A Key Role for Orphan Nuclear Receptor Liver Receptor Homologue-1 in Activation of Fatty Acid Synthase Promoter by Liver X Receptor

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Liver X receptor (LXR) activates fatty acid synthase (FAS) gene expression through binding to a DR-4 element in the promoter. We show that a distinct nuclear receptor half-site 21 bases downstream of the DR-4 element is also critical for the response of FAS to LXR but is not involved in LXR binding to DNA. This half-site specifically binds liver receptor homologue-1 (LRH-1) in vitro and in vivo, and we show LRH-1 is required for maximal LXR responsiveness of the endogenous FAS gene as well as from promoter reporter constructs. We also demonstrate that LRH-1 stimulation of the FAS LXR response is blocked by the addition of small heterodimer partner (SHP) and that FAS mRNA is overexpressed in SHP knock-out animals, providing evidence that FAS is an in vivo target of SHP repression. Taken together, these findings identify the first direct lipogenic gene target of LRH-1/SHP repression and provide a mechanistic explanation for bile acid repression of FAS and lipogenesis recently reported by others.

Fatty acids subserve a large number of specialized cellular functions including cholesterol esterification, production of lung surfactant, mammary gland secretions, and signaling molecules. Fatty acids are also fundamental components of all biological membranes and the primary biochemical form of energy storage. Despite being a multistep enzymatic process, basic fatty acid biosynthesis in higher eukaryotes is accomplished through the catalytic activities of only two gene products, acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS). ACC catalyzes the first step in which condensation of two acetyl-CoA molecules forms malonyl-CoA; FAS then catalyzes the remaining steps required to produce the fully saturated 16-carbon fatty acid palmitate.

Although ACC is generally considered the rate-limiting enzyme in the fatty acid biosynthetic pathway, in mammals FAS appears to be independently regulated at the transcription level by a large number of nutritional, hormonal, and cellular signals, including insulin (1), fatty acids (2, 3), thyroid hormone (4), sterols (5), oxysterols (6), glucocorticoids (7), growth factors (8), and cyclic AMP (9, 10). Extensive FAS promoter analyses have identified key binding sites for E-box-binding proteins USF1 and 2 (11), sterol regulatory element-binding proteins (SREBPs) (12), thyroid hormone receptor (TR) (13), liver X receptor (LXR) (6), and carbohydrate response element-binding protein (14) as well as for general transcription factors, which play important roles in mediating the cellular response to the various signals (15–17).

Regulation of FAS by oxysterols was reported by Joseph et al. (6) who identified a DR-4-type nuclear receptor response element for the oxysterol-responsive RXR/LXR heterodimer in the FAS promoter. This DR-4 was identified in a region of the FAS promoter that was also shown to be required for TR regulation (13).

On further analysis of the FAS promoter, we identified an additional nuclear receptor half-site 21 bases downstream of the DR-4 element (Fig. 1). The region surrounding this putative nuclear receptor site did not suggest the existence of an additional DR-4-type element. However, because of its proximity to the DR-4, we hypothesized that it might play a role in the activation of FAS by either LXR or TR. In the study reported here, we show that the additional half-site binds the orphan nuclear receptor liver receptor homolog-1 (LRH-1)/fetoprotein transcription factor and that LRH-1 binding to this site specifically augments the LXR response. In contrast, LRH-1 has no effect on thyroid hormone activation of FAS.

MATERIALS AND METHODS

Cell Culture—HepG2 human hepatoma cells were obtained from ATCC, cultured in Dulbecco’s minimum essential medium (Invitrogen) with penicillin/streptomycin (100 μg/ml), L-glutamine (2 mM), nonessential amino acids (100 μM), sodium pyruvate.

S-transferase; siRNA, small interfering RNA; CMV, cytomegalovirus; CMX, cytomegalovirus X.
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(1 mM), 5 mM Hapes, pH 7.2, and 10% fetal bovine serum, and maintained at 5% CO₂. One day before transfection, cells were plated at a density of 3.5 × 10⁶ cells/well in a 6-well plate in 3 ml of normal culture medium. HEK293T human embryonic kidney cells were obtained from ATCC, cultured in Dulbecco’s modified Eagle’s medium high glucose (Irvine Scientific) with penicillin/streptomycin (100 μg/ml), 1-glutamine (10 mM), nonessential amino acids (100 mM), 5 mM Hapes pH 7.2, and 10% fetal bovine serum, and maintained at 5% CO₂. One day before transfection, cells were plated at a density of 3.5 × 10⁵ cells/well in 6-well plates with 3 ml of normal culture medium.

Transient DNA Transfections—12–24 h after plating, cells were transfected by the standard calcium phosphate method as described previously (18). Where indicated, expression vectors for CMX-LXRα (0.1 μg/well), CMX-RXRα (0.1 μg/well), CMX-TRβ (0.4 μg/well), pCI-LRH-1 (0.1–0.5 μg/well), or CMX-SHP (50 ng/well) plus individual luciferase reporters (1 μg/well) and control CMV-β-galactosidase (1 μg/well) were used. 6–8 h post-transfection, cells were glycerol-shocked for 2 min and then washed 3 times with sterile phosphate-buffered saline. Medium was replaced with defined serum-free medium (Dulbecco’s minimum essential medium, penicillin/streptomycin (100 μg/ml), 1-glutamine (2 mM), nonessential amino acids (100 μM), 1 mM sodium pyruvate, 5 mM Hapes, pH 7.2, insulin/transferrin/selenite (5 μg/ml; 5 μg/ml; 5 ng/ml) (Sigma), 4% bovine serum albumin (Sigma, A-3803), and 25-hydroxycholesterol (0.1 μg/ml) plus one or more of the following: dimethyl sulfoxide (Me₅SO) (0.1%) as a vehicle control, GW39665 (1 mM), LG268 (100 mM), 22-R-hydroxycholesterol (2.5 μg/ml), T0901317 (1 μM), or T₃ (100 nM or 1 μM). Cells were allowed to incubate at 37 °C, 5% CO₂ for an additional 36–48 h before harvesting.

Plasmids—The FAS-700/+65 pg2 luciferase reporter construct was subcloned by PCR from the rat FAS-1594/+65 pg2 and the FAS-700/+65 MUT1 have been described previously (5, 6). All other FAS mutant reporter constructs were generated using the QuikChange site-directed mutagenesis kit (Stratagene) by replacing the individual half-site sequences with an equal number of adenine residues. The remaining reporter constructs were generated by PCR amplification and subcloning. pSynTATALuc is a reporter vector containing a minimal promoter region of the hydroxymethylglutaryl-CoA synthase promoter (−28/+39) and has been described previously (19). The SREBP-1c promoter used here contains a sequence from −937/+29 fused to the luciferase coding sequence in pg2 basic. The following expression vectors were provided by other laboratories: CMX-hTRβ (Barry Forman), CMX-mLXRα (Peter Tontonoz), CMX-hRXRα (Ron Evans and Bruce Blumberg), pCI-LRH-1 (Gregorio Gil), CMX-SHP (David Mangelsdorf).

Enzyme Assays—At the time of harvest cells were washed once with phosphate-buffered saline and then lysed in a reporter lysis buffer (25 mM Gly-Gly, 15 mM MgSO₄, 4 mM EGTA, 0.25% Triton). Luciferase activity of the lysates was measured in an Analytical Luminescence Monolight 2010 luminometer using 20–40 μl of cell extract plus 100 μl of luciferase assay reagent (Promega) and expressed in relative light units. β-Galactosidase activity was measured by a standard colorimetric assay at 420 nm absorbance using 50–100 μl of cell lysate and 2-nitrophenyl β-galactopyranoside as the substrate. Luciferase activity for each sample was divided by the β-galactosidase activity to yield normalized relative light units. -Fold activation was determined by dividing the normalized relative light units for a given sample by the normalized relative light units for the control sample (no activators plus Me₅SO). Each transfection was performed at least twice with similar results.

Electrophoretic Mobility Shift Assay—In vitro transcribed and translated LXRα and RXα proteins were generated using the T7 Tnt rabbit reticulocyte lysate (Promega). One or two microliters of each translation were added to each binding mixture (containing 10 mM Hapes, pH 7.6, 50 mM NaCl, 2.5 mM MgCl₂, 0.1 μM/μl poly(d/dC), 0.05% (v/v) Nonidet P-40, 10% glycerol) in a final volume of 20 μl. For an electrophoretic mobility shift assay using GST-LRH-1, 20 ng of purified bacterially expressed GST-LRH-1 were added per reaction. A 57-base double-stranded oligo containing the wild type sequence of the three nuclear receptor half-sites was 5′ end-labeled using T₄ polynucleotide kinase (U.S. Biochemical Corp.) and added to the binding mixtures (1–2 ng/reaction). Binding mixtures were incubated at 4 °C for 1–2 h. Samples were then run on 5% polyacrylamide: bisacrylamide (19:1) gels at room temperature for 1.5 h, fixed in a solution of 10% methanol, 10% acetic acid, and dried onto Whatman No. 3MM chromatography paper at 80 °C for 1 h. Dried gels were exposed to x-ray film for 12–48 h. DNA sequences (one strand from 5′-3′ only) were as follows; note that mutant bases are underlined. DNA sequences at all three half-sites were: wild type, KM 42, 5′-CTAGCACCTGAGTACTACCGCCTGAGGCGCCCTCCGCCAGGGTCAACGACCC; mut 1, KM 45, 5′-CTAGCACCTGAGTACTACCGCCTGAGGCGCCCTCCGCCAGGGTCAACGAC; mut 2, KM 46, 5′-CTAGCACCTGAGTACTACCGCCTGAGGCGCCCTCCGCCAGGGTCAACGAC; mut 3, KM 47, 5′-CTAGCACCTGAGTACTACCGCCTGAGGCGCCCTCCGCCAGGGTCAACGAC; Triple Mut, KM 48, 5′-CTAGCACCTGAGTACTACCGCCTGAGGCGCCCTCCGCCAGGGTCAACGAC; T₃ 100 nM or 1 μM), Cells were allowed to incubate at 37 °C, 5% CO₂ for an additional 36–48 h before harvesting.

Animal Studies—SHP knock-out mice described previously (20) and wild type C57B1/129sv mice were maintained on a normal chow diet. Adult animals were sacrificed, and liver RNA was extracted and analyzed for FAS expression by Q-PCR as described (21).
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plus 1 mM EDTA and 1 mM EGTA. The tissue was disrupted in a Tekmar Tissumizer at the lowest setting. Formaldehyde was added from a 37% stock (v/v) to a final concentration of 1%, and samples were rotated on a shaker for 6 min followed by the addition of glycine to a final concentration of 0.125 M. Samples were returned to the shaker for an additional 5 min, and then cells were collected by centrifugation (2000 rpm in Sorvall RC3B) at 4 °C. The cell pellet was washed once with homogenization buffer A (10 mM Hepes, pH 7.6), 25 mM KCl, 1 mM EDTA, 1 mM EGTA, 2 mM sucrose, 10% glycerol, 0.15 mM spermine, plus protease inhibitors as above. The final pellet was resuspended in buffer A and homogenized in a Dounce homogenizer with a pestle to release nuclei. The solution was layered over buffer A and centrifuged in a Beckman ultracentrifuge (1 h at 26,000 rpm, 4 °C), and the nuclear pellet was resuspended in buffer A and homogenized in a Dounce homogenizer plus 1 mM EDTA and 1 mM EGTA. The tissue was disrupted in a Tekmar Tissumizer at the lowest setting. Formaldehyde was added from a 37% stock (v/v) to a final concentration of 1%, and samples were rotated on a shaker for 6 min followed by the addition of glycine to a final concentration of 0.125 M. Samples were returned to the shaker for an additional 5 min, and then cells were collected by centrifugation (2000 rpm in Sorvall RC3B) at 4 °C. The cell pellet was washed once with homogenization buffer A (10 mM Hepes, pH 7.6), 25 mM KCl, 1 mM EDTA, 1 mM EGTA, 2 mM sucrose, 10% glycerol, 0.15 mM spermine, plus protease inhibitors as above. The final pellet was resuspended in buffer A and homogenized in a Dounce homogenizer with a pestle to release nuclei. The solution was layered over buffer A and centrifuged in a Beckman ultracentrifuge (1 h at 26,000 rpm, 4 °C), and the nuclear pellet was resuspended in nuclei lysis buffer (1% SDS, 50 mM Tris, pH 7.6, 10 mm EDTA). Nuclei were disrupted using an Ultrasonic model W-220F sonicator for 5 × 10 s to shear chromatin. Chromatin size was checked by agarose electrophoresis to ensure that the average size was between 200 and 500 bp. Aliquots were used in immunoprecipitation experiments with an antibody to LRH-1 (Santa Cruz; sc25389X) and processed for the rest of the chromatin immunoprecipitation protocol as described (22, 23). Final DNA samples were analyzed by quantitative PCR in triplicate with a standard dilution curve of the input DNA performed in parallel. Oligo pairs for the FAS LRH-1 binding region or exons 4 from the YY1 gene used in the Q-PCR are as follows: FAS-700 (5′), ATCTGGTCTCAGGGT; FAS-534 (3′), TAGGCAATTAGGTGTAGGG; YY1, (5′) TCTGACGGAGAGGTGAC; YY1, (3′) CTGAAGGGCTTTTCTCCAGTATG.

RESULTS

In our analysis of the FAS promoter, we noted a highly conserved sequence 21 bases downstream of the RXR/LXR response element bearing a strong resemblance to other nuclear receptor half-sites (Fig. 1). To determine whether this sequence played a role in the FAS LXR response, we first compared LXR activation of the wild type FAS promoter to LXR activation of similar reporter constructs containing mutations in each of the three half-sites (Fig. 2A). As expected, mutations of either half-site 1 or 2 (comprising the known LXR DR-4 sequence) played a role in the FAS LXR response, we first compared LXR activation of the wild type FAS promoter to LXR activation of similar reporter constructs containing mutations in each of the three half-sites (Fig. 2A). As expected, mutations of either half-site 1 or 2 (comprising the known LXR DR-4 sequence) resulted in a significant decrease in LXR activation. Surprisingly, however, mutation of the third half-site alone also resulted in a substantial defect in LXR activation, suggesting that the third half-site played an important and previously unappreciated role in LXR activation of FAS. Furthermore,
mutation of all three half-sites simultaneously resulted in the most significant defect. Even with all three sites mutated there was still a 6-fold activation by LXR/RXR. This residual activity is due to LXR induction of SREBP-1c, which subsequently activates the FAS promoter through binding to sites in the proximal promoter (6).

To better understand the mechanism through which the third half-site exerts its effect on LXR activation of FAS, we examined binding of in vitro translated RXRα and LXRα proteins to a 32P-labeled DNA probe containing all three nuclear receptor half-sites (Fig. 2B). The addition of both RXRα and LXRα proteins resulted in a single band (lane 1) that migrated in parallel with a known RXRα/LXRα heterodimer on a consensus DR-4. The addition of a 200-fold excess of unlabeled wild type competitor DNA effectively competed for RXRα/LXRα heterodimer binding (lane 2); however, competitor DNAs containing mutations in the first and/or second half-sites (lanes 3, 4, 6–9) were unable to compete significantly for heterodimer binding. In contrast, competitor DNA containing a mutation in the third half-site alone effectively competed for RXRα/LXRα heterodimer binding (lane 5). Thus, the third half-site did not play a significant role in RXRα/LXRα binding to this region of the promoter despite being an important determinant of the FAS LXR response.

Two other known LXR target genes, murine CYP7A1 (24) and human CETP (25), require binding of the monomeric nuclear receptor LRH-1 (also known as fetoprotein transcription factor and CYP7A1 promoter binding factor) at a site proximal to the LXR element, for efficient LXR activation. To determine whether LRH-1 could bind to the FAS third half-site and potentiate the LXR response of the FAS gene, we first assessed binding of recombinant LRH-1 protein to the FAS third half-site by electrophoretic mobility shift assay (Fig. 3A). Incubation of 20 ng of GST-LRH-1 with the labeled third half-site resulted in a single shifted band (lane 2) that migrated in parallel with GST-LRH-1 bound to a known LRH-1 binding site from the CYP8B1 promoter (lane 10). The addition of a 50- and 200-fold excess of unlabeled competitor DNA containing the wild type third half-site sequence effectively competed for GST-LRH-1 binding to the FAS half-site in a dose-dependent manner (lanes 3 and 4) as did competitor DNA containing the LRH-1 binding site from the CYP8B1 promoter (lanes 7 and 8). In contrast, the addition of competitor DNA containing a mutation in the third half-site did not compete for LRH-1 binding (lanes 5 and 6).

We next investigated direct binding of LRH-1 to the endogenous FAS promoter by chromatin immunoprecipitation in normal mouse liver using an antibody to LRH-1 and oligonucleotides that amplify the relevant region of the FAS promoter (Fig. 3B). Here, there was significant enrichment of the FAS promoter by the LRH-1 antibody relative to an IgG control, and this effect was specific as there was no enrichment of DNA from the YY1 locus analyzed in parallel.

To determine whether LRH-1 might activate the FAS promoter and augment the LXR response, we turned to HEK293T cells because HepG2 cells contain a significant level of endogenous LRH-1. To eliminate any indirect effects mediated through LXR activation of SREBP-1c (an LXR target gene and direct activator of FAS) (Fig. 2 and Refs. 5 and 6), a truncated wild type FAS promoter construct was constructed that contained the three nuclear receptor half-sites but lacked the downstream SREBP recognition motifs (FAS-700/-574) fused to a minimal TATA box containing promoter. This was transfected into HEK293T human embryonic kidney cells with an expression vector for LRH-1 along with the FAS promoter construct containing the three nuclear receptor half-sites (Fig. 4A).

Because the RXR/LXR heterodimer is permissive and, thus, can be activated simultaneously by an RXR ligand and an LXR ligand (26), we compared activation of the FAS promoter by a synthetic RXR agonist (LG268), an oxysterol known to activate LXR (22-R-hydroxycholesterol) or both in combination. In the absence of transfected LRH-1, the addition of each ligand or the combination of both resulted in 2–5-fold increases in FAS activation, as is consistent with the FAS being an RXR/LXR target gene. In the presence of transfected LRH-1, however, this modest response increased to 20.5-fold, whereas LXR ligands or LRH-1 alone activated FAS 4.4- and 2.9-fold, respectively. Furthermore, the effect of LRH-1 on the LXR response was specific.
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To better understand the contribution of each half-site to the LRH-1 potentiation effect, we used mutant versions of this FAS reporter construct containing either a double mutation of both halves of the DR-4 or a mutation in the putative LRH-1 (third) half-site (Fig. 4B). As in the previous experiment, LXR activation of the corresponding wild type FAS promoter was dramatically increased in the presence of LRH-1. Furthermore, the addition of an expression vector for SHP, a novel non-DNA binding nuclear receptor known to be a potent inhibitor of LRH-1 activity in other promoters, significantly blunted this response.

The FAS promoter construct containing a mutation in the DR-4 (MUT1/2) was defective for RXR/LXR signaling irrespective of the presence of LRH-1. Importantly, the magnitude of activation by LRH-1 alone on this mutant construct was similar to that seen on the wild type promoter, demonstrating an intact LRH-1 response.

Activation of the promoter construct containing a mutation in the third half-site (MUT 3) by RXR/LXR alone was comparable with that seen on the wild type construct; however, activation by LRH-1 alone was lost as was the concerted activation by both LXR and LRH-1. The residual inhibitory effect of SHP in the absence of the LRH-1 site was likely due to its known ability to inhibit LXR activation (27). Thus, SHP likely inhibits FAS gene expression through interactions with both LRH-1 and LXR.

We next used an siRNA approach to evaluate the effects of specifically reducing endogenous LRH-1 expression on the LXR-mediated induction of FAS in HepG2 cells (Fig. 5A). We used HepG2 cells because an siRNA approach has been used previously to silence LRH-1 gene expression (28). The siRNA specifically reduced endogenous LRH-1 mRNA by 30–50%, which resulted in a significant blunting of the response of FAS to LXR activation. To rule out off-target effects of the siRNA,
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FIGURE 6. A, LRH-1 enhancement of the LXR response is specific to the FAS promoter. HEK293T cells were transfected with pCI-LRH-1, CMX-SHP, CMV-β-galactosidase, and either SREBP-1c (~937/+29), pGL2, or rFAS-700/+65 as noted in the figure. Cells were treated with either Me$_2$SO (DMSO, as a vehicle control) or TO901317 (1 μM) (a synthetic LXR agonist) for 16–18 h and then harvested and assayed for luciferase and β-galactosidase activity. B, LRH-1 does not affect the T$_3$ T$_r$ response. HEK293T cells were transfected with CMX-TR$_β$, CMX-RXR$_α$, pCI-LRH-1, CMV-β-galactosidase, and rat FAS-700/+65 as noted in the figure and under “Materials and Methods.” Cells were treated with either Me$_2$SO (as a vehicle control) or T$_3$ (triiodothyronine) (100 nM) for 40 h and then harvested and assayed for luciferase and β-galactosidase activity. Error bars represent the mean ± S.E. of triplicate reactions.

we also measured mRNA expression of LXRα in these cells. As expected, the addition of the siRNA did not decrease LXRα levels.

Although LRH-1 knock-out mice die early in embryogenesis and are, thus, unavailable for study (29), SHP knock-out mice are viable (20). Given that SHP is a potent inhibitor of LRH-1 (24, 30, 31), it follows that if FAS is indeed an LRH-1 target gene, FAS mRNA expression would be elevated in SHP knock-out mice. Consistent with this hypothesis, our animal studies showed that FAS mRNA expression in SHP knock-out mice was 6.8-fold higher than in matched wild type controls (Fig. 5B).

In a previous report LRH-1 was shown to augment the LXR response of SREBP-1c (43). However, despite localization of the effect to the proximal 300 bases of the SREBP-1c promoter, an LRH-1 site could not be identified, and no binding studies were performed. To determine the effect of LRH-1 on LXR activation of SREBP-1c in our model system, we transfected HEK293T cells with an SREBP-1c promoter fragment encompassing the LXR element and the putative LRH-1 response region in the presence and absence of expression vector for LRH-1 and compared the LXR response with that of the FAS promoter (Fig. 6A). Both SREBP-1c and FAS showed a 3-fold induction by LXR ligand T0901317. However, the addition of increasing amounts of LRH-1 expression vector had no effect on the SREBP-1c LXR response, whereas FAS expression increased 10-fold. Also of note, no change in SREBP-1c activation was observed by LRH-1 alone, whereas FAS activation increased to 3-fold in the presence of transfected LRH-1 alone. Thus, in our system, LRH-1 does not play a direct role in the SREBP-1c LXR response.

The region of the FAS promoter containing the DR-4 has been previously shown to mediate the FAS response to TR (13). Because RXR/LXR and RXR/TR both preferentially bind DR-4 type nuclear receptor elements, we investigated whether LRH-1 binding to the third half-site also played a role in FAS activation by TR and T$_3$. Using HEK293T cells transfected with expression vectors for TR$_β$, RXR$_α$, and LRH-1, we discovered that, although TR and T$_3$ clearly activate the FAS promoter, the FAS T$_3$ response was not enhanced by the addition of increasing amounts of an expression vector for LRH-1 (Fig. 6B). Thus, LRH-1 potentiation of FAS is specific to the RXR/LXR response.

DISCUSSION

Liver receptor homolog-1 was described as CYP7A1 promoter binding factor, a liver-enriched transcription factor essential for expression of the CYP7A1 gene (32). Interestingly, although a required component of CYP7A1 gene expression, LRH-1 had very little activation potential of its own, and it was subsequently shown to significantly augment the response of the murine CYP7A1 promoter to LXR/oxysterol transactivation (24, 31). These studies also identified the LRH-1 central role in bile acid homeostasis by demonstrating that LRH-1 is a key target of SHP inhibition. LRH-1 is now recognized as an important component in negative feedback regulation of bile acid biosynthesis.

LRXα was also originally identified as a liver-enriched nuclear receptor (33, 34). It is activated through the binding of oxysterol agonists (35) and is an important regulator of cholesterol homeostasis (36). When cellular cholesterol levels are elevated, oxysterol levels rise and serve as physiologic agonists for LXR (37). In response, the expression of genes involved in cholesterol storage as well as cholesterol metabolism and excretion are activated. Key LXR target genes involved in cholesterol storage include SREBP-1c (35) and FAS (6). LXR target genes involved in cholesterol metabolism and excretion include murine CYP7A1 (38) and ABCA1 (39), ABCG5 and ABCG8 (40), apoE (41), and human CETP (42).

Because the identification of LRH-1 as a key factor for the LXR/oxysterol response of murine CYP7A1, the LXR responses of two other genes have been shown to be augmented by LRH-1: human CETP (25) and mouse SREBP-1c (43). CETP plays an important role in balancing human lipoprotein metabolism, and SREBP-1c is a key transcription factor responsible for orchestrating the lipogenic gene program (44).

The LXR and LRH-1 sites are conserved in the human, mouse, and chicken FAS promoters (Fig. 1). In contrast, the LXR site is not present in the human CYP7A1 gene (45), and the mouse genome does not encode a functional CETP gene. Thus, FAS is unique in that activation by both LXR and LRH-1 is conserved across species.

In our studies the LXR response of the SREBP-1c promoter was not altered by LRH-1 (Fig. 6). The LRH-1-responsive elements in murine CYP7A1, human CETP, and human and murine FAS are within 50 bases of the LXR response element. The SREBP-1c promoter used here contains several hundred base pairs surrounding the LXR site. Thus, it is unlikely that the lack of response is due to not using a large enough promoter fragment in the assay. It is possible, however, that the lack of
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LRH-1 responsiveness of the SREBP-1c promoter in our studies is due to the absence of some transcription factor in HEK293T cells that is present in the primary hepatocytes and cultured hepatoma cells used in the previous study. Alternatively, the effect on the SREBP-1c promoter might be indirect and/or require higher levels of LRH-1 than is required for the direct effect on the FAS promoter.

Why a distinct subset of LXR target genes depends upon LRH-1 for efficient activation in the presence of oxysterols is a compelling question. Because LRH-1 is selectively expressed in a handful of tissues, it has been postulated that the requirement for LRH-1 may allow for tissue specificity of the LXR/oxysterol response (24). In support of this hypothesis, CYP7A1, CETP, SREBP-1c, and FAS are all expressed at high levels in the liver but in a fairly restricted pattern in most other tissues. Interestingly, this group of LXR target genes is also sensitive to bile acid repression through SHP inhibition. Thus, regulation of a subset of LXR target genes involved in cholesterol metabolism by LRH-1 also provides an effective mechanism for selectively coupling their expression to bile acid homeostasis.

A recent report implicated FXR-mediated bile acid regulation of lipogenesis as the mechanism behind the triglyceride-lowering effect of bile acids (43). In this study supplementing the chow diet of mice with cholic acid for 1 day resulted in a significant increase in SHP mRNA and a corresponding decrease in SREBP-1c mRNA levels (43). Expression of FAS (as well as several other lipogenic genes) also declined and was attributed to the decrease in SREBP-1c. They further analyzed the SREBP-1c promoter and noted a SHP-dependent decrease in SREBP-1c. As a result, both triglyceride synthesis and bile acid synthesis (in rodents) are stimulated. When signaling through the LXR pathway is allowed to continue over 7 days, elevated flux of cholesterol into bile acids eventually triggers expression of SHP and other FXR target genes. In this case FAS would be subject to SHP repression, which would limit hepatic fatty acid biosynthesis and triglyceride accumulation. In this way, uncontrolled LXR-induced lipogenesis is effectively kept in check.

Interestingly, in a companion study, we showed that the nuclear receptor half-site in the FAS promoter that binds LRH-1 is the 5′ half of a nuclear receptor IR-1 element that directly binds the FXR/RXR heterodimer and stimulates FAS in response to bile acids (23). Although the precise rationale for the overlapping nuclear receptor sites is impossible to determine definitively, it is interesting to note that, whereas LRH-1 binding to this site would in effect make FAS sensitive to bile acid-induced repression by SHP, FXR/RXR binding to this site would induce FAS expression under conditions of elevated bile acids. Ironically, the opposing effects of bile acids on FAS promoter activation may actually be adaptive. When cholesterol levels are elevated, oxysterol levels increase and result in elevated signaling through the LXR pathway. FAS expression increases under these conditions to facilitate esterification of cholesterol. In this way, the initiating stimulus (e.g. excess cholesterol) turns on the biochemical pathway that mitigates its toxic effects. When bile acid flux is acutely elevated, bile acid-mediated up-regulation of SHP results in a drop in FAS (and CYP7A1) due to inhibition of LRH-1 even in the presence of activating oxysterol ligands for LXR. SHP inhibition of CYP7A1 blocks de novo biosynthesis of bile acids, thus preventing further bile acid accumulation. However, if bile acid flux remains elevated, chronic repression of bile acid biosynthesis eventually leads to accumulation of cholesterol. This prediction is supported by Watanabe et al. (43), who reported an increase in hepatic cholesterol levels in mice fed 0.5% cholic acid for 3 weeks. Although inhibition of bile acid biosynthesis is favorable for minimizing the toxic effects of bile acids, increased fatty acid biosynthesis is necessary under these conditions to esterify the excess cholesterol no longer permitted to enter the bile acid biosynthetic pathway. Thus, bile acid activation of FAS (via direct FXR/RXR binding to the IR-1) provides an SHP-independent mechanism for re-initiation of cholesterol esterification in the context of chronically elevated bile acids.

Acknowledgments—We thank Drs. Bruce Blumberg, Ronald Evans, Barry Forman, Gregorio Gil, David Mangelsdorf, and Peter Tontonoz for plasmid reagents and Timothy Willson for the GW3965. We also thank Peter Tontonoz for suggestions during the early stages of this work and Tae Il Jeon for assistance with the RNAi experiments.

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