Regulation of 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase Promoter by Nuclear Receptors Liver Receptor Homologue-1 and Small Heterodimer Partner

A MECHANISM FOR DIFFERENTIAL REGULATION OF CHOLESTEROL SYNTHESIS AND UPTAKE*

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Cholesterol homeostasis in mammals involves pathways for biosynthesis, cellular uptake, and hepatic conversion to bile acids. Key genes for all three pathways are regulated by negative feedback control. Uptake and biosynthesis are directly regulated by cholesterol through its inhibition of the proteolytic activation of the sterol regulatory element binding proteins. The conversion of cholesterol into bile acids in the liver is regulated through the bile acid-dependent induction of the negatively acting small heterodimer partner nuclear receptor. In this report, we have shown that the small heterodimer partner also directly regulates cholesterol biosynthesis through inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase but has no effect on low density lipoprotein receptor expression. This has significant metabolic significance, as it provides both a mechanistic mechanism to independently regulate cholesterol synthesis from uptake and a pathway for direct regulation of cholesterol biosynthesis by bile acids. This latter feature ensures that the early phase of bile acid synthesis (pre-cholesterol) is in metabolic communication with the later stages of the pathway to properly regulate whole pathway flux. This highlights an important regulatory feature that is shared with other key branched, multienzyme pathways, such as glycolysis, where pathway outflow through pyruvate kinase is regulated by the concentration of a key early intermediate, fructose 1,6-bisphosphate.

In the mammalian liver, cholesterol serves as a precursor in the synthesis of bile acids and as metabolite flow increases through the cholesterol pathway, bile acid production is increased. Bile acids act as feedback regulators of their biosynthesis by inhibiting the nuclear receptor-dependent activation of key bile acid biosynthetic target genes (1). The nuclear receptor FXR binds bile acids and induces the expression of genes involved in bile acid export and the gene encoding another nuclear receptor, SHP (2, 3). SHP lacks a DNA binding domain but interacts with the carboxyl-terminal activation domain of other DNA-bound nuclear receptors and inhibits their activity. The best documented target of SHP repression is the LRH-1/FTF nuclear receptor, which binds DNA as a monomer and activates expression of the bile acid biosynthetic genes CYP7A1 (2, 3) and CYP8B1 (4). Thus, as SHP levels rise in response to FXR-dependent activation, bile acid production is repressed through the negative effect of SHP.

Cholesterol is an essential component of mammalian membranes, and its production is tightly controlled through the negative effects of cholesterol directly on the endoplasmic reticular membrane proteins SREBPs and HMG-CoA reductase (5). As cholesterol accumulates, SREBP trafficking to and proteolytic activation in the Golgi are repressed, and the proteolytic release and subsequent degradation of HMG-CoA reductase from the endoplasmic reticular membrane is enhanced. The net effect is a decrease in both enzyme levels and metabolite flow through the pathway. This regulatory process is common to all mammalian cells.

However, bile acid production from cholesterol is unique to the mammalian liver, and it has been known for decades that bile acid feeding results in a similar down-regulation of cholesterol production in this organ (6). Traditionally, this has been attributed to the indirect effect that bile acids have through inhibiting CYP7A1, which would result in an increase in cholesterol followed by the predictable decline in mature SREBPs and HMG-CoA reductase activity.

Because the pathway leading to cholesterol in the liver corresponds to the "early" steps of bile acid synthesis, it would be more efficacious if a mechanism for the direct regulation of the early steps of the pathway directly through bile acids had evolved. In the current report, we present evidence supporting a direct mechanism for regulating cholesterol production by bile acids through the LRH-1/FTF and SHP nuclear receptors. We show that LRH-1/FTF activates and SHP represses HMG-CoA reductase transcription specifically, with no effect on LDL receptor expression. Overall, these studies define both a mechanism to independently regulate cholesterol synthesis from uptake, a key regulatory feature that has been documented in prior whole animal studies by Dietschy and co-workers (7). Additionally, these results also reveal a pathway for direct regulation of an early step in cholesterol biosynthesis by bile acids. This latter feature ensures that the early phase of bile acid synthesis (pre-cholesterol) is in metabolic communication with the later stages of the pathway to properly regulate whole pathway flux.
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MATERIALS AND METHODS

FLAG-tagged LRH-1/FTF and AF2 Domain Deletion (ΔAF-2)—PCR oligos containing EcoRI and XbaI restriction enzyme sites were used to amplify human FTF (1–500 amino acids) from pCI FTF (a gift from Dr. Gregorio Gil, Virginia Medical College). A PCR oligo including base pairs to amino acid 466 of FTF along with the wild-type amino-terminal oligo were used to construct the AF-2 domain mutant. Digested PCR product was cloned into EcoRI- and XbaI-digested 2xFLAG pCDNA3.1 vector described previously (8). All sequences were confirmed by DNA sequencing. The construction of the FLAG-tagged SREBPs and documentation that the predicted fusion proteins are efficiently expressed in the transfected cells was also reported previously (8). CMV-SHP was from Dr. David Mangelsdorf (University of Texas Southwestern Medical Center) and CMV-HNF-4 was from Dr. Frances Sladek (University of California Riverside).

FTT DNA Binding Domain Mutant—The Stratagene QuikChange site-directed mutagenesis kit was used to introduce two point mutations, substituting an alanine for cysteine 1 in the “P-box” (9) of the DNA binding domain of FTF using the 2xFLAG FTF construct as template and oligos containing the relevant base pair change. The incorporation of the point mutation was confirmed by DNA sequencing. For glutathione S-transferase-FTF, PCR oligos containing EcoRI sites were used to amplify human FTF from pCI FTF, and the digested product was cloned into EcoRI-digested pGEX-2T. HMG-CoA reductase and LDL receptor amplifying human FTF from pCI FTF, and the digested product was cloned into EcoRI-digested pGEX-2T.

Promoter Activation Studies—Wild-type and SHP−/− mice were housed, fed, and used in experiments as previously described (12), except as indicated in the figure legends. RNase protection assay and Northern blotting were performed with the indicated probes as previously described (12).

Immunoblotting Analysis of Protein Expression—These experiments were performed essentially as described previously (13). 293T cells were plated in normal medium (Dulbecco’s modified Eagle’s medium plus 10% (v/v) fetal bovine serum plus penicillin/streptomycin and glutamine) on day 0 at 450,000 cells/60-mm dish. On day 1, the cells were transfected with the appropriate plasmid construct along with a constitutive promoter luciferase reporter construct (4) was a gift from Dr. Gregorio Gil (Virginia Medical College).

Mouse Studies and RNA Analyses—Wild-type and SHP−/− mice were harvested using cell lysis buffer (13), and cell extracts were used to measure activity for luciferase and β-galactosidase.

Recombinant FTF Protein Purification—Escherichia coli cells expressing glutathione S-transferase-FTF (4) were grown at 37 °C to an OD of 0.6 and then induced with 1 mM isopropyl-1-thio-β-D-galactopyranoside at 37 °C for 3 h. The cells were harvested by sonication in NETN buffer (100 mM NaCl, 1 mM EDTA, 20 mM Tris, pH 8.0, 0.5% Nonidet P-40), and the soluble lysate was fractionated over a glutathione-agarose column and eluted with 10 mM glutathione. FTF protein fractions were identified by SDS-PAGE and Coomassie Blue staining, pooled, and dialyzed against 20 mM Tris, pH 8.0, 0.2 mM EDTA, and 50 mM KCl.

Electrophoretic Mobility Shift Assay—Single-stranded DNA oligos containing the potential FTF site at −300 were annealed for 1 h at 65 °C and then end-labeled with [32P] for 1 h at 37 °C. Purified FTF protein (25 ng) was incubated with 0.2 pmol of labeled HMG-CoA reductase probe on ice for 20 min and loaded onto a 5% acrylamide gel and run in 1× Tris borate-EDTA for 1–2 h. The gel was fixed for half an hour in 10% (v/v) acetic acid and 10% (v/v) methanol, dried, and then exposed to film. For competition experiments, purified FTF protein (25 ng) was incubated on ice for 15 min with cold probe containing the indicated DNA sequence in 50- or 200-fold molar excess of [32P]-labeled HMG-CoA reductase probe. Labeled HMG-CoA reductase probe (0.2 pmol) was then added to the reactions, followed by a further incubation on ice for an additional 20 min. The reactions were loaded onto gels as described above.

Chromatin Immunoprecipitation Analysis—Chromatin immunoprecipitation was performed essentially as previously described (14) with the following minor modifications. 293T cells were transfected with an expression plasmid for Myc-tagged LRH-1 by Lipofectamine (Invitrogen). 5 h post-transfection, the cells were transfected with defined serum-free medium as described above (minus sterols) to induce SREBP expression for 24 h. Formaldehyde cross-linking (1% (v/v)) was done for 9 min. After processing, the sonicated chromatin was obtained as described previously (14), and samples were preincubated with protein A-agarose beads and purified mouse IgG (50 μg) for 1 h at 4 °C on a rotator. Non-specifically bound material was removed by pelleting the agarose beads, and supernatant fractions were incubated overnight at 4 °C with 50 μg of anti-LRH-1 antibody (Santa Cruz Biotechnology catalog number SC-25389x) for the + Ab sample or 50 μg of purified mouse IgG for the −Ab sample, followed by incubation with blocked protein A beads for 2 h at 4 °C on a rotator. After washing and reversing the cross-linking (14), the samples were analyzed by PCR. For the PCRs, 5 μl of DNA from the LRH-1 precipitation was used, and PCR oligonucleotides that amplify a 250-bp fragment from the human HMG-CoA reductase or a 120-bp fragment from the human LDL receptor promoter were used, respectively. Amplification reactions were performed in triplicate at 30 cycles and monitored for amplification to ensure that the signals were in the linear range of the PCR. To analyze specific immunoprecipitation of Myc-tagged LRH-1, an immunoblot using an anti-Myc antibody (Santa Cruz Biotechnology catalog number SC-40) was performed on the material recovered after each immunoprecipitation.

RESULTS

Previous studies showed that bile acid-dependent inhibition of CYP7A1 is compromised in SHP−/− mice (12, 15, 16). Additionally, one of these studies also provided evidence that bile acid-dependent regulation of cholesterol metabolic genes, such as HMG-CoA reductase and the LDL receptor, might also be altered in SHP−/− mice (12). To more directly evaluate the effect of SHP on HMG-CoA reductase and...
LDL receptor gene expression, we compared their mRNA levels in wild-type and SHP−/− mice fed diets with and without cholic acid (CA) supplementation. The results in Fig. 1A demonstrate that treatment of wild-type mice with CA reduced the expression of mRNAs for both HMG-CoA reductase and the LDL receptor. Interestingly, in animals fed a normal chow diet, there was an increase in HMG-CoA reductase mRNA in SHP−/− compared with wild-type mice, and the suppression by CA feeding was blunted in the SHP−/− animals. In contrast, expression and CA suppression of LDL receptor mRNA were indistinguishable in wild-type and SHP−/− mice. These data suggest that SHP specifically inhibits HMG-CoA reductase but not the LDL receptor.

Bile acid feeding induces SHP through the bile acid-activated nuclear receptor FXR, but bile acids also have pleiotropic effects. Therefore, to more directly evaluate SHP and FXR in the regulation of HMG-CoA reductase, we analyzed the effects of a synthetic FXR agonist on HMG-CoA reductase mRNA in wild-type and SHP−/− mice (Fig. 1B). In wild-type animals, the synthetic FXR agonist GW4064 decreased HMG-CoA reductase expression, but the effect was lost in the SHP−/− animals (Fig. 1B, compare lanes 1, 2 and 5, 6 with lanes 3, 4 and 7, 8). In contrast, the FXR agonist had no effect on the expression of LDL receptor mRNA in either wild-type or SHP−/− animals. As an additional control, similar administration of a synthetic agonist for retinoid X receptor (RXR) or LRH-1 (LRH-1) did not affect mRNA levels for either HMG-CoA reductase or the LDL receptor (lanes 9–12).

These results suggest that SHP specifically inhibits HMG-CoA reductase expression. For known SHP target genes, such as CYP7A1 and CYP8B1, inhibition occurs by interfering with the activation by the nuclear receptor LRH-1/FTF. To determine whether a similar mechanism was functioning for HMG-CoA reductase, we first evaluated whether LRH-1/FTF could bind to the endogenous HMG-CoA reductase promoter in cellular chromatin using a chromatin immunoprecipitation assay. An LRH-1 expression vector was transfected into 293 cells, and formaldehyde cross-linked chromatin was treated with control IgG or with an antibody to LRH-1. The LRH-1 antibody did precipitate the LRH-1 protein specifically (immunoblot in Fig. 2), and the DNA associated with the immunoprecipitation pellets was analyzed by PCR for the presence of the promoters for either HMG-CoA reductase or the LDL receptor as a control. The PCR results demonstrated that LRH-1 protein bound specifically to the endogenous HMG-CoA reductase promoter, and it was not associated with the LDL receptor promoter chromatin (Fig. 2).

In transient transfection assays, the activation of HMG-CoA reductase by SREBPs was always significantly lower compared with other target genes analyzed in parallel, suggesting there was some additional...
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shown is sterol regulation. 293T cells were transfected with expression constructs for LRH-1/FTF (0.1 μg) or SHP (at 1 or 3 μg) along with the indicated luciferase reporters for the LDL receptor (LDLR), HMG-CoA reductase (RED) or CYP8B1 luciferase, and all contained an internal control β-galactosidase expression plasmid. After transfection, dishes were cultured in medium in the presence (+) or absence (−) of regulatory sterols. Fold regulation was calculated as the ratio of normalized luciferase activity for cells cultured in the absence versus presence of regulatory sterols. Samples were analyzed in duplicate, and error bars are included to indicate the range for the two duplicate values.

protein required that was missing (17). Based on the results presented above, we reasoned that this missing protein might be LRH-1/FTF. To test this idea, we performed a transient transfection assay in 293 cells using a culture protocol that activates the processing of endogenous SREBPs (14). Here, companion dishes of transfected cells are cultured with medium containing or lacking regulatory sterols, and endogenous SREBPs are cleaved from their membrane location and accumulate in the nucleus in the sterol-depleted samples (18, 19).

Consistent with our earlier studies, the HMG-CoA reductase promoter was activated ~2-fold by this sterol depletion protocol (Fig. 3 compare lane 5 with 6). When an expression plasmid for LRH-1/FTF was co-transfected under sterol-depleted conditions, there was a significant increase in expression of the HMG-CoA reductase promoter (Fig. 3, lane 7), and this stimulation was specifically inhibited when an SHP expression plasmid was added on top of the LRH-1/FTF construct (lanes 8–9). However, transfection of the SHP expression plasmid alone had no effect on the modest activation by endogenous SREBPs (Fig. 3, lane 6 with 10 and 11). This result suggests that SHP does not inhibit SREBP-mediated activation but only affects the promoter stimulation mediated by LRH-1/FTF.

For controls, we also analyzed the LDL receptor and CYP8B1 promoters (Fig. 3, lanes 1–4 and 12–17). Similar to our previous studies (17), the sterol depletion protocol resulted in a higher degree of activation of the LDL receptor promoter, and consistent with the studies in the SHP−/− mice, there was no effect of LRH-1/FTF or SHP on the LDL receptor promoter activity. However, as a positive control, LRH-1/FTF addition stimulated the CYP8B1 promoter, and this was inhibited by the addition of SHP.

In the above experiments using sterol depletion, all three SREBPs were released from the membrane and accumulated in the nucleus; therefore, it was unclear whether LRH-1/FTF functions to enhance the activation of all three SREBPs or whether there is a preference for one of the three SREBP isoforms. To address this, we performed transient promoter activation experiments in cells cultured in the presence of exogenous sterols to suppress the activation of endogenous SREBPs.
FIGURE 6. Conserved LRH-1/FTF DNA sites in HMG-CoA reductase promoter are required for efficient promoter stimulation. A, a schematic showing the positions of the two putative LRH-1 sites relative to already characterized sites for SREBP, nuclear factor-Y (NF-Y), and cAMP-response-element binding protein (CREB) (10). At the bottom is a lineup of the genomic DNA from the −300 region of the hamster, human, and mouse promoters showing the conservation of this putative LRH-1 site. The human site was also shown to bind directly to the LRH-1/FTF protein (S. Datta and T. F. Osborne, unpublished data). B, the wild-type and two deletion reporter constructs for the HMG-CoA reductase promoter (see A, B, and C in panel A) were analyzed for activation by SREBP-2 and LRH-1/FTF as indicated and as described in the previous figure legends. C, purified recombinant FTF (25 ng) was used in an electrophoretic mobility shift assay with 32P-labeled probe containing the putative LRH-1/FTF response element from −300 in the HMG-CoA reductase promoter, and where indicated (lanes 3–8), a molar excess (50 or 200×) of unlabeled, competitor (Comp.) DNAs were included in the binding reaction as described under “Materials and Methods.” wt, wild-type hamster HMG-CoA reductase LRH-1/FTF site; mt, HMG-CoA reductase mutant competitor with a single base mutation that alters the putative LRH-1/FTF site response element; 8, oligonucleotides containing a known LRH-1/FTF site promoter, the mouse CYP8B1 promoter.

promoter by itself, consistent with previous reports where SREBP-1c is a weak activator compared with SREBP-1a or -2 (8). However, the addition of the LRH-1/FTF expression construct resulted in a 3-fold stimulation. This was consistently above the small stimulation that resulted by the addition of the LRH-1/FTF expression plasmid alone (Fig. 4A, compare lanes 8 and 9 with 11).

Additionally, regardless of which SREBP was analyzed, the addition of the shp expression construct inhibited only the LRH-1/FTF-mediated effect, because the magnitude of activity after repression by shp was equal to that stimulation by each SREBP alone. These results indicate that LRH-1/FTF can function with all three SREBPs to activate the HMG-CoA reductase promoter and that SHP inhibition only affects the LRH-1/FTF stimulatory effect.

Because SHP is known to inhibit activation by other nuclear receptors, such as HNF-4 (20), and because overexpression of transcription factors in transient assays may exaggerate normal physiological effects, as a control, we analyzed whether HNF-4 could activate the HMG-CoA reductase promoter along with SREBPs (Fig. 4B). Transfection of an HNF-4 expression construct in place of LRH-1/FTF had no effect on SREBP-dependent activation of the HMG-CoA reductase promoter, whereas it efficiently activated the HNF-4 target gene CYP8B1 (Fig. 4B).

LRH-1/FTF, similar to other nuclear receptors, requires its carboxy-terminal AF-2 activation domain and a zinc finger DNA binding motif to activate target genes. To determine whether these critical functions are required for activation of HMG-CoA reductase, we deleted the AF-2 domain or introduced a point mutation at a critical cysteine residue of the DNA binding domain P-box (9) to alanine to inhibit DNA binding. Despite the fact that both of these mutant proteins were expressed efficiently in the transfected cells, neither one was able to activate the HMG-CoA reductase promoter like the wild-type protein (Fig. 5). Thus, both of the crucial nuclear receptor functional domains are required and suggest that LRH-1/FTF likely binds directly to the HMG-CoA reductase promoter.

In scanning the DNA sequence of the HMG-CoA reductase promoter used in these studies, we noted two putative recognition sites that are conserved between the hamster, mouse, and human promoters (Fig. 6A). To test whether these sites are important for LRH-1/FTF-mediated activation, we deleted them from the luciferase reporter construct. The activation studies shown in Fig. 6B show that deletion of the two sites resulted in a severe blunting of LRH-1/FTF-mediated activation, but these truncations had little effect on overall promoter activity or on stimulation by SREBPs alone (Fig. 6B) (21). Next, the DNA site at −300 was tested for DNA binding directly using recombinant LRH-1/FTF protein in an electrophoretic mobility shift assay (Fig. 6C). Recombinant LRH-1/FTF bound to this HMG-CoA reductase promoter site specifically, and a mutation that changed the sequence away from the predicted consensus recognition site failed to compete efficiently for binding. Additionally, oligonucleotides containing the LRH-1/FTF site from the CYP8B1 promoter or the cold wild-type HMG-CoA reductase DNA oligos used for the electrophoretic mobility shift assay competed efficiently for binding. Thus, DNA binding directly to the HMG-CoA reductase promoter is required for the LRH-1/FTF stimulatory effect.

DISCUSSION

In our previous studies, we noted that the magnitude of stimulation by sterol depletion or the addition of exogenous SREBP expression constructs was very modest for the HMG-CoA reductase promoter compared with the activation achieved with other SREBP target genes ana-
lyzed in parallel (17). In contrast, HMG-CoA reductase gene expression was activated very robustly when SREBP1c was overexpressed in mice (22). Although there might be additional differential post-initiation regulatory actions on the mRNAs that may partially account for these differences, the results are also consistent with a model where an additional protein was missing in our transient transfection assays for the HMG-CoA reductase promoter.

The current studies were initiated when we noted that regulation of HMG-CoA reductase was aberrant in SHP−/− mice. Additional studies presented here further support this idea and suggest that LRH-1/FTF is the missing protein. Additionally, the SHP effect exhibits specificity for HMG-CoA reductase, because neither the FXR agonist nor SHP itself had any effect on LDL receptor expression in any of the assays we utilized. Thus, the direct regulation through bile acids and SHP (Fig. 7, left) is specific to the cholesterol synthetic pathway. Our studies have focused on HMG-CoA reductase, because it is considered the classic rate-controlling enzyme of the pathway and because our earlier studies suggest there was a missing component in our transient expression assays. Whether additional enzymes of the pathway are similarly regulated remains to be determined.

When bile acid levels rise, CYP7A1 is inhibited and pathway flux is repressed. Without any alteration to earlier steps in the pathway, cholesterol levels would rise, which would inhibit SREBP maturation (Fig. 7, right). However, because the LRH-1/FTF activation of HMG-CoA reductase is inhibited by SHP, the studies presented here provide the first evidence that, in addition to the indirect effect of bile acids through cholesterol, they also have a direct inhibitory action on the expression of HMG-CoA reductase (Fig. 7, left). This indicates that the early and late sectors of the pathway are in metabolic communication with each other to more quickly adapt to changes in pathway influx and outflow. There is a similar regulatory mechanism in glycolysis, where the product of phosphofructokinase, fructose 1,6 bisphosphate, which measures early flux into the pathway, is a positive regulator of pyruvate kinase, which controls pathway outflow (23).

Spady et al. (7) report that endogenous cholesterol biosynthesis and cholesterol uptake through the LDL receptor pathway are independently regulated in the livers of mice. In these studies, the addition of the bile acid sequestrant cholestyramine to the diet significantly increased hepatic sterol synthetic rates, whereas LDL clearance rates were not altered relative to control fed mice. Cholestyramine reduces bile acid reabsorption and would effectively deplete the endogenous hepatic FXR agonist pool, which would be predicted to decrease SHP levels. In fact, we have documented that SHP expression is repressed by feeding mice a similar bile acid sequestrant (24). In another study (24), Sheng et al. mentioned that feeding mice a bile acid sequestrant alone was ineffective at increasing nuclear levels of SREBPs in mice.

Taken together, these two studies indicate that the mechanism by which bile acid sequestrants increase sterol biosynthesis cannot solely be explained by an increase in nuclear SREBP levels. The current studies demonstrating that expression of HMG-CoA reductase is activated by LRH-1/FTF and repressed by SHP, without any change in LDL receptor expression, provides a reasonable molecular explanation that connects both of these important earlier studies together.

The LRH-1/FTF DNA sites are conserved in the human HMG-CoA reductase promoter. Therefore, our results also suggest that FXR agonists might be effective when combined with statins to treat hypercholesterolemia in humans. Statin therapy results in a compensatory up-regulation of HMG-CoA reductase gene expression as the liver attempts to compensate for the decreased sterol production. The addition of an FXR ligand may work synergistically with statins to prevent this response through inhibiting activation by LRH-1/FTF.

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REFERENCES

1. Russell, D. W. (2003) Annu. Rev. Biochem.
2. Lu, T. T., Makishima, M., Repa, J. J., Schoonjans, K., Kerr, T. A., Auwerx, J., and Mangelsdorf, D. J. (2000) Mol. Cell, 507–515
3. Goodwin, B., Jones, S. A., Price, R. R., Watson, M. A., McKeever, D. D., Moore, L. B., Galarad, C., Wilson, J. G., Lewis, M. C., Roth, M. E., Maloney, P. R., Wilson, T. M., and Klee, K. S. (2000) Mol. Cell, 517–526
4. del Castillo-Olivares, A., and Gil, G. (2001) Nucleic Acids Res. 29, 4035–4042
5. Horton, J. D., Goldstein, J. L., and Brown, M. S. (2002) J. Clin. Invest. 109, 1125–1131
6. Shefer, S., Hauser, S., Lapar, V., and Mosbach, E. H. (1973) J. Lipid Res. 13, 573–580
7. Spady, D. K., Turley, S. D., and Dietschy, J. M. (1985) J. Lipid Res. 26, 465–472
8. Toth, J. I., Datta, S., Athanikar, J. N., Freedman, L. P., and Osborne, T. F. (2004) Mol. Cell. Biol. 24, 8288–8300
9. Umenson, K., and Evans, R. M. (1989) Cell 57, 1139–1146
10. Ngo, T. T., Bennett, M. K., Bourgeois, A. L., Toth, J. I., and Osborne, T. F. (2002) J. Biol. Chem. 277, 33901–33905
11. Athanikar, J. N., Sanchez, H. B., and Osborne, T. F. (1997) Mol. Cell. Biol. 17, 5193–5200
12. Wang, L., Lee, Y.-K., Bundman, D., Han, Y., Thevananther, S., Kim, C.-S., Chur, S., Wei, P., Heyman, R. A., Karin, M., and Moore, D. D. (2002) Dev. Cell 2, 721–731
13. Datta, S., and Osborne, T. F. (2005) J. Biol. Chem. 280, 3338–3345
14. Bennett, M., Toth, J. I., and Osborne, T. F. (2004) J. Biol. Chem. 279, 37360–37367
15. Kerr, T. A., Saeki, S., Schneider, M., Schaefer, K., Berdy, S., Redder, T., Shan, B., Russell, D. W., and Schwarz, M. (2002) Dev. Cell 2, 713–720
16. Watanabe, M., Houten, S. M., Wang, L., Moschetta, A., Mangelsdorf, D. J., Heyman, R. A., Moore, D. D., and Auwerx, J. (2004) J. Clin. Investig. 113, 1408–1418
17. Mullinder-Valletti, S., Sanchez, H. B., Rosenfeld, J. M., and Osborne, T. F. (1996) J. Biol. Chem. 271, 12247–12253
18. Rosenfeld, J. R., and Osborne, T. F. (1998) J. Biol. Chem. 273, 16112–16121
19. Sanchez, H. B., Yieh, L., and Osborne, T. F. (1995) J. Biol. Chem. 270, 1161–1169
20. Lee, Y. K., Dell, H., Dowhan, D. H., Hazdoupolou-Claradaras, M., and Moore, D. D. (2000) Mol. Cell. Biol. 20, 187–195
21. Osborne, T. F., Goldstein, J. L., and Brown, M. S. (1985) Cell 42, 203–212
22. Sheng, Z., Horton, J. D., Hamann, R. E., Shimomura, I., Brown, M. S., and Goldstein, J. L. (1996) J. Clin. Investig. 98, 1575–1584
23. Pilkis, S. J., and Claus, T. H. (1991) Annu. Rev. Nutr. 11, 465–515
24. Sheng, Z., Ota, H., Brown, M. S., and Goldstein, J. L. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 935–938

4 D.-J. Shin and T. F. Osborne, unpublished data.