2001

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THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 276, No. 2, Issue of January 12, pp. 974–983, 2001

Printed in U.S.A.

Thyroid Hormone Stimulates Acetyl-CoA Carboxylase-α Transcription in Hepatocytes by Modulating the Composition of Nuclear Receptor Complexes Bound to a Thyroid Hormone Response Element*

Received for publication, July 5, 2000, and in revised form, October 6, 2000
Published, JBC Papers in Press, October 10, 2000, DOI 10.1074/jbc.M005894200

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Triiodothyronine (T3) stimulates a 7-fold increase in transcription of the acetyl-CoA carboxylase-α (ACCα) gene in chick embryo hepatocytes. Here, we characterized an ACCα T3 response element (ACCα-T3RE) with unique functional and protein binding properties. ACCα-T3RE activated transcription both in the absence and presence of T3, with a greater activation observed in the presence of T3. In nuclear extracts from hepatocytes incubated in the absence of T3, ACCα-T3RE bound protein complexes (complexes 1 and 2) containing the liver X receptor (LXR) and the retinoid X receptor (RXR). In nuclear extracts from hepatocytes incubated in the presence of T3 for 24 h, ACCα-T3RE bound a different set of complexes. One complex contained LXR and RXR (complex 3) and another contained the nuclear T3 receptor (TR) and RXR (complex 4). Mutations of ACCα-T3RE that inhibited the binding of complexes 1 and 2 decreased transcriptional activation in the absence of T3, and mutations of ACCα-T3RE that inhibited the binding of complexes 3 and 4 decreased transcriptional activation in the presence of T3. The stimulation of ACCα transcription caused by T3 was closely associated with changes in the binding of complexes 1–4 to ACCα-T3RE. These data suggest that T3 regulates ACCα transcription by a novel mechanism involving changes in the composition of nuclear receptor complexes bound to ACCα-T3RE. We propose that complexes containing LXR/RXR ensure a basal level of ACCα expression for the synthesis of structural lipids in cell membranes and that complexes containing LXR/RXR and TR/RXR mediate the stimulation of ACCα expression caused by T3.

When the intake of dietary carbohydrate exceeds the immediate energy needs of the animal, excess carbohydrate is converted to triacylglycerols, which can be used for energy during periods of fasting. One of the enzymes that plays a pivotal role in mediating this response is acetyl-CoA carboxylase (ACC).[1] ACC catalyzes the ATP-dependent carboxylation of acetyl-CoA to malonyl-CoA, which is the donor of all but two (ω) of the carbon atoms for the synthesis of long-chain fatty acids. This reaction is the pace-setting step of the fatty acid synthesis pathway (1, 2). There are two ACC isoforms that are encoded by distinct genes. ACCα (260 kDa) is the principal isoform expressed in tissues that exhibit high rates of fatty acid synthesis such as liver, adipose tissue, and mammary gland. ACCβ (280 kDa) is the major isoform observed in heart and skeletal muscle, where it is thought to function primarily in the regulation of β-oxidation of fatty acids (3).

The concentration of ACCα in liver is subject to nutritional and hormonal regulation. For example, in starved animals, the concentration of hepatic ACCα is low; feeding a high carbohydrate, low fat diet stimulates an 8–20-fold increase in the amount of the enzyme (4–7). The effects of nutritional manipulation on ACCα concentration are mediated primarily by changes in the rate of transcription of the ACCα gene (8). Diet-induced changes in ACCα transcription are mimicked in primary cultures of chick embryo hepatocytes by manipulating the concentration of hormones and nutrients in the culture medium. The addition of 3,5,3′-triiodothyronine (T3) to the culture medium stimulates a 7-fold increase in ACCα transcription (9). Interestingly, a relatively long time (24 h) is required to achieve maximal rates of ACCα transcription after the addition of T3, suggesting that the accumulation of a rate-limiting intermediate is involved in mediating this response. The molecular mechanism by which T3 regulates ACCα transcription remains to be determined.

ACCα transcription is initiated from two promoters, resulting in mRNAs with heterogeneity in the 5′-untranslated region (2). The more upstream promoter (promoter 1) flanks exon 1, while the more downstream promoter (promoter 2) flanks exon 2. In livers of rats (10, 11) and chickens,[2] the increase in total ACCα mRNA abundance caused by the consumption of a high carbohydrate, low fat diet is mediated by alterations in the activities of promoter 1 and promoter 2, with the latter promoter playing a quantitatively greater role in mediating this response. Alterations in promoter 2 activity are also primarily responsible for the T3-induced stimulation in total ACCα mRNA abundance in chick embryo hepatocytes.[2]

Thyroid hormone action is initiated by the binding of T3 to nuclear receptors. Nuclear T3 receptors (TRs) are members of a superfamily of ligand-dependent transcription factors that include the receptors for steroid hormones, vitamin A deriva-

* This work was supported by Cooperative State Research Service/United States Department of Agriculture Grant 980-3711. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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[1] The abbreviations used are: ACC, acetyl-CoA carboxylase; bp, base pair(s); COUP-TF, chicken ovalbumin upstream promoter transcription factor; CAT, chloramphenicol acetyltransferase; DR4, half-sites arranged as direct repeats with a 4-bp spacer; LXR, liver X receptor; RXR, retinoid X receptor; TK, thymidine kinase; T3, 3,5,3′-triiodothyronine; TR, nuclear T3 receptor; T3RE, T3 response element; CBP, CREB-binding protein.

[2] L. Yin, Y. Zhang, T. Charron, and F. B. Hillgartner, manuscript in preparation.

tives, vitamin D3, oxysterols, prostanoids, and a large family of receptor-like proteins with unknown ligands (orphan receptors) (12, 13). TRs bind to DNA sequences denoted as T3 response elements (T3REs). A wide diversity of T3RE structures have been reported in T3-responsive genes (14). In general, T3REs consist of multiple copies of a hexameric sequence related to a consensus RGGWMA arranged as inverted repeats, everted repeats, direct repeats, or extended single copies of the hexamer. The nucleotide sequence of the hexameric half-sites and flanking DNA and the spacing of the half-sites influence the binding of TR and its interactions with other proteins (15–18). Consequently, the structure of the T3RE is an important factor influencing the transcriptional activity of TR. TRs can bind T3REs as monomers, homodimers, or heterodimers with retinoid X receptor (RXR) (13, 14). TR/RXR heterodimers are thought to be the principal species of TR bound to T3REs *in vivo*, since this complex binds DNA with higher affinity and modulates transcription more effectively than TR monomers and TR homodimers (19–21). In addition to TR and RXR, other members of the nuclear hormone receptor family can regulate transcription by binding to T3REs. For example, the α and β isoforms of the liver X receptor (LXR) heterodimerize with RXR on an artificial T3RE composed of directly repeated half-sites separated by a 4-bp spacer (22). Binding of LXR/RXR to this T3RE causes a stimulation of transcription in the absence and presence of ligands for LXR and RXR. Evidence that LXR/RXR heterodimers modulate transcription through T3REs of native genes is presently lacking.

TRs bound to T3REs exhibit two regulatory activities. In the absence of T3, TRs repress transcription (23–26). The addition of T3 causes a derepression of transcription and, in some instances, a further activation of transcription above that observed in the absence of TR (23). This dual regulatory activity of TR arises, in part, from the ability of TR to recruit auxiliary regulatory proteins referred to as corepressors and coactivators. In the absence of T3, TR binds to corepressors such as silencing mediator for retinoic and thyroid hormone receptors (27) and nuclear receptor corepressor (28). The presence of T3 causes the release of corepressors and the subsequent association of TR with coactivators (29, 30). Examples of coactivators of TR include CREB-binding protein (CBP) (31, 32), steroid receptor coactivator-1 (33), CBP-interacting protein (34, 35), and p300/CBP associated factor (36). Corepressors and coactivators may mediate the transcriptional effects of TR by directly interacting with the basal transcriptional machinery, by modulating interactions between TR and the basal transcriptional machinery, and by modifying chromatin structure (13, 29, 37).

In the present report, we have investigated the mechanism by which T3 regulates ACCα transcription in hepatocytes. A strongly active T3RE in the 5′-flanking region of ACCα promoter 2 has been characterized. An interesting feature of this ACCα T3RE that distinguishes it from T3REs of other T3-responsive genes is that it activates transcription both in the absence and presence of T3. In addition, this ACCα T3RE binds multiple protein complexes in hepatic nuclear extracts, and several of these complexes contain LXR/RXR heterodimers. We also show that the T3-induced stimulation of ACCα transcription in hepatocytes is closely associated with dramatic changes in the binding of LXR/RXR complexes and TR/RXR complexes to the ACCα T3RE. From these data, we propose that T3 regulates ACCα transcription by a novel mechanism involving changes in the binding of nuclear receptor complexes to a T3RE.

EXPERIMENTAL PROCEDURES

Reporter Plasmids—An 18-kilobase pair ACCα genomic clone was obtained by screening a λ chicken genomic library (CLONTECH) with a polymerase chain reaction-generated DNA fragment extending from −1500 to −855 bp relative to the start site of transcription of chicken ACCα promoter 2. The sequence of the primers used to generate the −1500 to −855 bp fragment was derived from the published ACCα promoter 2 sequence (38). ACCα 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. To construct p[ACC−4900/+274]CAT (HindIII/*Xho*I), p[ACC−2054/+274]CAT (*Bam*HI/*Xho*I), p[ACC−854/+274]CAT (*Pst*I/*Xho*I), p[ACC−391/+274]CAT (*Bsm*I/*Xho*I), p[ACC−212/+274]CAT (*Msc*I/*Xho*I), p[ACC−136/+274]CAT (*Eag*I/*Xho*I), and p[ACC−94/+274]CAT (*Bst*EII/*Xho*I), ACCα restriction fragments indicated in brackets were inserted upstream of the chloramphenicol acetyltransferase (CAT) gene in KSCAT (39). To construct p[ACC−108/+274]CAT, p[ACC−84/+274]CAT, p[ACC−59/+274]CAT, p[ACC−41/+274]CAT, and p[ACC−30/+274]CAT, ACCα fragments were amplified by polymerase chain reaction and inserted upstream of the CAT gene in KSCAT. pBLCAT2 (pTKCAT) was obtained from B. Luckow and G. Schutz (German Cancer Research Center) (40). The cryptic activator protein-1 site located 5′ of the multiple cloning site in pBLCAT2 (41) was removed by excising the *Nde*I/*Hin*dIII fragment from this plasmid followed by religation. Fragments of the ACCα promoter/regulatory region were inserted into *Sph*I and *Sal*I site 5′ of the herpes simplex virus thymidine kinase (TK) promoter in pTKCAT to form ACC/TKCAT constructs. p[ACC−212/−82]TKCAT, p[ACC−171/−82]TKCAT, and p[ACC−212/−108]TKCAT were constructed by first amplifying the indicated ACCα fragments using polymerase chain reaction and then subcloning them into pTKCAT. p[ACC−136/−82]TKCAT, p[ACC−108/−82]TKCAT and pTKCAT constructs containing mutations in the −108 to −82 fragment were made by inserting annealed complementary synthetic oligonucleotides into pTKCAT. Structures of reporter plasmids were confirmed by restriction enzyme mapping and nucleotide sequence analyses.

The cDNAs for human RXRα and chicken TRα were provided by R. Evans (Salk Institute) and H. Samuels (New York University), respectively. D. Mangelsdorf (University of Texas Southwestern Medical Center) provided the cDNAs for human LXRα and LXRβ. Expression plasmids for RXRα, TRα, LXRα, and LXRβ were developed by subcloning the cDNAs for these receptors into pSV-SPORT1 (Life Technologies, Inc.).

Cell Culture and Transient Transfection—Primary cultures of chick embryo hepatocytes were prepared as described previously (42) and maintained in serum-free Waymouth's medium MD705/1 containing 50 nM insulin (gift from Lilly) and corticosterone (1 μM). Chick embryo hepatocytes were incubated on 60-mm Petri dishes (Fisher) at 40 °C in a humidified atmosphere of 5% CO_2 and 95% air. Cells were transfected 6 h after plating, using 20 μg of Lipofectin (Life Technologies), 2.5 μg of p[ACC−4900/+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, chick embryo hepatocytes were harvested, and cell extracts were prepared as described by Baillie *et al.* (43). CAT activity (44) and protein (45) were assayed by the indicated methods. All DNAs used in transfection experiments were purified using the Qiagen endotoxin-free kit.

Gel Mobility Shift Analysis—Twenty hours after being placed into culture, chick embryo hepatocytes were incubated in Waymouth's medium containing insulin and corticosterone with or without T3 for the times indicated in the figure legends. Cells were harvested, and nuclear extracts were prepared as described (46) except that the protease inhibitors, leupeptin (0.25 μg/ml), benzamidine (10 mM), aprotinin (8 μg/ml), and phenylmethylsulfonyl fluoride (0.5 mM) were added to the extraction buffer at the indicated concentrations. Chicken TRα, human LXRα, human LXRβ, and human RXRα were translated *in vitro* using the TNT SP6 coupled reticulocyte lysate system (Promega). To assess the relative efficiency of synthesis of the different receptor proteins, incorporation of [^{35}S]methionine into receptor proteins was measured in parallel reactions. Double-stranded oligonucleotides were prepared by combining equal amounts of the complementary single-stranded DNA in a solution containing 10 mM Tris, pH 8.0, 1 mM 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-^{32}P]dCTP and/or [α-^{32}P]dGTP. Binding reactions were carried out in 20 μl containing 18 mM HEPES, pH 7.9, 90 mM KCl, 0.18 mM EDTA, 0.45 mM dithiothreitol, 18% 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 ng) of labeled DNA and 10 μg of nuclear extract or 2 μl

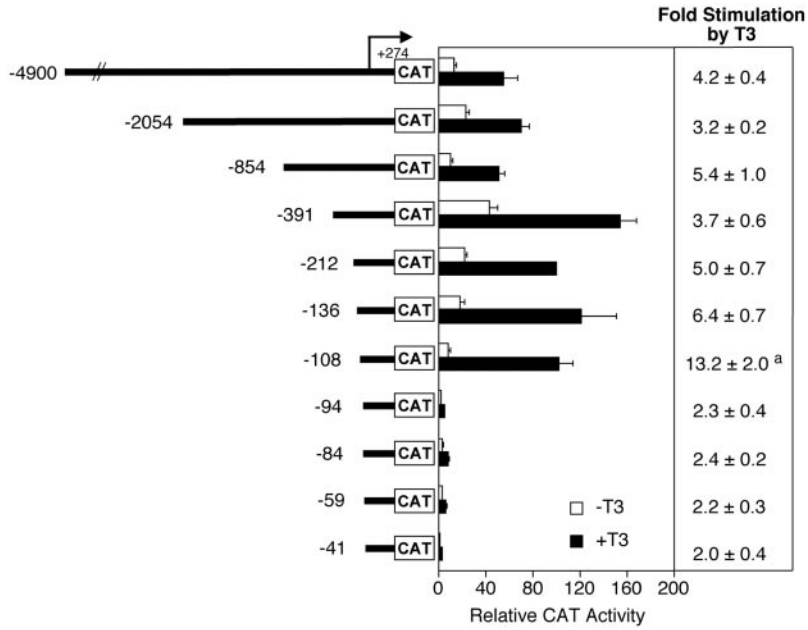


FIG. 1. **Effects of deletions of the 5′-flanking region of ACCα promoter 2 on transcriptional activity in the absence and presence of T3.** Chick embryo hepatocytes were transiently transfected with p[ACC−4900/+274]CAT or equimolar amounts of other plasmids as described under "Experimental Procedures." After transfection, cells were treated with or without T3 for 48 h. Cells were then harvested, extracts were prepared, and CAT assays were performed. *Left*, the constructs used in these experiments. The *number* at the *left* of each construct is the 5′-end of ACCα DNA in nucleotides relative to the transcription initiation site of promoter 2. The 3′-end of each construct was +274 bp. *Right*, CAT activity of cells transfected with p[ACC−212/+274]CAT and treated with T3 was set at 100, and the other activities were adjusted proportionately. The -fold stimulation by T3 was calculated by dividing the CAT activity for hepatocytes treated with T3 (*+T3*) by that for hepatocytes not treated with T3 (*−T3*). The -fold responses were calculated for individual experiments and then averaged. The results are the means ± S.E. of six experiments. CAT activity of T3-treated hepatocytes transfected with p[ACC−212/+274]CAT was 320 ± 42% conversion/h/mg of protein. *a*, the -fold stimulation by T3 for p[ACC−108/+274]CAT is significantly higher than that of any other construct ($p < 0.05$).

of *in vitro* translated proteins. The reaction was carried out on ice for 60 min. DNA and DNA-protein complexes were resolved on 6% nondenaturing 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 subject to storage phosphor autoradiography. For competition experiments, unlabeled competitor DNA was mixed with radiolabeled oligomer prior to the addition of nuclear extract. For antibody supershift experiments, nuclear extracts were incubated with antibodies for 30 min at 0 °C prior to the addition of the oligonucleotide probe. Monoclonal antibody that recognizes the α, β, and γ forms of RXR was generously provided by P. Chambon (Strasbourg, France). Polyclonal antibodies that recognized chicken ovalbumin upstream promoter-transcription factor I (N19), the α and β forms of chicken TR (FL-408), and the α and β forms of LXR (C19) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). The synthetic oligonucleotides that were used as probes or competitors in gel mobility shift assays are listed in Figs. 3*A* and 6*C*.

RESULTS

Identification of Sequences That Confer T3 Regulation on ACCα Promoter 2—Results from RNase protection analyses indicated that the T3-induced stimulation of ACCα expression in chick embryo hepatocytes is mediated primarily by changes in the activity of promoter 2 of the ACCα gene.[2] Transient transfection experiments were performed to identify the T3RE(s) mediating this regulation. In our initial experiments, chick embryo hepatocytes were transfected with a series DNA constructs containing 5′-deletions of ACCα promoter 2 linked to the CAT gene. In hepatocytes transfected with the longest construct, p[ACC−4900/+274]CAT, T3 caused a 4.2-fold increase in CAT activity (Fig. 1). Deletion of ACC-α sequences to −2054, −854, −391, and −212 bp had no effect on T3 responsiveness. Deletion of ACCα sequences from −136 to −108 bp caused a 2.1-fold increase T3 responsiveness, suggesting that the region between −136 and −108 bp contains a sequence that inhibits T3 responsiveness. Deletion of ACCα sequences from −108 to −94 bp caused a marked decrease in promoter activity in the absence and presence of T3. Because the extent of the decrease in promoter activity was greater in cells incubated in the presence of T3, T3 responsiveness was decreased by 83%. Further deletion of ACCα sequence to a 5′-end points of −84, −59, and −41 bp had no effect on residual T3 responsiveness (about a 2-fold increase in CAT activity). Further deletion of ACCα sequence to a 5′-end point of −30 bp abolished promoter activity in the absence and presence of T3 (data not shown). These data suggest that the region between −108 and −94 bp overlaps with a T3RE or an accessory element that augments T3 responsiveness. In addition, another T3RE of weaker activity is located downstream of −41 bp.

Analysis of 3′-deletions of ACCα DNA in the context of p[ACC−94/+274]CAT indicated the presence of a T3RE or T3RE accessory element between +179 and +151 bp.[3] However, this T3 regulatory sequence was not detected in 3′-deletions containing the T3 regulatory sequence between −212 and −94 bp. This observation suggests that sequences upstream of the transcription start site mediate most, if not all, of the T3 regulation of transcription initiated from ACCα promoter 2.

To obtain additional data indicating the presence of a T3RE in the 5′-flanking region of ACCα promoter 2, chick embryo hepatocytes were transfected with constructs containing ACCα DNA fragments linked to the minimal promoter of the herpes simplex virus TK gene. The TK promoter alone was unresponsive to T3 in chick hepatocytes (Fig. 2). Appending an ACCα DNA fragment from −212 to −82 bp to the TK promoter caused a 3.6-fold increase in T3 responsiveness. To further define the location of the T3RE in the −212 to −82 bp fragment, a series of deletions of p[ACC−212/−82]TKCAT were tested for their ability to confer T3 responsiveness. Deletion of the 5′-end to −171 bp caused a 36% increase in T3 responsiveness. Further

[3] Y. Zhang and F. B. Hillgartner, unpublished results.

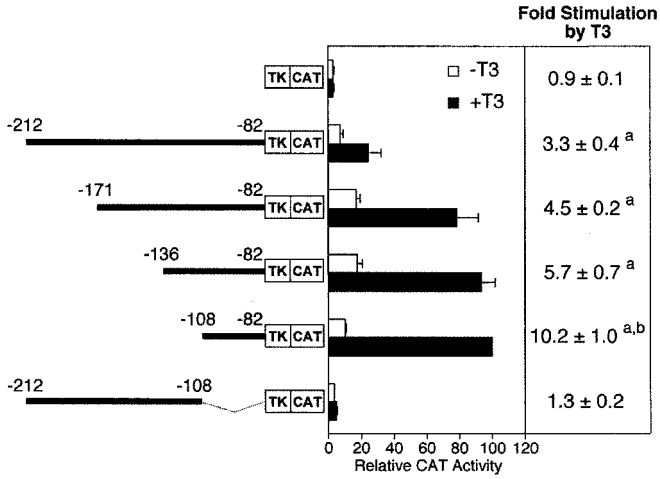
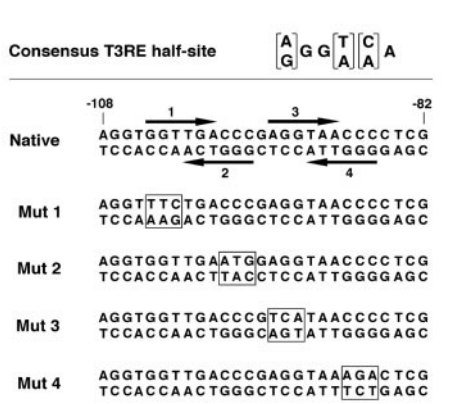


FIG. 2. **A strongly active T3RE is located between −108 and −82 bp of the ACCα gene.** Fragments of the ACC-α gene were linked to the minimal TK promoter in pTKCAT. Hepatocytes were transiently transfected with these constructs and treated with or without T3 as described in the legend of Fig. 1 and under "Experimental Procedures." *Left*, constructs used in these experiments. *Numbers* indicate the 5′ and 3′ boundaries of ACCα DNA relative to the transcription initiation site of promoter 2. *Right*, CAT activity in cells transfected with p[ACC−108/−82]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 four experiments. The CAT activity of extracts from T3-treated hepatocytes transfected with p[ACC−108/−82]TKCAT was 251 ± 36% conversion/h/mg of protein. Significant differences between means within a column ($p < 0.05$) are as follows. *a*, *versus* pTKCAT; *b*, *versus* any other construct.



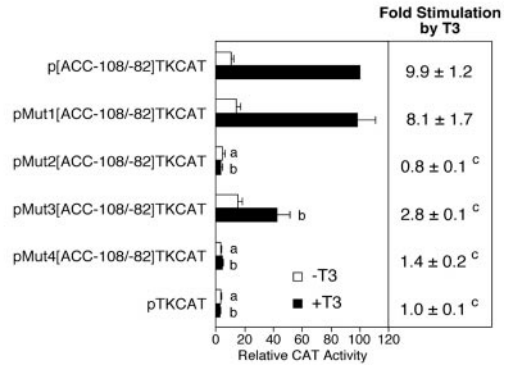


FIG. 3. **Delineation of the sequences in the −108 to −82 bp ACCα fragment that confer transcriptional activation in the absence and presence of T3.** *A*, native and mutant sequences of putative half-sites located between −108 and −82 bp of the ACCα gene. *Underlined* and *overlined* sequences in the native −108 to −82 bp fragment are motifs that are similar to the consensus T3RE half-site (*top*). The half-sites are numbered 1–4. The *arrows* indicate the orientation of the half-sites. Half-sites 2 and 3 conform perfectly to the consensus T3RE half-site sequence, whereas half-sites 1 and 4 are degenerate copies of the consensus sequence. Mutations were introduced into individual half-sites in the context of p[ACC−108/−82]TKCAT. mut 1 to mut 4 refer to the number of the half-site that was mutated in each construct. Mutated sequences are *boxed*. *B*, CAT activity of hepatocytes transfected with mutant reporter constructs. Hepatocytes were transiently transfected as described in the legend of Fig. 1 and under "Experimental Procedures." After transfection, cells were treated with or without T3 for 48 h. CAT activity in cells transfected with p[ACC−108/−82]TKCAT and treated with T3 was set at 100, and the other activities were adjusted proportionately. The results are the means ± S.E. of five experiments. The CAT activity of extracts from T3-treated hepatocytes transfected with p[ACC−108/−82]TKCAT was 238 ± 26% conversion/h/mg of protein. Significant differences between means within a column ($p < 0.05$) are as follows. *a*, *versus* p[ACC−108/−82]TKCAT treated without T3; *b*, *versus* p[ACC−108/−82]TKCAT treated with T3; *c*, *versus* p[ACC−108/−82]TKCAT.

deletion to −136 bp had no effect on T3 responsiveness. When the 5′-end was deleted to −108 bp, T3 responsiveness increased from 5.7- to 10.2-fold. This observation is consistent with data from 5′-deletion analysis in the context of p[ACC−4900/+274]CAT (Fig. 1), suggesting the presence of a T3 inhibitory element between −136 and −108 bp. When the 3′-end of the −212 to −82 fragment was deleted to −108 bp, T3 responsiveness was abolished. Thus, the sequence between −108 and −82 bp contains a strongly active T3RE. Interestingly, this T3RE confers T3 regulation by stimulating promoter activity both in the absence and presence of T3, with a greater stimulation observed in the presence of T3. These data support the results from 5′-deletion analysis in the context of p[ACC−4900/+274]CAT (Fig. 1) indicating the presence of a T3-dependent and T3-independent enhancer element between −108 and −94 bp.

Analysis of the sequences between −108 and −82 bp revealed the presence of four hexameric half-sites (Fig. 3*A*). Two of these half-sites (sites 2 and 3) conform perfectly to the consensus T3RE half-site sequence, RGGWMA. The other two half-sites (sites 1 and 4) are degenerate copies of the consensus half-site sequence. Half-sites 1 and 3 form an imperfect direct repeat with a 4-bp spacer (DR4) on the coding strand, and sites 2 and 4 form an imperfect DR4 on the noncoding strand. These putative DR4 elements partially overlap with each other. Other combinations of half-sites could form inverted or everted repeat structures. To investigate which of the putative T3RE half-sites were involved in mediating T3 regulation, hepatocytes were transfected with constructs that contained mutations of individual half-sites in the context of p[ACC−108/−82]TKCAT. Mutating the first 3 bp of half-site 1 (mut 1) had no effect on T3 responsiveness (Fig. 3*B*). In contrast, mutating the first 3 bp of half-site 2 (mut 2) or half-site 4 (mut 4) abolished T3 responsiveness. This effect was mediated by a decrease in promoter activity in the absence and presence of T3, with a greater effect in the presence of T3. The first 3 bp of half-site 3 also comprise part of the spacer separating half-sites 2 and 4. Mutating these 3 bp (mut 3) caused a 72% decrease in T3 responsiveness. This effect was mediated by a decrease in promoter activity in the presence of T3. These data demonstrate that T3 regulation conferred by the −108 to −82 bp fragment is mediated by two half-sites (sites 2 and 4) arranged as direct repeats on the noncoding strand. Both of these half-sites are required for the T3-dependent and T3-independent transcriptional activation functions of this T3RE. The T3-dependent enhancer function is also modulated by sequences in the spacer separating the two half-sites.

Characterization of Nuclear Proteins That Bind the ACCα

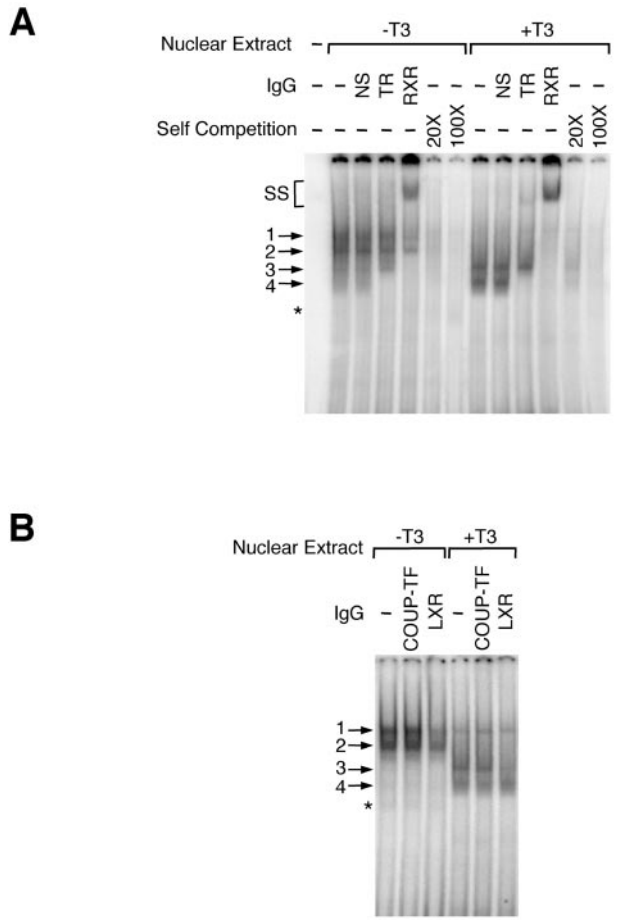


FIG. 4. **ACCα-T3RE binds hepatic protein complexes containing LXR/RXR and TR/RXR.** Gel mobility shift assays were performed using nuclear extracts prepared from hepatocytes incubated with or without T3 for 24 h. A double-stranded DNA fragment corresponding to -108 to -82 bp of the ACCα gene (ACCα-T3RE) was labeled with [α-^{32}P]dCTP using the Klenow fragment of *E. coli* DNA polymerase. The radiolabeled probe was incubated with 10 μg of nuclear protein as described under "Experimental Procedures." DNA and DNA-protein complexes were resolved on 6% nondenaturing polyacrylamide gels. *A*, nuclear extracts were incubated with antibodies against RXR and TR prior to the addition of the probe. Competition analysis was performed by mixing the labeled probe with a 20- and 100-fold molar excess of unlabeled ACCα-T3RE prior to the addition of nuclear extract. *B*, nuclear extracts were incubated with antibodies against COUP-TFI and LXR prior to the addition of the probe. Positions of the specific protein-DNA complexes (*arrows*), nonspecific complexes (*asterisk*), and supershifted complexes (*SS*) are indicated. These results are representative of four experiments employing independent preparations of nuclear extract. *NS*, normal serum.

T3RE between −108 and −82 bp—Gel mobility shift analyses were conducted to assess the binding of hepatic nuclear proteins to the T3RE between -108 and -82 bp. We will refer to this T3RE as ACCα-T3RE. Nuclear extracts were prepared from hepatocytes incubated in the absence and presence of T3 for 24 h. Incubation of nuclear extracts with a ^{32}P-labeled DNA probe containing ACCα-T3RE resulted in the formation of four protein-DNA complexes designated 1–4 in the order of increasing mobility (Fig. 4, *A* and *B*). The abundance of complex 1 and complex 2 was markedly increased in nuclear extracts from hepatocytes incubated in the absence of T3 relative to nuclear extracts from hepatocytes incubated in the presence of T3. Conversely, the abundance of complex 3 and complex 4 was markedly elevated in nuclear extracts from hepatocytes incubated in the presence of T3 compared with nuclear extracts from hepatocytes incubated in the absence of T3. A 100-fold molar excess of unlabeled ACCα-T3RE competed for the binding of complexes 1–4, suggesting that the binding of these protein-DNA complexes was specific (Fig. 4*A*).

Antibody supershift experiments were performed to identify the proteins that interacted with ACCα-T3RE. Antibodies directed against α, β, and γ isoforms of RXR completely disrupted the formation of complex 3 and complex 4 (Fig. 4*A*). This result was more readily observed in nuclear extracts from T3-treated hepatocytes in which the abundance of complex 3 and complex 4 was elevated. RXR antibodies partially disrupted the formation of complex 1 and complex 2 in nuclear extracts from hepatocytes incubated without T3. The disruption of complexes 1, 2, 3, and 4 by RXR antibodies was associated with the appearance of a supershifted complex of high intensity. These results suggest that most of the protein-DNA complexes that interact with ACCα-T3RE contain RXR or a protein highly related to RXR. Antibodies directed against the α and β isoforms of TR completely disrupted the formation of complex 4 but had no effect on the formation of complexes 1–3. The disruption of band 4 by TR antibody was associated with the appearance of a supershifted complex. Thus, complex 4 appears to contain TR/RXR heterodimers. Other proteins that can heterodimerize with RXR on DR4 elements include the α and β isoforms of LXR (22, 47). LXRα and LXRβ are expressed abundantly in liver (47–49). Preincubation of nuclear extracts with antibodies directed against LXRα and LXRβ partially disrupted the formation of complexes 1 and 2 in nuclear extracts from hepatocytes incubated without T3 and disrupted the formation of complex 3 in nuclear extracts from hepatocytes incubated with T3 (Fig. 4*B*). Thus, complexes 1–3 appear to contain LXR/RXR heterodimers. The orphan receptor, chicken ovalbumin upstream promoter-transcription factor (COUP-TF), also has been reported to bind T3REs (50, 51). Antibodies against COUP-TFI had no effect on protein binding to ACCα-T3RE.

The ability of the ACCα-T3RE to bind heterodimers containing LXR/RXR and TR/RXR was confirmed by gel mobility shift experiments employing *in vitro* synthesized receptors. Incubation of *in vitro* translated LXRα, LXRβ, RXRα, or TRα with the ACCα-T3RE probe resulted in little or no DNA binding activity (Fig. 5*A*). Inclusion of RXRα in the binding reactions with LXRα, LXRβ, and TRα stimulated the formation of high affinity protein-DNA complexes containing LXRα/RXRα, LXRβ/RXRα, and TRα/RXRα, respectively. Competition analyses indicated that the affinities of LXRα/RXRα, LXRβ/RXRα, and TRα/RXRα for ACCα-T3RE were similar (Fig. 5*B*).

Results from DNA binding analyses suggest that complex 1 and complex 2 mediate the enhancer activity of ACCα-T3RE in the absence of T3 and that complex 3 and complex 4 mediate the increase in enhancer activity of ACCα-T3RE caused by the addition of T3. To obtain further data supporting this hypothesis, competition experiments were performed using unlabeled DNA fragments containing native or mutant forms of ACCα-T3RE. These ACCα-T3RE fragments were the same sequences assayed in the transfection experiments described in Fig. 3. Unlabeled ACCα-T3RE, ACCα-T3RE mut 1, and ACCα-T3RE mut 3 were more effective than ACCα-T3RE mut 2 and ACCα-T3RE mut 4 in competing for the binding of complexes 1 and 2 in nuclear extracts from hepatocytes incubated in the absence of T3 and complex 3 in nuclear extracts from hepatocytes incubated in the presence of T3 (Fig. 6*A*). Thus, ACCα-T3RE mutants that abolished enhancer activity in the absence and presence of T3 (*i.e.* ACCα-T3RE mut 2 and ACCα-T3RE mut 4) bound proteins in complex 1, complex 2, and complex 3 with reduced affinity. These findings are consistent with a role of complexes 1 and 2 in mediating the enhancer activity of ACCα-T3RE in the absence of T3 and a role of complex 3 in mediating

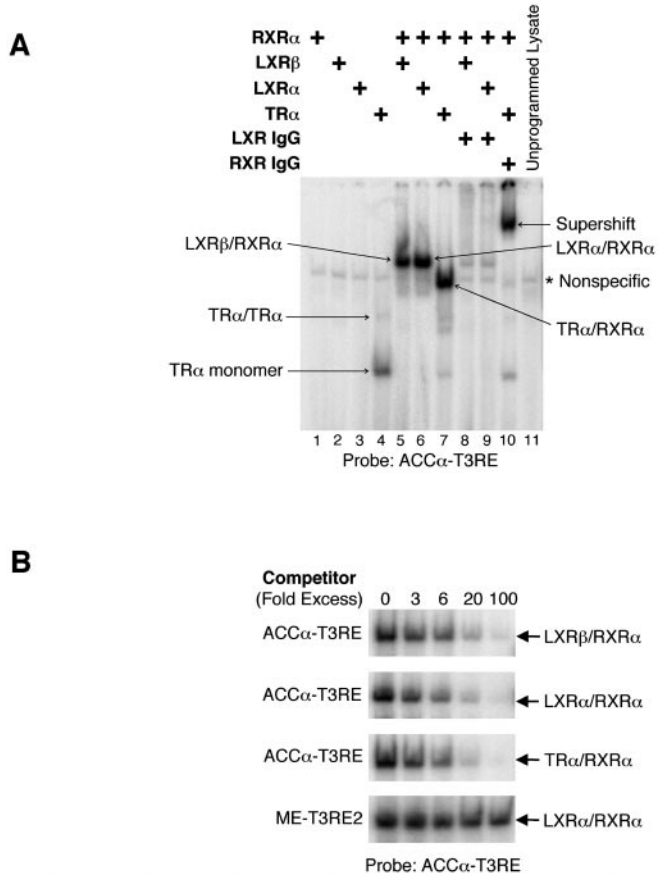


FIG. 5. **ACCα-T3RE binds LXRα, LXRβ, and TRα as heterodimers with RXRα.** Gel mobility shift assays were performed as described under "Experimental Procedures" using *in vitro* synthesized nuclear receptors and ^{32}P-labeled ACCα-T3RE (-108 to -82 bp) as the probe. *A*, equimolar amounts of *in vitro* synthesized LXRα, LXRβ, or TRα were incubated with the radiolabeled probe in the absence or presence of RXRα as indicated. In *lanes 8–10*, receptor preparations were incubated with antibodies against LXR or RXR prior to the addition of the probe. Positions of heterodimeric, homodimeric, monomeric, and supershifted receptor complexes are indicated by *arrows*. Nonspecific complexes are indicated by an *asterisk*. *B*, the radiolabeled ACCα-T3RE probe was incubated with a 3-, 6-, 20-, and 100-fold molar excess of unlabeled ACCα-T3RE or ME-T3RE2 prior to the addition of *in vitro* synthesized LXRα/RXRα, LXRβ/RXRα, and TRα/RXRα. The sequence of ME-T3RE2 is shown in Fig. 6*C*.

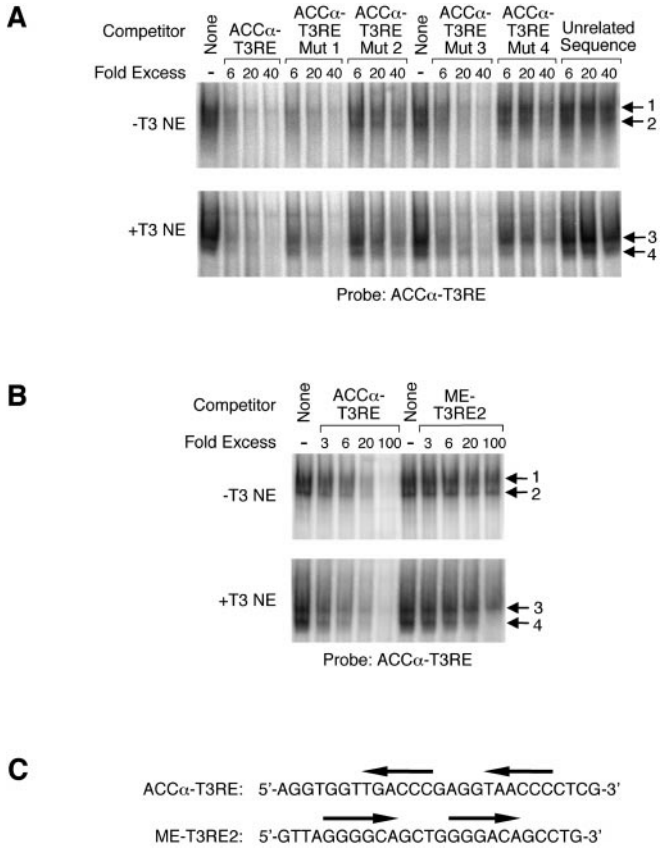


FIG. 6. **The ability of native and mutant T3REs to compete for the binding of hepatic nuclear proteins to ACCα-T3RE.** Gel mobility shift experiments were performed as described under "Experimental Procedures" using ^{32}P-labeled ACCα-T3RE as a probe and nuclear extracts prepared from hepatocytes incubated with or without T3 for 24 h. Nuclear extracts were incubated with the ACCα-T3RE probe in the presence of different concentrations of unlabeled competitor DNAs. Each reaction contained 10 μg of nuclear protein. DNA and DNA-protein complexes were resolved on 6% nondenaturing polyacrylamide gels. Specific protein-DNA complexes are indicated by *arrows*. *Bands 1* and *2* and *bands 3* and *4* are the predominant protein-DNA complexes observed with nuclear extracts from hepatocytes incubated in the absence of T3 and presence of T3, respectively. *A*, competition analysis with native and mutant forms of ACCα-T3RE. The sequences of the ACCα-T3RE competitors are shown in Fig. 3*A*. Unlabeled competitor DNAs (6-, 20-, or 40-fold molar excess) were mixed with the radiolabeled probe prior to the addition of nuclear extract. *B*, competition analysis with the major T3RE of the chicken malic enzyme gene (ME-T3RE2). Unlabeled ME-T3RE2 and ACCα-T3RE (3-, 6-, 20-, or 100-fold molar excess) were mixed with the radiolabeled probe prior to the addition of nuclear extract. *C*, comparison of the sequence of ME-T3RE2 with that of ACCα-T3RE. The *arrows* indicate the position and orientation of the half-sites. *$-$T3 NE*, nuclear extracts from hepatocytes treated without T3; *+T3 NE*, nuclear extracts from hepatocytes treated with T3. These data are representative of four experiments employing independent preparations of nuclear extract.

enhancer activity in the presence of T3. The competition profile for complex 4 was different from that of complexes 1–3. In nuclear extracts from hepatocytes incubated with T3, ACCα-T3RE and ACCα-T3RE mut 1 were more effective than ACCα-T3RE mut 2 and ACCα-T3RE mut 3 in competing for complex 4 binding activity. Thus, the inhibition of T3-induced transcriptional activity caused by ACCα-T3RE mut 2 and ACCα-T3RE mut 3 is associated with a decrease in the binding of complex 4. However, ACCα-T3RE mut 1 and ACCα-T3RE mut 4 exhibited a similar ability to compete for the binding of complex 4 despite the fact that these mutations caused markedly different effects on T3-induced transcriptional activity. Thus, the elimination of T3-dependent enhancer activity caused by mutation of the more upstream half-site of the ACCα-T3RE (*i.e.* ACCα-T3RE mut 4) is not associated with changes in the binding of TR/RXR. Previous studies have shown that variations in the structure of the upstream half-site of DR4-type T3REs can profoundly alter T3-dependent enhancer activity without causing changes in TR/RXR binding affinity (16). The mechanism mediating the alteration in T3-dependent enhancer activity involves changes in the conformation of TR/RXR heterodimers bound to DNA (52). This mechanism may explain the effect of ACCα-T3RE mut 4 on T3-dependent enhancer activity.

The major T3RE mediating the T3 regulation of the chicken malic enzyme gene is a DR4-type element located between -3883 and -3858 bp and is referred to as ME-T3RE2 (39, 53). In contrast to the transcriptional enhancer function of ACCα-T3RE in chick embryo hepatocytes incubated in the absence of T3 (Figs. 1, 2, and 3*B*), ME-T3RE2 functions as a strong repressor of transcription in chick embryo hepatocytes incubated under the same experimental conditions (53). Both ACCα-T3RE and ME-T3RE2 activate transcription in hepatocytes incubated in the presence of T3. The different activities of ACCα-T3RE and ME-T3RE2 in hepatocytes incubated in the absence of T3 may be due to differences in the binding affinities

of these T3REs for complexes 1 and 2. To investigate this possibility, we examined the ability of different concentrations of unlabeled ME-T3RE2 to compete for protein binding to ^{32}P-labeled ACCα-T3RE. A 100-fold molar excess of ME-T3RE2 was not effective in competing for the binding of complex 1 and complex 2 in nuclear extracts from hepatocytes incubated without T3 (Fig. 6*B*). The inability of ME-T3RE2 to bind the proteins in complex 1 and complex 2 is consistent with these complexes functioning as activators of transcription in the absence of T3. To investigate whether common factors were involved in mediating the T3-dependent enhancer activities of ACCα-T3RE and ME-T3RE2, competition experiments were performed using nuclear extracts from hepatocytes incubated in the presence of T3. ME-T3RE2 was effective in competing for the binding of complex 4 but was not effective in competing for complex 3. This observation suggests that the proteins comprising complex 4 (*i.e.* TR/RXR heterodimers) mediate the T3-dependent enhancer activities of both ACCα-T3RE and ME-T3RE2. The inability of ME-T3RE2 to bind complexes containing LXR/RXR heterodimers (*i.e.* complexes 1–3) was confirmed by experiments demonstrating that ME-T3RE2 was not effective in competing for binding of *in vitro* synthesized LXRα/RXRα (Fig. 5*B*).

Previous work from our laboratory has shown that in hepatocytes incubated in the presence of insulin, the stimulation of ACCα transcription by T3 occurs in two phases (Fig. 7*A*) (9). Incubating hepatocytes with T3 for 1 h causes a small increase (1.9-fold) in ACCα transcription. Between 1 and 5 h of T3 treatment, there is no change in the rate of ACCα transcription. After 5 h of T3 treatment, the rate of ACCα transcription increases again. A maximal rate of ACCα transcription is reached after 24 h of T3 treatment and is about 7 times the transcription rate observed before the addition of T3. To investigate the temporal relationship between ACCα transcription and the binding of nuclear proteins to ACCα-T3RE, the time course of the effects of T3 on protein binding to ACCα-T3RE was determined. Nuclear extracts from hepatocytes incubated with or without T3 for different times were subjected to gel mobility shift analysis using ACCα-T3RE as a probe. As observed earlier in Fig. 4, ACCα-T3RE primarily bound complexes 1 and 2 in nuclear extracts from hepatocytes incubated in the absence of T3 (Fig. 7*B*). Incubation of hepatocytes with T3 for 1 and 5 h had no effect on the pattern of protein binding to ACCα-T3RE. Between 5 and 24 h of T3 treatment, the binding of complexes 3 and 4 increased and the binding of complexes 1 and 2 decreased. The pattern of protein binding to ACCα-T3RE at 48 h of T3 treatment was similar to that at 24 h of T3 treatment (data not shown). Thus, T3-induced alterations in protein binding to ACCα-T3RE are closely correlated with the large increase in ACCα transcription between 5 and 24 h of hormone treatment. This observation provides further evidence that alterations in the binding of complexes 1–4 are involved mediating the T3-induced increase in ACCα transcription.

Effects of Expression of Exogenous LXR, RXR, and TR on ACCα-T3RE Function—Results of DNA binding analyses (Figs. 4 and 5) suggest that complexes containing LXR/RXR heterodimers mediate the transcriptional enhancer activity of ACCα-T3RE in hepatocytes incubated in the absence of T3. To obtain additional data supporting this hypothesis, we determined the effects of expression of exogenous LXRα, LXRβ, RXRα, LXRα plus RXRα, or LXRβ plus RXRα on transcription directed by the ACCα-T3RE. Chick embryo hepatocytes were transiently transfected with p[ACC−108/−82]TKCAT and expression plasmids containing or lacking the genes for LXRα, LXRβ, or RXRα. Overexpression of RXRα alone had no effect on

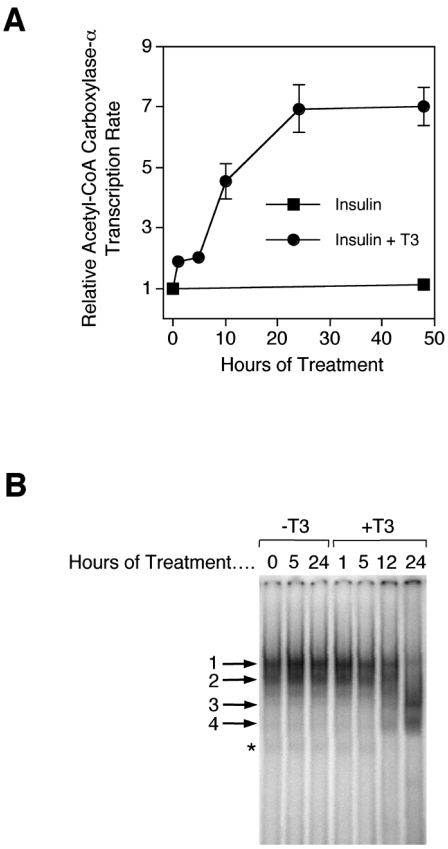


FIG. 7. **Time course for T3-induced changes in the binding of hepatic nuclear proteins to ACCα-T3RE.** *A*, previously published data for the time course of the T3-induced increase in ACCα transcription. Nuclear run-on assays were performed using nuclei isolated from hepatocytes incubated in the absence or presence of T3 for the indicated time periods. These data were taken from Hillgartner *et al.* (9). *B*, time course for T3-induced changes in protein binding to ACCα-T3RE. Gel mobility shift assays were performed using nuclear extracts prepared from hepatocytes incubated in the absence or presence of T3 for the indicated time periods. Nuclear extracts (10 μg of protein) were incubated with ^{32}P-labeled ACCα-T3RE as described under "Experimental Procedures." DNA and DNA-protein complexes were resolved on 6% nondenaturing polyacrylamide gels. Specific protein-DNA complexes are indicated by *arrows*. Nonspecific complexes are indicated by an *asterisk*. These data are representative of four experiments employing independent preparations of nuclear extract.

p[ACC−108/−82]TKCAT activity in hepatocytes incubated in the absence or presence of T3 (Fig. 8). In contrast, overexpression of LXRα or LXRβ alone or in combination with RXRα stimulated a 2.5–3-fold increase in p[ACC−108/−82]TKCAT activity in the absence of T3; overexpression of these receptors had no effect on p[ACC−108/−82]TKCAT activity in the presence of T3. When hepatocytes were transfected with a reporter plasmid lacking the ACCα-T3RE (pTKCAT), overexpression of LXRα, LXRβ, RXRα, LXRα plus RXRα, or LXRβ plus RXRα had no effect on promoter activity in the absence or presence of T3 (data not shown). These data are consistent with a role of LXR/RXR heterodimers in mediating the enhancer activity of ACCα-T3RE in the absence of T3.

We also investigated the effects of overexpression of TRα on p[ACC−108/−82]TKCAT activity in chick embryo hepatocytes. In contrast to the results for LXRα and LXRβ, overexpression of TRα and TRα plus RXRα caused an 88 and 91% decrease in p[ACC−108/−82]TKCAT activity, respectively, in hepatocytes incubated in the absence of T3; overexpression of these receptors had no effect on p[ACC−108/−82]TKCAT activity in hepatocytes incubated in the presence of T3 (Fig. 8). These data provide further evidence that TR/RXR heterodimers are not

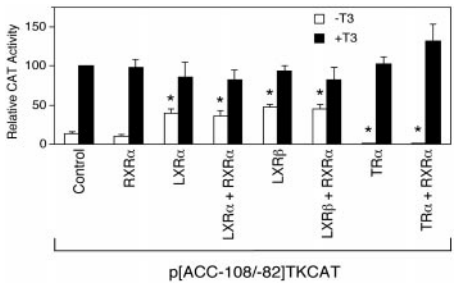


FIG. 8. **Effects of expression of exogenous LXRα, LXRβ, RXRα, and TRα on transcription directed by ACCα-T3RE.** Chick embryo hepatocytes were transiently transfected with p[ACC−108/−82]TK-CAT (1 μg/plate) and pSV-SPORT1-based expression plasmids (0.1 μg/plate) expressing the genes for LXRα, LXRβ, RXRα, and TRα. In transfections containing two receptor expression plasmids, 0.1 μg of each plasmid was transfected per plate. Empty expression plasmid (pSV-SPORT1) was added to bring the total amount of expression plasmid transfected per plate to 0.2 μg. Control transfections contained 0.2 μg/plate of empty expression plasmid. After transfection, cells were treated with or without T3 for 48 h. CAT activity in cells transfected with p[ACC−108/−82]TKCAT plus empty expression plasmid (control) and treated with T3 was set at 100, and the other activities were adjusted proportionately. The results are the means ± S.E. of three experiments. The CAT activity of extracts from T3-treated hepatocytes transfected with p[ACC−108/−82]TKCAT and empty expression plasmid was 214 ± 33% conversion/h/mg of protein. *, mean is significantly different ($p < 0.05$) from that of cells transfected with p[ACC−108/−82]TKCAT plus empty expression plasmid (control) and incubated without T3.

involved in mediating the enhancer activity of ACCα-T3RE in the absence of T3.

DISCUSSION

Previous studies analyzing the functional properties of TRs *in vivo* and *in vitro* have shown that the unliganded form of TR represses transcription and that the binding of T3 to TR reverses this effect and, in some instances, stimulates transcription above that observed in the absence of TR (13, 23–26). Accordingly, several native and artificial T3REs have been shown to confer T3 responsiveness by repressing transcription in the absence of T3 and activating transcription in the presence of T3 (53–56). In the present report, we have identified a T3RE (ACCα-T3RE) with different functional properties. ACCα-T3RE confers T3 regulation on ACCα promoter 2 in chick embryo hepatocytes by stimulating transcription both in the absence and presence of T3, with a greater stimulation observed in the presence of T3. These transcriptional effects of ACCα-T3RE were observed in the presence of endogenous cellular proteins; overexpression of TR or other proteins was not required to elicit a T3 response from transfected genes in chick embryo hepatocytes. To our knowledge, this is the first report describing a T3RE that functions as a T3-independent enhancer of transcription during physiological conditions.

Results from DNA binding (Figs. 4, 5, and 7), competition (Fig. 6), and transient transfection (Fig. 8) analyses suggest that the T3-independent enhancer activity of ACCα-T3RE is mediated by protein complexes containing LXR/RXR heterodimers (complexes 1 and 2) and that the increase in ACCα-T3RE enhancer activity caused by T3 treatment is mediated by protein complexes containing TR/RXR heterodimers (complex 4) and LXR/RXR heterodimers (complex 3). This is the first time that LXR/RXR heterodimers have been shown to play a role in mediating the transcriptional activity of a physiologically relevant T3RE. The different LXR/RXR complexes that bind ACCα-T3RE in hepatocytes may contain LXR and/or RXR of different sizes. The binding of coregulatory proteins to LXR/RXR heterodimers may also account for the different LXR/RXR complexes.

LXRs were initially identified as orphan receptors by screening libraries for homologues of nuclear hormone receptors and were subsequently shown to be bound and activated by naturally occurring oxysterols at physiological concentrations (47–49, 57). LXR/RXR binding sites have been identified in the genes for cholesterol 7α-hydroxylase (58), cholesterol ester transfer protein (59), and ABC1 (60, 61). These binding sites resemble DR4 elements and confer transcriptional activation in both the absence and presence of oxysterols, with a greater activation observed in the presence of oxysterols. There are no reports that the LXR/RXR binding sites in the genes for cholesterol 7α-hydroxylase, cholesterol ester transfer protein, and ABC1 confer T3 regulation of transcription. LXR has been proposed to play a key role in the regulation of cholesterol excretion in animals, as cholesterol 7α-hydroxylase, cholesterol ester transfer protein, and ABC1 are proteins involved in the regulation of reverse cholesterol transport. Conclusive evidence supporting a role of LXRα in the regulation of cholesterol catabolism has come from the characterization of the LXRα knockout mice. LXRα ablation causes an accumulation of cholesterol esters in liver as a result of an inability of cholesterol 7α-hydroxylase to be induced by dietary cholesterol (62). Studies with LXRα knockout mice also indicate that LXRα regulates expression of genes involved in fatty acid metabolism. For example, ablation of LXRα in mice causes a marked decrease in the expression of fatty acid synthase and stearoyl-CoA desaturase in liver (62); the molecular mechanisms mediating these effects are not known. The results of the present study demonstrating that LXR binds and activates the ACCα gene in hepatocytes provide additional support for a role of LXR in the regulation of lipogenic enzyme expression.

Previous work from our laboratory has shown that a relatively long time (24 h) is required to reach maximal rates of ACCα transcription after the addition of T3 and that most of the increase in transcription occurs between 5 and 24 h of hormone treatment (9). Comparison of the time course of T3-induced changes in ACCα transcription with the time course of T3-induced changes in ACCα-T3RE binding activity indicates that the increase in ACCα transcription between 5 and 24 h of T3 treatment is closely associated with an increase in the binding of complexes 3 and 4 and a decrease in the binding of complexes 1 and 2 (Fig. 7). This observation suggests that regulation of ACCα transcription by T3 is mediated by novel mechanism involving alterations in the composition of nuclear receptor complexes bound to ACCα-T3RE. We propose the following model for the regulation of ACCα transcription by T3. The small increase in transcription observed within 1 h of T3 addition is mediated by a ligand-induced stimulation of the derepression/activation function of TR in complex 4. This initial stimulation of ACCα transcription by T3 is limited by the low binding activity of complex 4 to the ACCα-T3RE relative to that of complex 1 and complex 2. The large increase in ACCα transcription between 5 and 24 h of T3 treatment is mediated by the increase in binding of complex 3 and complex 4 and the decrease in binding of complex 1 and complex 2. In this model, complex 3 and T3-bound complex 4 are more potent activators of transcription than complex 1 and complex 2. The relatively long time (>5 h) required to detect T3-induced changes in protein binding to ACCα-T3RE suggests that alterations in the synthesis of a regulatory protein are involved in mediating this effect. While the identity of this regulatory protein is not known, it does not appear to be TR or RXR, since expression of TRα, TRβ, RXRα, and RXRγ is not affected by T3 treatment in chick embryo hepatocytes (63). Data from Western blot analyses indicate that the concentration of LXRα and LXRβ in chick embryo hepatocytes is also not affected by T3 treatment.[3] We

speculate that T3 regulates the expression of nuclear receptor accessory proteins that, in turn, modulate protein binding to ACCα-T3RE. Such accessory proteins may regulate ACCα-T3RE binding activity by physically interacting with complexes 1, 2, 3, and/or 4 or by altering the phosphorylation state of these protein complexes.

In contrast to ACCα-T3RE, the major T3RE of the malic enzyme gene, ME-T3RE2, functions as a potent repressor of transcription in the absence of T3 (53). If complex 1 and complex 2 mediate the T3-independent enhancer activity of ACCα-T3RE in hepatocytes, then what are the factors that mediate the T3-independent repressor activity of ME-T3RE2? In previous work, we have shown that ME-T3RE2 binds to four complexes in nuclear extracts from chick embryo hepatocytes incubated without T3 (63). In contrast to the protein binding profile for ACCα-T3RE, all four complexes that interact with ME-T3RE2 contain TR, and three of the four complexes contain RXR. Thus, the T3-independent repressor activity of ME-T3RE2 is likely to be mediated by one or more complexes containing unliganded TR. This supposition is consistent with previous studies demonstrating that unliganded TR functions as a repressor of transcription (26). Interestingly, in chick embryo hepatocytes incubated in the absence of T3, the transcription rate of the malic enzyme gene as determined by nuclear run-on analysis is 30% of that observed for the ACCα gene.[4] This difference in transcription rate between malic enzyme and ACCα is likely to be mediated, at least in part, by the contrasting T3-independent activities of ME-T3RE2 and ACCα-T3RE.

In theory, the ability of a T3RE to bind TR and repress transcription in the absence of T3 serves to amplify the activating effects of T3 on transcription. Indeed, the robust T3-induced stimulation of transcription conferred by ME-T3RE2 in chick embryo hepatocytes (>270-fold) (53) is consistent with this hypothesis. What is the physiological significance of the enhancer activity of ACCα-T3RE in the absence T3? In addition to its role in energy homeostasis, ACCα is required for the synthesis of structural lipids in cell membranes. Consequently, a basal level of ACCα expression is observed under conditions when the enzyme is not induced by hormonal and nutritional factors. We propose that the ACCα-T3RE also has a dual physiological role. In addition to mediating the hormonal regulation of ACCα expression, ACCα-T3RE ensures a basal level of ACCα expression for obligatory cellular processes. The latter function of the ACCα-T3RE is mediated by the binding of LXR/RXR complexes. ACCα-T3RE may also contribute to the basal expression of ACCα in extrahepatic tissues, as LXRβ is expressed in a wide variety of tissues (48, 49).

In summary, we have characterized a T3RE in the ACCα gene with unique functional and protein binding properties. In addition, we have developed data suggesting that the delayed actions of T3 on ACCα transcription are mediated by a novel mechanism involving alterations in the composition of nuclear receptor complexes bound to the ACCα-T3RE. The observation that the ACCα-T3RE co-localizes with a prominent DNase I-hypersensitive site in chromatin from livers of intact chickens supports a role for this cis-acting sequence in mediating the regulation of ACCα transcription *in vivo*.[2] Future experimentation will be directed toward defining the mechanism by which T3 modulates the binding of nuclear receptor complexes to the ACCα-T3RE in hepatocytes.

Acknowledgments—We thank Drs. D. Mangelsdorf (LXRα and LXRβ), R. Evans (RXRα), and H. Samuels (TRα) for providing the indicated cDNAs. We thank Dr. P. Chambon for providing RXR antiserum.

[4] F. B. Hillgartner and T. Charron, unpublished results.

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