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

Endocrinology 147(9):4292–4302

doi: 10.1210/en.2006-0116

Mouse Sterol Response Element Binding Protein-1c Gene Expression Is Negatively Regulated by Thyroid Hormone

Koshi Hashimoto, Masanobu Yamada, Shunichi Matsumoto, Tsuyoshi Monden, Teturou Satoh, and Masatomo Mori

Department of Medicine and Molecular Science, Graduate School of Medicine, Gunma University, Maebashi, Gunma 371-8511, Japan

Sterol regulatory element-binding protein (SREBP)-1c is a key regulator of fatty acid metabolism and plays a pivotal role in the transcriptional regulation of different lipogenic genes mediating lipid synthesis. In previous studies, the regulation of SREBP-1c mRNA levels by thyroid hormone has remained controversial. In this study, we examined whether T_3 regulates the mouse SREBP-1c mRNA expression. We found that T_3 negatively regulates the mouse SREBP-1c gene expression in the liver, as shown by ribonuclease protection assays and real-time quantitative RT-PCR. Promoter analysis with luciferase assays using HepG2 and Hepa1–6 cells revealed that T_3 negatively regulates the mouse SREBP-1c gene promoter (−574 to +42) and that Site2 (GCCTGACAGGTGAAATCGGC) located around the transcriptional start site is responsible for the negative regulation by T_3. Gel shift assays showed that retinoid X receptor-α/thyroid hormone receptor-β heterodimer bound to Site2, but retinoid X receptor-α/liver X receptor-α heterodimer could not bind to the site. *In vivo* chromatin immunoprecipitation assays demonstrated that T_3 induced thyroid hormone receptor-β recruitment to Site2. Thus, we demonstrated that mouse SREBP-1c mRNA is down-regulated by T_3 *in vivo* and that T_3 negatively regulates mouse SREBP-1c gene transcription via a novel negative thyroid hormone response element: Site2. (*Endocrinology* 147: 4292–4302, 2006)

STEROL REGULATORY ELEMENT-BINDING proteins (SREBPs) are transcription factors that belong to the basic helix-loop-helix leucine zipper family (1, 2). The mammalian genome encodes three SREBP isoforms, designated SREBP-1a, SREBP-1c, and SREBP-2 (1). SREBP-2 is encoded by a gene on human chromosome 22q13. Both SREBP-1a and -1c are derived from a single gene on human chromosome 17p11.2 through the use of alternative transcription start sites that produce alternate forms of exon 1, designated 1a and 1c (1). SREBP-1a is a potent activator of all SREBP-responsive genes, including those that mediate the synthesis of cholesterol, fatty acids, and triglycerides. High-level transcriptional activation is dependent on exon 1a, which encodes a longer acidic transactivation segment than does the first exon of SREBP-1c. SREBP-1c preferentially enhances the transcription of genes required for fatty acid synthesis but not cholesterol synthesis (3). Liver X receptors (LXRs) bind to an LXR-binding site in the SREBP-1c promoter and activate SREBP-1c transcription in the presence of LXR agonists such as oxysterol (4, 5). Both thyroid hormone receptor β (TR-β) and LXRs form heterodimers with retinoid X receptor (RXR) and bind to the DNA binding site direct repeat 4 (DR-4) with identical geometry and polarity (6–8). We recently showed that TR-β and LXR-α interact on the mouse cholesterol 7α-hydroxylase (*CYP7A1*) gene promoter, suggesting cross talk between the two receptors (9).

To date it remains controversial how thyroid hormone regulates SREBP-1c gene expression. Viguerie *et al.* (10) reported that SREBP-1c mRNA is down-regulated by T_3 in human adipocytes, as shown by DNA microarray analysis, whereas Zhang *et al.* (11) reported that T_3 induces an increase of chicken SREBP-1 mRNA in chick embryo hepatocytes (CEH) under glucose administration. Furthermore, in a recent report, Kawai *et al.* (12) concluded that T_3 induces human SREBP-1c mRNA in HepG2 cells derived from human hepatocytes. To resolve this controversy and find possible differences among species, we studied mouse SREBP-1c gene regulation by T_3.

We initially hypothesized that TR-β could bind to the LXR-binding site in the SREBP-1c gene promoter, DR-4, and would positively regulate SREBP-1c gene transcription; however, the results of this study revealed that thyroid hormone down-regulates mouse SREBP-1c mRNA levels in the liver. We also showed here that T_3 negatively regulates the mouse SREBP-1c gene promoter through TR-β, and that its DNA binding site responsible for the negative regulation is not the LXR-binding site, but is Site2, which surrounds the transcriptional start site in the promoter. We confirmed the data above using Hepa1–6 cells, which are derived from mouse hepatocytes, and demonstrated that the human SREBP-1c gene promoter is also negatively regulated by thyroid hormone.

Materials and Methods

Animals

Four-week-old male C57/BL6 mice were employed for the study. All aspects of animal care were approved by the Institutional Animal Care

First Published Online June 22, 2006

Abbreviations: ChIP, Chromatin-immunoprecipitation; 5′DI, 5′-deiodinase; DR-4, direct repeat 4; LXR, liver X receptor; LXRE, LXR response element; MMI, methimazole; PTU, propylthiouracil; RPA, ribonuclease protection assay; RXR, retinoid X receptor; SRE, sterol response element; SREBP, SRE binding protein; TR, thyroid hormone receptor; TRE, thyroid hormone response element.

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

and Use Committee of Gunma University Graduate School of Medicine (Maebashi, Gunma, Japan). Animals were maintained on a 12-h light/12-h dark schedule (light on at 0600 h) and fed laboratory chow as indicated and given water *ad libitum*. The mice were rendered hypothyroid by the inclusion of 0.1% methimazole (MMI) in the drinking water and 1% (wt/wt) propylthiouracil (PTU) in the chow for 21 d (13, 14). To introduce a thyrotoxic status, the mice were injected daily with 10 μg per 100 g body weight T_3 for an additional 5-d period. The number of mice receiving each treatment was indicated in the figure legends. Serum free T_4 levels were determined using a GammaCoat RIA kit (DiaSorin, Inc., Stillwater, MN), and free T_3 levels were determined using an AMERLEX-MAB kit.

Plasmids

The mouse SREBP-1c promoter (−574/+42) plasmid, which contained the region from −574 to +42 bp of the mouse SREBP-1c gene, was generated by genomic PCR using 5′-GTGTAAGCTTGGATCCA-GAACTGGATCATCAGCCCC-3′ as a sense primer and 5′-GTGTA-AGCTTCCTAGGGCGTGCAGACGCTACCCCGA-3′ as an antisense primer (15). A *Hin*dIII restriction enzyme site was introduced into the primer sequences so that the PCR product could be subcloned into the pA3-Luc vector. The deletion constructs of the mouse SREBP-1c gene were generated using PCR site-directed mutagenesis (Expand 20kb Taq Long PCR system, Roche Molecular Biochemicals, Mannheim Germany) (16). All human TR-β1 and mutant cDNAs were placed into an SV40 expression construct, pSG5. All PCR-generated constructs were verified by sequencing the DNA. The human SREBP-1c promoter pGL4-Luc vector was a kind gift from Drs. E. J. Tarling and A. Bennett (University of Nottingham Medical School, Nottingham, UK) (17).

Transfections and luciferase assay

For the luciferase assay, we employed HepG2 cells, which were derived from human hepatocytes, or CV-1 cells, which were derived from the kidney of the African green monkey, or Hepa1–6 cells, which were derived from mouse hepatocytes. Hepa1–6 cells were purchased from RIKEN BioResource Center (Tsukuba, Ibaraki, Japan) (18). Two micrograms of the reporter plasmid and 0.1 μg of TR-β1 or its mutants in pSG5 were transfected per well of a six-well plate into HepG2 or Hepa1–6 cells using the calcium-phosphate method. Sixteen hours after transfection, cultures were treated with serum-free DMEM for 8 h in the absence or presence of 10^{-8} M of T_3. All transfections were equalized for the same total amount of expression vector using an empty vector as needed. We performed β-gal assays to confirm the transfection efficiency of the luciferase assay for each experiment at least once and found no significant difference in transfection efficiency among the plates. Data are presented as fold basal activation expressed as fold induction over vector (pSG5) in the absence of T_3 stimulation ± SEM. Luciferase activity was expressed as arbitrary light units per microgram of cellular protein. All transfection experiments were repeated at least twice with triplicate determination.

Western blotting

For analysis of the protein expression of TR-β1 and its mutant constructs, 3 μg of TR-β1 and its mutants in pSG5 were transfected per 10-cm-diameter plate into CV-1 cells using the calcium-phosphate method. Western blotting of whole cell lysates from CV-1 cells was performed using a rabbit anti-TR-β1 polyclonal antibody (06-539; Upstate Biotechnology, Inc., Lake Placid, NY).

RNA preparation, Northern blot analysis, and ribonuclease protection assay (RPA)

Total RNA was extracted from mouse liver using ISOGEN (Nippon Gene, Tokyo, Japan), and 20 μg of total RNA was subjected to Northern blot analysis or RPAs as indicated in the figure legends. Mouse SREBP-1a and -1c probes (19) were a gift from Dr. Iichiro Shimomura (Osaka University, Osaka, Japan). A rat cDNA probe for rat 5′ deiodinase(5′DI) was a gift from Dr. C. N. Mariash (University of Minnesota, Minneapolis, MN). The probe for cyclophilin was purchased from Ambion (pTRI-cyclophilin-mouse antisense control template; Ambion, Inc., Austin, TX). Northern blot analysis and RPAs were performed using of [α-^{32}P]UTP-labeled antisense riboprobes. RPAIII (Ambion) was employed for RPAs. The hybridization bands were quantitatively measured using Adobe Photoshop 4.0 (Adobe Systems Corp., San Jose, CA) and NIH Image (Scion Corp., Frederick, MD), and standardized against cyclophilin controls. All Northern blots and RPAs were repeated at least three times with similar results, and a representative result is shown.

Real-time quantitative PCR

Real-time quantitative PCR assays were performed using an ABI 7700 sequence detector (Applied Biosystems, Foster City, CA). Briefly, 1 μg of mouse liver total RNA was reverse transcribed with random hexamers using the Taqman Reverse Transcription Reagent kit (Applied Biosystems) according to the manufacturer's protocol. Mouse SREBP-1c mRNA expression was analyzed using SYBR Green PCR master mix (Applied Biosystems). The following primers were chosen to generate the PCR fragment: forward, 5′-ATCGGCGCGGAAGCTGTCGGGG-TAGCGTC-3′; reverse, 5′-ACTGTCTTGGTTGTTGATGAGCTGGAG-CAT-3′ (19). The PCR product was 116 bp. The primers were designed to be exon-spanning to avoid amplification of contaminating genomic DNAs (the PCR product should be 3194 bp in that case). To confirm that there was no genomic contamination, the bands were resolved on 1.5% agarose gels stained with ethidium bromide. The PCR results were normalized to mouse glyceraldehyde-3-phosphate dehydrogenase expression using a probe and primers from previously developed assays for glyceraldehyde-3-phosphate dehydrogenase (Applied Biosystems). The number of mice is indicated in the figure legends.

Gel-shift assays

EMSAs (gel-shift assays) were performed as described previously (17). Mouse LXR-α, human TR-β1, wild-type and human RXR-α recombinant proteins were synthesized from constructs in the pSG5 expression vector, using the TNT T7 quick coupled transcription/translation system (Promega, Madison, WI). Binding reactions contained 20 mM HEPES (pH 7.6), 50 mM KCl, 12% glycerol, 1 mM dithiothreitol, 1 μg of poly(dI-dC)·poly(dI-dC), and 4 μl of each of the synthesized nuclear receptors. Double-stranded oligonucleotides (DR-4 in rat cholesterol 7α-hydroxylase, CYP7A1):5′-TGTTTGCTTTGGTCACTCAAGTTCAA-3′, ΔSRE1–3 (see Fig. 5A); Site2 (as indicated in Fig. 5A); and LXR response element (LXRE):5′-TGACCGCCAGTAACCC-3′ in the mouse SREBP-1c promoter (5) were labeled with [α-^{32}P]deoxy-CTP by a fill-in reaction using a Klenow fragment of DNA polymerase. Binding reactions were performed at room temperature for 30 min, and the protein-DNA complexes were resolved on a 5% polyacrylamide gel in 0.5× TBE (45 mM Tris-base, 1 mM EDTA) on ice. T_3 was dissolved in 20 mM NaOH as a 1 mM stock solution and diluted to the indicated concentration in 20 mM Tris, pH 7.5. All gel-shift assays were repeated at least three times with similar results, and a representative result is shown.

In vivo chromatin-immunoprecipitation (ChIP) assay

In vivo ChIP assays were performed as we previously reported using a kit from Upstate Biotechnology (9, 22). We employed mouse liver tissue excised from nontreated or hypothyroid or thyrotoxic mice. Briefly, each sample of liver tissue (60–80 mg) was weighed and incubated in 1% formaldehyde (1 ml/20 mg tissue) at 37 C for 20 min with agitation. The tissue was then washed twice with ice-cold PBS buffer (PBS/1 mM PMSF/1 μg/ml aprotinin) and resuspended in 1000 μl of lysis buffer [1% sodium dodecyl sulfate/50 mM Tris-HCl (pH 8.1)/10 mM EDTA/1 mM phenylmethylsulphonylfluoride/1 μg/ml aprotinin) for 10 min at 4 C. The lysate was sonicated three times with 10-sec pulses using a sonicator set at 70% of maximum power to reduce the DNA length to between 200 and 1000 bp. Chromatin solution (500 μl) was used for each ChIP assay with 5 μl of a rabbit anti-TR-β1 polyclonal antibody (06-539; Upstate Biotechnology), goat anti-LXR-α polyclonal antibody (sc-1201; Santa Cruz Biotechnology, Santa Cruz, CA), or rabbit anti-RXR-α polyclonal antibody (sc-774; Santa Cruz Biotechnology). As a negative control, normal mouse IgG antibody (sc-2025; Santa Cruz Biotechnology) was used. PCR was performed in 50 μl with Ampli Taq (PerkinElmer, Wellesley, MA) for 30 cycles (annealing temperature, 60 C). The set of primers used for Site2 was as follows: forward,

5′-CATTCAGAGCACCGGGAGAAACCCG-3′; reverse 5′-TAGGGCG-TGCAGACGCTACCCCGACA-3′. The predicted PCR product length was 204 bp. The set of primers used for DR-4 was as follows: forward, 5′-TCCAGGCAAGTTCTGGGTGTGTGCG-3′; reverse, 5′-CGGGTT-TCTCCCGGTGCTCTGAATG-3′. The primers comprised two DR-4 sites in the mouse SREBP-1c promoter. The predicted PCR product length was 238 bp. The set of primers used for exon18, which is the 3′ exon for mouse SREBP-1c, was as follows: forward, 5′-TCTCAGGTATTCCTA-CATGAGGCCAC-3′; reverse, 5′-CGCTGATTTCTGTAAGTCAGCTC-TCA-3′. The predicted PCR product length was 201 bp. All PCR signals stained with ethidium bromide in 1.5% agarose gels were quantified with the Molecular Imager FX (Bio-Rad Laboratories, Hercules, CA). The values were corrected using the input values, and the relative OD is shown as a graph (see Fig. 6). All *in vivo* ChIP assays were repeated at least three times with similar results, and a representative result is shown.

Statistical analyses

Values are expressed as the mean ± SEM. The significance of differences between the mean values was evaluated using the unpaired Student's *t* test. Sample groups showing heterogeneity of variance were appropriately transformed.

Results

To examine whether thyroid hormone regulates mouse SREBP-1c gene expression, we performed RPAs using mouse liver total RNA. For this purpose, we rendered the mice thyrotoxic or hypothyroid. We measured serum free T_3 and T_4 levels and confirmed that T_3 and MMI/PTU treatment made the mice thyrotoxic and hypothyroid, respectively (Table 1). The 5′DI type 1 gene is positively regulated by thyroid hormone in the liver (23) and is a good indicator of thyroid status in mice. As shown in Fig. 1, during hypothyroidism, 5′DI mRNA levels were undetectable. However, 5′DI mRNA levels were strongly induced by T_3 up to 10-fold compared with the control level. These data demonstrated that the T_3 and MMI/PTU treatments for the mice had the expected effects.

As shown in Fig. 2A, T_3 reduced SREBP-1c mRNA levels by 40–50% compared with the control. In hypothyroid status, however, SREBP-1c mRNA levels were increased 1.5~2-fold compared with the control. In contrast, SREBP-1a mRNA levels were almost identical with both treatments. To confirm these data, we performed real-time quantitative PCR with mouse liver total RNA. As shown in Fig. 2B, SREBP-1c mRNA levels were increased in the hypothyroid status by about 1.5-fold compared with the control, and thyrotoxic treatment reduced the mRNA levels by about 50% compared with the control level. These data were fully compatible with the RPA data. Thus, these two lines of data indicate that mouse SREBP-1c gene expression in the liver is negatively regulated by thyroid hormone.

The mouse SREBP-1c promoter contains binding sites for specificity protein 1 and nuclear factor-Y (15). These sites

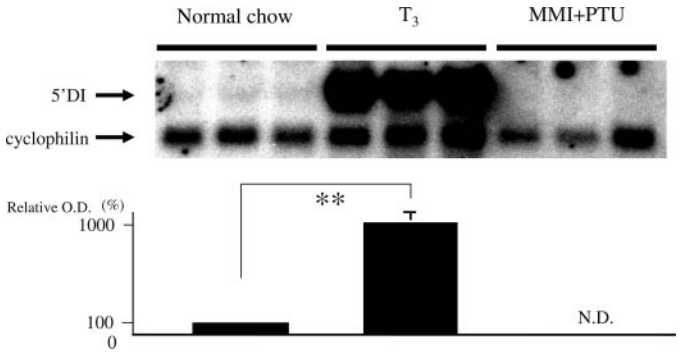

FIG. 1. 5′DI type 1 mRNA expression in the mouse liver. C57/B6 mice (4-wk-old male) were rendered thyrotoxic with T_3 and hypothyroid with a MMI/PTU diet. As a control, they were fed normal chow. Each treatment involved six mice. Liver total RNA was isolated, and 20 μg of total RNA were subjected to Northern blot analysis. Representative Northern blots of 5′DI type 1 are shown. Relative OD (mean ± SE) was controlled for cyclophilin mRNA levels using NIH image software. OD levels in mice fed normal chow were assigned a value of 100% for each group. N.D., Not detectable. The *asterisks* indicate that the difference between the denoted pairs is significant at a confidence level of $P < 0.001$ (**) by *t* testing.

together with Ebox and sterol response element 3 (SRE3) comprise the SRE complex, and the promoter also contains two DR-4 sites (Fig. 3; also see Fig. 5A). We subcloned mouse SREBP-1c promoter (−574/+42 bp) by genomic PCR and ligated it to the luciferase reporter (pA3-Luc). We also prepared deletion constructs of the SREBP-1c promoter by PCR mutagenesis and used these reporters together with the pSG5 vector or TR-β1 to transfect HepG2 cells. We also employed the TR-β1 Δ337T mutant, which is deficient in ligand binding, as a control (24). As shown in Fig. 3, the −574/+42 promoter fused with the luciferase reporter showed ligand-independent activation by TR-β1 (~2-fold). T_3 (10^{-8} M) reduced the −574/+42 promoter luciferase activity by 50%. In contrast, the TR-β1 Δ337T mutant did not show ligand-dependent repression. A shorter reporter (−381/+42) containing the DR-4 site was more active in the luciferase assay than the −574/+42 reporter. We speculated that this could have been due to the higher transfection efficiency of the shorter construct. The −381/+42 reporter also showed negative regulation by TR, as did the −574/+42 reporter. Interestingly, the −108/+42 reporter, which does not contain the DR-4 site, was also negatively regulated by TR, indicating that the DR-4 site was not required for negative regulation by TR. The −77/+42 and −50/+42 reporter activities were apparently reduced compared with that of the −574/+42 reporter, but these reporters were still negatively regulated by TR.

Next, we cotransfected several types of mutant TR-β1 and the −574/+42 reporter into CV-1 cells, which are known to be deficient for endogenous TRs (25). The GS125 mutant is

TABLE 1. Free T_4 and free T_3 at baseline (normal chow) T_3 treatment, and during T_3 deprivation (MMI + PTU)

	Normal chow	T_3	MMI + PTU
Free T_4 (ng/dl)	2.57 ± 0.18 (6)	2.04 ± 0.18 (7)	0.49 ± 0.07 (7)[a]
Free T_3 (pg/dl)	3.77 ± 0.16 (6)	86.79 ± 11.44 (7)[a]	1.01 ± 0.32 (7)[a]

Results are expressed as mean ± SEM. *Numbers in parentheses* are animals in each group.
[a] Statistical differences between normal chow and T_3 treatment (T_3) or during T_3 deprivation (MMI + PTU), $P < 0.0001$.

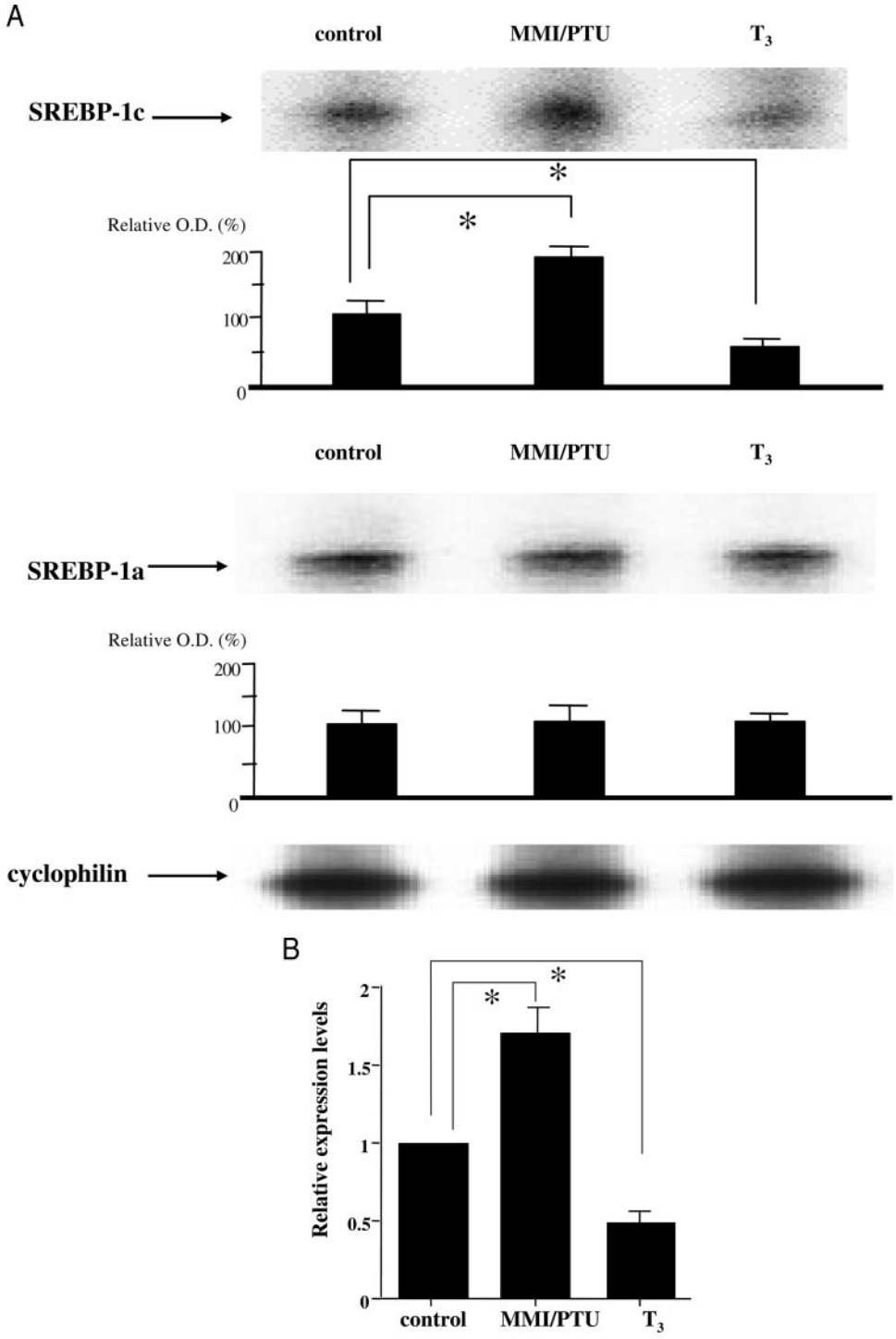

FIG. 2. T_3 suppresses SREBP-1c mRNA expression *in vivo*. A, C57/B6 mice (4-wk-old male) were rendered thyrotoxic with T_3 and hypothyroid with a MMI/PTU diet. Some of them were fed only normal chow (control). Each treatment involved six mice. RPAs were performed with liver total RNA using SREBP-1a- and/or SREBP-1c-specific riboprobes. The levels of mRNA expression were normalized to that of cyclophilin, and the results are expressed as the mean relative to the expression in control mice (mean ± SE), which was set to 100%. Representative RPA data are shown in this figure. The *asterisk* indicates that the difference between the denoted pairs is significant at a confidence level of $P < 0.01$ (*) by *t* testing. B, The expression of mouse SREBP-1c mRNA was monitored by real-time quantitative PCR assays. Each treatment involved six mice. Results (mean ± SE) are expressed as fold-activation relative to the expression in control mice, which was set to 1. The *asterisk* indicates that the difference between the denoted pairs is significant at a confidence level of $P < 0.01$ (*) by *t* testing.

deficient in binding DNA (26), and the E457A mutant is deficient in binding coactivators such as steroid receptor coactivator (SRC)-1 (27). As shown in Fig. 4, the E457A mutant negatively regulated the −574/+42 reporter, as did wild-type TR-β1. In contrast, GS125 as well as the Δ337T mutant did not demonstrate negative regulation of the mouse SREBP-1c promoter. These data indicate that both DNA and ligand binding are necessary for negative regulation of the SREBP-1c promoter by TR-β1.

As shown in Fig. 3, the −77/+42 and the −50/+42 SREBP-1c promoters were negatively regulated by T_3 and the activities of these short reporter constructs were apparently weaker than that of the −574/+42 reporter, and therefore we examined whether TR could bind to the SREBP-1c promoter around the transcriptional start site. For this purpose, we performed gel-shift assays using oligonucleotides (Fig. 5A, ΔSRE1–3) which divided the −50- to +40-bp region of the mouse SREBP-1c promoter sequence into three sites. As

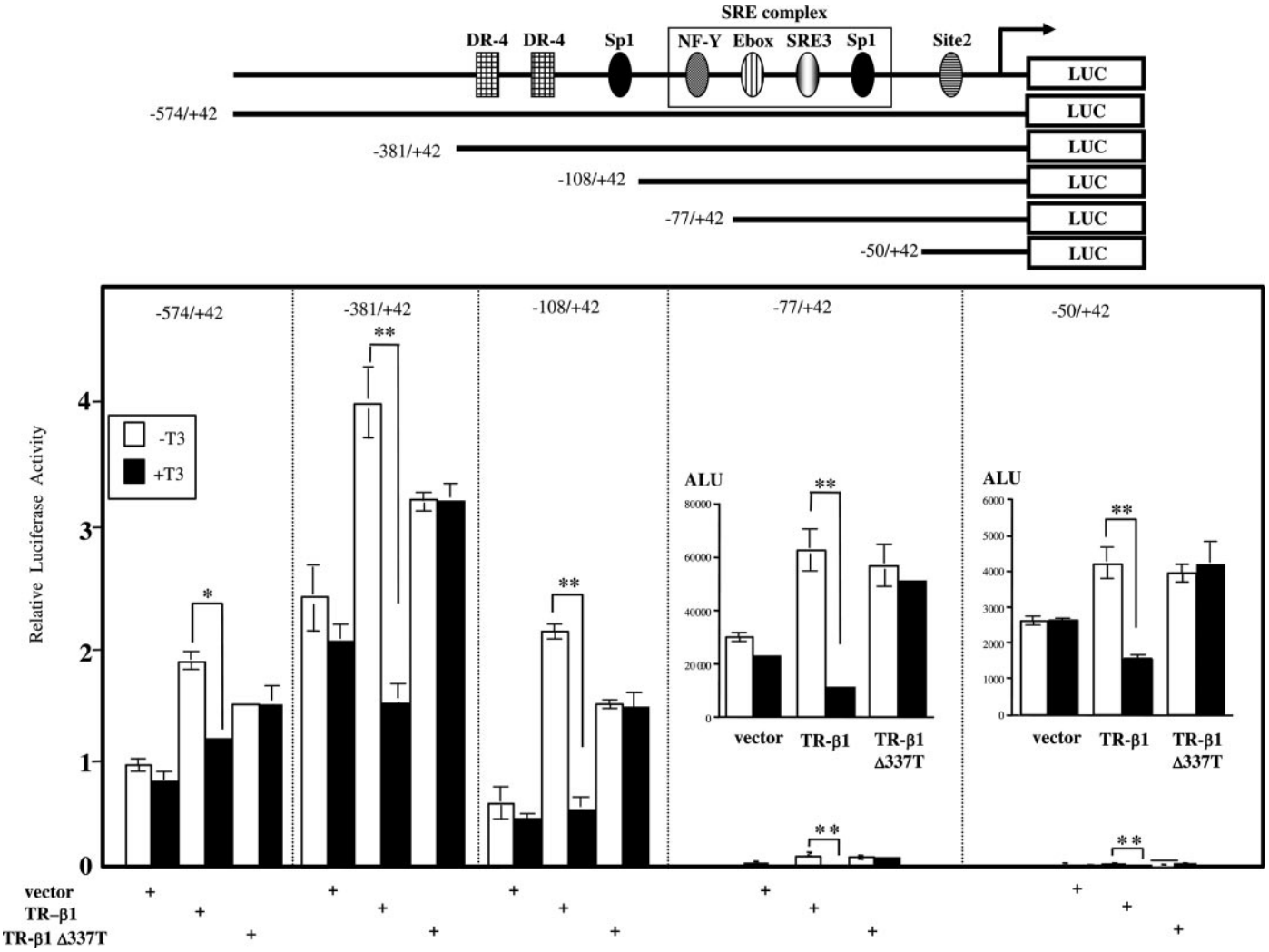

FIG. 3. TR-β negatively regulates transcription of the mouse SREBP-1c gene promoter. The mouse SREBP-1c promoter (−574/+42 bp) coupled to the luciferase reporter construct (pA3-Luc) or deletion mutants of the SREBP-1c promoter were cotransfected into HepG2 cells in the presence or absence of an expression vector for wild-type human TR-β1, Δ337T mutant (TR-β1Δ337T) or empty expression vector, pSG5 (vector). *Top*, Schematic representation of the deletion mutants of the SREBP-1c promoter. Relative luciferase activity (mean ± SEM, n = 3) represents the luciferase activity of the reporter construct (−574/+42 pA3 Luc) in the presence of the vector (pSG5) and in the absence of T_3 (10^{-8} M). Raw data are shown for the −77/+42 and the −50/+42 reporters (*inset*). ALU, Arbitrary light units. The *asterisk* indicates that the difference between the denoted pairs is significant at a confidence level of $P < 0.05$ (*) or $P < 0.01$ (**) by *t* testing.

shown in Fig. 5B, the RXR-TR heterodimer was formed on the ΔSRE2 probe, which contains the transcriptional start site, but not on the ΔSRE1 or ΔSRE3 probes. We then analyzed ΔSRE2 precisely and focused on the sequence 5′-GCCTGACAGGTGAAATCGGC-3′, referred to as 'Site2', which is located at the center of the ΔSRE2 probe. As a positive control, we employed the DR-4 (5′-TGTTTGCTTTGGTCACTCAAGTTCAA-3′, rat CYP7A1 promoter) oligonucleotide. We prepared two mutant probes for Site2 and performed gel-shift assays (Fig. 5C). As shown in Fig. 5C, TR-β1 and RXR-α formed a heterodimer on the DR-4, ΔSRE2, and Site2 probes, but the RXR-TR heterodimer did not bind to the m1 and m2 mutant probes. We also used cold Site2 oligonucleotide as a competitor, and found that the RXR-TR heterodimer band diminished in proportion to the dose of the competitor (data not shown). We also employed a native DR-4, which is a LXRE located at −234 to −219 bp in the promoter, as another positive control probe. As shown in Fig. 5C, the RXR-TR heterodimer clearly bound to this site.

Because LXRs share a DNA binding site with TR, we checked whether the RXR-LXR heterodimer could bind to ΔSRE2 and/or Site2. As shown in Fig. 5D, RXR-LXR heterodimer formation was not observed on ΔSRE2, Site2, or its mutant probes. These lines of data indicated that TR-β1 directly binds to Site2 in the mouse SREBP-1c promoter, forming a heterodimer with its partner, RXR-α.

To confirm our findings, we performed an *in vivo* ChIP assay using mouse liver tissue from nontreated (control), thyrotoxic, and hypothyroid, mice. As shown in Fig. 6 (*left panels*), in the thyrotoxic status, TR-β1 but not LXR-α was recruited to Site2, and in the hypothyroid status, the recruitment was totally inhibited. RXR-α, which is a heterodimerization partner for TR-β1, was also recruited to Site2, and T_3 administration significantly increased RXR-α recruitment to

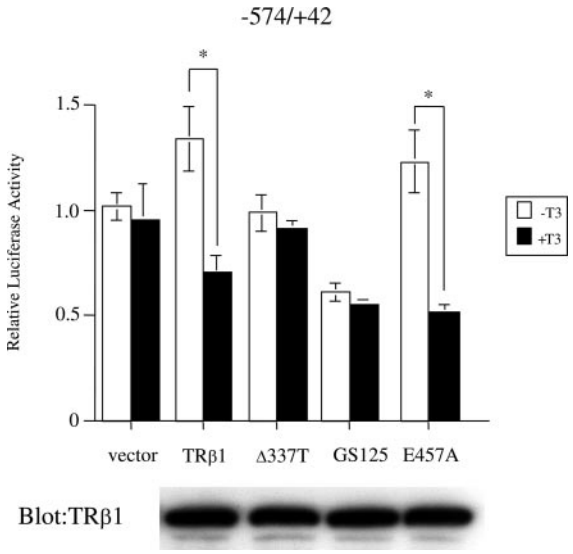

FIG. 4. DNA binding and ligand binding of TR-β1 are required for negative regulation of the SREBP-1c gene promoter by T_3. The $-574/+42$ pA3 Luc reporter plasmid and wild-type or mutant TR-β1 constructs were cotransfected into CV-1 cells. Relative luciferase activity (mean ± SEM, n = 3) represents luciferase activity of the reporter construct ($-574/+42$ pA3 Luc) in the presence of vector (pSG5) and in the absence of T_3 (10^{-8} M). The *asterisk* indicates that the difference between the denoted pairs is significant at a confidence level of $P < 0.05$ (*) by *t* testing. The expression of wild-type and mutant TR-β1s in CV-1 cells is shown in the *lower panel*. The characteristics of the mutant TR-β1s are as follows: Δ337T, ligand-binding defective; GS125, DNA-binding defective; E457A, coactivator-binding defective.

the mouse SREBP-1c promoter. On the other hand, immunoprecipitation with normal mouse IgG as a negative control showed only background levels as a negative control. We also performed *in vivo* ChIP assays using primers that contained two DR-4 sites on the mouse SREBP-1c promoter. Interestingly, thyroid hormone clearly induced TR-β1 recruitment to the DR-4 sites, and the recruitment was undetectable in the hypothyroid status (Fig. 6, *center panels*). RXR-α was bound to the DR-4 sites more efficiently than TR-β1 was in the euthyroid status. RXR-α recruitment to the DR-4 sites was induced by T_3 administration, and hypothyroid treatment diminished the recruitment. LXR-α was present on the DR-4 sites under all three treatment conditions, and its recruitment to the DR-4 sites was slightly but not significantly affected by T_3 administration. Normal mouse IgG demonstrated no specific bindings to the DR-4 sites (Fig. 6, *center panels*). We employed a set of primers that encompassed exon 18, which is the 3′ exon of the mouse SREBP-1c, as an additional control to verify the relevance of the *in vivo* ChIP assays. Expectedly, no binding of TR-β1, LXR-α, or RXR-α to the exon was observed (Fig. 6, *right panels*). These data indicated that TR-β1 was recruited to Site2 and the DR-4 sites on the mouse SREBP-1c promoter in a T_3 dose-dependent manner.

To confirm that Site2 is functionally responsible for the negative regulation of the mouse SREBP-1c promoter by TR-β1, we deleted Site2 from the $-574/+42$ luciferase reporter. As shown in Fig. 7, the mutant reporter did not show negative regulation by TR although its basal luciferase activity was almost identical to that of the wild-type reporter ($-574/+42$). We also used the mutant $-574/+42$ reporter, which harbored the same mutation as the gel-shift probes (m1, m2), and found no negative regulation by TR (data not shown).

To examine whether the human SREBP-1c promoter was negatively regulated by thyroid hormone, we employed the human SREBP-1c promoter ($-843/+62$) in pGL4-Luc. This construct was cotransfected with pSG5 vector or TR-β1 construct into HepG2 cells derived from human hepatocytes. As shown in Fig. 8A, the human promoter activity was significantly suppressed by T_3 via TR-β1. To examine whether there were differences among species, we used Hepa1–6 cells that were derived from mouse hepatocytes for the luciferase assays with the mouse SREBP-1c promoter ($-574/+42$) in pA3-Luc. As shown in Fig. 8B, the mouse promoter activity was significantly reduced by T_3 via TR-β1, as observed in HepG2 cells. We also confirmed that Site2 was important for the negative regulation of the gene by T_3 in Hepa1–6 cells (data not shown). These data indicate that there are no differences between mice and humans in SREBP-1c gene regulation by thyroid hormone.

Discussion

In this current study, we demonstrated that T_3 down-regulates mouse SREBP-1c mRNA both *in vivo* and *in vitro*. Until now it has been controversial how T_3 regulates the mouse SREBP-1c promoter. Viguerie *et al.* (10) reported that thyroid hormone represses SREBP-1c mRNA in human adipocytes by about 50% in human cDNA expression array and reverse transcription-competitive PCR assays. They speculated that the down-regulation of SREBP-1c might constitute a link between hyperthyroidism and insulin resistance (10). On the other hand, Kawai *et al.* (12) showed in a recent study that the mouse SREBP-1c gene promoter is up-regulated by T_3 through RXR-TR heterodimer binding to the DR-4 site. Their data are in direct opposition to our data regarding SREBP-1c gene regulation by T_3. Those authors constructed a mouse SREBP-1c promoter-TK (thymidine kinase proximal promoter) luciferase plasmid for their luciferase assays. They did not examine native mouse SREBP-1c promoter activity. Because TK is well known to be up-regulated by T_3 (28), their data, in which the mouse SREBP-1c gene promoter was up-regulated by T_3, are questionable. Moreover, they used CV-1 cells from the kidney of the African green monkey for the luciferase assay. Because SREBP-1c is expressed in human and rodent liver (19), it would be rational to employ hepatocytes for the reporter assay. The difference between our data and theirs might be due to the cell type difference. They also concluded that T_3 induces SREBP-1c mRNA in HepG2 cells using semiquantitative RT-PCR. In our study, we performed RPAs and real-time quantitative PCR using mouse liver total RNA and demonstrated that thyroid hormone repressed SREBP-1c mRNA expression *in vivo*. We speculate that the discrepancy between Kawai's data and ours is due to differences between conditions *in vitro* and *in vivo*. Zhang *et al.* (11) reported that T_3 induced chicken SREBP-1 gene expression in chick embryo hepatocytes, especially under glucose administra-

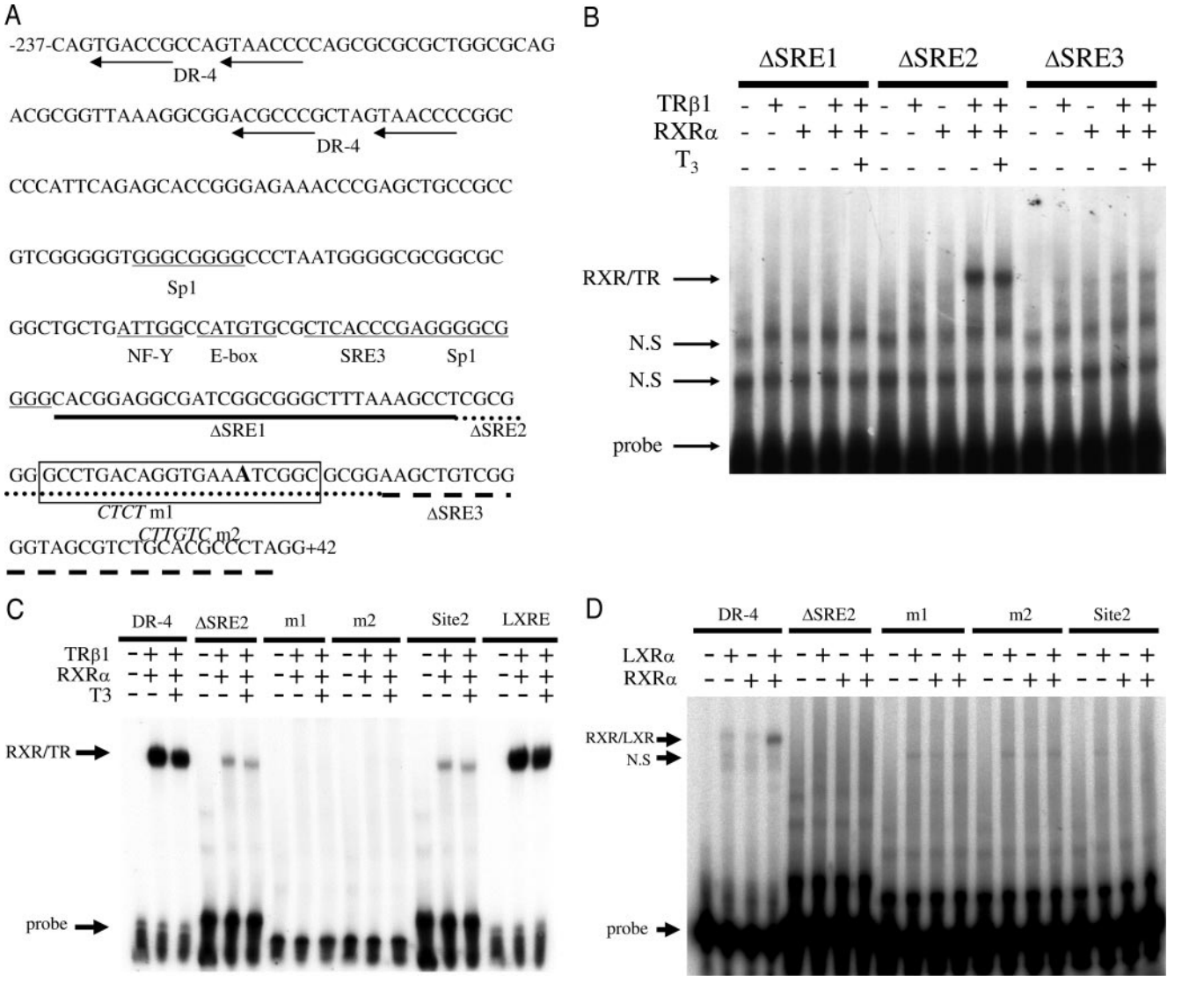

FIG. 5. RXR-TR heterodimer but not RXR-LXR binds to Site2. A, Mouse SREBP-1c promoter sequences surrounding a cognate transcription start site. We divided the sequences (−50/+42) into three parts (ΔSRE1–3). The Site2 sequence is *boxed*. The *bold letter* "A" is the cognate transcription start site. *Italics* represent mutations in Site2 (m1, m2). The two DR-4 (LXRE) sites are indicated by *arrows*. B, Four microliters of *in vitro*-translated TR-β1 and RXR-α protein generated from rabbit reticulocyte lysates were incubated with ^{32}P-radiolabeled DNA probes (ΔSRE1–3). T_3, 100 nM. C, Four microliters of *in vitro*-translated TR-β1 and RXR-α protein generated from rabbit reticulocyte lysates were incubated with ^{32}P-radiolabeled DNA probes (DR-4, ΔSRE2, m1, m2, Site2, and LXRE). T_3, 100 nM. D, Four microliters of *in vitro*-translated LXR-α and RXR-α protein generated from rabbit reticulocyte lysates were incubated with ^{32}P-radiolabeled DNA probes (DR-4, ΔSRE2, m1, m2, Site2). N.S., Nonspecific bands.

tion; however, they observed whole SREBP-1 mRNA, including both 1a and 1c isoforms, and did not examine SREBP-1c mRNA specifically.

Using several types of mutant TR, we showed that DNA binding of TR and ligand binding to TR are crucial to the negative regulation of the promoter. The necessity for DNA binding of TR for negative regulation by T_3 remains controversial, especially regarding the necessity of direct TR binding to promoter DNA (26, 29). Nevertheless, we concluded that DNA binding of TR (RXR-TR heterodimer) is required for negative regulation of the mouse SREBP-1c gene promoter based on the current data (Fig. 4). Another interesting observation was that a mutant TR (E457A) that was unable to interact with coactivators such as steroid receptor coactivator-1 was still able to negatively regulate the mouse SREBP-1c promoter. This is intriguing because the same mutant receptor has been shown to be unable to negatively regulate the pituitary TSH-β gene promoter (30). This divergence indicates that requirement of coactivators for negative regulation by TR could be tissue specific.

The mouse SREBP-1c promoter contains two DR-4 sites (LXREs), and LXRs stimulate promoter activity through binding to the sites. Because LXRs and TRs share the DR-4 site, we first speculated that TRs would also regulate the mouse SREBP-1c promoter through binding to the DR-4 site. In fact, our EMSA data clearly showed that the RXR-TR heterodimer bound to both the DR-4 site and LXRE.

However, this RXR-TR heterodimerization on the DR-4

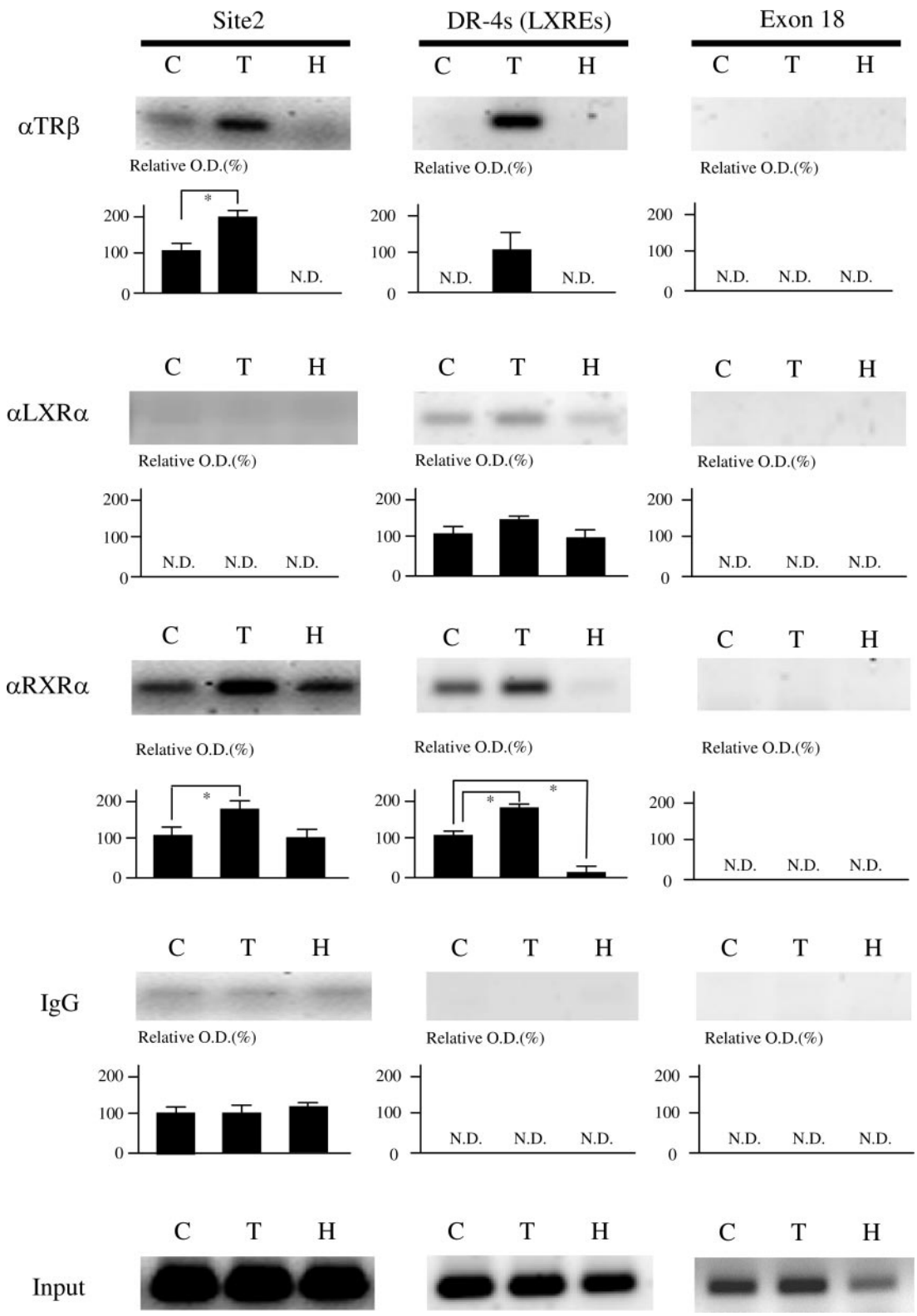

FIG. 6. TR-β1 together with RXR-α are recruited to Site2 and DR-4 (LXRE) sites in a T_3-dependent manner *in vivo*. *In vivo* ChIP assays were performed using mouse liver tissue for Site2 (*left panels*), DR-4 (LXRE) sites (*center panels*), and exon 18 (*right panels*). C57/B6 mice (4-wk-old male) were rendered thyrotoxic (T) with T_3 or hypothyroid (H) with a MMI/PTU diet. Some of them were fed normal chow only (controls, C). Each treatment involved six mice. Liver tissue homogenate from each treatment group was immunoprecipitated with a TR-β1 antibody (α TRβ) or an LXR-α antibody (α LXRα), or an RXR-α antibody (α RXRα), or mouse normal IgG as a negative control. The input was a nonimmunoprecipitated sample used as a positive control. Relative OD (mean ± SE) was controlled for the input level using NIH image software. N.D., Not detectable. The *asterisk* indicates that the difference between the denoted pairs is significant at a confidence level of $P < 0.01$ (*) by *t* testing.

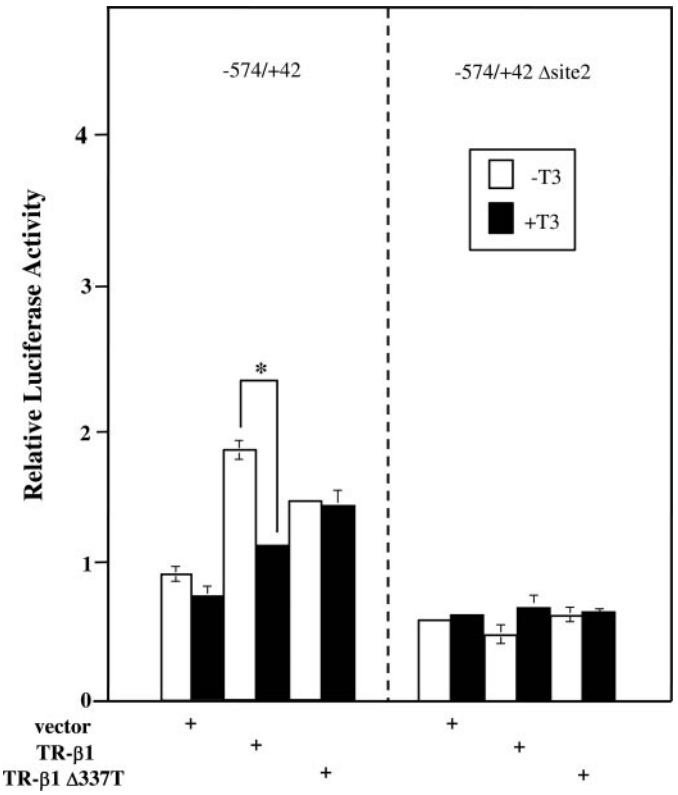

FIG. 7. Site2 is functionally responsible for the negative regulation of mouse SREBP-1c gene promoter by TR-β1. The mouse SREBP-1c promoter ($-574/+42$ bp) coupled to the luciferase reporter construct (pA3-Luc) or the Site2 deletion mutant was cotransfected into HepG2 cells in the presence or absence of an expression vector for wild-type human TR-β1, Δ337T mutant (TR-β1Δ337T), or empty expression vector, pSG5 (vector). Relative luciferase activity (mean $\pm$ SEM, n = 3) represents the luciferase activity of the reporter construct ($-574/+42$ pA3 Luc) in the presence of the vector (pSG5) and in the absence of T_3 (10^{-8} M). The *asterisk* indicates that the difference between the denoted pairs is significant at a confidence level of $P < 0.05$ (*) by *t* testing.

site was not used for gene regulation; that is, T_3 and TR did not transactivate the mouse SREBP-1c promoter through the DR-4 site. On the contrary, T_3 repressed the mouse SREBP-1c promoter activity through RXR-TR heterodimer binding to Site2 located around the transcription start site.

In vivo ChIP assays demonstrated that TR-β together with RXR-α are recruited to Site2 in a T_3 dose-dependent manner. We also observed that T_3 induced TR-β and RXR-α recruitment to the DR-4 sites (LXREs) in the mouse SREBP-1c promoter. These data are compatible with a recent report by Liu *et al.* (31) showing that TR-β was recruited to the TREs in a T_3-dependent manner in a time-course study. This induction by T_3 was not seen in EMSA; however, this divergence between the ChIP assay and EMSA could be due to differences between *in vivo* and *in vitro* conditions. In the control status, no detectable TR-β recruitment to the DR-4 sites (LXREs) was observed, although minimal binding of TR-β to Site2 was detected. This is also congruent with the report by Liu *et al.* (31) indicating that the requirement for TR-β binding to the TREs depends on the specific gene promoter. In *in vivo* ChIP assays, RXR-α was still bound to Site2 but not to the DR-4 sites (LXREs) under hypothyroid status. We think that this difference could be a key point in explaining how Site2 functions as a negative (TRE).

Differences in gene regulation by thyroid hormone among species are often seen. For example, thyroid hormone increases the rat hepatic enzyme cholesterol 7α-hydroxylase (CYP7A1) gene expression (32–35). However, very recently, Drover *et al.* (36) reported that T_3 represses the human CYP7A1 promoter. In this regard, we employed Hepa1–6 cells, which were derived from mouse hepatocytes, and a human SREBP-1c promoter construct to confirm our data (Fig. 8, A and B). We confirmed that the mouse SREBP-1c promoter was negatively regulated by thyroid hormone in the Hepa1–6 cells. Tarling *et al.* (17) reported that although the human and mouse SREBP-1c promoter sequences share conserved elements such as Sp1, SRE, NF-Y and LXRE sites, the two sequences are very different (42.0% similar). We found no similar sequences to Site2 in the human promoter sequence (GenBank accession no. NT_010718). However, negative regulation of the human SREBP-1c promoter by T_3 was also observed. Therefore, we concluded that no differences between human and mouse were seen in SREBP-1c gene regulation by thyroid hormone. Negative TREs distinct from Site2 may be present in the human promoter, and further analysis will be needed to examine this possibility.

TR seems to regulate lipid metabolism-related genes in various ways. Huuskonen *et al.* (37) reported that human ATP-binding cassette transporter A1 (ABCA1) gene promoter was negatively regulated by TR. They showed that RXR-TR heterodimer bound to the DR-4 site, and they concluded that T_3 represses gene promoter activity through the binding of RXR-TR heterodimer to the DR-4 site. Drover *et al.* (36) demonstrated that TR bound to two distinct sites on the human CYP7A1 gene promoter. They also showed that T_3 represses the human CYP7A1 promoter, but it requires only one of the two sites to which TR binds.

Site2 (GCCTGACAGGTGAAATCGGC) is recognized as a negative TRE (38). Although no consensus negative TRE has been identified to date, Sasaki *et al.* (39) studied the T_3-dependent repression of transcription mainly using TSH-α and -β, both of which contain a conserved sequence called the Z-element (CAAAG) (39, 40); however, Site2 has no Z-element motif. Moreover, no hexametric binding motif of the consensus sequence RGKTCA (R = A or G; K = G or T) (6, 42, 43) was seen in Site2. Our mutation study revealed that the RXR-TR heterodimer cannot bind to mutated Site2. Thus, it is clear that this site is responsible for RXR-TR binding, although, the molecular mechanism by which negative regulation by T_3 using Site2 must be clarified in future studies.

We speculate that the repression of SREBP-1c gene expression by T_3 is physiologically related to insulin resistance in the hyperthyroid state. Foretz *et al.* (44) reported that increased SREBP-1c expression overcomes the insulin dependency of glucokinase expression. Because SREBP-1c is a master gene for the regulation of lipid and glucose metabolism in the liver (45, 46), decreased endogenous activity of SREBP-1c in the hyperthyroid state should induce insulin resistance (21).

In this study, we have provided evidence that T_3 represses mouse SREBP-1c expression at the transcriptional level. It is noteworthy that RXR-TR heterodimer binding to the novel

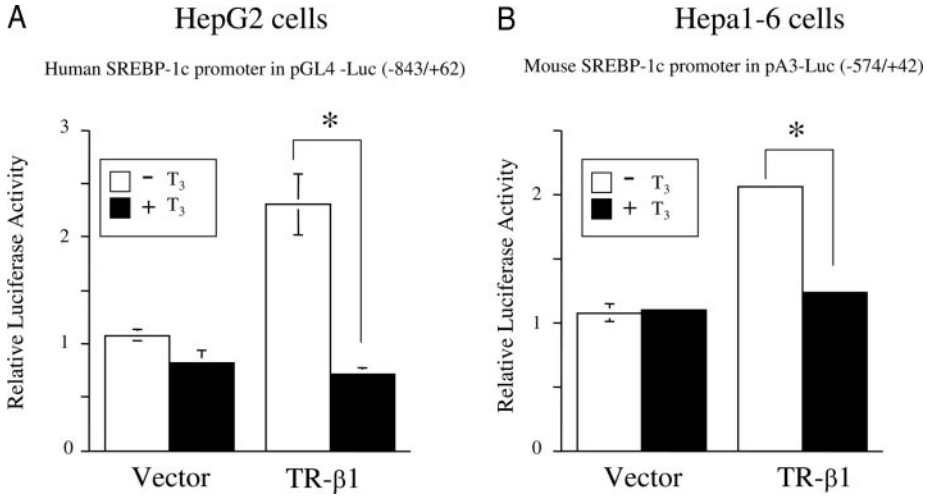

FIG. 8. No differences between human and mouse are observed in SREBP-1c gene regulation by thyroid hormone. A, The human SREBP-1c gene promoter is also negatively regulated by T_3. The human SREBP-1c promoter (−843/+62 bp) coupled to the luciferase reporter construct (pGL4-Luc) was cotransfected into HepG2 cells in the presence or absence of an expression vector for wild-type human TR-β1 or empty expression vector, pSG5 (vector). Relative luciferase activity (mean ± SEM, n = 3) represents the luciferase activity of the reporter construct (−843/+62 pGL4-Luc) in the presence of the vector (pSG5) and in the absence of T_3 (10^{-8} M). The *asterisk* indicates that the difference between the denoted pairs is significant at a confidence level of $P < 0.01$ (*) by *t* testing. B, The mouse SREBP-1c gene promoter is also negatively regulated by T_3 in Hepa1–6 cells. The mouse SREBP-1c promoter (−574/+42 bp) coupled to the luciferase reporter construct (pA3 Luc) was cotransfected into Hepa1–6 cells in the presence or absence of an expression vector for wild-type human TR-β1 or empty expression vector, pSG5 (vector). Relative luciferase activity (mean ± SEM, n = 3) represents luciferase activity of the reporter construct (−574/+42 pA3-Luc) in the presence of the vector (pSG5) and in the absence of T_3 (10^{-8} M). The *asterisk* indicates that the difference between the denoted pairs is significant at a confidence level of $P < 0.01$ (*) by *t* testing.

response element Site2, but not DR-4, is related to gene regulation by T_3. This means that although LXR and TR share a similar DNA binding element on the mouse SREBP-1c gene promoter, they do not compete because LXR does not bind to Site2. Although it has been said that TR does not appear to affect the lipid metabolic cascade (41), several recent reports indicated that TR and LXR cross talk mutually in lipid homeostasis (4, 9, 11, 37). Further studies of the SREBP-1c gene expression using TR knockout/knock-in animals that are mouse models of resistance to thyroid hormone should be of interest.

Acknowledgments

We thank Dr. Iichiro Shimomura (Osaka University, Osaka, Japan) for providing probes for mouse SREBP-1a and -1c. We also thank Dr. C. N. Mariash (University of Minnesota, Minneapolis, MN) for rat 5′DI probe. We appreciate Drs. E. J. Tarling and A. Bennett (University of Nottingham Medical School, Nottingham, UK) for the human SREBP-1c promoter pGL4-Luc plasmid.

Received January 27, 2006. Accepted June 9, 2006.

Address all correspondence and requests for reprints to: Koshi Hashimoto, M.D., Ph.D., Department of Medicine and Molecular Science, Graduate School of Medicine, Gunma University, 3-39-15 Showa-machi Maebashi, Gunma 371-8511, Japan. E-mail: khashi@med.gunma-u.ac.jp.

Current address for T.M.: Department of Endocrinology and Metabolism, Dokkyo Medical College, Mibu, Tochigi, Japan.

Disclosure statement: K.H., M.Y., S.M., T.M., T.S., and M.M. have nothing to declare.

References

1. **Brown MS, Goldstein JL** 1997 The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. Cell 89:331–340
2. **Brown MS, Goldstein JL** 1999 A proteolytic pathway that controls the cholesterol content of membranes, cells, and blood. Proc Natl Acad Sci USA 96:11041–11048
3. **Horton JD, Goldstein JL, Brown MS** 2002 SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. J Clin Invest 109:1125–1131
4. **Repa JJ, Liang G, Ou J, Bashmakov Y, Lobaccaro JM, Shimomura I, Shan B, Brown MS, Goldstein JL, Mangelsdorf DJ** 2000 Regulation of mouse sterol regulatory element-binding protein-1c gene (SREBP-1c) by oxysterol receptors, LXRα and LXRβ. Genes Dev 14:2819–2830
5. **Yoshikawa T, Shimano H, Amemiya-Kudo M, Yahagi N, Hasty AH, Matsuzaka T, Okazaki H, Tamura Y, Iizuka Y, Ohashi K, Osuga J, Harada K, Gotoda T, Kimura S, Ishibashi S, Yamada N** 2001 Identification of liver X receptor-retinoid X receptor as an activator of the sterol regulatory element-binding protein 1c gene promoter. Mol Cell Biol 21:2991–3000
6. **Umesono K, Murakami KK, Thompson CC, Evans RM** 1991 Direct repeats as selective response elements for the thyroid hormone, retinoic acid, and vitamin D3 receptors. Cell 65:1255–1266
7. **Quack M, Frank C, Carlberg C** 2002 Differential nuclear receptor signalling from DR4-type response elements. J Cell Biochem 86:601–612
8. **Berkenstam A, Farnegardh M, Gustafsson JA** 2004 Convergence of lipid homeostasis through liver X and thyroid hormone receptors. Mech Aging Dev 125:707–717
9. **Hashimoto K, Cohen RN, Yamada M, Markan KR, Monden T, Satoh T, Mori M, Wondisford, FE** 2006 Cross-talk between thyroid hormone receptor and liver X receptor regulatory pathways is revealed in a thyroid hormone resistance mouse model. J Biol Chem 281:295–302
10. **Viguerie N, Millet L, Avizou S, Vidal H, Larrouy D, Langin D** 2002 Regulation of human adipocyte gene expression by thyroid hormone. J Clin Endocrinol Metab 87:630–634
11. **Zhang Y, Yin L, Hillgartner FB** 2003 SREBP-1 integrates the actions of thyroid hormone, insulin, cAMP, and medium-chain fatty acids on ACCα transcription in hepatocytes. J Lipid Res 44:356–368
12. **Kawai K, Sasaki S, Morita H, Ito T, Suzuki S, Misawa H, Nakamura H** 2004 Unliganded thyroid hormone receptor-β1 represses liver X receptor α/oxysterol-dependent transactivation. Endocrinology 145:5515–5524
13. **Theodossiou C, Skrepnik N, Robert EG, Prasad C, Schapira DV, Hunt JD** 1999 Propylthiouracil-induced hypothyroidism reduces xenograft tumor growth in athymic nude mice. Cancer 86:1596–1601
14. **Wolf B, Aratan-Spire S, Czernichow P** 1984 Hypothalamo-pituitary regulation of thyrotrophin secretion in chronically catheterized Brattleboro rats. Endocrinology 114:1334–1337
15. **Amemiya-Kudo M, Shimano H, Yoshikawa T, Yahagi N, Hasty AH, Okazaki H, Tamura Y, Shionoiri H, Iizuka Y, Ohashi K, Osuga J, Harada K, Gotoda**

T, Sato R, Kimura S, Ishibashi S, Yamada N 2000 Promoter analysis of the mouse sterol regulatory element-binding protein-1c gene. J Biol Chem 275: 31078–31085
16. **Hashimoto K, Zanger K, Hollenberg AN, Cohen LE, Radovick S, Wondisford FE** 2000 cAMP response element-binding protein-binding protein mediates thyrotropin-releasing hormone signaling on thyrotropin subunit genes. J Biol Chem 275:33365–33372
17. **Tarling E, Salter A, Bennett A** 2004 Transcriptional regulation of human SREBP-1c (sterol-regulatory-element-binding protein-1c): a key regulator of lipogenesis. Biochem Soc Trans 32:107–109
18. **Kushida S, Peng BG, Uchimura E, Kuang M, Huang L, Miwa M, Ohno T** 2004 A tumour vaccine of fixed tumour fragments in a controlled-release vehicle with cytokines for therapy of hepatoma in mice. Dig Liver Dis 36:478–485
19. **Shimomura I, Shimano H, Horton JD, Goldstein JL, Brown MS** 1997 Elevated levels of SREBP-2 and cholesterol synthesis in livers of mice homozygous for a targeted disruption of the SREBP-1 gene. J Clin Invest 99:838–845
20. **Ren Y, Satoh T, Yamada M, Hashimoto K, Konaka S, Iwasaki T, Mori M** 1998 Stimulation of the preprothyrotropin-releasing hormone gene by epidermal growth factor. Endocrinology 139:195–203
21. **Cachefo A, Boucher P, Vidon C, Dusserre E, Diraison F, Beylot M** 2001 Hepatic lipogenesis and cholesterol synthesis in hyperthyroid patients. J Clin Endocrinol Metab 86:5353–5357
22. **Hashimoto K, Yamada M, Monden T, Satoh T, Wondisford FE, Mori M** 2005 Thyrotropin-releasing hormone (TRH) specific interaction between amino terminus of P-Lim and CREB binding protein (CBP). Mol Cell Endocrinol 229: 11–20
23. **Toyoda N, Zavacki AM, Maia AL, Harney JW, Larsen PR** 1995 A novel retinoid X receptor-independent thyroid hormone response element is present in the human type 1 deiodinase gene. Mol Cell Biol 15:5100–5112
24. **Usala SJ, Menke JB, Watson TL, Wondisford FE, Weintraub BD, Berard J, Bradley WE, Ono S, Mueller OT, Bercu BB** 1991 A homozygous deletion in the c-erbA β thyroid hormone receptor gene in a patient with generalized thyroid hormone resistance: isolation and characterization of the mutant receptor. Mol Endocrinol 5:327–335
25. **Satoh T, Yamada M, Iwasaki T, Mori M** 1996 Negative regulation of the gene for the preprothyrotropin-releasing hormone from the mouse by thyroid hormone requires additional factors in conjunction with thyroid hormone receptors. J Biol Chem 271:27919–27926
26. **Shibusawa N, Hollenberg AN, Wondisford FE** 2003 Thyroid hormone receptor DNA binding is required for both positive and negative gene regulation. J Biol Chem 278:732–738
27. **Flynn TR, Hollenberg AN, Cohen O, Menke JB, Usala SJ, Tollin S, Hegarty MK, Wondisford FE** 1994 A novel C-terminal domain in the thyroid hormone receptor selectively mediates thyroid hormone inhibition. J Biol Chem 269: 32713–32716
28. **Park HY, Davidson D, Raaka BM, Samuels HH** 1993 The herpes simplex virus thymidine kinase gene promoter contains a novel thyroid hormone response element. Mol Endocrinol 7:319–330
29. **Tagami T, Park Y, Jameson JL** 1999 Mechanisms that mediate negative regulation of the thyroid-stimulating hormone α gene by the thyroid hormone receptor. J Biol Chem 274:22345–22353
30. **Ortiga-Carvalho TM, Shibusawa N, Nikrodhanond A, Oliveira KJ, Machado DS, Liao XH, Cohen RN, Refetoff S, Wondisford FE** 2005 Negative regulation by thyroid hormone receptor requires an intact coactivator-binding surface. J Clin Invest 115:2517–2523
31. **Liu Y, Xia X, Fondell JD, Yen PM** 2006 Thyroid hormone-regulated target genes have distinct patterns of coactivator recruitment and histone acetylation. Mol Endocrinol 20:483–490
32. **Pandak WM, Heuman DM, Redford K, Stravitz RT, Chiang JY, Hylemon PB, Vlahcevic ZR** 1997 Hormonal regulation of cholesterol 7α-hydroxylase specific activity, mRNA levels, and transcriptional activity in vivo in the rat. J Lipid Res 38:2483–2491
33. **Hylemon PB, Gurley EC, Stravitz RT, Litz JS, Pandak WM, Chiang JY, Vlahcevic ZR** 1992 Hormonal regulation of cholesterol 7 α-hydroxylase mRNA levels and transcriptional activity in primary rat hepatocyte cultures. J Biol Chem 266:16866–16871
34. **Ness GC, Pendleton LC, Li YC, Chiang JY** 1990 Effect of thyroid hormone on hepatic cholesterol 7 α hydroxylase, LDL receptor, HMG-CoA reductase, farnesyl pyrophosphate synthetase and apolipoprotein A-I mRNA levels in hypophysectomized rats. Biochem Biophys Res Commun 172:1150–1156
35. **Ellis E, Goodwin B, Abrahamsson A, Liddle C, Mode A, Rudling M, Bjorkhem I, Einarsson C** 1998 Bile acid synthesis in primary cultures of rat and human hepatocytes. Hepatology 27:615–620
36. **Drover VAB, Norman CW, Agellon LB** 2002 A distinct thyroid hormone response element mediates repression of the human cholesterol 7α-hydroxylase (CYP7A1) gene promoter. Mol Endocrinol 16:14–23
37. **Huuskonen J, Vishnu M, Pullinger CR, Fielding PE, Fielding CJ** 2004 Regulation of ATP-binding cassette transporter A1 transcription by thyroid hormone receptor. Biochemistry 43:1626–1632
38. **Braverman LE, Utiger RD** 2000 Werner & Ingbar's the thyroid. 8th ed. Philadelphia: Lippincott Williams, Wilkins; 178–180
39. **Sasaki S, Lesoon-Wood LA, Dey A, Kuwata T, Weintraub BD, Humphrey G, Yang WM, Seto E, Yen PM, Howard BH, Ozato K** 1999 Ligand-induced recruitment of a histone deacetylase in the negative-feedback regulation of the thyrotropin β gene. EMBO J 18:5389–5398
40. **Nygard M, Wahlstrom GM, Gustafsson MV, Tokumoto YM, Bondesson M** 2003 Hormone-dependent repression of the E2F-1 gene by thyroid hormone receptors. Mol Endocrinol 17:79–92
41. **Chawla A, Repa JJ, Evans RM, Mangelsdorf DJ** 2001 Nuclear receptors and lipid physiology: opening the X-files. Science 294:1866–1870
42. **Naar AM, Boutin JM, Lipkin SM, Yu VC, Holloway JM, Glass CK, Rosenfeld MG** 1991 The orientation and spacing of core DNA-binding motifs dictate selective transcriptional responses to three nuclear receptors. Cell 65:1267–1279
43. **Mangelsdorf DJ, Evans RM** 1995 The RXR heterodimers and orphan receptors. Cell 83:841–850
44. **Foretz S, Guichard C, Ferre P, Foufelle F** 1999 Sterol regulatory element binding protein-1c is a major mediator of insulin action on the hepatic expression of glucokinase and lipogenesis-related genes. Proc Natl Acad Sci USA 96:12737–12742
45. **Foretz M, Pacot C, Dugail I, Lemarchand P, Guichard C, Le Liepvre X, Berthelier-Lubrano C, Spiegelman BM, Kim JB, Ferre P Foufelle F** 1999 ADD1/SREBP-1c is required in the activation of hepatic lipogenic gene expression by glucose. Mol Cell Biol 19:3760–3768
46. **Shimomura I, Shimano H, Korn BS, Bashmakov Y, Horton JD** 1998 Nuclear sterol regulatory element-binding proteins activate genes responsible for the entire program of unsaturated fatty acid biosynthesis in transgenic mouse liver. J Biol Chem 273:35299–35306

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