0013-7227/06/$15.00/0
Printed in U.S.A.

Endocrinology 147(5):2481–2489

doi: 10.1210/en.2005-1166

Indirect Regulation of Human Dehydroepiandrosterone Sulfotransferase Family 1A Member 2 by Thyroid Hormones

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Thyroid hormone, T_3, regulates cell metabolism, differentiation, and development. cDNA microarrays were performed to study the mechanism of target gene regulation after T_3 treatment in a thyroid hormone receptor-α (TRα)-overexpressing hepatoma cell line (HepG2-TRα). The differentially expressed target genes are several metabolic enzymes, including dehydroepiandrosterone-sulfotransferase family 1A member 2 (SULT2A1). Enzyme SULT2A1 was elevated roughly 5-fold at the protein level and 9-fold increase at the mRNA level after 48 h T_3 treatment in HepG2-TRα cells. Cycloheximide inhibited T_3-induced *SULT2A1* expression, suggesting that regulation was indirect. SULT2A1 has been reported to be regulated by the two transcription factors, steroidogenic factor 1 (SF1) and GATA, in the human adrenal gland. T_3 induced a 2.5- to 3.5-fold elevation of SF1 at the protein level and a 6.2-fold increase at the RNA level in HepG2-TRα cells. About seven SF1 binding sites exist on the *SULT2A1* gene. To identify and localize the critical SF1 binding site, series of deletion mutants of *SULT2A1* promoter fragments in pGL2 plasmid were constructed. The promoter activity of the *SULT2A1* gene was enhanced about 2.8- to 7.1-fold by T_3. The −228 SF1 binding site was identified as the most critical site because deleting this region reduced T_3-induced expression. Transcription factor SF1 application enhanced the −228 but not −117 reporter plasmid activities. SULT2A1 and SF1 up-regulation at protein and RNA levels in thyroidectomized rats occurred after T_3 application. In summary, this work demonstrated that the *SULT2A1* gene was mediated by SF1 and indirectly regulated by T_3. Further study is required to elucidate the physiological importance of SULT2A1 induction mediated by T_3. (*Endocrinology* 147: 2481–2489, 2006)

THE ACTIONS OF thyroid hormones, which regulate growth, development, and differentiation, are mediated by thyroid hormone nuclear receptors (TRs) (1). These TRs are derived from two genes, TRα and TRβ, which are located on chromosomes 17 and 3, respectively (2). Each gene expresses two receptor isoforms, α1 and α2 and β1 and β2, as a result of alternate splicing of their primary transcripts (3). The gene-regulating activity of TRs is mediated by binding to specific DNA sequences, known as thyroid hormone response elements (TREs), located at the promoter regions of thyroid hormone target genes (3). The transcriptional activity of TRs depends on not only T_3 but also the type of TREs. Recent studies showed that the transcriptional activity of TRs is further modulated via interaction with four cellular protein groups: 1) members of the nuclear receptor superfamily, notably retinoid X receptors (4); 2) corepressors, including p270/nuclear receptor corepressor (5), silencing mediator of retinoid and thyroid receptors (6), and T_3 receptor-associating cofactors (7); 3) coactivator steroid receptor coactivator-1 (8); and, 4) tumor suppressor p53 (9).

Mammalian sulfotransferase (SULT) has been classified into at least two groups (SULT1 and SULT2 families) based on the similarities in their amino acid sequences and enzymatic properties (10). Enzymes included in the SULT1 and SULT2 families transfer sulfonate to hydroxy groups of phenols and alcohols, respectively (11, 12). SULT2A1 is a cytosolic enzyme that mediates the sulfate conjugation of many hydroxysteroid substrates including bile acids, pregnenolone, estrogens, androgens, androgen precursor dehydroepiandrosterone, benzylic alcohol procarcinogens, and other hormonal or xenobiotic substrates (13, 14). Human SULT2A1 expression occurs predominantly in the liver, intestine, and adrenal glands (13, 15–17). Sulfonation typically generates stable and relatively polar conjugates that are recognized and eliminated from a cell (detoxification) through the facilitated action of ATP-binding cassette membrane transporters expressed on hepatocyte membranes (18). Although the enzymatic activity of SULT2A1 has been examined in detail, the regulation of human SULT2A1 expression in tissues remains unclear. Identifying the mechanisms regulating *SULT2A1* gene expression may help elucidate the mechanisms of steroid hormones and xenobiotic detoxification.

First Published Online February 9, 2006

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Abbreviations: CAR, Constitutive androstane receptor; D1, type I deiodinase; DHEA, dehydroepiandrosterone; DHEA-S, DHEA sulfate; ERR, estrogen-related receptor; FXR, farnesoid X receptor; Q-RT-PCR, quantitative RT-PCR; SF1, steroidogenic factor 1; SULT, sulfotransferase; Td, T_3 depleted; TR, thyroid hormone nuclear receptor; TRE, thyroid hormone response element; TX, thyroidectomy.

***Endocrinology* is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**

Previous studies have demonstrated that *TR* gene expression and regulation in nine human hepatoma cell lines (19). However, the mechanisms behind the selective maintenance of liver-specific gene transcription by TRα1 remain unclear. The liver is a target organ for TRs and is the principal site of blood protein synthesis during coagulation. Notably, Chamba *et al.* (20) by Western blot analysis identified the abundance of both TRα1 and TRβ1 in normal human liver is 0.8:1.08 absorbance units. Their results revealed significant amounts of TRα1, TRα2, and TRβ1 proteins in human hepatocytes (20). The well-differentiated hepatocellular carcinoma cell-line HepG2 secretes all 15 plasma proteins. Thus, using HepG2 cell lines can serve as an *in vitro* model system for investigating cell type-specific and TR isoform-specific regulation of T_3 target genes in the liver.

Previously Shih *et al.* (21) demonstrated that the T_3-enhanced up-regulation expression of 148 genes is time course dependent. Among these genes are those involved in metabolism, detoxification, signal transduction, cell adhesion, and cell cycles. Notably, several of these genes are essential in cell metabolism, which is traditionally associated with thyroid hormone function. Thus, this investigation focused on the *SULT2A1* gene and further verified its response to T_3 treatment at RNA and protein levels. Human SULT2A1 is a metabolic enzyme; however, the role of TR in the process of sulfonation by SULT2A1 is currently unknown.

Materials and Methods

Cell culture

Human hepatoma cell lines, HepG2-TRα1#1, HepG2-TRα1#2, HepG2-TRβ1, and HepG2-Neo were routinely grown in DMEM supplemented with 10% (vol/vol) fetal bovine serum. Three TR-overexpressing lines, and the control cell line HepG2-Neo, have been elucidated previously (22). The serum T_3 was depleted (Td) as in the manner described by Samuels *et al.* (23). Cells were cultured at 37 C in a humidified atmosphere of 95% air-5% CO_2.

Immunoblot analysis

Total cell lysates were prepared and protein concentration was measured using a Bradford assay kit (Pierce Biotechnology, Rockford, IL). Equal amounts of protein per lane were fractioned by SDS-PAGE on a 10% gel. Separated proteins were transferred to a nitrocellulose membrane and subsequently visualized via chemiluminescence using an ECL detection kit (Amersham Inc., Piscataway, NJ) as described previously (21). Antibodies used were rabbit polyclonal antibodies to SULT2A1 (Oxford Biomedical Research, Oxford, MI) and steroidogenic factor 1 (SF1; Upstate, Waltham, MA) (both at 1:1000 dilution in PBS). The intensities of immunoreactive bands were quantified using Image Gauge software (Fuji Film, Tokyo, Japan).

Northern blot analysis

Total RNA was extracted from the cells with TRIzol Reagent (Life Technologies, Rockville, MD). Equal amounts of total RNA (20 μg) were analyzed on a 1.2% agarose-formaldehyde gel as described previously (21, 22, 24, 25). Separated RNA molecules were then transferred to a nitrocellulose membrane and subjected to Northern blot analysis as described previously (21, 26).

Quantitative RT-PCR (Q-RT-PCR)

Total RNA was extracted from cells using TRIzol as described. Subsequently cDNA was synthesized using the Superscript II kit for RT-PCR (Life Technologies) as described previously (21). The genomic DNA contamination was eliminated using ribonuclease-free deoxyribonuclease (Promega Corp., Madison, WI) digestion.

The primer pairs for SULT2A1, SULT1A1, SULT1A3, SULT1B1, GATA, and SF1 were designed using Primer Express software (Applied Biosystems, Foster City, CA). Real-time Q-RT-PCR was conducted in a 15-μl reaction mixture containing 25 nM forward and reverse primers, 1× Syber Green reaction mix (Applied Biosystems), and varying quantities of template as described previously (21). Syber Green fluorescence was measured with the ABI PRISM 7000 sequence detection system (Applied Biosystems), as described previously (21, 24). The genes were normalized against the ribosomal binding protein (*RiboL35A*) gene, as specified in user bulletin no. 2 (Applied Biosystems).

Determine the stability of SULT2A1 mRNA

HepG2-TRα1#1 cells were treated with or without T_3 for 48 h to determine the stability of *SULT2A1* mRNA. Thereafter cells were incubated with 2 μg/ml actinomycin D for up to 9 h. At the indicated times, they were harvested and total cellular RNA was prepared and analyzed by Q-RT-PCR.

Cloning the SULT2A1 5′-flanking region and promoter activity assay

Fragments of the *SULT2A1* promoter (nucleotides −1463/+1; the translational start site was +1) were amplified via PCR, according to the published nucleotide sequence (27), and then inserted into the pGL2 vector (Promega). The promoter construct sequence was verified by automated DNA sequencing. To measure the influence of T_3 on the transcriptional activity of the *SULT2A1* promoter, HepG2-TRα1#1 cells (1×10^5 per 12-well plate) were cotransfected via a Lipofectamine protocol using 1 μg/well of pGL2 vector containing *SULT2A1* promoter sequences (Invitrogen Corp., Carlsbad, CA) as described previously (21). SF1 expression plasmid was a generous gift from K. L. Parker (University of Texas Southwestern Medical Center, Dallas, TX).

Preparation of nuclear extracts

Nuclear extracts were prepared as previously described with minor modifications (28). Briefly, HepG2-TR cells or rat liver tissues were collected and washed once in 10 volumes of PBS. The cells were then suspended in five volumes of cold buffer A [10 mM Tris (pH 7.4); 2 mM $MgCl_2$; 5 mM dithiothreitol; 1 mM phenylmethylsulfonyl fluoride; 2 μM leupeptin, and aprotinin] and incubated on ice for 15 min. Cells were lysed using 20 strokes of a Dounce homogenizer. The lysated cells were centrifuged at $1300 \times g$ for 10 min. The pelleted nuclei were washed three times in buffer A without Nonidet P-40, centrifuged again as described above, resuspended in one volumes of cold buffer B [20 mM Tris (pH 8.0), 2 mM EDTA, 1 mM dithiothreitol, 400 mM KCl, 5% glycerol, 0.5 mM phenylmethylsulfonyl fluoride], and incubated on ice for 60 min. The solution was then centrifuged at $48{,}000 \times g$ for 1 h. The supernatant was collected for further study.

Animals

Twelve male Sprague Dawley rats underwent thyroidectomies (TX) at 6 wk of age according to method used in previous reports (29). Each rat was given 1% calcium lactate in drinking water after surgery. Two weeks after surgery, each rat was injected ip with T_3 at 10 μg per 100 g body weight or a control vehicle (2.5 mM NaOH in PBS) daily for 2 further weeks. Rats were killed at the end of the experiment, and their serum was used for T_3 and TSH determination. Expression levels of several proteins in the liver were analyzed by Q-RT-PCR or a Western blot analysis. All animal experiments in this study were performed in accordance with National Institutes of Health guidelines and the Chang-Gung Institutional Animal Care and Use Committee Guide for Care and Use of Laboratory Animals.

Results

SULT2A1 was regulated by T_3 in HepG2-TR cell lines

Previously the authors of this study used cDNA microarrays to identify genes induced by T_3 in HepG2-TR cells. The

gene *SULT2A1* was selected for the following reasons: control of its expression by T_3 and TR has not been studied; and T_3 induced an 8-fold expression of *SULT2A1* (microarray result, data not shown). The *SULT2A1* gene is crucial to the rapid detoxification of xenobiotic and sterol hormone metabolism. However, elucidation of the underlying mechanisms by which T_3 regulates this gene requires further study. Although T_3 also induced *SULT1A1* expression, by microarray data, the result was verified by Q-RT-PCR. The *SULT1A1* mRNA levels were induced by 6- to 7-fold after the incubation of HepG2-TRα 1#1 cells with 10 nM T_3 for 48 h (data not shown). However, the mRNA levels of other SULTs, such as *SULT1A3* and *SULT1B1*, were not induced by T_3 (data not shown).

To further examine the regulation of the *SULT2A1* gene by T_3, isogenic HepG2 cell lines that consistently express wild-type TRα1 (HepG2-TRα1 clones 1 and 2) and TRβ1 (HepG2-TRβ1) were used. As a control, HepG2 cells were transfected with the empty vector, yielding a cell line expressing the Neo protein (HepG2-Neo cells). In the three HepG2 cell lines (HepG2-TRα1 clones 1 and 2 and HepG2-TRβ1), the TR protein was overexpressed to approximately 10-, 3-, and 2-fold the expression in the HepG2-Neo control cell line. The transactivation activities were reported previously (21), suggesting that the isogenic cell lines overexpress TRα1 or TRβ1, and the levels of expression are strongly correlated with their functional capacity to transactivate expression of downstream genes (21).

Effects of T_3 on the abundance of SULT2A1 protein and mRNA in HepG2-TRα1 and TRβ1 cell lines

Of interest next was the effect of TRs on the degree of SULT2A1 protein expression when HepG2 isogenic cell lines were incubated in media containing varying levels of T_3 across different time points (Fig. 1). Incubation results indicated that T_3 significantly ($P < 0.01$) increased the amount of SULT2A1 protein in the HepG2-TRα1#1, #2, and -TRβ1 stable cell lines. The SULT2A1 levels were enhanced by roughly a factor of 1.5- to 3.0-fold after incubation of HepG2-TRα1#1, #2, and TRβ1 cells with 1 nM T_3 for 24 h. Furthermore, cells incubated in 10 nM of T_3 for 24 h gave a slightly greater and more significant ($P < 0.01$) induction (2.0- to 3.5-fold) of SULT2A1 protein (Fig. 1, A–C). Moreover, after 48 h incubation in 10 nM of T_3, SULT2A1 expression was further enhanced (2.8- to 5.0-fold) (Fig. 1, A–C). These results demonstrated that the effect of T_3, on the protein level of SULT2A1, in TRα1 and -β1 overexpressing cells is time and dose dependent. Additionally, immunoblot analysis showed that exposure of control HepG2-Neo cells expressing endogenous levels of TR proteins, incubated to 10 nM T_3 for 12–48 h, did not significantly increase of SULT2A1 protein levels (Fig. 1D). Thus, the degree of SULT2A1 protein induction by T_3 correlated with the level of TR protein expression.

Northern blot analysis was used to assess the response of *SULT2A1* mRNA expression to the exogenous addition of T_3. A 1.9-kb *SULT2A1* transcript was identified in HepG2-

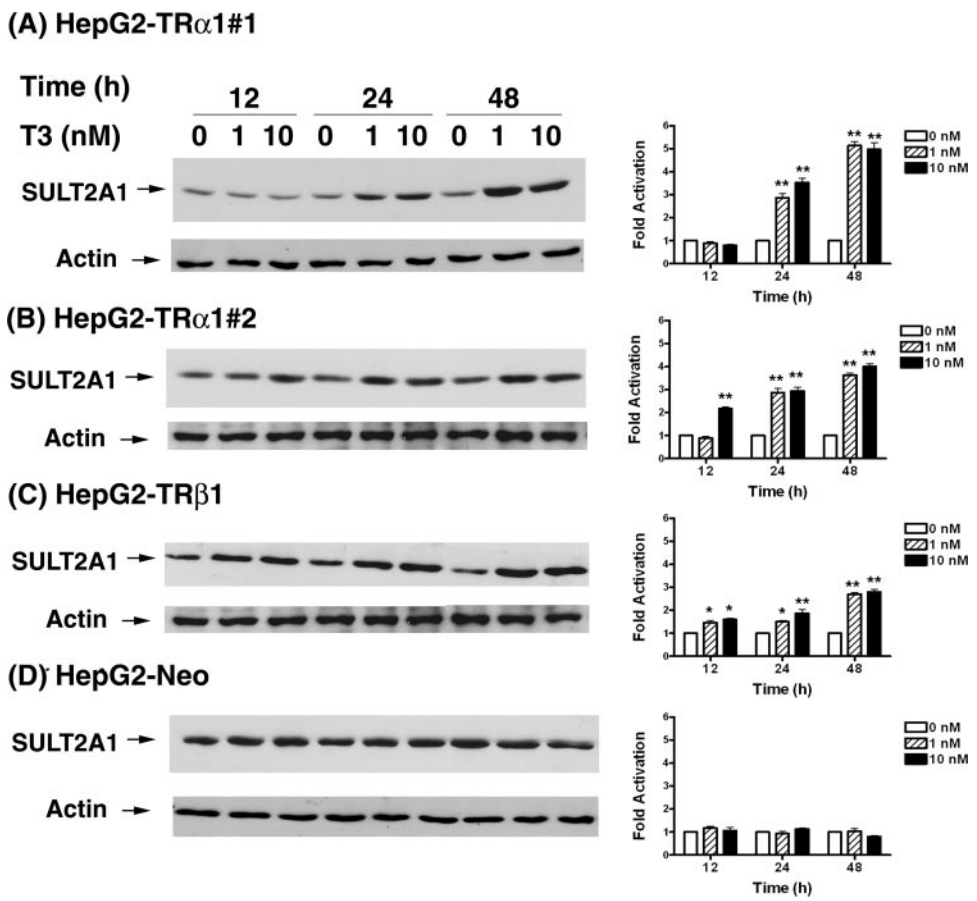

FIG. 1. Effect of T_3 on SULT2A1 protein expression in HepG2 cell lines. The SULT2A1 protein expression level in four TR stable lines, HepG2-TRα1#1 (A), -TRα1#2 (B), -TRβ1 (C), and HepG2-Neo cells (D). Three TR stable lines and Neo cells were incubated in Td medium in the absence or presence of 1–10 nM T_3 for 12–48 h, after which cell lysates (20 μg protein) were subjected to immunoblot analysis with polyclonal antibodies to SULT2A1 (Oxford Biomedical Research). The 34-kDa SULT2A1 band is shown. β-Actin was used as an internal control. The intensities of the SULT2A1 on blots were quantified, and the increase in SULT2A1 expression was measured at each time point. Measurements are presented as the number of folds the activations exceeds those under control (0 nM T_3) conditions. Data are presented as means ± SE obtained from at least three independent experiments. Student's *t* test. **, $P < 0.01$; *, $P < 0.05$, T_3 *vs.* Td treated.

TRα1#1 cells (Fig. 2A). Figure 2B presents quantification results (indicated by fold activation). Thereafter expression of *SULT2A1* mRNA in HepG2-TRα1#2, -TRβ1, and -Neo cells was determined by quantitative PCR (Fig. 2, C–E). Exposure of these cell lines to 10 nM T_3 for 24 h induced expression of *SULT2A1* mRNA in HepG2-TRα1#1, #2, and -TRβ1 cells, with increases of 2.1-, 1.5-, and 1.8-fold, respectively. This quantitative finding demonstrates that the enhanced expression of *SULT2A1* mRNA in response to T_3 correlates with the amount of TR in individual cell lines. The highest levels of T_3 (10 nM) used in this experiment, increased slightly higher than the level of *SULT2A1* expression already displayed in

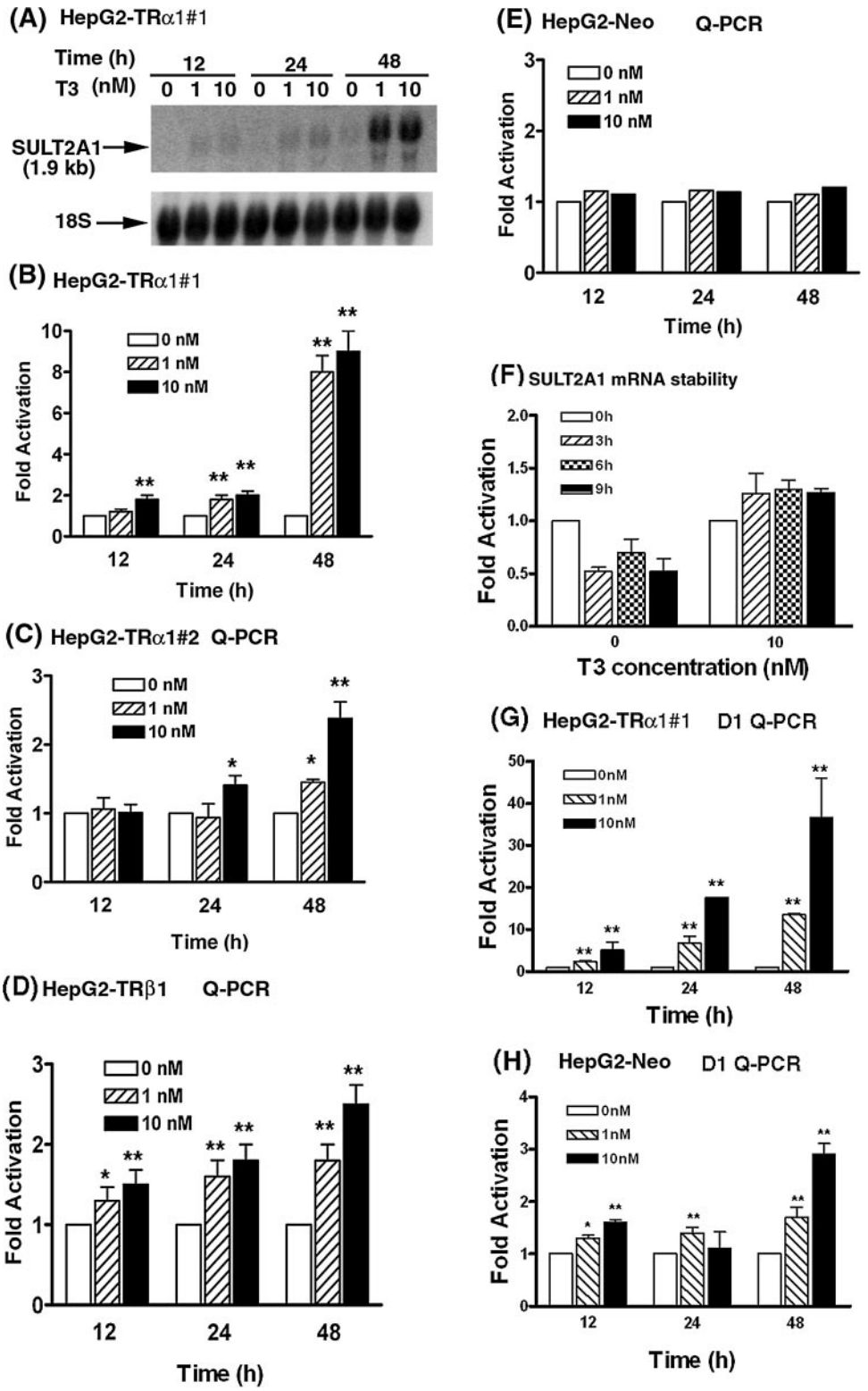

FIG. 2. Effect of T_3 on the amount of *SULT2A1* mRNA in HepG2 cell lines. A, HepG2-TRα 1#1 cells were incubated for 12–48 h in the absence or presence of 1 and 10 nM T_3, after which total RNA was isolated and subjected (20 μg per lane) to Northern blot analysis with ^{32}P-labeled *SULT2A1* and 18S RNA probes. Positions of the 1.9-kb *SULT2A1* and *18S* RNAs are indicated. B, Intensities of the *SULT2A1* mRNA bands on blots similar to that shown in A were quantified, and the degree of the T_3-induced increase in *SULT2A1* transcripts was determined at each point. Induction of *SULT2A1* transcripts was also determined by Q-RT-PCR a described in *Materials and Methods*. Section for HepG2-TRα1#2 (C), HepG2-TRβ1 (D), and HepG2-Neo (E) cells is shown. F, Actinomycin D was used as described in *Materials and Methods* to determine the stability of *SULT2A1* mRNA after T_3 application. The mRNA in the Td or T_3 group, which was treated with actinomycin D for 0 h, was labeled 1. *D1* gene induction by T_3 was used as a control in HepG2-TRα1#1 (G) or HepG2-Neo cells (H). Data were expressed as means ± SE from at least three independent experiments. Student's *t* test. **, $P < 0.01$; *, $P < 0.05$, T_3 *vs.* Td treated.

the 1 nM T_3 experiment (Fig. 2). These experimental results confirm that *SULT2A1* gene expression is very sensitive to small amounts of T_3 (1 nM) in the medium. Incubating the HepG2-TRβ1 cell line in 1 nM T_3 for 24 h and 48 h increased *SULT2A1* levels 1.7- and 1.8-fold, respectively (Fig. 2D). However, when the cells were exposed to 10 nM T_3 for 24 and 48 h, *SULT2A1* mRNA expression increased 1.8- and 2.4-fold, respectively. These measurement results indicate that the action of T_3 in TRα1 was similar to that in the TRβ1 stable cell line. The level of *SULT2A1* induced depends on the degree of expression of TR proteins. Furthermore, T_3 had minimal effect on the amount of *SULT2A1* mRNA in HepG2-Neo cells (Fig. 2E). Thus, the effect of T_3 on SULT2A1 protein expression appears to be mediated, at least in part, at the mRNA level.

Actinomycin D was used after cells were incubated in the Td (0 nM T_3) or 10 nM T_3 for 48 h to determine further the stability of *SULT2A1* mRNA in HepG2-TRα1#1 cells. Interestingly, *SULT2A1* mRNA in the T_3-treated group was much more stable than that in the Td group (Fig. 2F). In fact, *SULT2A1* mRNA levels in the T_3-treated group did not decline after 9 h of actinomycin D treatment. The increase in *SULT2A1* mRNA levels was caused at least in part by the decrease in their degradation after T_3 treatment. Human *type I deiodinase* (*D1*) gene was used as the control. D1 is stimulated by T_3 and is involved in thyroid hormone metabolism (30). Indeed, T_3 up-regulated *D1* expression in both HepG2-TRα1#1 and -Neo cells (Figs. 2, G and H). However, the degree of induction of the former considerably exceeded that of the latter.

Effects of T_3 and cycloheximide on the amount of SULT2A1 mRNA

To further delineate the regulatory action of T_3 on *SULT2A1* mRNA expression, cycloheximide, a protein synthesis inhibitor, was used. Expression of *SULT2A1* mRNA induced by T_3 in the presence or absence of cycloheximide was determined in HepG2-TRα1#1 cells for 12 and 24 h. Transcriptional response of *SULT2A1* mRNA to T_3 over 12- and 24-h periods was substantially reduced in the presence of cycloheximide, suggesting that regulation was indirect (Fig. 3). Similar results were observed for both HepG2-TRα1#2 and HepG2-TRβ1 (data not shown). These analytical results indicated that blocking protein synthesis almost completely inhibits T_3-induced *SULT2A1* transcription. After this experimental result, *de novo* protein synthesis may be critical to *SULT2A1* transcription.

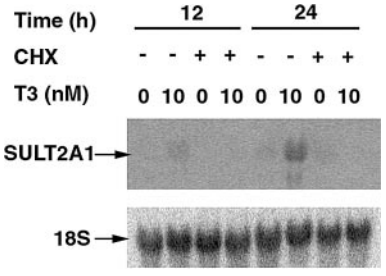

FIG. 3. Cycloheximide (CHX) reduced the response of *SULT2A1* to T_3 activation. The HepG2-TRα1#1 cells were treated (as described in Fig. 2) with or without 10 μg/ml CHX. After T_3 activation for varying times, total RNA was isolated and subjected (20 μg per lane) to Northern blot analysis.

T_3-induced SF1

To clarify which transcription factor was induced by TR and subsequently stimulated *SULT2A1* expression, transcription factors GATA-6 and SF1 were examined based on a previous report (27). Q-RT-PCR (Fig. 4) analysis showed that T_3-induced *SF1* expression at the mRNA level was 2.1- to 6.2-fold in HepG2-TRα1#1 cells (Fig. 4B) with no induction in Neo cells (data not shown). Additionally, T_3 did not enhance *GATA-6* mRNA expression (Fig. 4A). Induction of SF1 by T_3 was also examined at the protein level: T_3 application increased SF1 expression from 2.5- to 3.5-fold (Fig. 5) in the HepG2-TRα1#1. However, this phenomenon was not observed in HepG2-Neo cells (Fig. 5).

SF1 induced the expression of SULT2A1 at the transcriptional level

Promoter activity assays were conducted to determine whether the regulation of *SULT2A1* expression by T_3 occurred at the transcriptional level. The 1463 bp *SULT2A1* promoter and its serially deleted mutants were constructed. Increased promoter activity stimulated by T_3 ranged from 2.8- to 7.1-fold (HepG2-TRα1 cell) in the $-1463/+1$ (translational start site design set at +1), $-332/+1$, and $-228/+1$ reporter plasmids (Fig. 6, B and C). β-Galactosidase was used

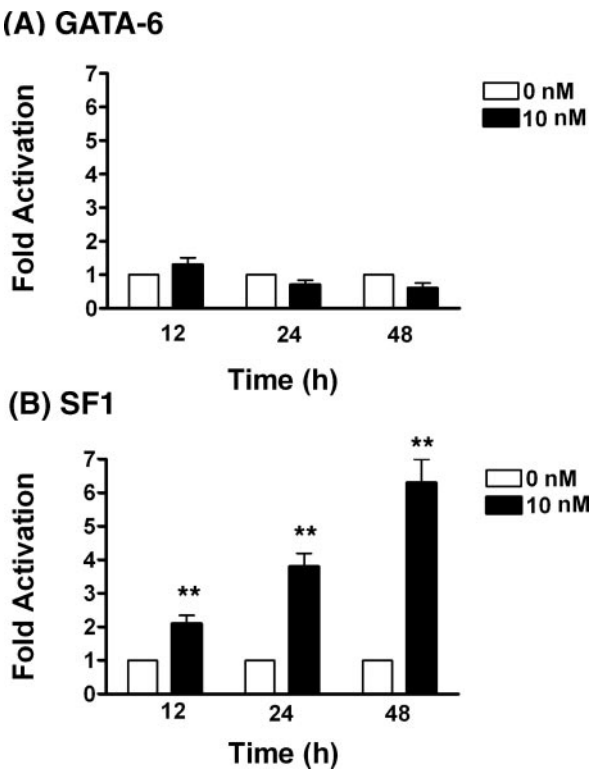

FIG. 4. Influence of T_3 on *GATA-6* and *SF1* expressions at the RNA level. HepG2-TRα1#1 cells were incubated for 12–48 h in the absence and presence of 10 nM T_3, after which total RNA was isolated and subjected to Q-RT-PCR analysis. Data were expressed as means ± SE from at least three independent experiments. Student's *t* test. **, $P < 0.01$, T_3 *vs.* Td treated.

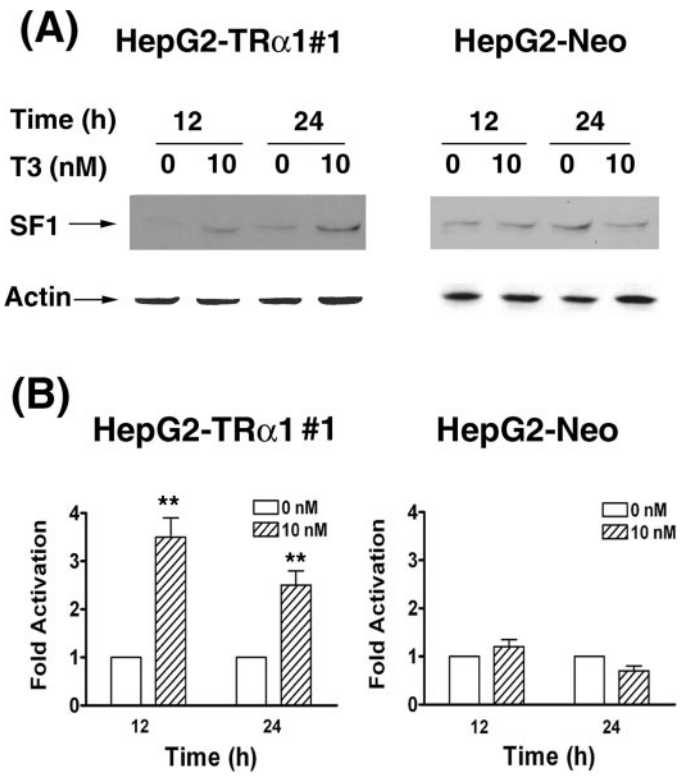

FIG. 5. The SF1 protein was-induced by T_3 in HepG2-TRα1 cells. A, HepG2-TRα1#1 or Neo cells were incubated with Td medium in the absence and presence of 10 nM T_3 for 12 and 24 h, after which nuclear extracts (100 μg protein) were subjected to immunoblot analysis with polyclonal antibody to SF1 (Upstate). The 34-kDa SF1 is shown. The SF1 protein in HepG2-Neo cells was not induced by T_3. β-Actin was the loading control. B, The intensities of the SULT2A1 on blots were quantified, and the increase in SULT2A1 expression was measured at each time point. Measurement results are presented as fold activations, compared with those in control (Td) conditions. Data were expressed as means ± SE from at least three independent experiments. Student's *t* test. **, $P < 0.01$, T_3 *vs.* Td treated.

to control transfection efficiency. There are at least seven SF1 binding sites indicated by a *triangle* in the −1463 to +1 *SULT2A1* upstream promoter region in Fig. 6A. However, if the SF1 binding site at −228 is deleted, T_3-induced transcription activity at the −117/+1 or −50/+1 constructs diminished (Fig. 6, B and C). This analytical result was further confirmed by exogenous transfection of the SF1 expression vector into HepG2-TRα1 cells. The transfection of SF1 expression vector in T_3-depleteled medium increased −228/+1 reporter activities 9.5-fold and did not increase −117/+1 reporter activities in Td medium (Fig. 7). However, both T_3 and SF1 on the minimal promoter region (−228/+1) exhibited slightly increased reporter activities but no synergistic effect (Fig. 7). The experimental data clearly demonstrated that T_3 mediates SF1 by specifically increasing the number of *SULT2A1* transcripts in HepG2-TRα1#1 cells (Fig. 6B) and, to a lesser extent, -TRβ1 cells (Fig. 6C).

T_3-induced SULT2A1 and SF1 proteins expression in vivo

Two groups of 6-wk-old male Sprague Dawley rats (n = 6) underwent surgical TX to determine the *in vivo* response of SULT2A1 or SF1 to T_3 treatment. One group (three rats per group) was injected with T_3 daily for 2 wk (TX + T_3), the second group did not receive T_3 injections (TX), and the third group was control sham-operated rats (n = 3). The rats were killed at experiment end, and their serum was collected for T_3 and TSH determination. Their livers were removed for Western blot analysis. The T_3 serum levels in the TX group were about 0.016-fold (7.6 *vs.* 489 ng/dl) those in the group that underwent T_3 treatment (TX + T_3). The levels of TSH in the TX group were around 226-fold (3.16 *vs.* 0.014 mg/ml) those in the T_3-treated group. The T_3 and TSH serum levels in the sham group were around 40 ng/dl and 2.23 mg/ml, respectively. Western blot analysis of rats administered T_3 demonstrated that SULT2A1 and SF1 proteins levels were elevated, compared with those in the TX group. The SULT2A1 protein level in the T_3 group was approximately 2-fold higher than that in the TX group (Fig. 8A). The expression of SF1 protein was approximately 4- or 2-fold higher in the TX + T_3 or sham group (N), respectively, than in the TX group (Fig. 8B). The levels of SF1 mRNA were about 7.8- or 4.2-fold higher in the TX + T_3 or sham group, respectively than in the TX group (Fig. 8C). These measurement results further validate the data obtained by *in vitro* Q-RT-PCR and Northern and Western blot analyses that elucidate the regulation of SULT2A1 expression by thyroid hormones at the mRNA and protein levels.

Discussion

This study investigated treatment of human liver cancer cells (HepG2) with T_3-induced human *SULT2A1* at the mRNA and protein levels. Subsequent cycloheximide study showed that regulation of *SULT2A1* by T_3 is indirect and mediated by SF1. Further study demonstrated that T_3 upregulates SF1. Additionally, exogenous transfection of SF1 expression vector into HepG2-TRα1 cells increased *SULT2A1* promoter activity 9.5-fold. Furthermore, transfection analyses demonstrated that an important SF1 site in the upstream human *SULT2A1* 5′-flanking-228 region was responsible for T_3-induced human *SULT2A1* gene transcription. Supporting these findings were a similar regulation observed in TX rats. Whereas this study investigated T_3-induced SULT2A1 in human hepatoma cell lines, similar results have been obtained in animal studies.

The SULT2A1 catalyzes sulfonation of dehydroepiandrosterone (DHEA) to DHEA sulfate (DHEA-S) (31). The stability of the DHEA molecule in circulation is enhanced by sulfonation (32). The sulfatase in peripheral tissues returns DHEA-S to DHEA and provides it as a precursor for local steroid hormone (32). T_3 is metabolized in liver mainly by conjugation with glucuronic acid or sulfate. In contrast to T_3 itself and the stable glucuronide, T_3 sulfate is rapidly degraded by successive deiodination of the tyrosyl and phenolic rings. In humans, deiodination, glucuronidation, and perhaps sulfation are important pathways of thyroid hormone metabolism. Sulfation increases the hydrophilicity and the biliary excretion of the hormone. However, the main goal of the sulfation of the thyroid hormone is to facilitate its solubility and degradation by D1 (33, 34). The hypothalamic-pituitary-thyroid-negative feedback control axis regulates the excess of T_3/T_4 (35). In this work, another negative feed-

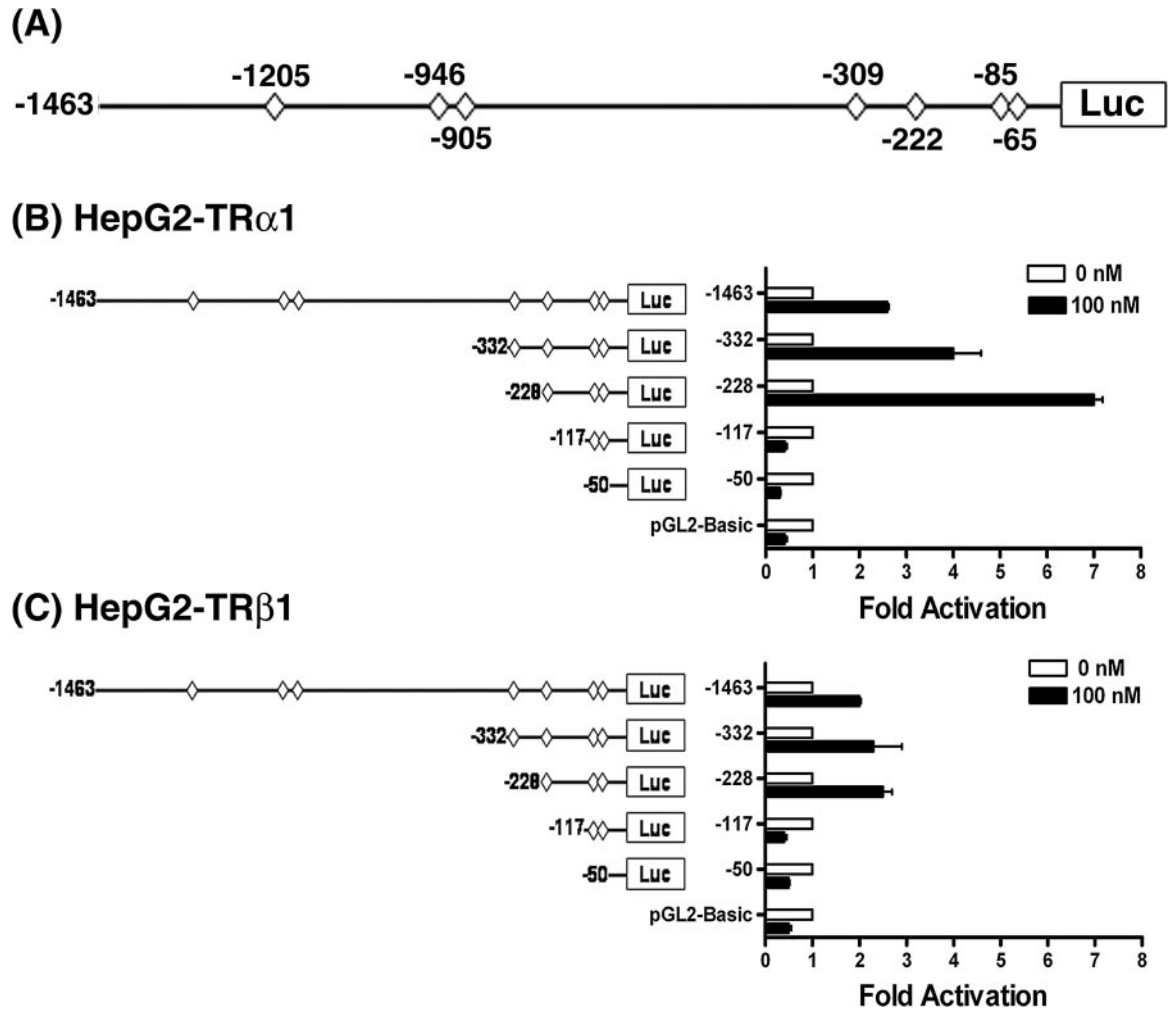

FIG. 6. Regulation of *SULT2A1* expression by T_3 at the transcriptional level. A, Schematic representation of *SULT2A1* promoter with potential SF1 binding sites indicated by a *diamond* (◇). The *numbers* above or below indicate the base pair at which the site starts based on the translational start site (+1). A series of pGL2-basic reporter constructs containing progressively smaller amounts of *SULT2A1* 5′-flanking DNA (1 μg/well) were cotransfected in HepG2-TRα1#1 (B) and HepG2-TRβ1 (C) cells. After transfection, cells were harvested after 24 h T_3 treatment. Data were normalized to cotransfected β-galactosidase, and fold-activation was calculated relative to each Td control. Results are presented as means ± SD of data from three independent experiments performed in triplicate.

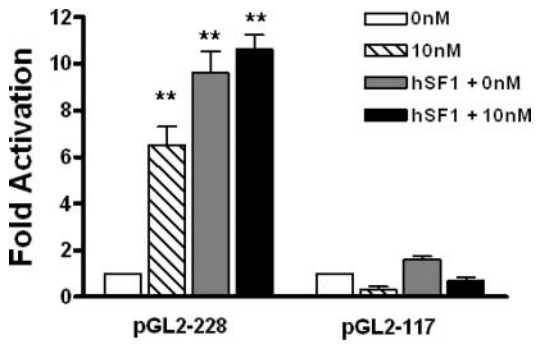

FIG. 7. Enhancement of *SULT2A1* expression by SF1. Two reporter constructs (−228/+1, −117/+1) DNA (1 μg/well) individually were transfected into HepG2-TRα1 cells. The reporters activities were assayed by cotransfection with or without SF1 expression vector into HepG2-TRα1 cells in the presence or absence of T_3. Data were normalized to cotransfected β-galactosidase, and fold activation was calculated relative to each Td control. Results are presented as means ± SD of data from three independent experiments performed in triplicate. Student's *t* test. **, $P < 0.01$, T_3 *vs.* Td treated.

back control mechanism, based on T_3 up-regulation of SULT2A1 expression, facilitates the degradation of T_3. Recently Maglich *et al.* (36) reported that thyroid hormones are regulated by the orphan nuclear receptor constitutive androstane receptor (CAR) during fasting. In addition, CAR also mediates the induction of hepatic drug metabolism in response to various xenobiotics (37). Sulfotransferase genes, including SULT2A1, SULT1A1, N-sulfotransferase (SULTN), and uridine diphosphoglucuronosyltransferase-1A, were induced in a CAR-dependent manner. Most of them are also involved in thyroid hormones metabolism.

Except by thyroid hormones, SULT2A1 is regulated by several other nuclear hormone receptors: estrogen-related receptor (ERR) (11), glucocorticoid (12), peroxisome proliferators activated receptor-α (38), vitamin D receptor (39), farnesoid X receptor (FXR) (40), all-trans retinoic acid (vitamin A) receptor (41), and pregnane X receptor (42). Promoter activity of *SULT2A1* was induced 2.6-fold by the ERR (11). Contrary to experimental results obtained in this study, ERR

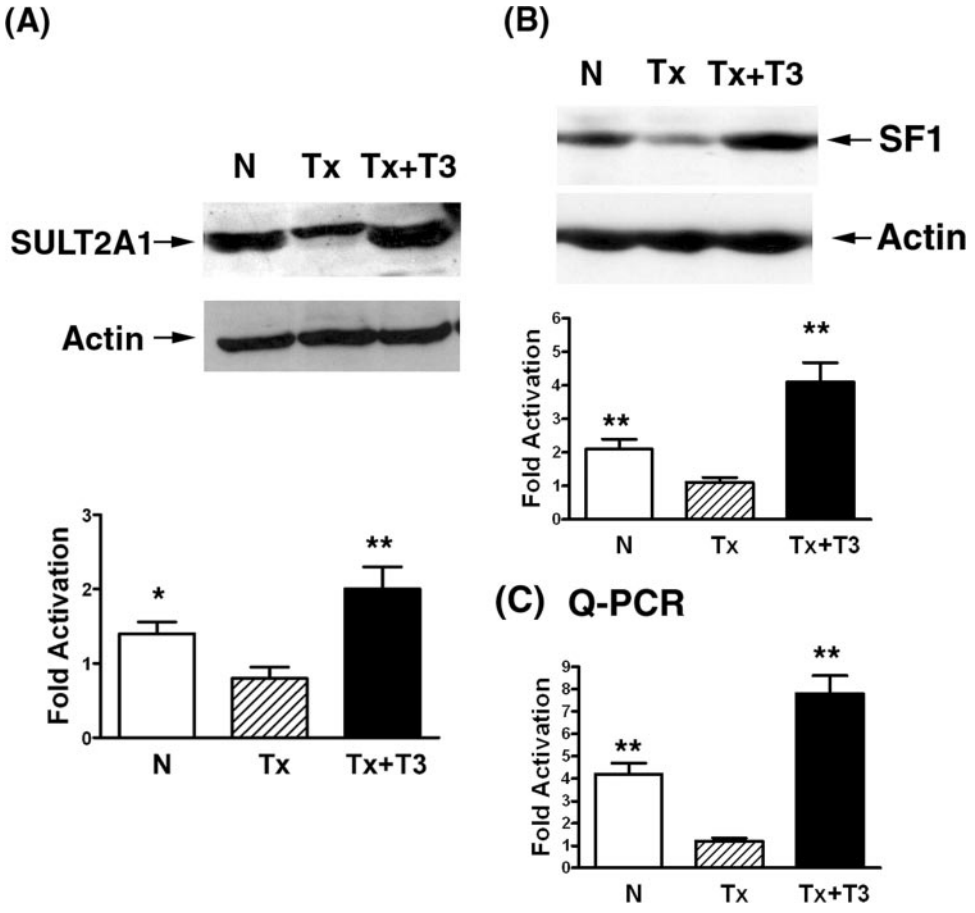

FIG. 8. Induction of SULT2A1 and SF1 expression by thyroid hormone in rat liver. The expression of SULT2A1 (A) and SF1 (B) in normal (N, sham-operated), TX, or TX+T_3 male Sprague Dawley rat liver was determined by Western blot analysis, as presented in *Materials and Methods*. Data are presented as means ± SE from two independent experiments (three rats per group). The intensities of the SULT2A1, SF1, and β-actin bands on the blots were quantified. These results are displayed as the folds of activation and exceed those in the TX group. C, Expression of *SF1* mRNA in the three groups of rat liver, assayed by Q-RT-PCR. Student's *t* test. **, $P < 0.01$; *, $P < 0.05$, TX+T_3 or N *vs.* TX treated.

effects on SULT2A1 were more significant than the stimulation in response to SF1 only (11). However, three potential ERR binding sites (−1191, −85, −65) were critical to regulating SULT2A1 expression, a finding different from that obtained in this study. Experimental results in this study indicated that SF1 binding site at −228 on the *SULT2A1* promoter is the most critical site. Furthermore, the upstream region at nucleotides −5949 to −5929 relative to the transcription start site of *SULT2A1* contain the peroxisome proliferator response element (38). Song *et al.* (40) demonstrated that chenodeoxycholic acid, the primary bile acid and a known ligand for FXR, markedly induces rat *SULT2A1* promoter and, furthermore, this induction is mediated by a response element of the inverted hexanucleotide repeat (IR-0) palindrome (GGGTCATGAACT) sequence. Because T_3 indirectly regulated SULT2A1, no TRE was identified. Notably, not all steroid hormones activate SULT2A1 expression. Song *et al.* (43) showed that androgen receptor exerts its negative regulatory effect indirectly through transcriptional interference of octamer transcription factor-1 and CCAAT/enhancer-binding protein, rather than through direct interaction of DNA and androgen receptor. Transcription factors other than SF1, such as GATA-6, octamer transcription factor-1, and CCAAT/enhancer-binding protein, also regulated SULT2A1 expression (27). However, the regulation of GATA-6 by T_3 was not observed and, consequently, GATA-6 did not play a role in T_3 stimulation. Competition among TR, vitamin D receptor, all-trans retinoic acid (vitamin A) receptor, ERR, and FXR suggests that the intracellular ligand/receptor/transcription factor availability may correlate with the extent that a specific nuclear receptor pathway influenced thyroid/steroid/xenobiotic metabolism by SULT2A1.

Tagawa *et al.* (44) showed that serum DHEA-S levels were decreased in patients with hypothyroidism and increased in patients with hyperthyroidism. Thyroid hormones may enhance the synthesis of these steroids, and DHEA sulfotransferase levels may be increased in hyperthyroidism. Experimental findings by Tagawa *et al.* strongly support findings obtained in this study. In summary, patients with hyperthyroidism usually generate increased SULT2A1 levels, indicating that modulation of T_3 levels is critical to controlling SULT2A1 *in vivo*.

In conclusion, this investigation demonstrated that ligand-activated TR indirectly transactivates *SULT2A1* gene transcription through a critical SF1 binding site located in the distal portion of the 5′-flanking −228 region of the gene. Although such genes were isolated from human tumor cell lines, their regulation was similar to that of those in rats. However, the physiological role of this regulatory pathway remains unclear. Experimental results suggest that T_3 is crucial to *in vitro* and *in vivo* regulation of steroid production, stability, metabolism, and detoxification. Further characterization of TR signaling in *SULT2A1* gene expression is likely of considerable physiological significance.

Acknowledgments

Received September 12, 2005. Accepted January 30, 2006.

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This work was supported by Chang-Gung University, Taoyuan, Taiwan (CMRP 1332, CMRPD 34013, NMRP 1074), Chang-Gung Molecular Medicine Research Center Taoyuan, Taiwan (CMRP 140041), and the National Science Council of the Republic of China, Taiwan (NSC 91-2320-B-182-041).

All authors have nothing to declare.

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