Sp1 elements in *SULT2B1b* promoter and 5′-untranslated region of mRNA: Sp1/Sp2 induction and augmentation by histone deacetylase inhibition

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Abstract The steroid/sterol sulfotransferase gene (*SULT2B1*) encodes for two isozymes of which one (SULT2B1b) sulfonates cholesterol and is selectively expressed in skin. The human *SULT2B1* gene contains neither a TATAAA nor a CCAAT motif upstream of the coding region for SULT2B1b; however, this area is GC-rich. Of five Sp1 elements identified two had regulatory activity utilizing immortalized human keratinocytes: one element is located above the ostensible transcription initiation site, whereas the other is located within the 5′-untranslated region of the SULT2B1b mRNA. Sp1 and Sp2 transcription factors identified by supershift analyses induced reporter gene activity, an effect markedly augmented by histone deacetylase inhibition.
Published by Elsevier B.V. on behalf of the Federation of European Biochemical Societies.

Keywords: Sulfonation; SULT2B1 promoter; Cis-element; Trans-factor; Chromatin structure

1. Introduction

Enzymes that catalyze the sulfoconjugation of hormones and neurotransmitters comprise a superfamily of cytosolic sulfotransferases (SULT), of which the SULT2 family, consisting of two subfamilies (SULT2A1 and SULT2B1), is engaged in the sulfoconjugation of steroids/sterols [19]. The gene for human *SULT2B1*, as a result of an alternative exon 1 and differential splicing, encodes for two mRNAs, i.e., SULT2B1a and SULT2B1b [10]. The use of exon 1A produces SULT2B1a, whereas to produce SULT2B1b exon 1B plus a portion of exon 1A is required. SULT2B1b efficiently sulfonates cholesterol,

while SULT2B1a avidly sulfonates pregnenolone [6]. Cholesterol sulfate, quantitatively the most significant sterol sulfate in human plasma, has emerged as a multifaceted molecule with noteworthy physiologic actions [26]. In the realm of keratinocyte development and barrier formation, cholesterol sulfate will activate isozymes of protein kinase C [5], inhibit cholesterol synthesis [29], induce the gene for transglutaminase I, an essential cross-linking enzyme involved in barrier formation [14], and regulate the gene for involucrin, a major cross-linked protein constituent of the insoluble cornified cell envelope [9]. Interestingly, cholesterol sulfate is a ligand for the retinoic acid-related orphan nuclear receptor α (RORα), whereby it significantly increases transcriptional activity [13]; furthermore, RORα is highly expressed in skin [25]. We previously reported on the selective expression of the SULT2B1b isoform in human skin as well as primary cultures of normal human epidermal keratinocytes undergoing calcium-induced differentiation [11], and in this communiqué present initial studies regarding transcriptional control of expression of the *SULT2B1* gene in human keratinocytes.

2. Materials and methods

2.1. Cell culture
Immortalized but highly differentiated human keratinocytes (HaCaT cells) {originally developed by Boukamp et al. [2] and a generous gift of Dr. Shyh-Ing Jang at the NIAMS, NIH, Bethesda, MD} were grown in Dulbecco's modified essential medium (DMEM) supplemented with antibiotics and 10% fetal bovine serum unless otherwise specified.

2.2. Isolation of human SULT2B1b 5′-flanking region and preparation of reporter gene constructs
DNA sequence of human *SULT2B1* gene obtained from the human genome project (GenBank Accession No. AC008403) was used to produce the 5′-flanking region of the SULT2B1b isoform by employing the Advantage Genomic PCR kit (BD Biosciences Clontech), human genomic DNA (BD Biosciences Clontech) as template and the appropriate sense and antisense primers flanked by *Kpn*1 and *Xho*1, respectively. PCR was performed as follows: denature at 95 °C for 30 s followed by 40 cycles of denaturation at 95 °C for 15 s then annealing/elongation at 68 °C for 3 min. The −3389 to −27 PCR product was gel purified (Qiagen) and sub-cloned into the pCR2.1 TA cloning vector (Invitrogen) prior to ligation with the pGL3 luciferase reporter gene expression vector (Promega) at *Kpn*1 and *Xho*1 restriction sites to produce the pGL3(−3389/−27) construct. Nota bene: the nucleotide (nt) numbering system employed throughout this manuscript complies with recommended practice [4]; thus, the nt immediately upstream of the translation initiation codon (ATG) has been denoted as −1.

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Abbreviations: SULT, Sulfotransferase; nt, nucleotide; TSS, transcription start site; UTR, untranslated region; RT, reverse transcription; EMSA, electrophoresis mobility shift assay; HDAC, histone deacetylase; TSA, trichostatin A

Various segments of SULT2B1b 5′-flanking region, i.e., −3189/−27, −951/−27, −401/−27, −331/−27, −212/−27, −179/−27, −148/−27 and −125/−27 were obtained by performing nested-PCR using the pGL3(−3389/−27) construct as template, appropriate sense primers and the common antisense primer (−55/−27) designed to be located well downstream of the 5′-end of the 5′-untranslated region (UTR) of SULT2B1b mRNA. Nested PCR was performed with pfu-Ultra DNA polymerase (Stratagene) as follows: denature at 95 °C for 1 min followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 62 °C for 30 s, and elongation at 72 °C for appropriate durations (1 min/kb of the expected length of each PCR product). PCR products were subcloned into the firefly luciferase expression vector at Kpn1 and Xho1 restriction sites. The additional constructs pGL3(−2940/−27), pGL3(−2286/−27), pGL3(−1740/−27) and pGL3(−548/−27) were generated by double digestions of the pGL3(−3389/−27) construct at, respectively, Kpn1/Spe1, Kpn1/Sma1, Kpn1/AatII, and Kpn1/Mlu1 restriction sites followed by blunting and ligation.

2.3. Reverse transcription (RT) PCR analysis

Total RNA was extracted from HaCaT cells using Absolutely RNA RT-PCR Miniprep kit (Stratagene). RT was performed using the ThermoScript RT-PCR system (Invitrogen). Briefly, the first cDNA strand was synthesized at 50 °C for 60 min using 1 μg of total RNA as template and random hexamer primers. PCR was performed using 2 μl cDNA reaction mix as template and gene-specific primer pairs using platinum Taq DNA polymerase (Invitrogen) under the conditions of denaturing at 94 °C for 2 min, followed by 30 cycles of denaturizing at 94 °C for 30 s, annealing at 58 °C for 30 s and extension at 72 °C for 30 s.

2.4. Transient transfection and reporter gene assay

HaCaT cells were seeded in 6-well plates at 2.5×10^5 cells/well 1 day prior to transfections that were performed with a selective pGL3/SULT2B1b upstream construct with or without $Renilla$ luciferase plasmid (Promega) as indicated in figure legends. Transfections were carried out using a 3-fold excess of SuperFect (Qiagen). Cells were harvested 48 h after transfection and both firefly luciferase and $Renilla$ luciferase activities were measured (Promega). All experiments were carried out in triplicate and repeated twice. In some experiments, 0.3 μM trichostatin A (TSA; Sigma) was added to the incubation medium 12 h prior to harvesting.

2.5. Site-directed mutagenesis

Transcription factor binding elements were altered using Quick Change Site-Directed Mutagenesis (Stratagene). Briefly, PCR was performed using 50 ng of the pGL3(−548/−27) construct and 10 pmol of sense and antisense primers (Table 1) under the conditions of denaturation at 95 °C for 30 s, followed by 18 cycles of denaturation at 95 °C for 30 s, annealing at a temperature determined by a formula supplied with instructions, and extension at 68 °C for 10 min. Reaction mixtures were treated with Dpn1. PCR products were amplified with XL10-Gold competent cells (Stratagene) and sequenced.

2.6. Electrophoresis mobility shift assay (EMSA)

EMSA was carried out as follows: double stranded oligonucleotides (Table 1) were end-labeled with ^{32}P using [γ-^{32}P] ATP (3000 Ci/mmol, Perkin–Elmer Life Sciences) and T4 polynucleotide kinase [22] and column-purified with a NucTrap probe purification column (Stratagene). EMSA was performed using labeled oligonucleotides ($\sim130 \times 10^3$ cpm), HaCaT cell nuclear extract (~5 μg) from Active Motive and a commercially available kit (Promega). Samples were subjected to electrophoresis using 6% (w/v) polyacrylamide gels in $0.5\times$ Tris/borate/EDTA buffer (0.178 M Tris/borate and 4 mM EDTA) at 4 °C for 2 h. For supershift analyses, nuclear extracts were preincubated with ~2 μg of antibody to human Sp1, Sp2, Sp3 or Sp4 (Santa Cruz Biotechnology) for 2 h at 4 °C prior to incubation with the probes and then subjected to electrophoresis using 3.5% polyacrylamide gels. Following electrophoresis, gels were dried and analyzed by autoradiography.

2.7. DNA sequence analysis

DNA sequence was analyzed for potential transcription factor binding using the TESS (Transcription Element Search System) web tool (http://www.cbil.upenn.edu/cgi-bin/tess/tess?RQ=WELCOME).

Table 1
Primers used for constructing, substitution mutants, RT-PCR and oligonucleotides for EMSA

Name	Sequence	Position
Primers for site-directed mutagenesis		
Sp1.2/M2(S)	5′-CACTGCTCCTCtttGCCCTCAGAGCAGGGTGGCTCC-3′	(−2327/−197)
Sp1.2/M2(A)	5′-GGAGCCACCCTGCTCTGAGGGCaaaGAGGAGCAGTG-3′	(−232/−197)
Sp1.5/M5(S)	5′-GAGAACCGGCTGGGTGCTGaaaCTCCCCTTGGGC-3′	(−154/−121)
Sp1.5/M5(A)	5′-GCCCAAGGGGAGtttCAGCACCCAGCCGGTTCTC-3′	(−154/−121)
Primers for RT-PCR		
RTP1(S)	5′-CTGCCCCTCCCCTTGGGCCGGGCACGGAGTAG-3′	(−138/−107)
RTP2(S)	5′-CTGGGTGCTGCCCCTCCCCTTGGGCCGGGCAC-3′	(−145/−114)
RTP3(S)	5′-AGAACCGGCTGGGTGCTGCCCCTCCCCTTGG-3′	(−153/−124)
RTP(S)	5′-AGCTGGGAGAACCGGCTGGGTGCTGCCCCTC-3′	(−160/−130)
RTP5(S)	5′-TAGCAGCTGGGAGAACCGGCTGGGTGCTG-3′	(−164/−136)
RTP6(S)	5′-TTGGAGGCGTGGATAGCAG-3′	(−177/−159)
RTP7(S)	5′-TGTTGGAGGCGTGGATAGCAGCTGGGAG-3′	(−179/−152)
RTP8(S)	5′-TGTTGGAGGCGTGGATAGC-3′	(−179/−161)
RTP9(S)	5′-GCCTCTCCCCGCTGTTGG-3′	(−191/−174)
RTP10(S)	5′-AGAGCAGGGTGGCTCCCTCTGGCCTCTC-3′	(−212/−185)
RTP11(A)	5′-AGATGATCTCGATCATCCAGGTCGTGCCTG-3′	(+212/+242)
Oligonucleotides for EMSA		
WT/Sp1.2	5′-ACTGCTCCTCCCCGCCCTCA-3′	(−231/−212)
	3′-IGACGAGGAGGGGCGGGAGT-5′	
M2/Sp1.2	5′-ACTGCTCCTttttCGCCCTCA-3′	(−231/−212)
	3′-TGACGAGGAaaaGCGGGAGT-5′	
WT/Sp1.5	5′-CGGCTGGGTGCTGCCCCTCCCCTTGGGCCG-3′	(−148/−119)
	3′-GCCGACCCACGACGGGGAGGGGAACCCGGC-5′	
M5/Sp1.5	5′-CGGCTGGGTGCTGaaaCTCCCCTTGGGCCG-3′	(−148/−119)
	3′-GCCGACCCACGACtttGAGGGGAACCCGGC-5′	

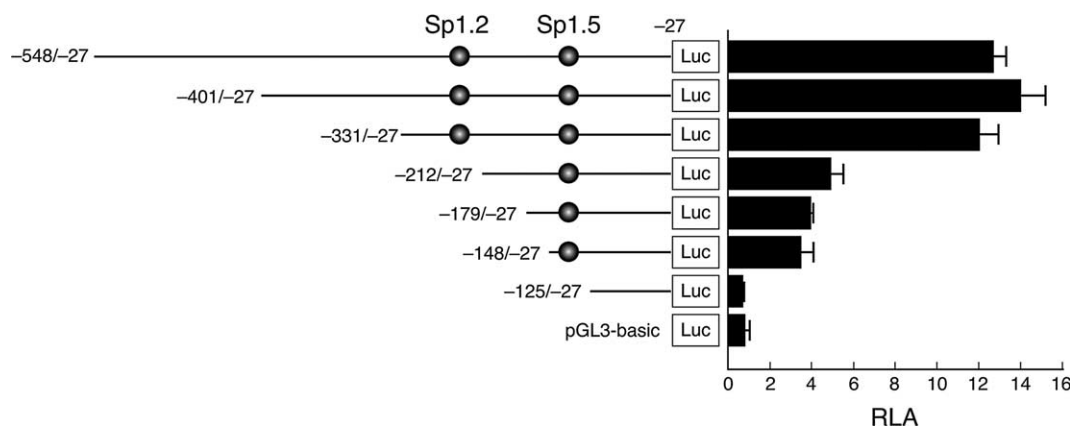Underlined and lower case letters indicate mutated bases. S, sense; A, antisense; WT, wild type.

Fig. 1. Various constructs (1.5 μg) of the 5′-flanking region fused to the firefly luciferase reporter gene vector were co-transfected along with *Renilla* luciferase expression vector (0.2 μg) into HaCaT cells. Firefly luciferase activity was normalized to *Renilla* luciferase activity and the relative luciferase activities (RLA) are presented as fold-increase over the promoterless pGL3 basic vector. The length of each 5′-flanking segment, which is relative to the translation initiation ATG codon (A is +1), is indicated to the left of each proportional line. The location of regulatory Sp1 sites is indicated with closed circles. Horizontal column lengths represent the average of multiple replicates and error bars are indicated.

3. Results

3.1. SULT2B1 promoter activity upstream of exon 1B

A 3363 base pair (bp) fragment of the human *SULT2B1* gene upstream of exon 1B and spanning nucleotides −3389 to −27 (relative to the ATG initiation codon) was cloned. When this sequence was examined for promoter activity by deletion analysis, a bimodal activity pattern was produced with a proximal peak in activity around nt −401 and a second peak near nt −3189 (data not presented). For our initial foray into regulation of this gene, we opted to concentrate on the more downstream or proximal promoter region, and a deletion analysis involving nucleotides −548 to −27 is depicted in Fig. 1.

3.2. Analysis of the proximal promoter region of human SULT2B1b

The DNA sequence of the SULT2B1b 5′-flanking region extending from −1 to −548 (relative to ATG) contains multiple potential transcription factor binding sites (Fig. 2), including AP-2(1), Ets-1(1), Ets-2(1), Pit-1a(4), NF-1(1), AP-1(1), AP-4(1) and Sp1(5) where numbers in parentheses denote the number of potential sites. Mutational analysis revealed that when these sites were individually altered and examined by transfection, either no effect on promoter activity (AP-1, AP-4 and NF-1) or modest decreases in activity ranging from ∼20% to ∼40% were noted (data not presented). Regarding the five Sp1 sites, mutation of three sites (Sp1.1, Sp1.3 and

Fig. 2. DNA sequence of the proximal promoter region of the human *SULT2B1* gene (+1 indicates the A in the ATG translation initiation codon). Potential transcription factor binding sites as revealed using the TESS Web Tool are underlined and labeled (multiple similar motifs are indicated by sequential numbering following a period).
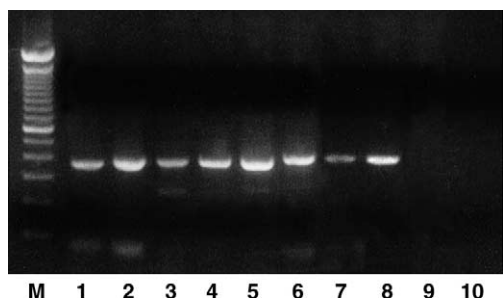
Fig. 3. RT-PCR products obtained using various sense primers located downstream and upstream of the purported TSS as previously reported [10]. Lanes 1–10 show results obtained using the correspondingly numbered sense primers RTP1, RTP2, RTP3, RTP4, RTP5, RTP6, RTP7, RTP8, RTP9 and RTP10 and a common antisense primer (RTP11) located in exon 2 (cf. Table 1).

Sp1.4) revealed little effect on promoter activity (data not presented), whereas mutation of Sp1.2 and Sp1.5 suggested regulatory activity, and altering the two sites concurrently led to complete loss in activity (vide infra). Combinations of either the Sp1.2 or Sp1.5 site with other Sp1 sites, as well as combinations of either of these Sp1 sites with other potential transcription factor binding sites noted above produced essentially the same result as mutating the sites individually (data not presented). It was noted that the Sp1.2 and Sp1.5 mutational results were consistent with results of the deletion analyses (cf. Fig. 1).

3.3. Transcription start site (TSS) of human SULT2B1b gene

In order to determine the relationship of regulatory Sp1.2 and Sp1.5 elements to transcription initiation, an examination of the TSS for SULT2B1b was undertaken by RT-PCR, a procedure that employed an antisense primer located in exon 2, and various overlapping sense primers ranging from nt −107

to nt −212 (Table 1). All sense primers up to primer 8 yielded PCR products, indicating that these sequences were part of SULT2B1b mRNA, whereas the 9th and 10th sense primers failed to yield PCR products indicating that these sequences were not part of SULT2B1b mRNA (Fig. 3). Based on the RT-PCR results, it was concluded the 5′-end of the 5′-UTR of SULT2B1b mRNA extends beyond nt −161, perhaps to nt-174 (cf. Fig. 2).

3.4. Electrophoresis mobility shift analysis

EMSA examination revealed that probes encompassing either the Sp1.2 or Sp1.5 site produced upshifted bands using HaCaT cell nuclear extracts (Fig. 4). The probe containing the Sp1.2 site produced 3 upshifted bands (Fig. 4A, lane 1), whereas the probe containing the Sp1.5 site produced 2 upshifted bands (Fig. 4B, lane 1). Specificity was ascertained when upshifted bands disappeared when radio-labeled probes were incubated with an excess of unlabeled counterparts (Fig. 4A/B, lane 2). When the Sp1 site within each probe was mutated, however, only the topmost upshifted bands disappeared (Fig. 4A/B, lane 3). That the topmost upshifted band represented both Sp1 and Sp2 protein was indicated by supershift analyses, i.e., antibody to either Sp1 or Sp2 produced supershifted bands (Fig. 4C/D, lanes 2,3). On the other hand, antibodies to Sp3 and Sp4 produced no supershifted bands (Fig. 4C/D, lanes 4 and 5).

3.5. Transfection of HaCaT cells with Sp1 and Sp2 expression vectors

The potential role of Sp1 and Sp2 on SULT2B1b proximal promoter activity was explored by co-transfecting HaCaT cells with Sp1 or Sp2 expression vectors singly or in combination along with the −548/−27 reporter gene construct. An increase in promoter activity with both Sp1 and Sp2 was consistently noted with Sp1 being more potent than Sp2, although a suboptimal combination of Sp1 and Sp2 produced a synergistic re-
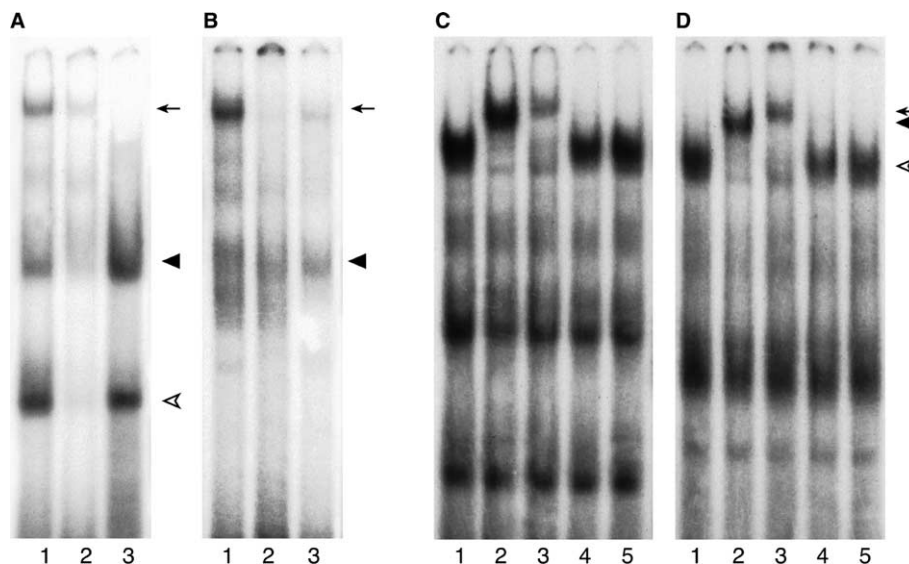

Fig. 4. EMSA involving probes containing either Sp1.2 (A and C) or Sp1.5 (B and D) motifs and HaCaT cell nuclear extracts. Panels A/B: lane 1, labeled probe; lane 2, labeled probe and 100-fold excess of unlabeled probe; lane 3, labeled mutant probe. Open arrowhead, closed arrowhead and small arrow denote upshifted bands. Panels C/D: lane 1, no antibody; lane 2, antibody to Sp1; lane 3, antibody to Sp2; lane 4, antibody to Sp3; lane 5, antibody to Sp4. Open arrowhead denotes band of Sp1/Sp2 protein–probe complex, whereas closed arrowhead and small arrow denote supershifted bands with antibodies to Sp1 and Sp2, respectively.
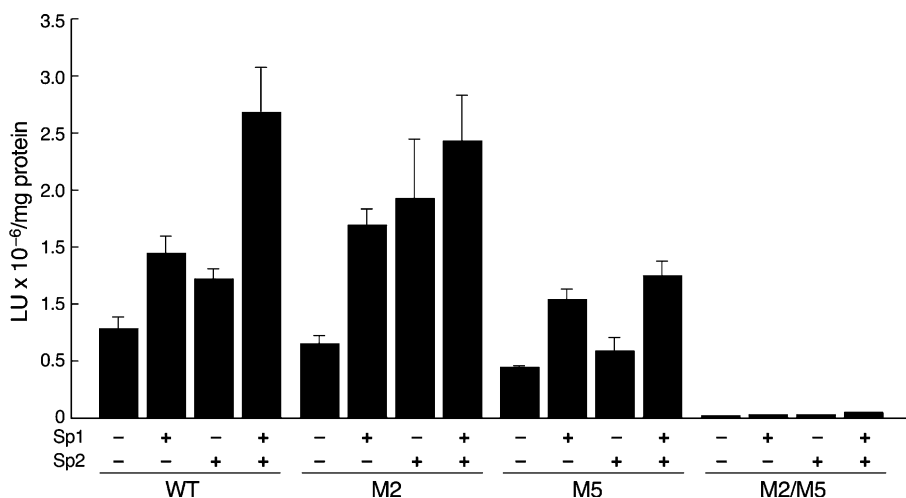
Fig. 5. Promoter activity of *SULT2B1* gene in response to cotransfection of HaCaT cells with Sp1 and Sp2 expression vectors. Cells were cotransfected with 1 μg of various forms of the proximal promoter construct pGL3(−548/−27), i.e., unmutated (WT), mutated Sp1.2 (M2), mutated Sp1.5 (M5) and combination of mutated Sp1.2 and Sp1.5 (M2/M5) along with 1.5 μg pRSVSp1 or 1.5 μg pCMVSp2 expression vectors (1 μg in combination experiments). M2 and M5 were obtained by site-directed mutagenesis using the WT pGL3(−548/−27) construct as a template and the site-directed mutagenesis primers listed in Table 1. The double mutant construct (M2/M5) was obtained using the M2 mutant construct as a template and M5 site-directed mutagenesis primer. Luciferase activity (LU) was determined 48 h after transfection and normalized to protein concentration. Basic pRSV vector was added to equalize the amount of DNA used. Column heights represent the average of multiple replicates, and error bars are indicated.

sponse (Fig. 5). Mutation of Sp1.2 produced only a modest decrease in promoter activity, whereas mutation of Sp1.5 produced a more significant decrease in promoter activity; however, when Sp1.2 and Sp1.5 were mutated simultaneously, promoter activity was lost (Fig. 5). Co-transfection of Sp1 and/or Sp2 with mutation of the Sp1.2 site yielded similar results as with wild type. In contrast, co-transfection of Sp1 and/or Sp2 with mutation of the Sp1.5 site resulted in a significant reduction in promoter activity compared to wild type (Fig. 5). When TSA, the histone deacetylase (HDAC) inhibtor, was added to the HaCaT cell culture medium, a striking augmentation in reporter gene activity occurred (Fig. 6). Furthermore, TSA plus Sp2 increased promoter activity 60%, whereas TSA plus Sp1 increased promoter activity 4-fold compared to TSA alone

(Fig. 6). The presence of TSA with a mutated Sp1.2 site yielded a paradoxical increase in promoter activity over wild type in contrast to TSA with the mutated Sp1.5 site, which resulted in a clear decrease in promoter activity (Fig. 6). As in the case when TSA was not present, mutation of the two Sp1 sites simultaneously resulted in a complete loss of promoter activity even in the presence of TSA (Fig. 6).

4. Discussion

The gene for human *SULT2B1* contains neither a canonical TATAAA nor a CCAAT motif in the upstream region flanking exon 1B that encodes for the SULT2B1b isoform; nor is
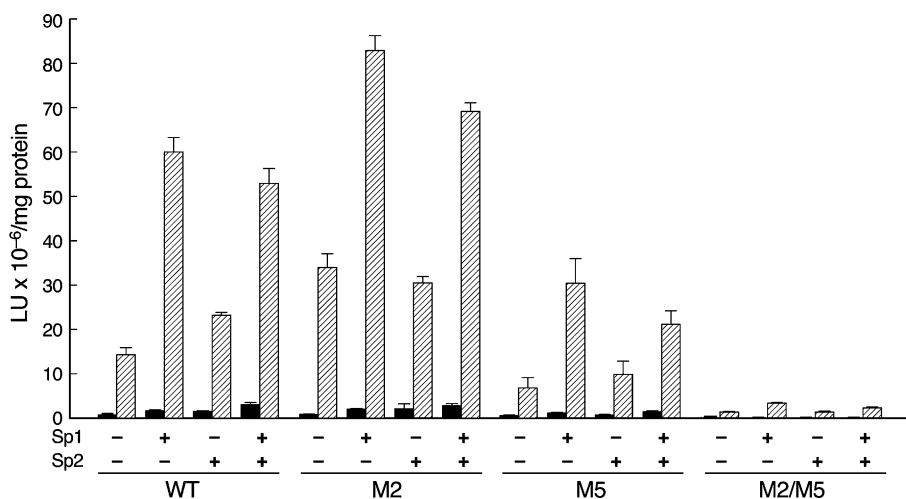

Fig. 6. The experiments described in Fig. 5 were repeated in the presence of 0.3 μM TSA, which was added 12 h prior to harvesting cells for determination of luciferase activity. For comparative purposes, data in Fig. 5 are included here using the same scale used for the (+)TSA results. Column heights represent the average of multiple replicates, and error bars are indicated.

there an initiator motif, an element overlapping the TSS of many TATA-lacking as well as TATA-containing promoters [24]. To establish the TSS for *SULT2B1* expression of the SULT2B1b isoform, a variety of methods were utilized, including primer-extension, RNase protection and the RNA ligase-mediated rapid amplification of cDNA ends (RACE). The sum result of these studies suggested the existence of multiple start sites. While some TATA-less promoters retain the ability to direct transcription initiation from a specific nt, others appear to direct transcription initiation from multiple sites [24]. Nevertheless, because of the variability in potential start sites, RT-PCR was employed to determine at least a minimal length for the dominant SULT2B1b mRNA 5′-UTR (assuming multiple species exist). These results suggested the 5′-end of the SULT2B1b mRNA extends beyond nt −161 and may extend to nt −174 upstream of the ATG initiation codon, a location creating a 5′-UTR length significantly longer than any size determined by the other methods employed. Placing the 5′-end at nt −174 is more in keeping with the original report of the 5′-end of the SULT2B1b mRNA being located at nt −149 upstream of the start of translation as determined by the standard 5′-RACE procedure [10]. While we do not have precise knowledge of the TSS for the SULT2B1b isoform, nevertheless, locating the TSS between nt −161 and nt −174 upstream of the translation initiation codon seems reasonable.

Many TATA-less promoters are characterized by the presence of multiple GC boxes, which bind the Sp1 transcription activator forming a central role in the assembly of the transcription complex of these promoters [21]. Regulation of the *SULT2B1* gene expressing the SULT2B1b isoform is at least partly under the influence of the Sp1 family of transcription factors, i.e., the area upstream of the coding region of SULT2B1b contains multiple GC/GT boxes, and mutational analyses indicated involvement of GC/GT boxes located between nt −215 and nt −221 (Sp1.2) and between nt −127 and nt −136 (Sp1.5) in transcriptional regulation. Furthermore, progressive shortening of the 5′-flanking region yielded results entirely consistent with the mutational experiments. Locating the TSS at nt −161 places the two regulatory Sp1 elements above and below the TSS, i.e., Sp1.2 is 23 nt upstream of the TSS, whereas Sp1.5 is 47 nt downstream of the TSS placing it within the 5′-UTR, and transcriptional regulation within the 5′-UTR is known [1,28]. Promoter activity within exon 1 represents a positive internal regulatory sequence [17], an effect that is commonly due to the presence of functional Sp1 binding elements [17,8,18,23,3].

Support for the Sp1 family regulating the human *SULT2B1* gene was also obtained when nuclear extracts from HaCaT cells expressing SULT2B1b were found to contain proteins that bound to probes containing Sp1 sites as well as to the presence of Sp1 and Sp2 proteins in those nuclear extracts. Additionally, co-transfection of HaCaT cells with Sp1 and/or Sp2 expression vectors produced dose-dependent increases in promoter activity, although transcriptional activation by Sp2 was less potent than that produced by Sp1. Interestingly, co-expression of Sp1 and Sp2 in suboptimal amounts produced a synergistic effect. Use of a HDAC inhibitor resulted in a dramatic augmentation in reporter gene activity induced by Sp1, an effect not seen with Sp2. Furthermore, the synergism produced by Sp1 and Sp2 in the absence of TSA treatment, was not demonstrable in the presence of TSA, i.e., the level of transcriptional activation with Sp1 and Sp2 in the presence of TSA

was no greater than that which occurred with Sp1 alone plus TSA. It was thus concluded that, whereas Sp1 functions as a transactivator of the *SULT2B1* gene regulating expression of the SULT2B1b isoform, the role of Sp2 is less clear despite its apparent ability to enhance the effect of Sp1 in experiments where HDAC inhibition was not employed. Stimulation of promoter activity seen with TSA alone in the absence of Sp1 co-transfection might have been due in part to the presence of endogenous Sp1 in HaCaT cells. That Sp1-stimulated SULT2B1b promoter activity by inducing histone acetylation was suggested when the effect of HDAC inhibition was lost with mutation of the regulatory Sp1-binding sites. Additionally, it is possible that TSA activation of promoter activity is mediated by stabilizing acetylation of Sp1 itself, which in turn could recruit other factors to the active site [12].

A major hurdle in activating transcription is the presence of a nucleosomal barrier that limits access of the transcription machinery to DNA templates [20]. Mechanisms exist, however, that enable cells to relieve nucleosomal repression such as modification of chromatin components, particularly histone acetylation [15], which promotes gene transcription by making promoter sequences accessible to transcription factors [7], whereas the association with HDAC contributes to the suppressive activity of several transcription factors [15]. Additionally, acetylation of nonhistone proteins, e.g., transcription factors as noted previously for Sp1, also occurs, which can have regulatory implications [15]. Activation of a given promoter requires multiple transcription factors that bind cooperatively to their cognate sites or possibly act synergistically by other mechanisms. A unique feature of Sp1 as a transcription factor is its synergistic activation and interaction with other transcription factors [16]. In this regard, there were two unidentified proteins, in addition to the Sp1 and Sp2 proteins that specifically bound to probes containing the Sp1 motifs, and an effort is currently under way to identify these proteins. It is known that Sp1 and related family members are not the only proteins to recognize GC/GT boxes. Several other zinc-finger proteins have been found to have a binding specificity similar to Sp1 [27]. Of course, this initial report on transcriptional regulation of the *SULT2B1* gene represents only the tip of the iceberg.

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