

The mechanism mediating the activation of acetyl-coenzyme A carboxylase- α gene transcription by the liver X receptor agonist T0-901317

Saswata Talukdar and F. Bradley Hillgartner¹

Department of Biochemistry and Molecular Pharmacology, School of Medicine, West Virginia University, Morgantown, WV 26506

Abstract In birds and mammals, agonists of the liver X receptor (LXR) increase the expression of enzymes that make up the fatty acid synthesis pathway. Here, we investigate the mechanism by which the synthetic LXR agonist, T0-901317, increases the transcription of the acetyl-coenzyme A carboxylase- α (ACC α) gene in chick embryo hepatocyte cultures. Transfection analyses demonstrate that activation of ACC α transcription by T0-901317 is mediated by a *cis*-acting regulatory unit (–101 to –71 bp) that is composed of a liver X receptor response element (LXRE) and a sterol-regulatory element (SRE). The SRE enhances the ability of the LXRE to activate ACC α transcription in the presence of T0-901317. Treating hepatocytes with T0-901317 increases the concentration of mature sterol-regulatory element binding protein-1 (SREBP-1) in the nucleus and the acetylation of histone H3 and histone H4 at the ACC α LXR response unit. These results indicate that T0-901317 increases hepatic ACC α transcription by directly activating LXR•retinoid X receptor (RXR) heterodimers and by increasing the activity of an accessory transcription factor (SREBP-1) that enhances ligand induced-LXR•RXR activity.—Talukdar, S., and F. B. Hillgartner. The mechanism mediating the activation of acetyl-coenzyme A carboxylase- α gene transcription by the liver X receptor agonist T0-901317. *J. Lipid Res.* 2006. 47: 2451–2461.

Supplementary key words fatty acid synthesis • sterol-regulatory element binding protein • thyroid hormone • chicken • histone acetylation

The first committed step of the fatty acid synthesis pathway is the ATP-dependent carboxylation of acetyl-CoA to form malonyl-CoA. This reaction, catalyzed by acetyl-coenzyme A carboxylase- α (ACC α), constitutes a key control point in the synthesis of long-chain fatty acids from carbohydrate (1, 2). Malonyl-CoA serves as a donor of C₂ units for the synthesis of palmitate catalyzed by fatty acid synthase. Malonyl-CoA is also a substrate of specific

elongases involved in the chain elongation of fatty acids to very-long-chain fatty acids (3). The essential role of ACC α in lipid biosynthesis has been confirmed by studies demonstrating that knockout of the ACC α gene disrupts embryonic development before day 7.5 (4).

In lipogenic tissues of birds and mammals, transcription of the ACC α gene is regulated by nutritional and hormonal factors. For example, ACC α transcription is low in livers of starved chicks, and feeding a high-carbohydrate, low-fat diet stimulates an 11-fold increase in ACC α transcription (5). Diet-induced changes in ACC α transcription are mimicked in primary cultures of chick embryo hepatocytes by manipulating the concentrations of hormones and nutrients in the culture medium (6). Incubating chick embryo hepatocytes with the active form of the thyroid hormone 3,5',3-triiodothyronine (T3) stimulates a 5- to 7-fold increase in ACC α transcription. The mechanism by which T3 increases ACC α transcription involves multiple processes. First, T3 interacts with the nuclear 3,5',3-triiodothyronine receptor (TR) bound to a 3,5',3-triiodothyronine response element (T3RE) on the more downstream promoter (promoter 2) of the ACC α gene (7). This T3RE (–101 to –86 bp) is composed of two hexameric half-sites arranged as direct repeats with 4 bp separating the half-sites (DR-4 element). Second, T3 treatment increases the binding of TR•retinoid X receptor (RXR) heterodimers to the ACC α T3RE. The mechanism for this effect has not been defined. Third, T3 treatment increases the binding of sterol-regulatory element binding protein-1 (SREBP-1) to a sterol-regulatory element (SRE) (–80 to –71 bp) located immediately downstream of the ACC α T3RE (8). SREBP-1 directly interacts with TR•RXR heterodimers and enhances the ability of this complex to activate ACC α transcription in the presence of T3 (9).

In our studies analyzing the regulation of ACC α transcription by T3, we observed that the ACC α T3RE not only bound protein complexes containing TR•RXR hetero-

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¹ To whom correspondence should be addressed.
e-mail: fbhillgartner@hsc.wvu.edu

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dimers but also bound protein complexes containing liver X receptor (LXR)•RXR heterodimers (7). LXRs are nuclear hormone receptors that are bound and activated by naturally occurring oxysterols (10, 11). Two isoforms of LXR, designated LXR α and LXR β , have been identified in birds and mammals. LXR β is expressed in a wide variety of tissues, whereas LXR α is selectively expressed in liver, adipose tissue, intestine, and macrophages (12, 13). LXRs play a key role in regulating cholesterol excretion by mediating the stimulatory effects of oxysterols on the transcription of genes involved in reverse cholesterol transport and bile acid synthesis. For example, naturally occurring oxysterols and synthetic, nonsteroidal LXR agonists activate the transcription of a battery of genes involved in cholesterol efflux (ABCA1, ABCG1, ABCG5, and ABCG8), cholesterol clearance (cholesteryl ester transfer protein and apolipoprotein E), and cholesterol catabolism (cholesterol 7 α -hydroxylase) (14–16). For each of these genes, regulation of transcription by LXR agonists is conferred by a liver X receptor response element (LXRE) that binds LXR•RXR heterodimers. Because oxysterols are produced in proportion to cellular cholesterol content, LXRs have been proposed to function as sensors in a feed-forward pathway that stimulates reverse cholesterol transport and cholesterol excretion in response to high cholesterol levels in the diet. Consistent with this proposal, mice lacking the LXR α and/or LXR β gene exhibit diminished cholesterol excretion and increased cholesterol levels in the blood and liver when fed a high-cholesterol diet (17, 18).

The ability of LXR agonists to activate genes involved in cholesterol excretion has led to an evaluation of the atheroprotective properties of these compounds in murine models of atherosclerosis. Oral administration of the synthetic LXR ligand/agonist, T0-901317, to mice lacking the LDL receptor or apolipoprotein E causes an increase in blood HDL levels and reverses the formation of atherosclerotic lesions in the aorta (19, 20). LXR agonists also improve insulin sensitivity in murine models of type 2 diabetes (21, 22). These exciting findings are tempered by the observation that treatment with T0-901317 also causes hypertriglyceridemia and the development of a fatty liver in rodents and chickens (23–26). These undesired effects of T0-901317 are caused by alterations in the expression of enzymes that make up the fatty acid synthesis pathway. For example, T0-901317 treatment increases the hepatic expression of the mRNAs encoding ACC α , FAS, ATP-citrate lyase (ATP-CL), and stearoyl-coenzyme A desaturase-1 (SCD1) (25–27). The aim of this study was to determine the mechanism by which T0-901317 regulates the expression of ACC α in avian liver. We show that T0-901317 acts directly on the liver to increase the expression of ACC α and that the extent of this effect is modulated by the presence of insulin and T3. We further show that T0-901317 increases ACC α transcription by activating LXR•RXR heterodimers bound to the ACC α LXRE/T3RE and that SREBP-1 interacts with LXR•RXR to enhance the stimulatory effects of T0-901317 on ACC α transcription.

EXPERIMENTAL PROCEDURES

Cell culture and analytical assays

Hepatocytes were isolated from livers of 19 day old chick embryos as described previously (28). Cells were incubated in serum-free Waymouth's medium MD752/1 containing penicillin (60 μ g/ml) and streptomycin (100 μ g/ml) on untreated Petri dishes at 40°C in a humidified atmosphere of 5% CO₂ and 95% air. Hormone and other additions were as described in the figure legends. The triacylglycerol concentration of the culture medium was measured using an enzymatic kit (Sigma).

Isolation of RNA and quantitation of mRNA levels

Medium was removed and RNA was extracted from hepatocytes by the guanidinium thiocyanate/phenol/chloroform method (29). Total RNA (15 μ g) was separated by size on 0.9% agarose, 0.7 M formaldehyde gels and then transferred to a Nytran membrane (Schleicher and Schuell) using a vacuum blotting apparatus (Pharmacia Biotechnology). The RNA was cross-linked to the membrane by ultraviolet light and baked at 80°C for 30–60 min. RNA blots were hybridized with ³²P-labeled DNA probes labeled by random priming (30). Hybridization and washes were as described (31). Membranes were subjected to storage phosphor autoradiography. Hybridization signals were quantified using ImageQuant software (Molecular Dynamics). cDNAs for chicken ACC α (5), FAS (32), SCD1 (33), SREBP-1 (34), ATP-CL (35), ABCA1 (25), LXR α (36), and malic enzyme (37) have been described.

Plasmids

Reporter plasmids are named by designating the 5' and 3' ends of the ACC α DNA fragment relative to the transcription start site of promoter 2. A series of 5' deletions and 3' deletions of ACC α promoter 2 in the context of p[ACC–2054/+274] chloramphenicol acetyltransferase (CAT) have been described (7). An ACC α promoter construct containing a mutation of the SRE between –79 and –72 bp in the context of p[ACC–108/+274]CAT has been described (9). p[ACC–108/–66]TKCAT, p[ACC–84/–66]TKCAT, and pTKCAT constructs, containing mutations in the –108 to –66 bp ACC α fragment, have been described (9).

Transient transfection

Chick embryo hepatocytes were transfected as described by Zhang, Yin, and Hillgartner (7). Briefly, cells were isolated and incubated on 60 mm Petri dishes. At 6 h of incubation, the medium was replaced with one containing 10 μ g of lipofectin (Invitrogen), 1.5 μ g of p[ACC–2054/+274]CAT or an equimolar amount of another reporter plasmid, and pBluescript KS(+) to bring the total amount of transfected DNA to 1.5 μ g per plate. At 18 h of incubation, the transfection medium was replaced with fresh medium with or without T0-901317 (6 μ M). At 66 h of incubation, chick embryo hepatocytes were harvested and cell extracts were prepared as described (38). CAT activity (39) and protein (40) were assayed by the indicated methods.

Western blot analysis

Nuclear extracts were prepared from chick embryo hepatocytes as described (9). The proteins of the nuclear extract were subjected to electrophoresis on 10% SDS-polyacrylamide gels and then transferred to polyvinylidene difluoride membranes (Millipore) using an electroblotting apparatus (Bio-Rad Laboratories, Hercules, CA). Immunoblot analysis was carried out using

a mouse monoclonal antibody against SREBP-1 (IgG-2A4; American Type Culture Collection, Manassas, VA). Antibody/protein complexes on blots were detected using enhanced chemiluminescence (Amersham Biosciences). Chemiluminescence on the blots was visualized using a FluorChem 8000 imager (Alpha Innotech Corp.), and signals for the mature form of SREBP-1 were quantified using FluorChem V200 software.

Histone acetylation

The extent of histone acetylation on ACC α promoter 2 was measured using a chromatin immunoprecipitation (ChIP) assay. The procedure for this assay was as described by Yin et al. (41). ChIP assays were carried out with antibodies against acetyl-histone H3 (06-599) and acetyl-histone H4 (06-866; Upstate Biotechnology). Precipitated DNA was analyzed by PCR using Taq DNA polymerase (New England Biolabs) and primers specific for the ACC α and SCD1 promoters. The cycling parameters were as follows: 1 cycle of 95°C for 4 min; 30 cycles of 95°C for 1 min, 61°C for 1 min, and 72°C for 1 min; and 1 cycle of 72°C for 10 min. The forward primer of the ACC α gene was 5'-TCC-CCTCCGTCAGCAGCCAAATGGG-3'; the reverse primer was 5'-ATCCCCGGTCCCCGCCCTCGGCTCC-3'. The forward primer of the SCD1 gene was 5'-AGCGAACAGCAGATTGCGGCAG-3'; the reverse primer was 5'-TCTCGGCGTGCCAGAAGGGAGGT-3'. Amplified products were subjected to electrophoresis on 2% agarose gels and visualized by ethidium bromide staining.

Statistical methods

Data were subjected to ANOVA, and statistical comparisons were made with Dunnett's test or Student's *t*-test. Statistical significance was defined as $P < 0.05$.

RESULTS

LXR activation increases the expression of ACC α in primary cultures of chick embryo hepatocytes

Oral administration of T0-901317 to chickens and rats causes a 2- to 3-fold increase in hepatic ACC α mRNA levels (25, 26). To investigate whether this phenomenon was attributable to a direct effect of T0-901317 in the liver, we determined whether T0-901317 regulated the expression of ACC α in primary cultures of chick embryo hepatocytes. Incubating hepatocytes with T0-901317 in the absence of other hormones for 24 h caused a 2.4-fold increase in the abundance of ACC α mRNA (**Fig. 1**). Treatment with the RXR agonist 9-*cis* retinoic acid had no effect on ACC α mRNA abundance in the absence or presence of T0-901317. Thus, LXR agonists but not RXR agonists regulate ACC α expression in hepatocyte cultures.

In chick embryo hepatocytes, insulin has no effect by itself but amplifies the increase in ACC α transcription caused by T3 (6). This observation prompted us to investigate whether insulin modifies the effects of T0-901317 on ACC α expression. Incubating hepatocytes with T0-901317 stimulated a greater increase in ACC α mRNA abundance in the presence of insulin (3.5-fold) than in the absence of insulin (2.4-fold) (**Fig. 1**). Thus, as observed for T3 regulation of ACC α , insulin enhances the stimulatory effects of T0-901317 on ACC α expression.

In previous work, we showed that both TR and LXR bind the ACC α T3RE as heterodimers with RXR (7). This observation raised the possibility that a common *cis*-acting regulatory sequence is involved in mediating the effects of T0-901317 and T3 on ACC α transcription. As a first step in investigating this possibility, we determined the effects of T0-901317 on the expression of ACC α in the presence of T3. Incubating hepatocytes with T3 and insulin caused a 4.6-fold increase in the abundance ACC α mRNA abundance (**Fig. 1**). Addition of T0-901317 in the presence of T3 and insulin caused a further increase in ACC α mRNA abundance, although the magnitude of this effect (39%) was substantially smaller than the effect of T0-901317 on ACC α expression in the presence of insulin alone (350%). Addition of T0-901317 in the presence of T3, insulin, and 9-*cis* retinoic acid had no effect on ACC α mRNA abundance. The nonadditive effects of T0-901317 and T3 on ACC α mRNA abundance support the proposal that a common *cis*-acting sequence(s) mediates the actions of T0-901317 and T3 on ACC α transcription.

We also investigated the effects of T0-901317 on the expression of other lipogenic enzymes. In hepatocytes incubated in the absence and presence of insulin, addition of T0-901317 to the culture medium increased the abundance of mRNAs encoding FAS, SCD1, and ATP-CL (**Fig. 1**). T0-901317-induced expression of FAS, SCD1, and ATP-CL was higher in the presence of insulin than in the absence of insulin. In hepatocytes incubated with T3 and insulin, addition of T0-901317 caused a small increase (32%) in the abundance of FAS mRNA but had no effect on the abundance of SCD1 mRNA or ATP-CL mRNA. Incubating hepatocytes with 9-*cis* retinoic acid in the absence or presence of T0-901317 had no effect on FAS, SCD1, and ATP-CL mRNA levels. Thus, regulation of the expression of FAS, SCD1, and ATP-CL by agonists of LXR and RXR is similar to that of ACC α .

In contrast to ACC α , FAS, SCD1, and ATP-CL, the ability of T0-901317 to increase the expression of malic enzyme was substantially lower than that of T3. Treatment with T0-901317 and insulin caused a 2.9-fold increase in malic enzyme mRNA abundance, whereas treatment with T3 and insulin caused a 35-fold increase in malic enzyme mRNA abundance (**Fig. 1**). These observations are consistent with previous work demonstrating that the major T3RE mediating T3 regulation of malic enzyme transcription lacks the ability to bind LXR•RXR heterodimers (7).

To determine whether the T0-901317-induced increase in lipogenic enzyme expression was associated with an increase in triacylglycerol production, the triacylglycerol concentration in the culture medium was monitored in hepatocytes treated with or without T0-901317. The triacylglycerol concentration of the culture medium increased progressively during a 48 h incubation period (**Fig. 2**). The extent of the increase in triacylglycerol concentration was higher in cells treated with T0-901317 relative to cells not receiving T0-901317. An increase in hepatic lipogenic enzyme expression and triacylglycerol secretion likely contributes to the hypertriglyceridemia observed in animals treated with T0-901317 (23, 24, 26).

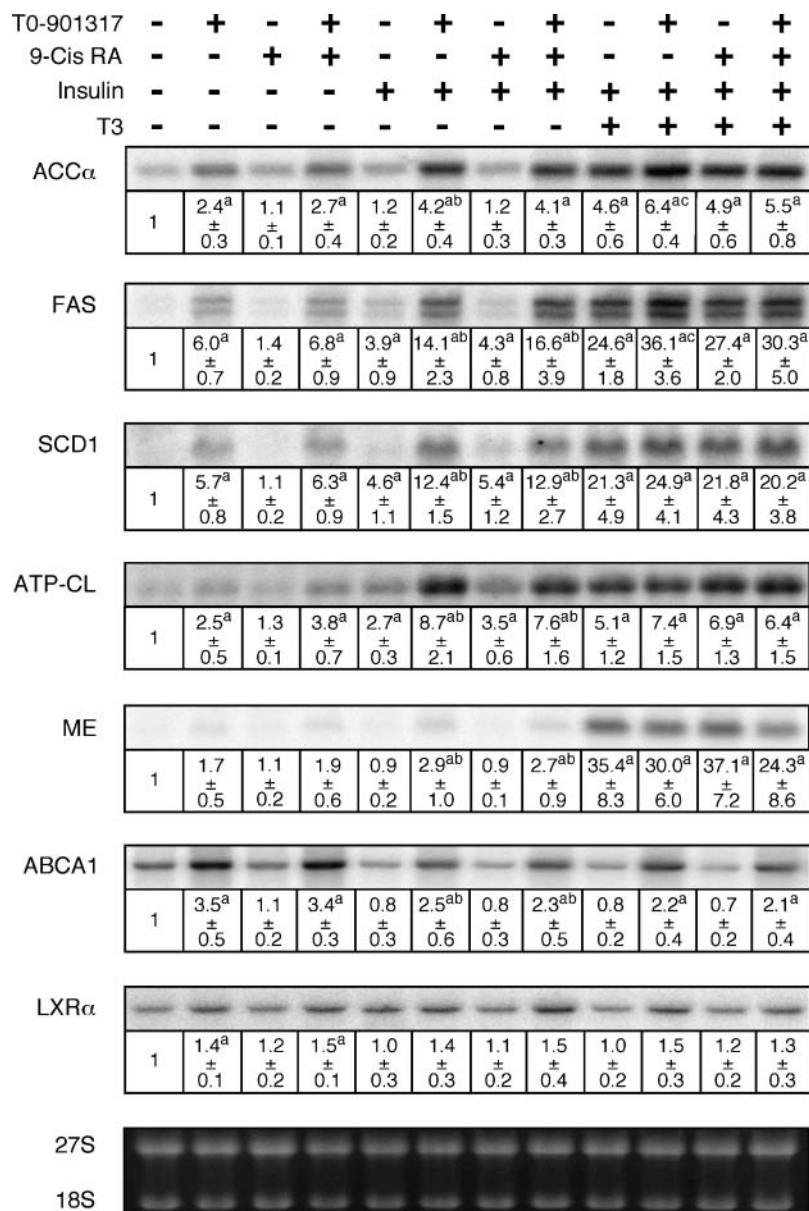


Fig. 1. The liver X receptor (LXR) agonist T0-901317 increases the expression of acetyl-coenzyme A carboxylase- α (ACC α) and other lipogenic enzymes in primary cultures of chick embryo hepatocytes. Hepatocytes were isolated as described in Experimental Procedures and incubated in serum-free Waymouth's medium. At 18 h of incubation, the medium was changed to one of the same composition supplemented with or without T0-901317 (6 μ M) in the absence or presence of insulin (50 nM), insulin and 9-*cis* retinoic acid (9-Cis RA; 100 nM), insulin and 3,5',3-triiodothyronine (T3; 1.5 μ M), or insulin, T3, and 9-*cis* retinoic acid. After 28 h of treatment, total RNA was isolated and the abundance of mRNA encoding ACC α , FAS, stearoyl-coenzyme A desaturase-1 (SCD1), ATP-citrate lyase (ATP-CL), malic enzyme (ME), ABCA1, and LXR α was measured by Northern analysis. Levels of mRNA in cells treated without T0-901317, 9-*cis* retinoic acid, insulin, and T3 were set at 1, and the other values were adjusted proportionally. Values are means \pm SEM of four experiments. Hybridization signals from a representative experiment are shown for each mRNA. Ribosomal subunits (27S and 18S) stained with ethidium bromide are shown as controls for RNA loading. ^a Mean is significantly ($P < 0.05$) different from that of cells treated without T0-901317, 9-*cis* retinoic acid, insulin, and T3; ^b mean is significantly ($P < 0.05$) different from that of cells treated with insulin; ^c mean is significantly ($P < 0.05$) different from that of cells treated with insulin and T3.

Identification of a LXR response unit that mediates the effects of T0-901317 on ACC α transcription

Previous studies have shown that T3 regulation of ACC α transcription is mediated by a 23 bp region (-101 to -71 bp) in promoter 2 of the ACC α gene (7). This region

contains a DR-4 element (-101 to -86 bp) that binds heterodimers composed of TR•RXR and LXR•RXR and a SRE (-82 to -71 bp) that binds SREBP-1. To determine the role of these sequences and other sequences in the ACC α gene in mediating the stimulatory effects of T0-

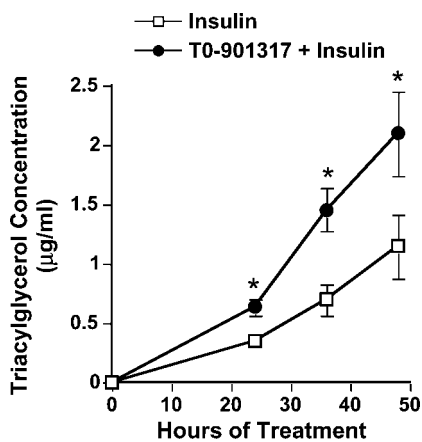


Fig. 2. Effect of T0-901317 on the accumulation of triacylglycerols in the culture medium of chick embryo hepatocytes. Hepatocytes were plated on 90 mm Petri dishes (1×10^7 cells/dish) in Waymouth's medium containing insulin. At 18 h of incubation, the medium was changed to one of the same composition supplemented with or without T0-901317. After 0, 24, 36, and 48 h of incubation with T0-901317, the concentration of triacylglycerol in the culture medium was measured using a spectrophotometric assay. Data are means \pm SEM of three experiments. * Mean is significantly ($P < 0.05$) different from that of cells incubated with insulin for the same time period.

901317 on ACC α transcription, transient transfection experiments were performed using reporter constructs containing portions of ACC α promoter 2 linked to the CAT gene. In chick embryo hepatocytes transfected with a reporter construct containing 2,054 bp of 5'-flanking DNA, T0-901317 caused a 2.9-fold increase in promoter activity (Fig. 3). 5' deletion of ACC α sequences to -391, -136, and -108 bp had no effect on T0-901317 responsiveness. Deletion of ACC α sequences containing the DR-4 element (-108 to -84 bp) abolished the stimulatory effect of T0-901317 on ACC α transcription. Further deletion to -41 bp had no effect on T0-901317 responsiveness. Mutation of the SRE (-80 to -71 bp) in the context of 108 bp of 5'-flanking DNA caused a 49% reduction in T0-901317 responsiveness. These results indicate that the DR-4 element (LXRE) is required for T0-901317 regulation of ACC α transcription and that the SRE enhances the ability of the LXRE to activate ACC α transcription in the presence of T0-901317.

To determine whether the functional interaction between the ACC α LXRE and SRE required the presence of additional *cis*-acting sequences, hepatocytes were transfected with constructs containing fragments of the ACC α gene linked to the minimal promoter of the herpes simplex virus thymidine kinase (TK) gene. The TK promoter alone was unresponsive to T0-901317 (Fig. 4). When a DNA fragment containing both the ACC α LXRE and the ACC α SRE (-108 to -66 bp) was linked to the TK promoter, treatment with T0-901317 caused a 5.5-fold increase in promoter activity. Mutation of the ACC α SRE in the context of the ACC α -108 to -66 bp fragment caused a 49% decrease in T0-901317 responsiveness. When a DNA frag-

ment containing the ACC α SRE alone (-84 to -66 bp) was appended to the TK promoter, T0-901317 treatment had no effect on promoter activity. These data demonstrate that the ACC α SRE can function alone to enhance T0-901317 regulation conferred by the ACC α LXRE. Thus, a region of the ACC α gene containing a LXRE and a SRE is responsible for mediating the effects of T0-901317 on ACC α promoter 2 activity. We refer to this region as the ACC α liver X receptor response unit (LXRU).

LXR activation increases the abundance of mature SREBP-1 in chick embryo hepatocytes

In our studies analyzing the regulation of the ACC α gene by T3, we showed that T3 treatment increased the abundance of the mature, active form of SREBP-1 in chick embryo hepatocytes and that this effect contributed to the activation of ACC α transcription by T3 (8). This observation prompted us to ask whether T0-901317 regulated SREBP-1 levels in chick embryo hepatocyte cultures. The time course of the effects of T0-901317 on the abundance of mature SREBP-1 protein, SREBP-1 mRNA, and ACC α mRNA was determined in hepatocytes cultured in the presence of insulin. Treatment with T0-901317 for 6 h caused a 1.5-fold increase in mature SREBP-1 concentration (Fig. 5). A larger increase in mature SREBP-1 concentration (2.2-fold) was observed after 24 h of treatment with T0-901317. In contrast to the time course for mature SREBP-1, the T0-901317-induced stimulation of ACC α mRNA levels occurred at a later time point (between 24 and 48 h of treatment). This observation is consistent with the proposal that alterations in SREBP-1 levels play a role in mediating the regulation of ACC α transcription by T0-901317. Treatment with T0-901317 increased the abundance of SREBP-1 mRNA, and this effect was maximal (1.9- to 2.1-fold) at or before 2 h of incubation. Thus, T0-901317-induced changes in mature SREBP-1 concentration appear to be mediated by a pretranslational mechanism.

Effect of LXR activation on histone acetylation of the ACC α gene

The T0-901317-induced increase in ACC α transcription may be mediated in whole or in part by alterations in histone acetylation. Previous studies have shown that the activation of LXR•RXR heterodimers by LXR ligands/agonists triggers the recruitment of coactivator complexes containing histone acetyltransferase activity (42, 43). Increased histone acetylation causes a chromatin decondensation that enhances the accessibility of the basal transcriptional machinery and other transcription factors to the target promoter. To investigate the role of histone acetylation in mediating the activation of ACC α transcription by T0-901317, ChIP experiments were performed in chick embryo hepatocytes incubated in the absence or presence of T0-901317. Hepatocytes were treated with 1% formaldehyde to cross-link DNA to associated proteins. Protein-DNA complexes were immunoprecipitated with an antibody against acetylated histone H3 or an antibody against acetylated histone H4. Immunoprecipitated DNA

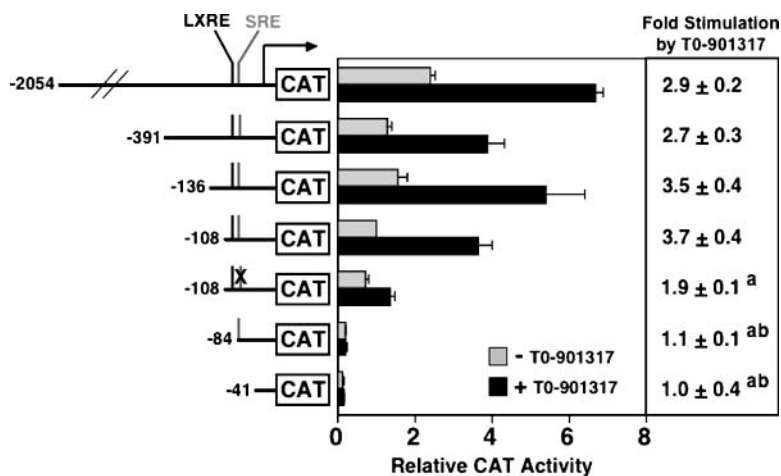


Fig. 3. Effect of mutations of the 5'-flanking region of ACC α promoter 2 on transcriptional activity in the absence and presence of T0-901317. Chick embryo hepatocytes were transiently transfected with p[ACC-2054/+274]chloramphenicol acetyltransferase (CAT) or equimolar amounts of other plasmids as described in Experimental Procedures. After transfection, cells were treated with or without T0-901317 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 the ACC α DNA in nucleotides relative to the transcription initiation site of promoter 2. The 3' end of each construct was +274 bp. The locations of the liver X receptor response element (LXRE) (-101 to -86 bp) and the sterol-regulatory element (SRE) (-80 to -71 bp) are indicated by vertical lines. A block mutation of the SRE is indicated by an X through the vertical line. Right: The CAT activity of cells transfected with p[ACC-108/+274]CAT and treated with T0-901317 was set at 1, and the other activities were adjusted proportionally. The fold stimulation by T0-901317 was calculated by dividing the CAT activity for hepatocytes treated with T0-901317 (+ T0-901317) by that for hepatocytes not treated with T0-901317 (- T0-901317). The fold responses were calculated for individual experiments and then averaged. The results are means \pm SEM of six experiments. ^a The fold stimulation by T0-901317 is significantly ($P < 0.05$) lower than that of p[ACC-108/+274]CAT; ^b the fold stimulation by T0-901317 is significantly ($P < 0.05$) lower than that of p[ACC-108/+274]CAT containing a block mutation of the SRE.

was analyzed by PCR using primers that flanked the ACC α LXRU. In hepatocytes incubated in the absence of T0-901317, acetylation of histone H3 and histone H4 was detected at the ACC α LXRU. Addition of T0-901317 to the culture medium stimulated a rapid increase (≤ 2 h) in the acetylation of histone H3 and H4 (Fig. 6, Table 1). Histone acetylation remained high for 6 h of T0-901317 treatment and then declined between 6 and 24 h of T0-901317 treatment. These results support the proposal that an increase in histone acetylation plays a role in mediating the effects of T0-901317 on ACC α transcription.

We also used ChIP to assess the effects of T0-901317 treatment on histone acetylation in an uncharacterized region of the SCD1 promoter using a primer set that amplified SCD1 sequences between -369 and -127 bp. In contrast to the data for the ACC α gene, treatment with T0-901317 had no effect on histone acetylation of this region of the SCD1 gene (Fig. 6). This observation indicates that the effects of T0-901317 on histone acetylation are sequence-specific.

DISCUSSION

In previous work analyzing the effects of T3 on ACC α transcription in avian hepatocytes, we identified a T3RE

that conferred T3 regulation on ACC α promoter 2 (7). Interestingly, this T3RE not only bound protein complexes containing TR•RXR heterodimers but also bound protein complexes containing LXR•RXR heterodimers. In this report, we provide functional evidence that LXR•RXR heterodimers regulate ACC α transcription. A specific ligand/agonist of LXR (T0-901317) activates ACC α transcription, and this effect is mediated by the LXRE/T3RE in ACC α promoter 2. We also demonstrate that SREBP-1 is an accessory factor that enhances the ability of LXR•RXR to increase ACC α transcription in the presence of T0-901317 and that LXR activation by T0-901317 increases the concentration of the mature, active form of SREBP-1 in chick embryo hepatocytes.

How does SREBP-1 enhance the stimulatory effects of T0-901317 on ACC α transcription? One possibility is that SREBP-1 facilitates the recruitment of coactivators to T0-901317-bound LXR•RXR complexes. LXR α , LXR β , and SREBP-1 interact with several coactivator proteins, including CREB binding protein (CBP) and the TRAP/ARC/DRIP complex (42–47). We postulate that the presence of SREBP-1 on ACC α promoter 2 provides additional coactivator interaction sites that stabilize the binding of CBP, TRAP/ARC/DRIP, and other coactivators to T0-901317-bound LXR•RXR. In support of this model, SREBP-1 and nuclear hormone receptors interact with separate peptide se-

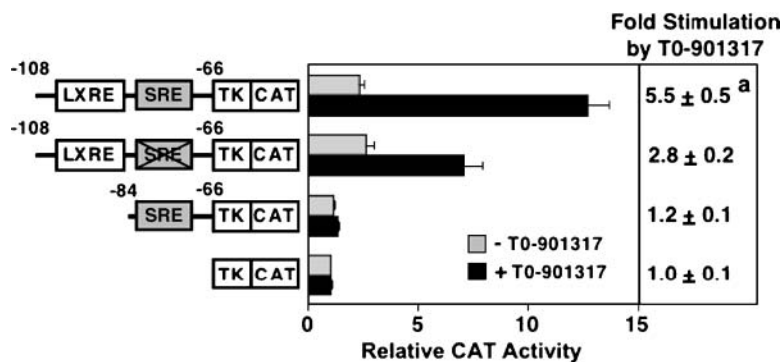


Fig. 4. The ACC α SRE alone enhances the transcriptional activity of the ACC α LXRE in the presence of T0-901317. Fragments of the ACC α gene containing the LXRE and/or SRE were linked to the minimal thymidine kinase (TK) promoter in TKCAT. Chick embryo hepatocytes were transiently transfected with these constructs and treated with or without T0-901317 as described in the legend to Fig. 3 and in Experimental Procedures. Left: The constructs used in these experiments. Numbers indicate the 5' and 3' boundaries of the ACC α DNA relative to the transcription initiation site of promoter 2. A block mutation of the SRE is indicated by an X across the box representing the SRE. Right: The CAT activity in hepatocytes transfected with TKCAT and treated without T0-901317 was set at 1, and the other activities were adjusted proportionally. The fold stimulation by T0-901317 was calculated as described in the legend to Fig. 3. The results are means \pm SEM of five experiments. ^a The fold stimulation by T0-901317 for p[ACC-108/-66] TKCAT is significantly ($P < 0.05$) higher than that of any other construct.

quences on CBP and separate subunits of the TRAP/ARC/DRIP complex (48–51).

SREBP-1 also enhances the ability of TR•RXR to activate ACC α transcription in the presence of T3 (9). In analyzing the mechanism mediating this effect, we showed that SREBP-1 interacted physically with TR and that binding of T3 to TR enhanced this interaction. We postulated that SREBP-1•SREBP-1 homodimers formed a tetrameric complex with TR•RXR heterodimers and that tetrameric complex formation enhanced the recruitment of coactivators to ACC α promoter 2. We also showed in these studies that LXR•RXR heterodimers do not interact physically with SREBP-1. Thus, in contrast to the mechanism by which SREBP-1 enhances TR•RXR activity, direct interactions between SREBP-1 and LXR•RXR do not play a role in mediating the stimulatory effects of SREBP-1 on LXR•RXR activity.

The mechanism by which LXR agonists regulate transcription has been analyzed for other lipogenic genes. In human hepatoma cells, T0-901317 activation of FAS transcription is mediated by a single LXRE in the FAS promoter (52). In primary rat hepatocyte cultures, the T0-901317-induced increase in SREBP-1c transcription is mediated by two LXREs in the SREBP-1c promoter (53, 54). Both the FAS promoter and the SREBP-1c promoter contain one or more SREs that are located \sim 110–540 bp downstream of the LXRE(s). In cells that express physiological levels of LXR, these SREs enhance basal transcription but have little or no effect on the regulation of transcription by T0-901317. In contrast to these findings, the SRE in ACC α promoter 2 enhances the T0-901317 regulation of transcription but has no effect on basal transcription in chick embryo hepatocytes (Figs. 3, 4). These gene-specific differences in SRE activity may be attributable to variations in

the proximity of the SRE relative to the LXRE and other *cis*-acting regulatory sequences. For example, the close association of the SRE with the LXRE/T3RE in ACC α promoter 2 may facilitate interactions between LXR•RXR and SREBP-1 and the ability of SREBP-1 to enhance T0-901317 responsiveness, whereas the wide separation of the SREs and LXREs in the FAS and SREBP-1c promoters may impede interactions between LXR•RXR and SREBP-1 and the ability of SREBP-1 to enhance T0-901317 responsiveness. Previous studies have shown that the SREs in the FAS and SREBP-1c promoters are closely linked to a binding site for nuclear factor-Y (NF-Y) and that SREBP-1 activity is dependent on interactions between SREBP-1 and NF-Y (53–56). These interactions enhance the ability of SREBP-1 to stimulate basal transcription. In contrast, the SRE in ACC α promoter 2 is not closely associated with binding sites for NF-Y. The lack of interaction of SREBP-1 with NF-Y on ACC α promoter 2 may explain why the ACC α SRE is not effective in modulating basal transcription.

Promoter 2 of the human ACC α gene and the rat ACC α gene contains two closely spaced SREs that mediate the effects of sterol depletion on ACC α transcription (57, 58). In contrast to promoter 2 of the chicken ACC α gene, a sequence resembling a DR-4 element is not present in the region flanking the SREs in the human and rat ACC α promoters. Thus, the role of SREBP-1 in mediating the stimulatory effects of T0-901317 on ACC α transcription may vary depending on the class of animals.

Another finding of this study is that LXR plays a permissive role in mediating the actions of insulin on ACC α transcription in chick embryo hepatocytes. Insulin stimulates ACC α expression in the presence of T0-901317 but has no effect on ACC α expression in the absence of T0-901317 (Fig. 1). In rat hepatocytes, insulin enhances

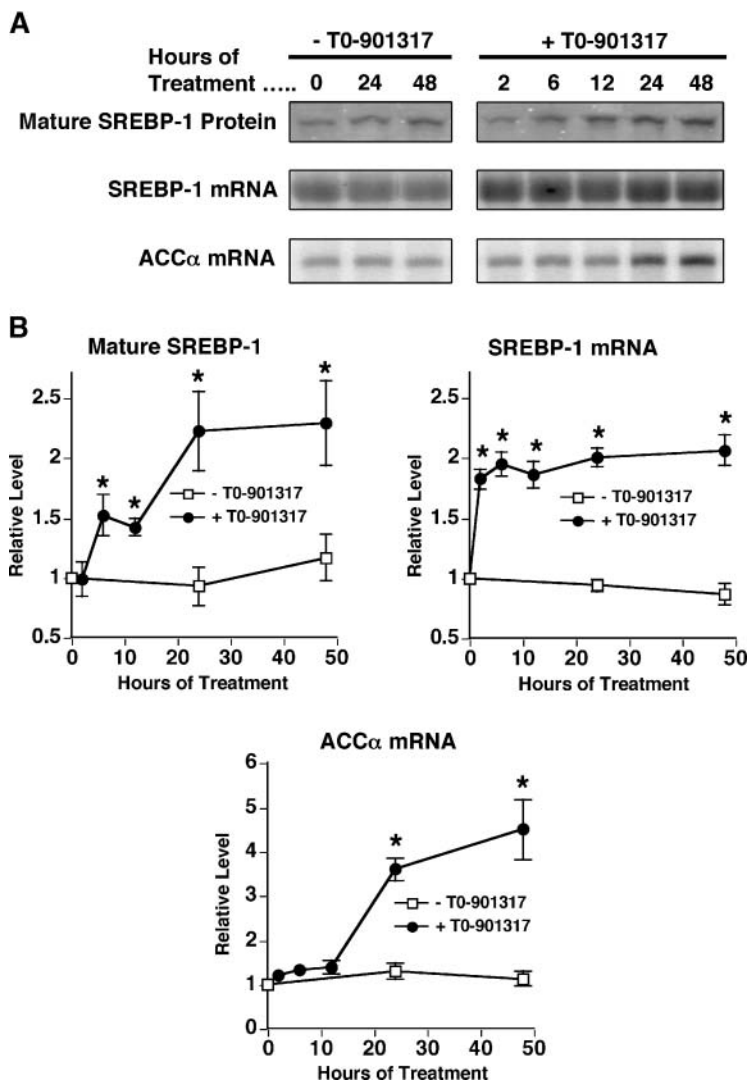


Fig. 5. T0-901317 increases the concentration of mature sterol-regulatory element binding protein-1 (SREBP-1) in chick embryo hepatocyte cultures. Chick embryo hepatocytes were isolated and incubated in serum-free Waymouth's medium containing insulin. At 18 h of incubation, the medium was changed to one of the same composition. T0-901317 was added at this time. After 2, 6, 12, 24, and 48 h of T0-901317 treatment, cellular extracts or total RNA were prepared as described in Experimental Procedures. A: The abundance of mature SREBP-1 in nuclear extracts was measured by Western analyses. The abundance of SREBP-1 mRNA and ACC α mRNA was measured by Northern analysis. These data are from a representative experiment. B: Signals for mature SREBP-1 protein from Western analyses and SREBP-1 mRNA and ACC α mRNA from Northern analyses were quantitated. Levels of mature SREBP-1 protein from Western analyses, and SREBP-1 mRNA, and ACC α mRNA in hepatocytes treated with T0-901317 for 0 h were set at 1. Values are means \pm SEM of four experiments. * The mean is significantly ($P < 0.05$) different from that of cells incubated with T0-901317 for 0 h or without T0-901317 for 24 or 48 h.

the ability of T0-901317 to increase mature SREBP-1 concentration as a result of a stimulatory effect of insulin on the processing of precursor SREBP-1 to mature SREBP-1 (59). We have confirmed this finding in chick embryo hepatocytes (data not shown). Because SREBP-1 enhances the LXR activation of ACC α transcription in chick embryo hepatocytes, we postulate that alterations in mature SREBP-1 abundance mediate the stimulatory effects of insulin on ACC α transcription. In support of this hypothesis, insulin does not increase the expression of ABCA1 in the presence of T0-901317 (Fig. 1). Previous work has shown that the ABCA1 gene is not a target of SREBP-1 (60).

Previous studies performed in rat hepatocytes indicate that insulin induces ACC α expression by increasing the activity of LXR. For example, Tobin et al. (61) have shown that insulin stimulates a 10-fold increase in the expression of LXR α mRNA and that ablation of the LXR α gene abolishes the stimulatory effect of insulin on ACC α expression. Others have shown that insulin increases the transcription of SREBP-1c and that this effect is mediated by two LXR•RXR binding sites in the SREBP-1c promoter (53, 54). SREBP-1c is a key factor mediating the effects of insulin

on ACC α transcription in rat hepatocytes (27, 62). These findings contrast with our data indicating that LXR plays a permissive role in mediating the effects of insulin on ACC α transcription in chick embryo hepatocytes. The reason for the differences between birds and rodents in the mechanism by which insulin regulates ACC α transcription is not clear. They may reflect subtle class-dependent differences in the role of insulin in the control of lipogenesis and/or other metabolic processes in liver.

The ACC α LXRE/T3RE enhances ACC α transcription in both the absence and presence of T0-901317 and T3, with a greater activation observed in the presence of T0-901317 and T3 (Figs. 3, 4) (7). We previously hypothesized that the enhancer activity of the ACC α LXRE/T3RE in the absence of T0-901317 and T3 was mediated by LXR•RXR heterodimers, as the primary protein complexes that bind the ACC α LXRE/T3RE in the absence of T0-901317 and T3 contain LXR•RXR heterodimers (7). Recent studies have shown that the unliganded form of LXR•RXR represses gene transcription as a result of its ability to interact with corepressor proteins and that the addition of LXR ligand increases gene transcription by

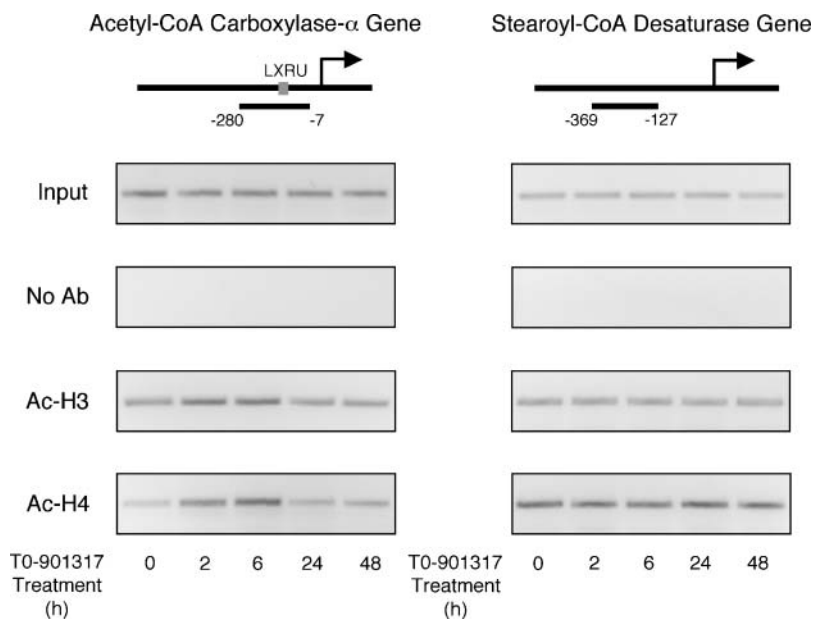


Fig. 6. Treatment of chick embryo hepatocyte cultures with T0-901317 causes a transient increase in histone acetylation at the ACC α liver X receptor response unit (LXRU). Chick embryo hepatocytes were isolated and incubated in serum-free Waymouth's medium containing insulin. At 18 h of incubation, the medium was changed to one of the same composition. T0-901317 was added at this time. After 2, 6, 24, and 48 h of T0-901317 treatment, the association of acetylated histone H3 (Ac-H3) and acetylated histone H4 (Ac-H4) with ACC α and SCD1 genomic sequences was measured. Chromatin immunoprecipitation assays were performed as described in Experimental Procedures. Immunoprecipitates were analyzed by PCR using primers that flanked the LXRU of ACC α promoter 2 and an uncharacterized region of the SCD1 promoter. The regions of the ACC α and SCD1 genes that were amplified by PCR are indicated at top. Chromatin samples that were processed in parallel without the application of primary antibody served as controls (No Ab). The input lanes show the results of PCR using chromatin samples taken before the immunoprecipitation step. Results are representative of three independent experiments.

causing the release of corepressors and the recruitment of coactivators to LXR•RXR (63). In view of these observations, we further hypothesize that LXR•RXR complexes associated with the ACC α LXRE/T3RE in the absence of T0-901317 and T3 are bound by endogenous LXR and/or RXR ligands. Several lines of evidence support this proposal. First, treatment of chick embryo hepatocytes with naturally occurring agonists of LXR [22-(*R*)-hydroxy

cholesterol] and RXR (9-*cis* retinoic acid) has little or no effect on ACC α mRNA abundance (Fig. 1 and data not shown). Second, the ACC α LXRE/T3RE is not associated with the corepressor protein, nuclear receptor corepressor, in the absence of T0-901317 and T3 (41). Last, the ACC α LXRE/T3RE is associated with acetylated histone H3 and acetylated histone H4 in the absence of T0-901317 and T3 (Fig. 6). The ability of T0-901317 to increase the transcriptional activity and histone acetylation of the ACC α LXRE/T3RE is likely attributable to the fact that this synthetic agonist is more effective than endogenous LXR agonists in stimulating the recruitment of histone acetyltransferase-containing coactivators to LXR•RXR (42).

In summary, we show that T0-901317 activates ACC α transcription by increasing the activity of LXR•RXR and SREBP-1 and the acetylation of histone H3 and histone H4 on ACC α promoter 2. The identification of small molecules that selectively inhibit one or more of these processes represents a potential strategy to enhance the utility of T0-901317 in the treatment of atherosclerosis and type 2 diabetes. The feasibility of this approach is supported by recent studies demonstrating that suppression of ACC α expression reverses hepatic steatosis and insulin resistance in rats fed a high-fat diet (64). ■

TABLE 1. Effect of T0-901317 treatment on the acetylation of histones at the acetyl-CoA carboxylase- α liver X receptor response unit

Histone	T0-901317 Treatment				
	0 h	2 h	6 h	24 h	48 h
Acetylated histone H3	1.0	1.4 \pm 0.1 ^a	1.8 \pm 0.1 ^a	1.1 \pm 0.2	1.1 \pm 0.1
Acetylated histone H4	1.0	1.7 \pm 0.1 ^a	2.2 \pm 0.2 ^a	1.0 \pm 0.1	1.1 \pm 0.1

Signals from chromatin immunoprecipitation assays using the indicated antibodies were quantitated. The data are expressed as fold differences relative to samples from hepatocytes treated with T0-901317 for 0 h. Values are means \pm SEM of three experiments. Results from a representative experiment are shown in Fig. 6.

^aSignificantly different ($P < 0.05$) from cells treated with T0-901317 for 0 h.

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REFERENCES

1. Barber, M. C., N. T. Price, and M. T. Travers. 2005. Structure and regulation of acetyl-CoA carboxylase genes of metazoa. *Biochim. Biophys. Acta.* **1733**: 1–28.
2. Brownsey, R. W., A. N. Boone, J. E. Elliott, J. E. Kulpa, and W. M. Lee. 2006. Regulation of acetyl-CoA carboxylase. *Biochem. Soc. Trans.* **34**: 223–227.
3. Leonard, A. E., S. L. Pereira, H. Sprecher, and Y. S. Huang. 2004. Elongation of long-chain fatty acids. *Prog. Lipid Res.* **43**: 36–54.
4. Abu-Elheiga, L., M. M. Matzuk, P. Kordari, W. Oh, T. Shaikenov, Z. Gu, and S. J. Wakil. 2005. Mutant mice lacking acetyl-CoA carboxylase 1 are embryonically lethal. *Proc. Natl. Acad. Sci. USA.* **102**: 12011–12016.
5. Hillgartner, F. B., T. Charron, and K. A. Chesnut. 1996. Alterations in nutritional status regulate acetyl-CoA carboxylase expression in avian liver by a transcriptional mechanism. *Biochem. J.* **319**: 263–268.
6. Hillgartner, F. B., T. Charron, and K. A. Chesnut. 1997. Triiodothyronine stimulates and glucagon inhibits transcription of the acetyl-CoA carboxylase gene in chick embryo hepatocytes: glucose and insulin amplify the effect of triiodothyronine. *Arch. Biochem. Biophys.* **337**: 159–168.
7. Zhang, Y., L. Yin, and F. B. Hillgartner. 2001. 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. *J. Biol. Chem.* **276**: 974–983.
8. Zhang, Y., L. Yin, and F. B. Hillgartner. 2003. SREBP-1 integrates the actions of thyroid hormone, insulin, cAMP, and medium-chain fatty acids on ACC α transcription in hepatocytes. *J. Lipid Res.* **44**: 356–368.
9. Yin, L., Y. Zhang, and F. B. Hillgartner. 2002. Sterol regulatory element-binding protein-1 interacts with the nuclear thyroid hormone receptor to enhance acetyl-CoA carboxylase- α transcription in hepatocytes. *J. Biol. Chem.* **277**: 19554–19565.
10. Lehmann, J. M., S. A. Kliewer, L. B. Moore, T. A. Smith-Oliver, B. B. Oliver, J. L. Su, S. S. Sundseth, D. A. Winegar, D. E. Blanchard, T. A. Spencer, et al. 1997. Activation of the nuclear receptor LXR by oxysterols defines a new hormone response pathway. *J. Biol. Chem.* **272**: 3137–3140.
11. Janowski, B. A., P. J. Willy, T. R. Devi, J. R. Falck, and D. J. Mangelsdorf. 1996. An oxysterol signalling pathway mediated by the nuclear receptor LXR α . *Nature.* **383**: 728–731.
12. Teboul, M., E. Enmark, Q. Li, A. C. Wikstrom, M. Pelto-Huikko, and J. A. Gustafsson. 1995. Or-1, a member of the nuclear receptor superfamily that interacts with the 9-cis-retinoic acid receptor. *Proc. Natl. Acad. Sci. USA.* **92**: 2096–2100.
13. Willy, P. J., K. Umesonu, E. S. Ong, R. M. Evans, R. A. Heyman, and D. J. Mangelsdorf. 1995. LXR, a nuclear receptor that defines a distinct retinoid response pathway. *Genes Dev.* **9**: 1033–1045.
14. Repa, J. J., and D. J. Mangelsdorf. 2002. The liver X receptor gene team: potential new players in atherosclerosis. *Nat. Med.* **8**: 1243–1248.
15. Edwards, P. A., H. R. Kast, and A. M. Anisfeld. 2002. BAREing it all: the adoption of LXR and FXR and their roles in lipid homeostasis. *J. Lipid Res.* **43**: 2–12.
16. Zelcer, N., and P. Tontonoz. 2006. Liver X receptors as integrators of metabolic and inflammatory signaling. *J. Clin. Invest.* **116**: 607–614.
17. Peet, D. J., S. D. Turley, W. Ma, B. A. Janowski, J. M. Lobaccaro, R. E. Hammer, and D. J. Mangelsdorf. 1998. Cholesterol and bile acid metabolism are impaired in mice lacking the nuclear oxysterol receptor LXR α . *Cell.* **93**: 693–704.
18. Alberti, S., G. Schuster, P. Parini, D. Feltkamp, U. Diczfalusy, M. Rudding, B. Angelin, I. Bjorkhem, S. Pettersson, and J. A. Gustafsson. 2001. Hepatic cholesterol metabolism and resistance to dietary cholesterol in LXR β -deficient mice. *J. Clin. Invest.* **107**: 565–573.
19. Joseph, S. B., E. McKilligin, L. Pei, M. A. Watson, A. R. Collins, B. A. Laffitte, M. Chen, G. Noh, J. Goodman, G. N. Hagger, et al. 2002. Synthetic LXR ligand inhibits the development of atherosclerosis in mice. *Proc. Natl. Acad. Sci. USA.* **99**: 7604–7609.
20. Terasaka, N., A. Hiroshima, T. Koicyama, N. Ubukata, Y. Morikawa, D. Nakai, and T. Inaba. 2003. T-0901317, a synthetic liver X receptor ligand, inhibits development of atherosclerosis in LDL receptor-deficient mice. *FEBS Lett.* **536**: 6–11.
21. Laffitte, B. A., L. S. Chao, J. Li, R. Waczak, S. Hummasti, S. B. Joseph, A. Castrillo, D. C. Wilpitz, D. J. Mangelsdorf, J. L. Collins, et al. 2003. Activation of liver X receptor improves glucose tolerance through coordinate regulation of glucose metabolism in liver and adipose tissue. *Proc. Natl. Acad. Sci. USA.* **100**: 5419–5424.
22. Grefhorst, A., T. H. van Dijk, A. Hammer, F. H. van der Sluijs, R. Havinga, L. M. Havekes, J. A. Romijn, P. H. Groot, D. J. Reijngoud, and F. Kuipers. 2005. Differential effects of pharmacological liver X receptor activation on hepatic and peripheral insulin sensitivity in lean and *ob/ob* mice. *Am. J. Physiol. Endocrinol. Metab.* **289**: E829–E838.
23. Grefhorst, A., B. M. Elzinga, P. J. Voshol, T. Plosch, T. Kok, V. W. Bloks, F. H. van der Sluijs, L. M. Havekes, J. A. Romijn, H. J. Verkade, et al. 2002. Stimulation of lipogenesis by pharmacological activation of the liver X receptor leads to production of large, triglyceride-rich very low density lipoprotein particles. *J. Biol. Chem.* **277**: 34182–34190.
24. Chisholm, J. W., J. Hong, S. A. Mills, and R. M. Lawn. 2003. The LXR ligand T0901317 induces severe lipogenesis in the *db/db* diabetic mouse. *J. Lipid Res.* **44**: 2039–2048.
25. Mulligan, J. D., M. T. Flowers, A. Tebon, J. J. Bitgood, C. Wellington, M. R. Hayden, and A. D. Attie. 2003. ABCA1 is essential for efficient basolateral cholesterol efflux during the absorption of dietary cholesterol in chickens. *J. Biol. Chem.* **278**: 13356–13366.
26. Schultz, J. R., H. Tu, A. Luk, J. J. Repa, J. C. Medina, L. Li, S. Schwendner, S. Wang, M. Thoolen, D. J. Mangelsdorf, et al. 2000. Role of LXRs in control of lipogenesis. *Genes Dev.* **14**: 2831–2838.
27. Liang, G., J. Yang, J. D. Horton, R. E. Hammer, J. L. Goldstein, and M. S. Brown. 2002. Diminished hepatic response to fasting/refeeding and liver X receptor agonists in mice with selective deficiency of sterol regulatory element-binding protein-1c. *J. Biol. Chem.* **277**: 9520–9528.
28. Goodridge, A. G. 1973. Regulation of fatty acid synthesis in isolated hepatocytes prepared from the livers of neonatal chicks. *J. Biol. Chem.* **248**: 1924–1931.
29. Chomczynski, P., and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162**: 156–159.
30. Feinberg, A. P., and B. Vogelstein. 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* **132**: 6–13.
31. Amasino, R. M. 1986. Acceleration of nucleic acid hybridization rate by polyethylene glycol. *Anal. Biochem.* **152**: 304–307.
32. Yuan, Z. Y., W. Liu, and G. G. Hammes. 1988. Molecular cloning and sequencing of DNA complementary to chicken liver fatty acid synthase mRNA. *Proc. Natl. Acad. Sci. USA.* **85**: 6328–6331.
33. Lefevre, P., C. Diot, P. Legrand, and M. Douaire. 1999. Hormonal regulation of stearoyl coenzyme-A desaturase 1 activity and gene expression in primary cultures of chicken hepatocytes. *Arch. Biochem. Biophys.* **368**: 329–337.
34. Zhang, Y., and F. B. Hillgartner. 2004. Starvation and feeding a high-carbohydrate, low-fat diet regulate the expression sterol regulatory element-binding protein-1 in chickens. *J. Nutr.* **134**: 2205–2210.
35. Daval, S., F. Pitel, C. Le Nigen, M. Douaire, A. Signal, and S. Lagarrigue. 2000. Cloning and mapping of the ACLY gene to a chicken microchromosome. *Anim. Genet.* **31**: 412–413.
36. Handschin, C., M. Podvinec, R. Amherd, R. Looser, J. C. Ourlin, and U. A. Meyer. 2002. Cholesterol and bile acids regulate xenosensor signaling in drug-mediated induction of cytochromes P450. *J. Biol. Chem.* **277**: 29561–29567.
37. Winberry, L. K., S. M. Morris, Jr., J. E. Fisch, M. J. Glynias, R. A. Jenik, and A. G. Goodridge. 1983. Molecular cloning of cDNA sequences for avian malic enzyme. Nutritional and hormonal regulation of malic enzyme mRNA levels in avian liver cells in vivo and in culture. *J. Biol. Chem.* **258**: 1337–1342.
38. Baillie, R. A., S. A. Klautky, and A. G. Goodridge. 1993. Transient transfection of chick-embryo hepatocytes. *J. Nutr. Biochem.* **4**: 431–439.

39. Gorman, C. M., L. F. Moffat, and B. H. Howard. 1982. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. *Mol. Cell. Biol.* **2**: 1044–1051.
40. Sedmak, J. J., and S. E. Grossberg. 1977. A rapid, sensitive, and versatile assay for protein using Coomassie Brilliant Blue G250. *Anal. Biochem.* **79**: 544–552.
41. Yin, L., Y. Wang, S. Dridi, C. Vinson, and F. B. Hillgartner. 2005. Role of CCAAT/enhancer-binding protein, histone acetylation, and coactivator recruitment in the regulation of malic enzyme transcription by thyroid hormone. *Mol. Cell. Endocrinol.* **245**: 43–52.
42. Albers, M., B. Blume, T. Schlueter, M. B. Wright, I. Kober, C. Kremoser, U. Deuschle, and M. Koepl. 2006. A novel principle for partial agonism of liver X receptor ligands. Competitive recruitment of activators and repressors. *J. Biol. Chem.* **281**: 4920–4930.
43. Wiebel, F. F., K. R. Steffensen, E. Treuter, D. Feltkamp, and J. A. Gustafsson. 1999. Ligand-independent coregulator recruitment by the triply activatable OR1/retinoid X receptor- α nuclear receptor heterodimer. *Mol. Endocrinol.* **13**: 1105–1118.
44. Huuskonen, J., P. E. Fielding, and C. J. Fielding. 2004. Role of p160 coactivator complex in the activation of liver X receptor. *Arterioscler. Thromb. Vasc. Biol.* **24**: 703–708.
45. Lee, S. K., S. Y. Jung, Y. S. Kim, S. Y. Na, Y. C. Lee, and J. W. Lee. 2001. Two distinct nuclear receptor-interaction domains and CREB-binding protein-dependent transactivation function of activating signal cointegrator-2. *Mol. Endocrinol.* **15**: 241–254.
46. Naar, A. M., P. A. Beaurang, S. Zhou, S. Abraham, W. Solomon, and R. Tjian. 1999. Composite co-activator ARC mediates chromatin-directed transcriptional activation. *Nature*. **398**: 828–832.
47. Oliner, J. D., J. M. Andresen, S. K. Hansen, S. Zhou, and R. Tjian. 1996. SREBP transcriptional activity is mediated through an interaction with the CREB-binding protein. *Genes Dev.* **10**: 2903–2911.
48. Belakavadi, M., and J. D. Fondell. 2006. Role of the mediator complex in nuclear hormone receptor signaling. *Rev. Physiol. Biochem. Pharmacol.* **156**: 23–43.
49. Chakravarti, D., V. J. LaMorte, M. C. Nelson, T. Nakajima, I. G. Schulman, H. Juguilon, M. Montminy, and R. M. Evans. 1996. Role of CBP/p300 in nuclear receptor signalling. *Nature*. **383**: 99–103.
50. Ericsson, J., and P. A. Edwards. 1998. CBP is required for sterol-regulated and sterol regulatory element-binding protein-regulated transcription. *J. Biol. Chem.* **273**: 17865–17870.
51. Toth, J. I., S. Datta, J. N. Athanikar, L. P. Freedman, and T. F. Osborne. 2004. Selective coactivator interactions in gene activation by SREBP-1a and -1c. *Mol. Cell. Biol.* **24**: 8288–8300.
52. Joseph, S. B., B. A. Laffitte, P. H. Patel, M. A. Watson, K. E. Matsukuma, R. Walczak, J. L. Collins, T. F. Osborne, and P. Tontonoz. 2002. Direct and indirect mechanisms for regulation of fatty acid synthase gene expression by liver X receptors. *J. Biol. Chem.* **277**: 11019–11025.
53. Cagen, L. M., X. Deng, H. G. Wilcox, E. A. Park, R. Raghov, and M. B. Elam. 2005. Insulin activates the rat sterol-regulatory-element-binding protein 1c (SREBP-1c) promoter through the combinatorial actions of SREBP, LXR, SP-1 and NF-Y cis-acting elements. *Biochem. J.* **385**: 207–216.
54. Chen, G., G. Liang, J. Ou, J. L. Goldstein, and M. S. Brown. 2004. Central role for liver X receptor in insulin-mediated activation of SREBP-1c transcription and stimulation of fatty acid synthesis in liver. *Proc. Natl. Acad. Sci. USA.* **101**: 11245–11250.
55. Magana, M. M., S. H. Koo, H. C. Towle, and T. F. Osborne. 2000. Different sterol regulatory element-binding protein-1 isoforms utilize distinct co-regulatory factors to activate the promoter for fatty acid synthase. *J. Biol. Chem.* **275**: 4726–4733.
56. Xiong, S., S. S. Chirala, and S. J. Wakil. 2000. Sterol regulation of human fatty acid synthase promoter requires nuclear factor-Y and SP-1-binding sites. *Proc. Natl. Acad. Sci. USA.* **97**: 3948–3953.
57. Magana, M. M., S. S. Lin, K. A. Dooley, and T. F. Osborne. 1997. Sterol regulation of acetyl coenzyme A carboxylase promoter requires two interdependent binding sites for sterol regulatory element binding proteins. *J. Lipid Res.* **38**: 1630–1638.
58. Mao, J., S. S. Chirala, and S. J. Wakil. 2003. Human acetyl-CoA carboxylase 1 gene: presence of three promoters and heterogeneity at the 5'-untranslated mRNA region. *Proc. Natl. Acad. Sci. USA.* **100**: 7513–7520.
59. Hegarty, B. D., A. Bobard, I. Hainault, P. Ferre, P. Bossard, and F. Foufelle. 2005. Distinct roles of insulin and liver X receptor in the induction and cleavage of sterol regulatory element-binding protein-1c. *Proc. Natl. Acad. Sci. USA.* **102**: 791–796.
60. Schmitz, G., and T. Langmann. 2005. Transcriptional regulatory networks in lipid metabolism control ABCA1 expression. *Biochim. Biophys. Acta.* **1735**: 1–19.
61. Tobin, K. A., S. M. Ulven, G. U. Schuster, H. H. Steineger, S. M. Andresen, J. A. Gustafsson, and H. I. Nebb. 2002. Liver X receptors as insulin-mediating factors in fatty acid and cholesterol biosynthesis. *J. Biol. Chem.* **277**: 10691–10697.
62. Stoeckman, A. K., and H. C. Towle. 2002. The role of SREBP-1c in nutritional regulation of lipogenic enzyme gene expression. *J. Biol. Chem.* **277**: 27029–27035.
63. Wagner, B. L., A. F. Valledor, G. Shao, C. L. Daige, E. D. Bischoff, M. Petrowski, K. Jepsen, S. H. Baek, R. A. Heyman, M. G. Rosenfeld, et al. 2003. Promoter-specific roles for liver X receptor/corepressor complexes in the regulation of ABCA1 and SREBP1 gene expression. *Mol. Cell. Biol.* **23**: 5780–5789.
64. Savage, D. B., C. S. Choi, V. T. Samuel, Z. X. Liu, D. Zhang, A. Wang, X. M. Zhang, G. W. Cline, X. X. Yu, J. G. Geisler, et al. 2006. Reversal of diet-induced hepatic steatosis and hepatic insulin resistance by antisense oligonucleotide inhibitors of acetyl-CoA carboxylases 1 and 2. *J. Clin. Invest.* **116**: 817–824.