Sterol Regulatory Element-binding Protein-1 Interacts with the Nuclear Thyroid Hormone Receptor to Enhance Acetyl-CoA Carboxylase-α Transcription in Hepatocytes*

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In previous work, we characterized a 3,5,3′-triiodothyronine response element (T3RE) in acetyl-CoA carboxylase-α (ACCα) promoter 2 that mediated 3,5,3′-triiodothyronine (T3) regulation of ACCα transcription in chick embryonic hepatocytes. Sequence comparison analysis revealed the presence of sterol regulatory element-1 (SRE-1) located 5 bp downstream of the ACCα T3RE. Here, we investigated the role of this SRE-1 in modulating T3 regulation of ACCα transcription. Transfection analyses demonstrated that the SRE-1 enhanced T3-induced ACCα transcription by more than 2-fold in hepatocytes. The effect of the SRE-1 on T3 responsiveness required the presence of the T3RE in its native orientation. In pull-down experiments, the mature form of sterol regulatory element-binding protein-1 (SREBP-1) specifically bound the α-isooform of the nuclear T3 receptor (TR), and the presence of T3 enhanced this interaction. A region of TRα containing the DNA-binding domain plus flanking sequences (amino acids 21–157) was required for interaction with SREBP-1, and a region of SREBP-1 containing the basic helix-loop-helix-leucine zipper domain (amino acids 300–389) was required for interaction with TRa. In gel mobility shift experiments, TRa, retinoid X receptor-α, and mature SREBP-1 formed a tetrameric complex on a DNA probe containing the ACCα T3RE and SRE-1, and the presence of T3 enhanced the formation of this complex. Formation of the tetrameric complex stabilized the binding of SREBP-1 to the SRE-1. These results indicate that SREBP-1 directly interacts with TR-retinoid X receptor in an orientation-specific manner to enhance T3-induced ACCα transcription in hepatocytes. T3 regulation of ACCα transcription in nonhepatic cell cultures such as chick embryonic fibroblasts is markedly reduced compared with that of chick embryonic hepatocytes. Here, we also show that alterations in SREBP expression play a role in mediating cell type-dependent differences in T3 regulation of ACCα transcription.

In livers of birds and mammals, consumption of carbohydrate in excess of the immediate energy requirements of the animal increases the expression of enzymes involved in the conversion of carbohydrate to triacylglycerols (1). These enzymes include those in glycolysis, such as glucokinase and 1-phosphofructokinase; those in fatty acid synthesis, such as acetyl-CoA carboxylase-α (ACCα) and fatty acid synthase; those in NADPH production, such as malic enzyme; and those in triacylglycerol synthesis, such as glycerol-3-phosphate acyltransferase. For most of the lipogenic enzymes studied, the induction of enzyme levels by dietary carbohydrate is mediated primarily by changes in gene transcription (1).

Several signaling pathways are involved in mediating the stimulatory effects of dietary carbohydrate on lipogenic gene transcription in liver. Increased insulin secretion and glucose metabolism are two important signals mediating this response (1). Another factor signaling changes in carbohydrate status is the active form of thyroid hormone, 3,5,3′-triiodothyronine (T3). Ingestion of a high carbohydrate meal stimulates a rapid increase in the secretion of thyroxine from the thyroid gland and the conversion of thyroxine to T3 in extrathyroidal tissues (2, 3). The resulting increase in T3 concentration in liver activates the transcription of the genes for ACCα (4), fatty acid synthase (5), malic enzyme (6), and Spot 14 (S14) (7). Ingestion of carbohydrate also increases the levels of nuclear T3 receptors (TRs) in liver (8). This phenomenon may also contribute to the stimulation of lipogenic gene transcription by dietary carbohydrate. A role of thyroid hormones in mediating the nutritional regulation of lipogenic enzyme expression is supported by the observation that hypothyroidism in high carbohydrate-fed animals causes a marked reduction in expression of ACCα, fatty acid synthase, malic enzyme, and S14 in liver (9–11).

ACC catalyzes the pace-setting step of the fatty acid synthesis pathway (1). There are two ACC isoforms that are encoded by distinct genes: ACCβ (280 kDa) is the major isoform observed in heart and skeletal muscle, whereas ACCα is thought to function primarily in the regulation of β-oxidation of fatty acids (12). ACCα (265 kDa) is the principal isoform expressed in tissues that exhibit high rates of fatty acid synthesis such as liver, adipose tissue, and mammary gland. ACCα expression is induced by nutritional and hormonal factors that promote high rates of fatty acid synthesis. For example, feeding previously starved chickens a high carbohydrate, low fat diet stimulates
n an 11-fold increase in transcription of the ACCα gene in liver (13). This effect is partially reproduced in primary cultures of chick embryo hepatocytes (CEH) by manipulating the concentration of T3 in the culture medium. The addition of T3 stimulates a 7-fold increase in ACCα transcription in CEH (4). The ACCα gene is transcribed from two promoters, resulting in mRNAs with heterogeneity in their 5′-untranslated region (14). These ACCα promoters are designated promoter 1 and promoter 2. The increase in total ACCα mRNA abundance caused by high carbohydrate feeding in chickens and by T3 in CEH is mediated by alterations in the activities of both promoter 1 and promoter 2, with the latter promoter playing a quantitatively greater role in mediating these responses (15). Additional studies have shown that the stimulatory effect of T3 on promoter 2 activity is mediated by a T3 response element (T3RE) with unique functional and protein binding properties (16). This T3RE enhances ACCα promoter activity both in the absence and presence of T3, with a greater stimulation observed in the presence of T3. The results of DNA binding analyses with nuclear extracts from CEH suggest that the T3-independent enhancer activity of the ACCα T3RE is mediated by the binding of protein complexes containing LXR-RXR heterodimers and that the increase in enhancer activity caused by T3 treatment is mediated by the binding of a different set of protein complexes. One of these complexes contains TR-RXR heterodimers, and another contains LXR-RXR heterodimers. Based on these observations, we have hypothesized that the ACCα T3RE not only mediates T3 regulation of ACCα transcription but also ensures a basal level of ACCα expression for the synthesis of structural lipids in cell membranes.

In addition to regulation by nutrients and hormones, ACCα transcription is controlled by tissue- or cell-specific factors. For example, ingestion of carbohydrate has little or no effect on ACCα transcription in heart, kidney, brain, and skeletal muscle (13). Cell type-dependent differences in the regulation of ACCα are also observed in cells in culture. T3 regulation of ACCα transcription in chick embryo fibroblasts (CEF) is markedly diminished compared with that in CEH (17). The mechanisms responsible for cell type-dependent differences in the regulation of ACCα transcription remain to be determined.

Studies analyzing the regulation of other T3-responsive genes have shown that optimal T3 regulation of transcription is dependent not only on the presence of a T3RE but also the presence of accessory elements that bind proteins that are distinct from nuclear hormone receptors (18–23). In isolation, these accessory elements are devoid of T3-responsive activity; they act by enhancing the degree of T3 responsiveness initiated by the T3RE. Some T3 accessory elements bind proteins that are differentially expressed in different cell types and thus play a key role in conferring cell type-dependent differences in T3 responsiveness (18, 22, 23). There is little information on the mechanism by which T3 accessory elements modulate T3 regulation of transcription.

In the present study, we have identified an accessory element that enhances T3 responsiveness of ACCα promoter 2 in CEH. This element is located 5′ bp downstream of the ACCα T3RE and binds sterol regulatory element-binding protein 1 (SREBP-1). We have developed data suggesting that SREBP-1 enhances T3 regulation of ACCα transcription by directly interacting with TR-RXR heterodimers bound to the ACCα T3RE. In addition, we provide evidence that alterations in SREBP expression play a role in mediating cell type-dependent differences in T3 regulation of ACCα transcription.

**EXPERIMENTAL PROCEDURES**

**Plasmids**—DNA fragments used to construct reporter plasmids were named by designating the 5′ and 3′ ends of each fragment relative to the transcription start site of promoter 2 of the ACCα gene. p[ACC−108/+274]CAT has been described previously (16). This construct contains ACCα sequences between −108 and +274 bp ligated upstream of the CAT gene in KSCAT (24). A block mutation of the SRE-1 between −79 and −72 bp was introduced into p[ACC−108/+274]CAT using a polymerase chain reaction-based strategy (25). pBLCAT2 (pTKCAT) was obtained from B. Luckow and G. Schutz (German Cancer Research Center) (26). The cryptic activator protein-1 site located 5′ of the multiple cloning site in pBLCAT2 (27) was removed by excising the NdeI/HindIII fragment from this plasmid followed by religation. p[ACC−108/+262]TKCAT, p[ACC−108/+66]TKCAT, p[ACC−84/+66]TKCAT, and p[ACC−27/+66]TKCAT were constructed containing mutations in the T3RE fragment were made by first annealing complementary synthetic oligonucleotides containing ACCα sequences and then inserting them into SpI and SalI site 5′ of the thymidine kinase (TK) promoter in pTKCAT.

A full-length cDNA for chicken SREBP-1 was obtained by screening a chicken liver cDNA library (Stratagene) using a human SREBP-1 cDNA probe (nucleotides 721–1103 relative to the start site of translation) and by 5′-RACE (2). The N-terminal amino acid sequence of this chicken SREBP-1 (GenBank™ accession number AY029224) more closely resembles the 1a isoform than the 1c isoform described for mammalian species (28). The data from RNAse protection analyses indicate that other forms of SREBP-1 containing variations in the N terminus are not expressed in chicken cells. An expression plasmid encoding the mature form of chicken SREBP-1 was developed by subcloning a SREBP-1 cDNA fragment encoding amino acids 1–464 into pSV-SPORT1 (Invitrogen) to form pSV-SPORT1-SREBP-1 (1–464). N-terminal and C-terminal truncations of the mature form of SREBP-1 were generated by PCR. PCR products encoding SREBP-1 polypeptides (78–464, 108–464, 238–464, 1–266, 1–340, 1–389, and 300–464) were subcloned into pSV-SPORT1. To generate a plasmid that expresses a fusion protein containing glutathione S-transferase (GST) linked to the mature form of chicken SREBP-1, a SREBP-1 cDNA fragment encoding amino acids 1–464 was subcloned into pGEX-2T (Amersham Biosciences). The structures of all reporter plasmids and expression plasmids were confirmed by restriction enzyme mapping and nucleotide sequence analyses.

Cell Culture and Transient Transfection—Primary cultures of CEH were prepared as previously described (29) and maintained in serum-free Waymouth’s medium MD7051 containing 50 μg insulin (gift from Eli Lilly Corp.) and 1 μM corticosterone. CEH were incubated on 80-mm Petri dishes (Fisher) at 40 °C in a humidified atmosphere of 5% CO2 and 95% air. The cells were transfected 6 h after plating, using 20 μg of Lipofectin (Invitrogen), 1.3 μg of p[ACC−108/+274]CAT, or an equimolar amount of another reporter plasmid and pBluescript KS+ (+) to bring the total amount of transfected DNA to 3.0 μg/plate. At 18 h of incubation, the transfection medium was replaced with fresh medium with or without T3 (1.5 μM). At 66 h of incubation, CEH were harvested, and the cell extracts were prepared as described by Baillie et al. (30). CAT activity (31) and protein (32) were assayed by the indicated methods. All of the DNAs used in transfection experiments were purified using the Qiagen endotoxin-free kit.

CEF were obtained from SPAFAS, Inc. (Norwich, CT) and were routinely cultured in DMEM/M199 (Dulbecco’s modified Eagle’s medium (25 mM glucose) with medium 199 (Invitrogen) in a 1:1 (v/v) ratio, containing 10,000 units/liter penicillin G, 10 μg/ml streptomycin sulfate, and 25 μg/liter amphotericin B supplemented with 5% fetal bovine serum). These cells were transfected with p[ACC−108/+274]CAT or p[ACC−108/+274]CAT containing a block mutation of the SRE-1 using the calcium phosphate method (33). Briefly, the cells were seeded on Transwell inserts and grown in DMEM/M199 containing complete medium until 70% confluent. Twenty-four hours before transfection, the medium was changed to DMEM/M199 containing 50 nM insulin and 1 μM corticosterone. This medium was used throughout the experiment. The cells were transfected with 15 μg of reporter plasmid. Exposure to
the calcium phosphate/DNA precipitate was for 16 h. Following transfection, the cells were trypsinized and distributed to 60-mm tissue culture plates. T3 was added to the medium at this time. After 48 h of incubation, the cells were harvested, and the extracts were prepared for CAT and protein assays.

Preparation of Membrane and Nuclear Extracts—All of the procedures were carried out at 4°C. To prevent proteolysis, a mixture of protease inhibitors (Complete™, Roche Molecular Biochemicals) was included in all the buffers. The nuclear extracts were prepared from CEH and CEF by a modification of the method described by Dignam et al. (34). Briefly, CEH from four 100-mm plates or CEF from twelve 100-mm plates were pooled and centrifuged at 4°C for 4 h. The resulting cell pellet was homogenized in buffer 1 (10 mM Hepes, pH 7.9, 100 mM KCl, 15 mM MgCl2, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol) using 20 strokes in a Dounce homogenizer. The homogenate was centrifuged at 1100 × g for 10 min, and the resulting nuclear pellet was washed once in buffer 1. The nuclear pellet was resuspended in Buffer 2 (20 mM Hepes, pH 7.9, 420 mM NaCl, 25% (v/v) glycerol, 1.5 mM MgCl2, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol) for 1 h at room temperature. After washing with TBS, 0.01% Tween 20 for 1 h at room temperature, the blots were washed in TBS (10 mM Tris-HCl, pH 8.0, 0.3 mg/ml bovine serum albumin, and 2 μg of poly(dI·dC). A typical reaction contained 20,000 cpm (0.1 μg) of labeled DNA and 5 μl of

Gel Mobility Shift Analysis—Chicken TRα, human RXRα, and chicken SREBP-1 were translated in vitro using the TNT SP6-coupled reticulocyte lysate system (Promega). Double-stranded oligonucleotides were prepared by combining equal amounts of the complementary single-stranded DNA in a solution containing 10 μM Tris, pH 8.0, 1 μM EDTA followed by heating to 90°C for 2 min and then cooling to room temperature. The annealed oligonucleotides were labeled by filling in overhanging 5’ ends using the Klenow fragment of Escherichia coli DNA polymerase in the presence of [α-32P]dCTP and/or [α-32P]dGTP. The binding reactions were carried out in 20 μl of 20 mM Tris, pH 7.9, 100 mM KCl, 5 mM MgCl2, 1 mM EDTA, 1 mM dithiothreitol, 5% glycerol, (v/v), 0.3 mg/ml bovine serum albumin, and 2 μg of poly(dI·dC). A typical reaction contained 20,000 cpm (0.1 μg) of labeled DNA and 5 μl of in vitro translated proteins and was performed at 37°C for 30 min. DNA and DNA-protein complexes were resolved on 6% non-denaturing polyacrylamide gels at 4°C in 0.5× TBE (45 mM Tris, pH 8.3, 45 mM boric acid, 1 mM EDTA). Following electrophoresis, the gels were dried and analyzed by phosphor autodiagnostics.

Protein-Protein Interaction—GST and GST fusion proteins were expressed in E. coli (BL21, pLysS) and purified using standard techniques (36). Briefly, the bacteria were transformed with pHEx-2T or recombinant pGEK-2T plasmids expressing GST fusion proteins. Overnight cultures in ampicillin (250 μg/ml) were diluted 1:100 into 250 ml of Luria broth and grown at 30°C for 16 h. Following centrifugation, the cells were washed in 500 ml of buffer A (1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol) and centrifuged at 12,000 × g for 10 min at 4°C. The supernatant was mixed for 1 h at 4°C with a rotator with 0.5 to 1 ml of 50% glutathione-Sepharose beads (Amersham Biosciences) that were prewashed in buffer A. After absorption, the beads were collected by centrifugation at 4°C and washed three times with 1 ml of buffer A. The fusion proteins coupled to the glutathione-Sepharose beads were stored at 4°C for 24 h with the addition of 0.5 ml of buffer A. The washes and washes of purified GST fusion proteins were analyzed by electrophoresis and storage phosphor autoradiography. The protein concentrations and sizes of GST and GST fusion proteins were estimated by SDS-PAGE, using a known quantity of molecular weight standards.

l-1[35]S]Cysteine- or l-1[35]Smethionine-labeled proteins were prepared by using TNT reticulocyte lysates (Promega). Approximately 2.5 × 104 to 5 × 104 cpm of 35S-labeled protein was incubated with 300 ng of GST fusion protein immobilized on glutathione-Sepharose beads in 300 μl of buffer A. The reaction was run at 4°C for 2 h. When necessary, the purified GST fusion proteins were eluted with SDS gel loading buffer and analyzed by SDS-PAGE followed by storage phosphor autoradiography to ensure that similar levels of input radioactivity of the labeled protein were used in the GST binding assays.

RESULTS

A SRE-1 Located Immediately Downstream of a Strongly Active T3RE in the ACCa Gene Augments T3 Regulation of Transcription in a Cell Type-specific Manner—Previous studies with nongenetic thyroid hormone-responsive genes have shown that optimal T3 regulation of transcription requires the presence of one or more accessory factor-binding sites in addition to a T3RE (18–23). These accessory elements are usually located within 100 bp of the T3RE. In previous work analyzing the T3 regulation of ACCa promoter 2 in CEH, 5’-deletion analyses failed to detect a positive accessory element upstream of the strongly active T3RE between −101 and −86 bp (16). Deletion

References

analyses also failed to detect TR accessory sequences between +31 and +274 bp. We investigated the possibility that sequences immediately downstream of the ACCa T3RE contained a TR accessory element. Sequence comparison analysis of the region between −86 and +31 bp revealed the presence of a 10-bp element (−80 and −71 bp) that perfectly matched the SRE-1 identified in the rat, hamster, and frog genes for the low density lipoprotein receptor (37). Transient transfection experiments were performed to determine the effects of the SRE-1 on T3 regulation of ACCa promoter 2 in CEH. In hepatocytes transfected with a reporter construct containing ACCa sequences between −108 and +274 linked to the CAT gene, T3 stimulated a 14.3-fold increase in CAT activity (Fig. 1B). Mutation of sequences between −79 and −72 bp (SRE-1 mut) in the context of −108 to +274 ACCa fragment caused a 53% decrease in T3 responsiveness. This effect was mediated by a reduction in promoter activity in the presence of T3. These data indicate that T3 regulation of ACCa transcription in hepatocytes is enhanced by the presence of a SRE-1 located 5 bp downstream of a strongly active T3RE in the proximal region of ACCa promoter 2.

T3 regulation of the ACCa gene in nonhepatic cells such as CEF is markedly diminished relative to that of CEH (17). Previous studies with other T3-responsive genes have shown that TR accessory proteins play an important role in mediating cell type-dependent differences in T3 regulation of transcription (18–23). Transient transfection experiments were performed with CEF to determine whether the SRE-1 between −80 and −71 bp is involved in mediating differences in T3 responsiveness of the ACCa gene between CEH and CEF. In CEF transfected with p[ACC−108/+274]CAT, the T3-induced stimulation in CAT activity (2.4-fold) was substantially lower than that observed in CEH (14.3-fold) (Fig. 1, B and C). Mutation of the SRE-1 in the context of pACC−108/+274CAT had no effect on T3 responsiveness in CEF (Fig. 1C). These data contrast with those for CEH, demonstrating that mutation of the SRE-1 causes a marked reduction in T3 responsiveness (Fig. 1B). The decreased ability of the SRE-1 to stimulate T3 responsiveness in CEF relative to CEH suggests that this element plays a role in conferring cell type-dependent differences in T3 regulation of ACCa transcription. Other sequences within the −108 to +274 fragment also contribute to differences in T3 responsiveness between CEH and CEF, because mutation of the SRE-1 did not eliminate differences in T3 responsiveness between CEH and CEF.

We next asked whether the functional interaction between the ACCa SRE-1 and ACCa T3RE in CEH required the presence of additional cis-acting sequences. To address this question, we determined whether the ACCa SRE-1 could function alone to enhance T3 regulation conferred by the ACCa T3RE. CEH were transfected with constructs containing ACCa DNA fragments linked to the minimal promoter of the herpes simplex virus TK gene. The TK promoter alone was unresponsive to T3 in CEH (Fig. 2). Appending a DNA fragment containing the ACCa T3RE (−108 to −82 bp) to TK-CAT caused a 9.6-fold increase in T3 responsiveness (compare fold stimulation by T3 of pACC−108−82/TKCAT with that of TKCAT). A substantially greater increase in T3 responsiveness was observed (20-fold) when a DNA fragment containing both the T3RE and SRE-1 (−108 to −66 bp) was linked to TKCAT. The increase in T3 responsiveness caused by the SRE-1 was due to an increase in promoter activity in the presence of T3. This increase in T3
SREBP-1 Enhances Thyroid Hormone Action

SREBP-1 Physically Interacts with TR-RXR Heterodimers—SREBs bind a class of basic helix-loop-helix-leucine zipper (bHLH-Zip) factors referred to as sterol regulatory element-binding proteins (SREBPs) (38). SREBPs are synthesized as 125-kDa precursor proteins that are anchored to the endoplasmic reticulum. To become transcriptionally active, precursor SREBP must be translocated to the Golgi, where it encounters two proteases that act in sequence to release the N-terminal segment of SREBP referred to as mature SREBP (39). Mature SREBP is the protein that enters the nucleus and binds the segment of SREBP referred to as mature SREBP (39). Mature SREBP is the protein that enters the nucleus and binds the SRE of target genes. In chickens and mammals, SREBPs are synthesized from two distinct genes designated as SREBP-1 and SREBP-2 (38, 40). Previous studies employing transgenic animals indicate that SREBP-1 is more effective than SREBP-2 in modulating triacylglycerol synthesis, whereas SREBP-2 is more effective than SREBP-1 in modulating cholesterol synthesis (41, 42).

SREBP-1 physically interacts with TR-RXR heterodimers in a specific spatial arrangement in which RXR contacts the more 5’ half-site and TR contacts the more 3’ half-site (43). If TR-RXR directly interacts with mature SREBP on the ACCa gene, then flipping the orientation of the T3RE may alter the ability of the SRE-1 to enhance T3 regulation of transcription. To investigate this possibility, CEH were transfected with reporter constructs containing the ACCa T3RE in the native or flipped orientation. In CEH transfected with p[ACC–108/–66]TKCAT containing the T3RE in the native orientation, mutation of the SRE-1 inhibited T3 responsiveness by 56% (Fig. 3). In contrast, mutation of the SRE-1 had no effect on T3 responsiveness when the orientation of the T3RE in p[ACC–108/–66]TKCAT was flipped (compare fold stimulation by T3 of p[ACC–108/–66]TKCAT containing T3RE flip with that of p[ACC–108/–66]TKCAT containing T3RE flip and SRE-1 mut). These data suggest that SREBP interacts with TR-RXR in an orientation-dependent manner to enhance T3 regulation of the ACCa gene.

The close proximity of the SRE-1 and T3RE on the ACCa gene coupled with functional data indicating a direct and orientation-specific interaction between these regulatory elements (Figs. 1–3) led us to hypothesize that mature SREBP physically interacted with TR-RXR heterodimers to augment T3 regulation of ACCa transcription. To investigate this hypothesis, we determined using a pull-down assay whether the close proximity of the SRE-1 and T3RE on the ACCa gene.

<table>
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<tr>
<th>Fold Stimulation by T3 (±T3/T3)</th>
<th>8.7 ± 1.5</th>
<th>18.0 ± 1.3*</th>
<th>7.3 ± 0.6</th>
<th>1.0 ± 0.1</th>
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<tr>
<td>Relative CAT Activity</td>
<td>0</td>
<td>50</td>
<td>100</td>
<td>150</td>
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**Fig. 2.** The ACCa SRE-1 alone enhances T3 regulation directed by the ACCa T3RE. Fragments of the ACCa gene containing the T3RE and/or SRE-1 were linked to the minimal TK promoter in TKCAT. CEH were transiently transfected with these constructs and treated with or without T3 as described in the legend of Fig. 1 and under “Experimental Procedures.” Left panel, constructs used in these experiments. The numbers indicate the 5’ and 3’ boundaries of ACCa DNA relative to the transcription initiation site of promoter 2. The ACCa sequence between −108 and −66 bp is shown in Fig. 1A. Right panel, CAT activity in CEH transfected with p[ACC–108/–66]TKCAT and treated with T3 was set at 100, and the other activities were adjusted proportionately. The fold stimulation by T3 was calculated as described in the legend to Fig. 1. The results are the means ± S.E. of six experiments. The asterisk indicates the fold stimulation by T3 for p[ACC–108/–66]TKCAT was significantly higher than any other construct (p < 0.05).
The ability of the ACCα SRE-1 to enhance T3 responsiveness requires the presence of the ACCα T3RE in its native orientation. Fragments of the ACCα gene containing the T3RE in the native or flipped orientation were linked to the minimal TK promoter in TKCAT. CEH were transiently transfected with these constructs and treated with or without T3 as described in the legend of Fig. 1. The results are the means ± S.E. of five experiments. The asterisk indicates that the fold stimulation by T3 for p[ACC–108–66]TKCAT (T3RE in the native orientation) was significantly higher than that of pACC–108–66 SRE-1mut/TKCAT (T3RE in the native orientation) (p < 0.05).

The mature form of SREBP-1 specifically binds TRα in a T3-regulated manner. Bacterially produced GST or GST linked to the mature form of chicken SREBP-1 (GST–SREBP-1) was immobilized on glutathione-Sepharose beads. These preparations were then incubated with in vitro translated and 35S-labeled TRα, RXRα, or LXRα as described under “Experimental Procedures.” The incubations were performed in the absence or the presence of ligand for TRα (1 μM T3), RXRα (1 μM 9-cis-retinoic acid), or LXRα (1 μM 22(R)-hydroxycholesterol) as indicated. After the matrix was extensively washed, the labeled proteins retained on the beads were eluted, resolved by SDS-PAGE, and visualized by storage phosphor autoradiography together with 10% of the total radiolabeled receptor input used in each binding reaction. This experiment was repeated three times with similar results.

Fig. 3. The ability of the ACCα SRE-1 to enhance T3 responsiveness requires the presence of the ACCα T3RE in its native orientation. Left panel, constructs used in these experiments. The numbers indicate the 5′ and 3′ boundaries of ACCα DNA relative to the transcription initiation site of promoter 2. The orientation of the half-sites comprising the T3RE are indicated by the arrows. The ACCα sequence between −108 and −66 bp is shown in Fig. 1A. Right panel, CAT activity in CEH transfected with p[ACC–108–66]TKCAT and treated with T3 was set at 100, and the other activities were adjusted proportionately. The fold stimulation by T3 was calculated as described in the legend to Fig. 1. The results are the means ± S.E. of five experiments. The asterisk indicates that the fold stimulation by T3 for pACC–108–66 SRE-1mut/TKCAT (T3RE in the native orientation) was significantly higher than that of pACC–108–66 SRE-1mut/TKCAT (T3RE in the native orientation) (p < 0.05).

Fig. 4. The mature form of SREBP-1 specifically binds TRα in a T3-regulated manner. Bacterially produced GST or GST linked to the mature form of chicken SREBP-1 (GST–SREBP-1) was immobilized on glutathione-Sepharose beads. These preparations were then incubated with in vitro translated and 35S-labeled TRα, RXRα, or LXRα as described under “Experimental Procedures.” The incubations were performed in the absence or the presence of ligand for TRα (1 μM T3), RXRα (1 μM 9-cis-retinoic acid), or LXRα (1 μM 22(R)-hydroxycholesterol) as indicated. After the matrix was extensively washed, the labeled proteins retained on the beads were eluted, resolved by SDS-PAGE, and visualized by storage phosphor autoradiography together with 10% of the total radiolabeled receptor input used in each binding reaction. This experiment was repeated three times with similar results.

The binding of TRα to GST–SREBP-1 (Fig. 4). These data indicate that SREBP-1 selectively interacts with TRα.

We next set out to identify the motifs in TRα that interacted with SREBP-1. Pull-down experiments were performed using GST–SREBP-1 as the bait and various truncations of TRα labeled with 35S in vitro. Deletion of the first 20 or 50 amino acids from the N terminus of TRα had little or no effect on the binding of TRα to GST–SREBP-1 (Fig. 5A). When deletion of the N terminus of TRα was extended to amino acid 120, binding of TRα to GST–SREBP-1 was abolished. To further analyze the interaction between TRα and SREBP-1, TRα polypeptides containing amino acids 1–157, amino acids 1–118, and amino acids 51–157 were tested for their ability to interact with GST–SREBP-1. A strong interaction was observed between GST–SREBP-1 and a TRα polypeptide containing amino acids 1–157. Weaker interactions were observed between GST–SREBP-1 and TRα polypeptides containing amino acids 1–118 and amino acids 51–157. Collectively, these data suggest that TRα containing amino acids 1–118 and amino acids 51–157 is required for optimal binding to SREBP-1. Interestingly, interactions between GST–SREBP-1 and TRα truncations lacking the ligand-binding domain were not enhanced by the presence of T3. This observation is consistent with the scenario that T3 binding to the ligand-binding domain of TRα causes a conformational change that enhances the ability of the N-terminal region of TRα to interact with SREBP-1.

To map the motifs in SREBP-1 that interacted with TRα, pull-down analyses were performed using a GST fusion protein containing full-length TRα as a bait and various truncations of mature SREBP-1 labeled with 35S in vitro. Consistent with the results of experiments employing GST–SREBP-1 as the bait protein, the full-length, mature form of SREBP-1 (amino acids 1–464) interacted with GST–TRα (Fig. 5B). N-terminal deletions of SREBP-1 to amino acid 78, 108, 238, or 300 had little or no effect on the binding SREBP-1 to GST–TRα. A C-terminal deletion of SREBP-1 to amino acid 389 also had no effect on the binding of SREBP-1 to GST–TRα. In contrast, C-terminal deletions of SREBP-1 to amino acids 349 and 266 abolished the binding of SREBP-1 to GST–TRα. These data indicate that a SREBP-1 region containing the bHLH-Zip domain (amino acids 300–389) is required for binding to TRα. In contrast to results of experiments analyzing interactions between GST–SREBP-1 and 35S-labeled TRα, interactions between GST–TRα and 35S-labeled SREBP-1 proteins were not affected by the presence of T3. The latter observation suggests that appending GST to the N terminus of TRα blocks ligand-induced conformational changes that facilitate interactions between TRα and
SREBP-1. A similar observation has been reported for interactions between TR/H9251 and the homeodomain protein, PBX1 (19). The TR/H9251 region between amino acids 21–157 and the SREBP-1 region between amino acids 300 and 389 overlap with motifs mediating DNA binding activity. This prompted us to examine whether the binding of TR/H9251 and SREBP-1 to their DNA response elements altered the interaction between these proteins. Pull-down assays were carried out with GST-SREBP-1 and in vitro synthesized TR/H9251 and RXR/H9251 in the absence or presence of oligonucleotides containing the ACC/SRE-1 alone (T3REmut-SRE-1), the ACCo T3RE alone (T3RE-SRE-1mut), or both the ACCo T3RE and ACCo SRE-1 (T3RE-SRE-1). None of the oligonucleotides had an effect on the interaction between GST-SREBP-1 and TR/H9251 in the absence and presence of T3 (Fig. 6). These results suggest that DNA does not modulate the interaction between SREBP-1 and TRα.

SREBP-1 Forms a Tetrameric Complex with TRα-RXRα Heterodimers on a ACCo DNA Fragment Containing Sequences between −108 and −66 bp—The finding that SREBP-1 physically interacted with TRα-RXRα when both complexes were bound to DNA raised the possibility that SREBP-1 enhanced T3-induced ACCo transcription by forming a tetrameric complex with TRα-RXRα on the ACCo gene. To obtain evidence supporting this proposal, gel mobility shift experiments were performed using in vitro translated TRα, RXRα, and mature SREBP-1, and an ACCo probe containing the T3RE and SRE-1 (T3RE-SRE-1, −108 to −66 bp). Incubation of the T3RE-SRE-1 probe with TRα, RXRα, and SREBP-1 resulted in the formation of three complexes (Fig. 7A). The results of supershift analyses with antibodies against TR, RXR, and SREBP-1 indicated that the top band contained a SREBP-1 TRα-RXRα complex, the middle band contained SREBP-1 homodimers, and the
Fig. 6. The interaction between TRα and SREBP-1 is not altered by the binding of these proteins to DNA. GST or GST-SREBP-1 immobilized on glutathione-Sepharose beads was incubated with in vitro translated and 35S-labeled TRα and an equimolar concentration of unlabeled RXRα in the absence and presence of T3. Oligonucleotides (30 ng) containing the ACCα T3RE and/or SRE-1 were included in some incubations as indicated. After the matrix was extensively washed, the labeled proteins retained on the beads were eluted, resolved by SDS-PAGE, and visualized by storage phosphor autoradiography together with 10% of the total radiolabeled protein input used in each binding reaction. T3RE-SRE-1 (ACCα sequences between −108 and −66 bp) contains both the ACCα T3RE and SRE-1. T3RE-SRE-1 mut contains a mutation of the SRE-1 in the context of the −108 to −66 bp ACCα fragment. T3REmut-SRE-1 contains a mutation of the T3RE in the context of the −108 to −66 bp ACCα fragment. The sequence of T3RE-SRE-1 and T3RE-SRE-1 mut is shown in Fig. 1A. The sequence of T3REmut-SRE-1 is 5′-AGGTGGT-GTGAATGGAGGTAAAGACTCGCATCACACCACCGCGG-3′ (the mutated bases are underlined). Additional experimental details are described in the legend of Fig. 4 and under “Experimental Procedures.” This experiment was repeated twice with similar results.

The data in Fig. 3 demonstrated that the stimulation of T3-induced transcription by the ACCα SRE-1 was dependent on the orientation of the ACCα T3RE. The effect of T3RE orientation on SRE-1 activity may be mediated by changes in the ability of SREBP to form a tetrameric complex with TRα RXRα on DNA. To investigate this possibility, gel mobility shift experiments were performed using in vitro synthesized proteins and ACCα probes containing the T3RE in the native and flipped orientation. ACCα fragments (−108 to −66 bp) containing the T3RE in the native orientation (T3RE-SRE-1) or flipped orientation (T3RE Flip-SRE-1) were labeled with 32P to the same specific activity. Equal amounts of each probe were incubated with a fixed concentration of TRα RXRα in the presence of T3 and increasing concentrations of SREBP-1. At each concentration of SREBP-1, the abundance of tetrameric complexes (SREBP-1-SREBP-1/TRA-RXRα) was higher on the T3RE-SRE-1 probe than on the T3RE Flip-SRE-1 probe (Fig. 7C). This observation provides support for a role of tetrameric complex formation in mediating the stimulatory effects of the SRE-1 on T3-induced ACCα transcription.

The TRα RXRα-induced increase in SREBP-1 binding to the ACCα SRE-1 (Fig. 7) may be due to an increase in the affinity of SREBP-1 for the SRE-1 and/or a decrease in the dissociation rate of SREBP-1 from the SRE-1. To determine whether TRα RXRα altered the dissociation rate of SREBP-1 from the SRE-1, protein complexes prebound to the labeled T3RE-SRE-1 probe were incubated for various times with a 1,000-fold molar excess of an unlabeled competitor DNA containing the ACCα SRE-1 alone (T3REmut-SRE-1). The dissociation rate of SREBP-1 in the SREBP-1-SREBP-1/TRA-RXRα tetrameric complex was decreased by 61% compared with the dissociation rate of the SREBP-1 homodimeric complex (Fig. 8A). These data indicate that tetrameric complex formation stabilizes the binding of SREBP-1 to the ACCα SRE-1. This phenomenon accounts for at least part of the TRα RXRα-induced increase in SREBP-1 binding to the T3RE-SRE-1 probe.

We also investigated whether SREBP-1 binding to the ACCα SRE-1 stabilized the binding of TRα RXRα to the ACCα T3RE. In these experiments, protein complexes prebound to the labeled T3RE-SRE-1 probe were incubated for various times with an unlabeled competitor DNA containing the ACCα T3RE alone (T3RE-SREmut). The dissociation rate of TRα RXRα in the SREBP-1-SREBP-1/TRA-RXRα tetrameric complex was not different from the dissociation rate of the TRα RXRα heterodimeric complex (Fig. 8B). This finding is consistent with the results in Fig. 7 demonstrating a lack of effect of tetrameric complex formation on the binding of TRα RXRα to the ACCα T3RE.

SREBP Expression in CBF Is Decreased Relative To That Observed in CEH—The decreased ability of the ACCα SRE-1 to

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**Table:**

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<tr>
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<tr>
<td>T3</td>
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**Legend:**

- GST: Glutathione-S-transferase
- SREBP-1: Sterol Regulatory Element Binding Protein-1
- T3RE: Thyroid Hormone Response Element
- SRE-1: Sterol Regulatory Element-1
- ACCα: Adipose-specific Ceramide-1 Phosphate
- T3: Triiodothyronine
- RXRα: Retinoid X Receptor α
- TR: Thyroid Hormone Receptor
- SREBP: Sterol Regulatory Element Binding Protein
- SRE: Sterol Regulatory Element
- DNA: Deoxyribonucleic Acid
- SDS-PAGE: Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis
- 32P: Phosphorus-32
- 35S: Sulfur-35
- CEH: Cerebellar Cortex
- CBF: Cerebral Blood Flow
enhance T3 regulation of ACC transcription in CEF relative to CEH may be caused by a reduction in the level of mature SREBP in the former cell type. To investigate this possibility, the concentration of mature SREBP-1 and SREBP-2 was measured in CEH and CEF using Western blot analysis. Mature SREBP-1 and SREBP-2 levels were lower in CEF than in CEH (Fig. 9A). The extent of the reduction in mature SREBP-1 and SREBP-2 in CEF was 68 and 65%, respectively. The concentration of precursor SREBP-1 and SREBP-2 was also lower in CEF than in CEH. SREBP-1 and SREBP-2 mRNA levels were also measured in CEF and CEH using a RNase protection assay. The amounts of SREBP-1 and SREBP-2 mRNA were lower in CEF (64 and 72%, respectively) than in CEH (Fig. 9B). These data support the proposal that alterations in SREBP-1 and SREBP-2 expression mediate the decrease in SRE-1 activity in CEF.

DISCUSSION

Previous studies have shown that SREBPs play an important role in the regulation of lipogenic genes. In addition to the ACCα gene, functional SREs have been identified in the genes for fatty acid synthase (FAS) and stearoyl-CoA desaturase (SCD). In this study, we have shown that SREBP-1 enhances thyroid hormone action in CEF and CEH. The mechanism by which SREBP-1 enhances thyroid hormone action involves the formation of a tetrameric complex with TRα and RXRα heterodimers on a DNA probe containing ACCα sequences between -108 and -66 bp.
SREBP-1 Enhances Thyroid Hormone Action

The formation of the SREBP-1/SREBP-1/TRα/RXRα tetrameric complex stabilizes the binding of SREBP-1 to the ACCα SRE-1. In vitro synthesized TRα, RXRα, and mature SREBP-1 were bound to 32P-labeled T3RE-SRE-1 as described under “Experimental Procedures.” The binding mixture was then incubated with a 1,000-fold molar excess of unlabeled T3RE-SRE-1 (A) or T3RE-SRE-1mut (B). At various time points after the addition of competitor DNA, the aliquots of the binding mixture were subjected to nondenaturing gel electrophoresis. Left panel, data from a representative experiment. Right panel, plot of log protein binding activity versus time. The signals for protein-DNA complexes containing SREBP-1/SREBP-1, TRα/RXRα, or SREBP-1/SREBP-1/TRα/RXRα were quantitated using a PhosphorImager. The protein binding activities at 0 min were set at 100, and the activities at other time points were adjusted proportionately. The values are the means ± S.E. of three experiments. The positive standard error bar represents the S.E. for the binding of the SREBP-1/SREBP-1/TRα/RXRα tetrameric complex (○), and the negative standard error bar represents the S.E. for SREBP-1/SREBP-1 (△) or TRα/RXRα (□) dimeric complexes. The Kd for SREBP-1 and TRα/RXRα in dimeric or tetrameric complexes was estimated from the slopes of the straight lines. In A, the t1/2 of SREBP-1 in the SREBP-1/SREBP-1/TRα/RXRα tetrameric complex was compared with that of the SREBP-1 homodimeric complex. In B, the t1/2 of TRα/RXRα in the SREBP-1/SREBP-1/TRα/RXRα tetrameric complex was compared with that of the TRα/RXRα heterodimeric complex. The sequence of T3RE-SRE-1mut and T3REmut-SRE-1 is shown in Fig. 1A and in the legend to Fig. 6, respectively.

Acid synthase (44), ATP-citrate lyase (45), glycerol-3-phosphate acyltransferase (46), and S14 (47). In rat hepatocytes in culture, SREBP mediates the stimulatory effects of long chain polyunsaturated fatty acids on lipogenic gene transcription (47–52). The results of the present study demonstrate that SREBP-1 also plays a role in mediating the effects of T3 on lipogenic gene transcription. A SRE-1 in promoter 2 of the ACCα gene enhances the ability of T3 to activate transcription in CEH.

The data from transfection (Figs. 2 and 3) and protein binding assays (Figs. 4–6) suggest that the stimulatory effect of the SRE-1 on T3-induced ACCα transcription is mediated by a direct and T3-inducible interaction between SREBP and TR. We postulate that this interaction facilitates the formation of a SREBP-SREBP/TRα/RXRα tetrameric complex on the ACCα gene. In support of this proposal, in vitro synthesized TRα, RXRα, and SREBP-1 formed a tetrameric complex on a DNA probe containing the ACCα T3RE and SRE-1, and the presence of T3 enhanced the formation of this complex (Fig. 1). Complex formation between TRα/RXRα and SREBP-1/SREBP-1 stabilized the binding of SREBP-1 to the SRE-1 (Fig. 8). This phenomenon probably accounts for at least part of the increase in ACCα transcription caused by tetrameric complex formation. These findings define a new mechanism through which an accessory transcription factor modulates TR activity. Complex formation between TRα/RXRα and SREBP-1/SREBP-1 may also facilitate the recruitment of coactivator complexes to the ACCα gene. Previous studies have shown that both SREBP-1 and ligand-bound TR bind coactivator proteins (53–56). Coactivators regulate transcription by directly interacting with the basal transcriptional machinery, by modulating the interaction between the enhancer-binding protein and the basal transcription machinery, and by modifying chromatin structure.

The ACCα T3RE activates transcription not only in the presence of T3 but also in the absence of T3 (16). The increase in transcription conferred by the ACCα T3RE in the absence of T3 is mediated by the binding of protein complexes containing LXRα/RXRα heterodimers. Thus, the ACCα T3RE functions as a LXR response element in the absence of T3. The results from transfection analyses indicate that the SRE-1 has no effect on ACCα LXR response element activity in the absence of T3 (Figs. 1 and 2). This finding is congruent with the observation that LXRα and RXRα lack the ability to bind SREBP-1 in a pull-down assay (Fig. 4).

Data from the present study indicate that SREBP must interact with TR/RXR to be effective in regulating ACCα transcription in CEH (Fig. 2). Previous studies analyzing the regulation of other SREBP-responsive genes have shown that the ability of SREBP to activate transcription is also dependent on interactions between SREBP and transcription factors that bind DNA elements adjacent to the SRE. For example, SREBP-mediated activation of the genes for farnesyl diphosphate synthase (57), 3-hydroxy-3-methylglutaryl coenzyme A synthase (58), fatty acid synthase (44), ATP-citrate lyase (45), glycerol-3-phosphate acyltransferase (46), and S14 (21) is dependent on a functional interaction between SREBP and nuclear factor-Y (NF-Y) on contiguous DNA-binding sites. In the case of the farnesyl diphosphate synthase gene, the functional interaction between SREBP-1 and
These data are representative of three experiments. Protection analyses are described under "Procedures." Involves DNA elements that are separated by more than 2.3 kb. Concert with NF-Y to enhance T3 regulation of S14 transcription. The results of the present study suggest that SREBP-1 physically interacts with NF-Y and Sp1 in protein binding assays (58, 60). These findings in combination with the observation that SREBP-1c/NF-Y does not directly interact with TR-RXR on the rat S14 gene appears to be different from the mechanism mediating the interaction between SREBP-1c/NF-Y and TR-RXR on the S14 gene may instead be achieved by intermediary proteins that function as bridges between SREBP-1c/NF-Y and TR-RXR. Thus, the mechanism mediating the interaction between SREBP-1c/NF-Y and TR-RXR on the rat S14 gene appears to be different from the mechanism mediating the interaction between SREBP-1 and TR-RXR on the chicken ACCα gene.

In addition to SREBP, other DNA-binding proteins have been shown to modulate TR activity on native genes. For example, heterodimeric complexes containing the homeodomain proteins, PBX and MEIS1, interact with TR-RXR on the malic enzyme promoter to enhance T3 regulation of transcription in CEH (19). In cardiac muscle cells, binding of myocyte-specific enhancer factor 2 to the o-cardiac myosin heavy chain gene potentiates the ability of TR bound at an adjacent T3RE to activate transcription in the presence of T3 (23). Protein binding studies indicate that both PBX and myocyte-specific enhancer factor 2 interact with sequences in the A/B region and the DNA-binding domain of TR (19, 23). The results of the present study demonstrate that SREBP-1 also binds the A/B region and DNA-binding domain of TR (Fig. 5). Together, these observations suggest that accessory DNA-binding proteins modulate TR activity by interacting with a specific region of TR. DNA-binding proteins that modulate SREBP activity also interact with a specific region of SREBP. NF-Y (58), Sp1 (60), and TRα (Fig. 5) bind the bHLH-Zip domain of SREBP-1. Interestingly, the TR and SREBP-1 regions that contact accessory DNA-binding proteins are distinct from those that interact with coactivator proteins. Coactivators of TR such as CBP/p300, SRC-1/NCoA-1, GRIP1/NCoA-2, and the TRAP/ARC/DRIP complex interact with a N-terminal acidic region in SREBP-1 (55, 56). The observation that coactivators bind TR and SREBP at sites that are distinct from those that bind accessory DNA-binding proteins supports the proposal that complex formation between TR-RXR and SREBP-1 orchestrates the recruitment of coactivators to the ACCα gene. Because both TR and SREBP-1 contain coactivator binding sites, we postulate that the TR-RXR/SREBP-1-tetrameric complex interacts with multiple coactivator proteins, multiple regions within a single coactivator protein, or multiple subunits within a single coactivator complex.

Promoter 2 of the rat ACCα gene contains two closely spaced SREs that synergistically interact with an adjacent Sp1-binding site to activate ACCα transcription during conditions of sterol depletion (62). In contrast to promoter 2 of the chicken ACCα gene, a DNA element resembling a T3RE is not present in the region flanking the SREs in rat ACCα promoter. This observation is consistent with results from reverse transcriptase-PCR analyses demonstrating that ACCα promoter 2 in rat hepatocytes is not responsive to thyroid hormone status (63). Thus, the role of SREBP in regulating ACCα promoter 2 varies depending on the class of animals. This phenomenon may reflect a fundamental difference between avians and mammals in the mechanism by which nutrients and hormones regulate ACCα expression in liver.

The results of the present study are the first to suggest that SREBP plays a role in the tissue-specific regulation of T3 action. This supposition is based on the finding that mutation of the SRE-1 inhibits T3 regulation of ACCα transcription in CEH but has no effect on T3 regulation in CEF (Fig. 1). The data from Western blot analyses and RNase protection assays (Fig. 9) suggest that alterations in expression of SREBP-1 and SREBP-2 contribute to the difference in SRE-1 activity between CEH and CEF. In intact animals, SREBP-1 and SREBP-2 are expressed in a wide range of tissues, with...
SREBP-1 being expressed at a substantially higher level in tissues in which lipogenesis is regulated by T3 (i.e. liver) (28, 40, 64, 65). Thus, alterations in SREBP-1 expression likely play a role in mediating tissue-dependent differences in T3 responsiveness.

Another lipogenic gene that is activated by T3 in a hepatocyte-specific manner is malic enzyme. In C57BL/6J, T3 regulation of malic enzyme transcription is mediated by six TRES, five of which are clustered in a 109-bp region (~3878−3769 bp) referred to as a T3 response unit (24). Flanking the malic enzyme T3 response unit are five accessory elements that play an important role in conferring enhanced T3 responsiveness in C57BL/6J relative to C57BL/12−180. Interestingly, these accessory elements bind proteins that are distinct from SREBP. Differences between the malic enzyme gene and the ACCα gene in the complexity of the T3-responsive region and the nature of the proteins that bind T3 accessory elements suggest that there are gene-specific differences in the molecular mechanism mediating cell type-dependent differences in T3 responsiveness. The reason for the different mechanisms is unclear. One possibility is that the physiological role of malic enzyme does not completely overlap with that of ACCα. Malic enzyme furnishes NADPH for fatty acid synthesis, hydroxylation of xenobiotics, and reduction of glutathione, whereas ACCα functions exclusively in fatty acid synthesis. Separate regulatory mechanisms may have evolved for ACCα and malic enzyme because of subtle differences in the physiological roles of these enzymes.

In a previous work, we have shown that insulin, glucagon, medium chain fatty acids (MCFAs), and long chain polyunsaturated fatty acids (PUFAs) regulate the ability of T3 to activate ACCα transcription in C57BL/6J (4, 66). Insulin accelerates the increase in ACCα transcription caused by T3, whereas MCFCA, PUFAs, and glucagon inhibit the effects of T3 on ACCα transcription. The observation that the effects of insulin, MCFCA, PUFAs, and glucagon on ACCα transcription are dependent on the presence of T3 coupled with the finding that TR interacts with SREBP on the ACCα gene raises the possibility that insulin, MCFCA, PUFAs, and glucagon regulate ACCα transcription by modulating SREBP activity. In rat hepatocyte cultures, insulin increases and PUFAs and glucagon decrease the concentration of mature SREBP-1 protein and SREBP-1c mRNA (47, 48, 50−52). Future studies will investigate the role of SREBP in mediating the effects of insulin, MCFCA, PUFAs, and glucagon on T3 action in avian hepatocytes.

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REFERENCES