells were transfected as indicated under *Materials and Methods* with 5 μg of Ets factors and 1 μg of promoter constructs in pGL3Basic vector. Luciferase activity was assessed as stated above and is expressed relative to each promoter construct's activity cotransfected with empty pAC5.1 vector. Asterisks indicate significant differences to the activity of each promoter construct transfected with pAC5.1 (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

gene sequence as promoter. When the activities of the minimal homologous promoters were compared, the *SULT1A1* −112/+65 promoter was shown to have 3-fold higher activity than the *SULT1A3* −125/+43 promoter construct, although sharing >77% sequence identity in this region. This difference seemed to be caused by a lack of one EBS core sequence motif, GGAA. The importance of this binding site as a crucial element in the regulation of the *SULT1A1* promoter was highlighted when exchanging two bases in EBS1 could effectively reverse the activities of the two SULT1A promoters. Furthermore, it was shown that the adjacent EBS motifs were also important for *SULT1A1* promoter activity and determining optimal binding of Ets factors to this site. An EBS motif is generally made up of the purine-rich GGAA/T core, which is the binding site for the 85-amino acid ETS domain of Ets transcription factors (Graves and Petersen, 1998). More than 30 human members of this transcription factor family have been isolated, which have been shown to control a variety of genes, including viral genes, antigen receptors, transcription factors, cytokines, growth factors, myeloid- and lymphoid-specific genes, and genes involved in angiogenesis (Oikawa and Yamada, 2003). Because of the wide expression profile of SULT1A1, ubiquitously expressed Ets transcription factors were targeted as potential regulators of *SULT1A1* gene regulation. It was found that ubiquitously expressed Ets factors GABPα and β and Elf1 from hepatocarcinoma cell lines were able to bind the *SULT1A1* EBS probe and that recombinant forms of these factors could induce the *SULT1A1* promoter in S2 cells.

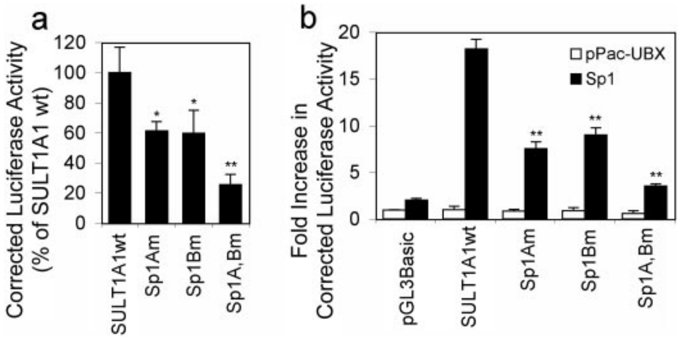

Fig. 8. a, influence of Sp1 site mutations on *SULT1A1* −112/+65 promoter activity in HepG2 cells. Sp1 site mutations were made using site-directed mutagenesis (Fig. 4a), transfected into HepG2 cells, and lysed cells were assayed for luciferase activity as stated under *Materials and Methods*. Results are corrected for pRLSV40 internal *R. reniformis* luciferase standard and expressed relative to the activity of the *SULT1A1* −112/+65 construct (mean ± S.D.; $n = 3$). Asterisks indicate significant differences to this construct (*, $p < 0.05$; **, $p < 0.01$). (b) Influence of Sp1 on *SULT1A1* −112/+65 promoter and Sp1 site mutants in S2 cells. S2 cells were transfected as indicated under *Materials and Methods* with *SULT1A1* promoter wild-type (wt) and Sp1 mutant constructs (Fig. 4a) in pGL3Basic and 2 μg of Sp1 transcription factor in pPac-UBX vector. Luciferase activity of lysed cells was normalized against total protein, and data represent the mean ± S.D. of three transfections. Luciferase activity was expressed relative to each promoter construct's activity cotransfected with empty pPac-UBX 5.1 vector. Asterisks indicate significant differences to the activity of the SULT1A1 −112/+65 wild-type promoter activity cotransfected with Sp1 (**, $p < 0.01$).

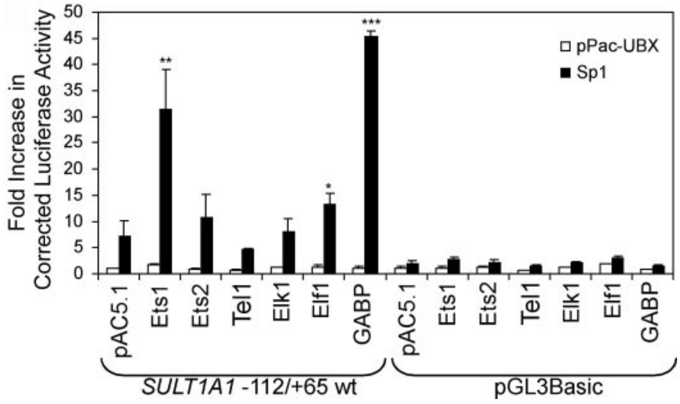

Fig. 9. Sp1 and Ets transcription factors GABP and Ets1 act synergistically on the *SULT1A1* −112/+65 promoter. *SULT1A1* −112/+65 promoter in pGL3Basic (wt) or the empty pGL3Basic vector were cotransfected with 5 μg of Ets transcription factors in pAC5.1 vector and either 2 μg of Sp1 or empty pPac-UBX vector into S2 cells as indicated under *Materials and Methods*. Luciferase activity of lysed cells was normalized against total protein, and data represent the mean ± S.D. of three transfections. Results are expressed as fold increases from the promoter activity of the *SULT1A1* −112/+65 construct cotransfected with empty Ets transcription vector pAC5.1 and empty Sp1 transcription vector pPac-UBX. Asterisks indicate significant differences to the activity of the SULT1A1 −112/+65 construct transfected with Sp1 and pAC5.1 (**, $p < 0.01$; ***, $p < 0.001$).

Ets factors have been shown to act in synergy with a variety of transcription factors, including Sp1 (Oikawa and Yamada, 2003). Mutations of the Sp1 consensus binding sites flanking the *SULT1A1* EBS were shown to influence the promoter's activity, and Sp1 was shown to significantly enhance the GABP induced activity of the SULT1A1 promoter in S2 cells. This synergistic effect between GABP and Sp1 has been observed in other promoters, such as those of the human *utrophin* and *heparanase-1* genes, which are also TATA-less promoters (Galvagni et al., 2001; Jiang et al.,

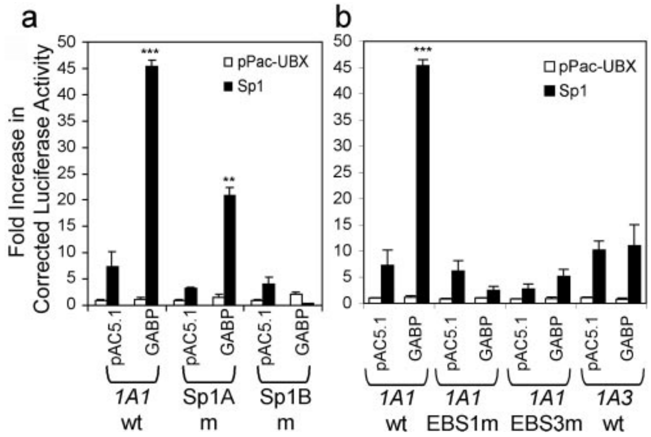

Fig. 10. a, influence of Sp1 site mutants on GABP-Sp1 synergy. *SULT1A1* promoter constructs were transfected with either GABP or empty vector pAC5.1 (5 μg) and Sp1 or empty vector pPac-UBX (2 μg) as indicated under *Materials and Methods*. Luciferase activity of lysed cells was normalized against total protein, and data represent the mean ± S.D. of three transfections, and are expressed as fold increase from the promoter activity of −112/+65 construct cotransfected with empty Ets transcription vector pAC5.1 and empty Sp1 transcription vector pPac-UBX. Asterisks indicate significant differences to the activity of each promoter construct transfected with Sp1 and the empty expression vector pAC5.1 (**, $p < 0.01$; ***, $p < 0.001$ b, the *SULT1A3* promoter cannot support GABP-Sp1 synergy as all EBS sites are required. *SULT1A1* and *SULT1A3* promoter constructs were transfected with either GABP or empty vector pAC5.1 (5 μg) and Sp1 or empty vector pPac-UBX (2 μg). Luciferase activity was assessed as described above with results expressed as fold increase from the promoter activity of −112/+65 construct cotransfected with empty Ets transcription vector pAC5.1 and empty Sp1 transcription vector pPac-UBX. Asterisks indicate significant differences to the activity of each promoter construct transfected with Sp1 and the empty Ets expression vector pAC5.1 (***, $p < 0.001$).

2002). It has been shown that Sp1 and Sp3 can directly interact with GABPα, but not GABPβ (Galvagni et al., 2001). Sp3 was also able to act in synergy with GABP to induce the *SULT1A1* promoter, although less effectively than Sp1 (data not shown). It was recently shown that Sp1 regulates the promoters of human *PAPS synthase 1* and *2*, encoding the enzyme responsible for the synthesis of the sulfonate donor PAPS (Shimizu et al., 2001, 2002). Sp1 is an important transcription factor that is necessary for development and cell cycle regulation. It also seems to be required for the regulation of the enzymes involved in sulfonation.

Interestingly, no synergy between GABP and Sp1 could be observed in the induction of the *SULT1A3* promoter, which lacks EBS1. Although individually Ets factors and Sp1 were able to activate this promoter, the presence of the three EBS repeats seems to be necessary for correct binding of Ets factors to enable interaction with Sp1. GABP was able to synergistically enhance the Sp1-induced activity of the *SULT1A1* promoter 3-fold, which can be correlated to the same difference in activity observed between wild-type *SULT1A1* and *SULT1A3* promoters in the hepatocarcinoma cell lines. These data provide an insight into the differential regulation between the *SULT1A1* and *SULT1A3* genes in the adult liver. mRNA levels of SULT1A1 seem to be approximately 3- to 4-fold higher than those of SULT1A3 in primary human hepatocytes, which correlates with the difference in promoter activities observed. SULT1A3 protein is generally not readily detectable in the adult liver and was not seen in hepatocyte cytosol, unlike SULT1A1. This suggests that post-transcriptional modifications or protein stability differences between SULT1A1 and SULT1A3 may also contribute to the ultimate expression level of SULT1A3 in the adult liver. The abundant expression pattern of SULT1A1 suggests that this carcinogen-converting enzyme plays an important role in the detoxification and metabolic activation of xenobiotics entering the body through tissues such as skin, gut, and liver (Windmill et al., 1998; Dooley et al., 2000). The role of ubiquitously expressed transcription factors in the regulation of SULT1A1 may ensure constant expression of this enzyme at portals of entry for these compounds, to facilitate their rapid metabolism and elimination from the body.

The third gene of the SULT1A subfamily, *SULT1A2*, was shown to have a highly active promoter flanking its distal 5′UTR, which shares >95% sequence identity with the minimal *SULT1A1* promoter described in this study (data not shown). This promoter contains the same EBS and Sp1 binding sites, suggesting that it shares a common mechanism of regulation with *SULT1A1*.

In conclusion, this study represents the first report elucidating the mechanisms of regulation of the human *SULT1A* sulfotransferase genes. The activity of the *SULT1A1* promoter seems to be dependent on a triplicate repeat of the EBS core sequence GGAA. The ubiquitously expressed Ets transcription factors Elf and the GABP heterodimer are able to bind and induce transcriptional activation of the *SULT1A1* gene, of which the activation by GABP was synergistically enhanced by the presence of Sp1. Moreover, comparison with the related *SULT1A3* sequence, which has a two-base pair mutation in one EBS core sequence revealed that the lack of this site abolished the synergy observed between GABP and Sp1.

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