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The orphan nuclear receptor LRH-1 activates the ABCG5/ABCG8 intergenic promoter

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Abstract The ATP binding cassette (ABC) half-transporters ABCG5 and ABCG8 facilitate biliary and intestinal removal of neutral sterols. Here, we identify a binding site for the orphan nuclear receptor liver receptor homolog-1 (LRH-1) at nt 134–142 of the ABCG5/ABCG8 intergenic region necessary for the activity of both the ABCG5 and ABCG8 promoters. Mutating this LRH-1 binding site reduced promoter activity of the human ABCG5/ABCG8 intergenic region more than 7-fold in HepG2 and Caco2 cells. Electrophoretic mobility shift assays with HepG2 nuclear extracts demonstrated specific binding of LRH-1 to the LRH-1 binding motif in the human ABCG5/ABCG8 intergenic region. LRH-1 overexpression increased promoter activity up to 1.6-fold and 3-fold in Caco2 and 293 cells, respectively. Finally, deoxycholic acid repressed the ABCG5 and ABCG8 promoters, consistent with bile acid regulation via the farnesoid X receptor-small heterodimeric partner-LRH-1 pathway. These results demonstrate that LRH-1 is a positive transcription factor for ABCG5 and ABCG8 and, in conjunction with studies on LRH-1 activation of other promoters, identify LRH-1 as a "master regulator" for genes involved in sterol and bile acid secretion from liver and intestine.—Freeman, L. A., A. Kennedy, J. Wu, S. Bark, A. T. Remaley, S. Santamarina-Fojo, and H. B. Brewer, Jr. **The orphan nuclear receptor LRH-1 activates the ABCG5/ABCG8 intergenic promoter.** ***J. Lipid Res.*** **2004.** 45: **1197–1206.**

Supplementary key words ATP binding cassette transporter • CPF • liver receptor homolog-1 • FTF • FXR • SHP • sitosterolemia • cholesterol • bile acids

The recent identification of ABCG5 and ABCG8 as the ATP binding cassette (ABC) half-transporters defective in sitosterolemia has led to major advances in understanding the molecular mechanisms of sterol transport (1, 2). Sitosterolemia is a rare autosomal recessive disorder characterized by increased plasma levels of plant, shellfish, and animal sterols, xanthomas, and increased risk of premature cardiovascular disease (3, 4). These defects have been attributed to enhanced intestinal absorption and decreased biliary excretion of sterols (3). In mice, ABCG5/ABCG8 deficiency increases plasma sitosterol and decreases bile cholesterol (5), whereas ABCG5/ABCG8 overexpression increases biliary cholesterol secretion and decreases dietary cholesterol absorption (6). Both genes are expressed in liver, small intestine, and gallbladder (1, 2, 7, 8), consistent with a role in biliary and intestinal secretion. ABCG5 and ABCG8 reside on the apical plasma membrane of polarized hepatocyte WifB cells and must be coexpressed for proper trafficking to the surface (9, 10). ABCG5/ABCG8 have also been localized to intestinal microvilli in the gut lumen (11). These findings establish ABCG5 and ABCG8 as key transporters that regulate the excretion of cholesterol and other sterols from the body.

Given their importance in decreasing cholesterol absorption, it is of interest to determine how these genes are regulated. Several lines of evidence argue that ABCG5 and ABCG8 may be coordinately regulated. They are both half-transporters that interact physically, as demonstrated by coimmunoprecipitation studies (9, 10), and functionally, as discussed above. Moreover, the two genes are arranged in a head-to-head configuration and the human 374 bp intergenic region has bidirectional promoter activity (1, 12). The two genes also have very similar tissue distributions. Thus, it is anticipated that the two genes will be coordinately regulated. However, no transcription factor binding motif mediating the activation of either promoter, much less coactivation of both promoters bidirectionally, has yet been identified. Thus, the process by which transcriptional activation of both genes occurs si-

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Abbreviations: ABC, ATP binding cassette; DCA, deoxycholic acid; EMSA, electrophoretic mobility shift assay; FCS, fetal calf serum; FXR, farnesoid X receptor; LRH-1, liver receptor homolog-1; LXR, liver X receptor; SF-1, steroidogenic factor-1; SHP, small heterodimeric partner.

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multaneously, leading to coregulation, has not yet been determined.

Here, we present data that indicate that the orphan nuclear receptor liver receptor homolog-1 (LRH-1), also called CYP7A1 promoter factor (CPF), B1F, α-fetoprotein transcription factor (FTF), PHR-1, and NR5A2, is a transcription factor that bidirectionally stimulates both the ABCG5 and ABCG8 promoters. These studies, in combination with studies on other genes regulated by LRH-1, implicate LRH-1 as a master regulator of genes that function to decrease cholesterol levels in liver and intestine.

MATERIALS AND METHODS

Plasmid construction

Mutations in the steroidogenic factor-1 (SF-1)/LRH-1 site [nt 134–142 of the ABCG5/ABCG8 intergenic promoter, lower strand, identified by MatInspector (13)] were created by overlap PCR using the full-length (nt 1–374) ABCG5/ABCG8 intergenic fragment (12) as a template. Numbering is as described previously (12), with nucleotides 1 and 374 adjacent to the ABCG8- and ABCG5-initiating ATGs, respectively. LRHm1 mutagenesis primers were 5′GGGCCCGTCTCTCCCTGGCAAGCCCACCTAC3′ and 5′GTAGGTGGGCTTGCCAGGGAGAGACGGGCCC3′, deleting the entire LRH-1 binding site (12 nt), as defined by MatInspector. LRHm2 mutagenesis primers were 5′TCTCCCAGCTCGATTTGGCAAGCCCACCTACAAAC3′ and 5′TTGCCAAATCGAGCTGGGAGAGACGGGCCCAGG, deleting nt 137–139. LRHm5 primers were TTGCCATCACACAGAGCTGGGAGAG3′ and CTCTCCCAGCTCTGTGTGATGGCAA3′, creating a nucleotide substitution mutant corresponding to mutant M5 in Fig. 2 of ref. (14), which eliminates the binding of LRH-1 to its binding site in the CYP7α promoter (14). Upstream and downstream primers were 5′TTTTTTTTTAGATCTGGGGCCCACAGGTCTGTG3′ and 5′TTTTTTTTTAGATCTGGCCAACAGGCAGCAAAG3′. *Bgl*II-digested promoter fragments were cloned bidirectionally into *Bgl*II-digested pGL3Basic, as in ref. (12). For the LRH-1 overexpression construct pCI-LRH, cDNA was synthesized as in ref. (12), and LRH-1 (NR5A2; NM_003822) was amplified with 5′TTTTTTTGCGGCCGCACTAAGAATGTCTTCTAATTCAGATACTGGG3′ and 5′TTTTTTTGCGGCCGCTATGCTCTTTTGGCATGCAACATTTC3′. The *Not*I-digested PCR product was cloned into *Not*I-digested pCI-neo (Promega, Madison, WI). Constructs were sequence verified.

Transfections and promoter activity assays

HepG2 and Caco2 cells (American Type Culture Collection, Manassas, VA) grown in 12-well plates with DMEM/5% fetal calf serum (FCS) were transiently cotransfected with test plasmid and pCMVβ, a β-galactosidase-expressing vector (Clontech, Palo Alto, CA), using ExGen 500 (MBI Fermentas, Hanover, MD). In some experiments, pCI-LRH or the corresponding empty vector pCI-neo was cotransfected. 293 cells were transfected similarly but with pRLSV40 and Fugene 6 (Roche, Indianapolis, IN). HepG2 and Caco2 cell lysates collected 24 h after transfection were assayed for luciferase and β-galactosidase, as described (15). The DualLuciferase Assay Kit (Promega) was used for 293 cell lysates. For bile acid addition, medium was replaced 24 h after transfection with Ham's F12 medium (Sigma, St. Louis, MO) containing 5% charcoal-stripped, delipidated FCS (Sigma) containing either vehicle (ethanol) or deoxycholic acid (DCA) (50, 100, or 150 μM); cells were incubated for an additional 24 h and then lysed as described above.

Electrophoretic mobility shift assay

Probes were end-labeled with [γ-^{32}P]ATP (3,000 Ci/mmol; NEN/Perkin-Elmer, Boston, MA) using the DNA 5′-End-Labeling Kit (Roche). HepG2 nuclear extracts were from Geneka/ActiveMotif (Carlsbad, CA). Electrophoretic mobility shift assays (EMSAs) were as described (15) with minor modifications. PolydI/polydC was from Amersham (Piscataway, NJ). Antibodies against LRH-1/B1F-2 (V14X, N15X, N16X), USF1 (H86X), and TFIID (SI-1X) were from Santa Cruz Biotechnology (Santa Cruz, CA).

Northern analysis

HepG2 cells were grown in six-well plates, and DCA or vehicle was added in Ham's F12 medium with charcoal-stripped, delipidated serum as described above. After 24 h, RNA was isolated with Ultraspec Reagent (Biotecx, Houston, TX). Northern blots were probed with ^{32}P-labeled cDNA probes [human ABCG5, full length; human ABCG8, bp 1,047–2,561; small heterodimeric partner (SHP), full-length (16); and cyclophilin (Ambion, Austin, TX)].

RESULTS

An SF-1/LRH-1 transcription factor binding motif is essential for promoter activity of the ABCG5/ABCG8 intergenic region

We scanned the ABCG5/ABCG8 intergenic region for transcription factor binding sites involved in sterol metabolism using MatInspector (13). A sequence at nt 134–142 (GCAAGGAAT) matched the binding motif (nCAAGgyc) for SF-1 (core similarity, 1.000; matrix similarity, 0.813). SF-1 is important for the transcription of steroid hydroxylases and sterol transport proteins in steroidogenic tissues (17, 18). Although SF-1 itself is not expressed in liver (17) or intestine (19), a closely related protein with identical binding specificity, LRH-1, is expressed in liver and intestine as well as in pancreas, preadipocytes, and portions of the ovary (20–24). LRH-1 regulates the transcription of a number of genes involved in sterol elimination (see below).

To investigate whether LRH-1 transcriptionally activates the ABCG5 and ABCG8 promoters, we generated luciferase reporter constructs of the full 374 bp ABCG5/ABCG8 intergenic promoter in both directions with a 12 nt deletion (LRHm1), a 3 nt deletion (LRHm2), or a nucleotide substitution mutation (LRHm5) (14) in the SF-1/LRH-1 binding site. **Figure 1** (top panels) demonstrates that mutating the LRH site to LRHm2 decreases ABCG5 and ABCG8 promoter activity by 12.6- and 15.4-fold, respectively, in HepG2 cells. Similarly, Fig. 1 (bottom panels) demonstrates that mutating the LRH site to LRHm2 decreases ABCG5 and ABCG8 promoter activity by 7.0- and 32-fold, respectively, in Caco2 cells. The decrease in promoter activity was even greater for LRHm1 and LRHm5.

Thus, the LRH-1 site strongly activates both the ABCG5 and ABCG8 promoters in HepG2 and Caco2 cells.

LRH-1 binds the LRH-1 site in the ABCG5/ABCG8 promoter

To demonstrate the binding of LRH-1 to its cognate site in the ABCG5/ABCG8 promoter, we performed EMSA (**Fig. 2**). Figure 2B demonstrates that incubation of a ra-

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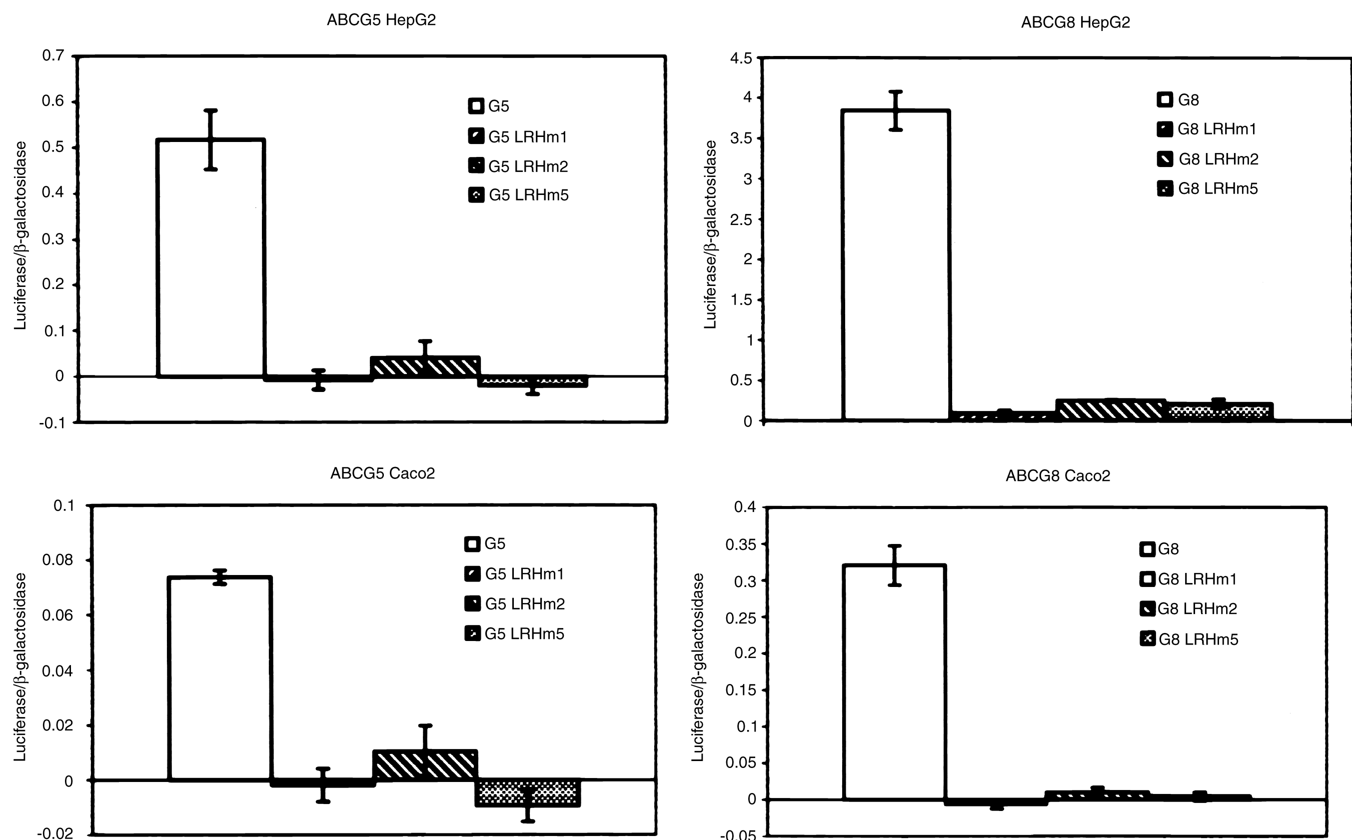

Fig. 1. Mutations of the liver receptor homolog-1 (LRH-1) binding site strongly decrease ABCG5/ABCG8 promoter activity in both directions. Three independent mutations of the LRH-1 binding site were introduced into a fragment containing the full-length (nt 1–374) ABCG5/ABCG8 intergenic region, and the resulting mutant fragments were cloned bidirectionally into pGL3Basic. The resulting wild-type and mutant reporter plasmids (G5, left panels; G8, right panels) were transfected into HepG2 cells (top panels) or Caco2 cells (bottom panels) along with a β-galactosidase vector (pCMVβ) to normalize for transfection efficiency. A total of 1.0 μg of reporter plasmid and 0.1 μg of pCMVβ were transfected per well. After 24 h, extracts were prepared and assayed for luciferase and β-galactosidase activity. The ratio of luciferase activity in relative light units was divided by the β-galactosidase activity to give a normalized luciferase value, as described previously (15). In a single transfection study, each plasmid was transfected in triplicate. Data from a representative experiment out of three independent experiments are shown. Note the different scales for ABCG5 and ABCG8 luciferase/β-galactosidase activity. Error bars represent 1 SD.

diolabeled probe spanning nt 86–188 (fragment A, Fig. 2A) with nuclear extract from HepG2 cells resulted in the formation of at least two gel-shifted complexes with lower electrophoretic mobility than the naked DNA probe. These shifts were abolished when a double-stranded oligonucleotide spanning the LRH-1 binding site (fragment B) was used as a competitor but not when the same oligonucleotide with the LRH-1 binding site deleted (fragment B_{del}) was used as a competitor. As expected, competition with unlabeled full-length fragment A abolished both gel shifts. Thus, the LRH-1 site in the ABCG5/ABCG8 intergenic promoter binds two complexes present in the HepG2 nuclear extract.

To establish that LRH-1 is the transcription factor binding to this site, we performed gel-shift analysis using LRH-1-specific antibodies (Fig. 2C). Preincubating the HepG2 nuclear extracts with anti-LRH-1 antibodies before adding radiolabeled probe resulted in the disappearance of the large and small DNA-protein complex seen with probe alone (Fig. 2C, lane 2) and the appearance of DNA-protein complexes of intermediate size (Fig. 2C, lane 3). These findings indicate the formation of two separate DNA-protein complexes containing LRH-1; binding of LRH-1 antibody to the higher molecular weight protein complex prevents it from binding DNA, whereas binding of LRH-1 antibody to the lower molecular weight complex causes a supershift. These findings are consistent with the binding of two separate complexes containing LRH-1 to the 102 bp fragment.

To rule out the possibility of a second binding site for LRH-1 in fragment A and to more specifically evaluate the binding of LRH-1 to its motif at nt 134–142, we generated a smaller (25 nt) probe (fragment C) that spanned nt 124–148 of the intergenic promoter and contained the intact LRH-1 binding site. EMSA with fragment C using as competitor either fragment C or fragment C_{mut}, which contained point mutations in the LRH-1 binding site, confirmed the binding of two complexes in the HepG2 nuclear extract to the LRH-1 binding site (Fig. 2D, left lanes). Consistently, using fragment C_{mut} as a probe did not result in a gel shift (Fig. 2D, right lanes). Moreover, preincubation of the HepG2 nuclear extract with three in-

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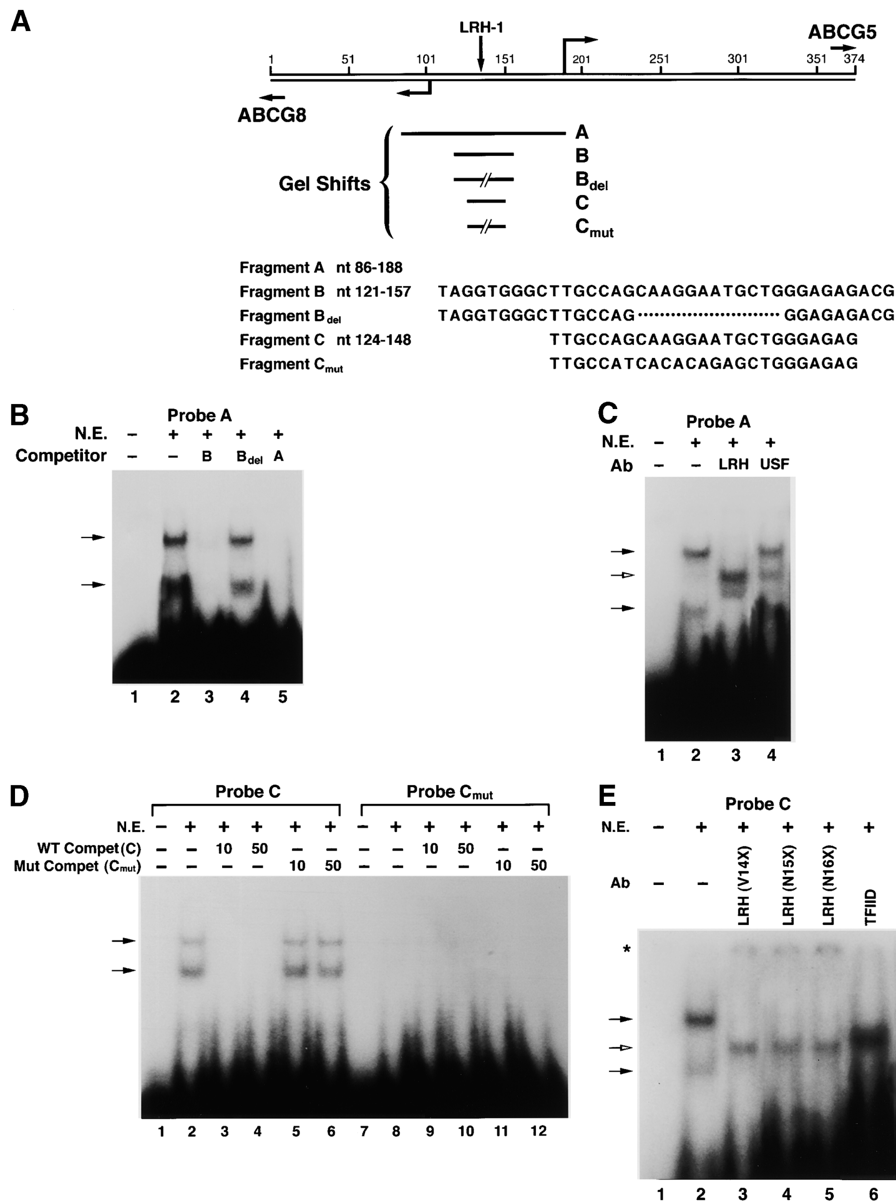

Fig. 2. The conserved steroidogenic factor-1 (SF-1)/LRH-1 site in the ABCG5/ABCG8 intergenic region binds LRH-1 in a HepG2 nuclear extract. A: At top, a scheme of the ABCG5/ABCG8 intergenic region. The location of the LRH-1 binding site at nt 134–142 is indicated by the down arrow. Start sites of transcription for ABCG8 and ABCG5 are indicated by left and right arrows, respectively. Below the scheme, ABCG5/ABCG8 fragments used for electrophoretic mobility shift assays. Fragment A (102 bp) spanned nt 86–188 of the intergenic region. Fragment B spanned nt 121–157. Fragment B_{del} was identical to fragment B except that the LRH-1 binding site identified by MatInspector was deleted. Fragment C (25 nt) spanned nt 124–148. Fragment C_{mut} was identical to fragment C except for the indicated point mutations, equivalent to those used in ref. (14). B: Oligonucleotide competition assay. A total of 2.7 μg of HepG2 nuclear extracts was preincubated for 1 h at room temperature in 20 μl of gel-shift buffer (15) and 1.5 μg of polydI/polydC with or without a 10-fold excess of double-stranded competitor for LRH-1. One nanogram of ^{32}P-labeled fragment A (15,000 cpm) was then added, and the reaction was incubated for 20 min at room temperature, followed by electrophoresis (15). Lane 1, radiolabeled probe with no nuclear extract added. Lane 2, radiolabeled probe with 2.7 μg of HepG2 nuclear extract. Lanes 3–5, as in lane 2 but nuclear extract was incubated with unlabeled fragment B (lane 3), B_{del} (lane 4), or A (lane 5) as competitors before the addition of probe. C: Antibody (Ab) competition assay. As in B, except that nuclear extracts were preincubated in the presence or absence of 5.0 μl of antibodies against LRH-1 (V14X) or USF1 for 2 h at room temperature before the addition of probe (^{32}P-labeled fragment A). Lane 1, radiolabeled probe with no nuclear extract added. Lane 2, radiolabeled probe with 2.7 μg of HepG2 nuclear extract. Lanes 3 and 4, as in lane 2 but nuclear extract was incubated with antibodies to LRH-1 (lane 3) or, as a negative control, USF-1 (lane 4). D: As in B, using fragment C (left) or fragment C_{mut} (right) as a probe and wild-type (WT) C or C_{mut} as competitors, as indicated. E: As in C, using fragment C as a probe after preincubation of nuclear extract with three independent anti-LRH-1 antibodies (V14X, N15X, and N16X) or anti-TFIID antibody. Closed arrows indicate the positions of the two LRH-1-DNA complexes; open arrows delineate intermediate-sized complexes formed after antibody addition. The asterisk indicates the position of a complex supershifted to the top of the gel. NE, nuclear extract.

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dependent LRH-1 antibodies followed by the addition of probe C led to the formation of intermediate-sized and supershifted complexes (Fig. 2E), again indicating the presence of two LRH-1-containing complexes in the HepG2 nuclear extract capable of binding the LRH-1 motif at nt 134–142 of the intergenic promoter [see also ref. (14)]. Consistent with previous evidence for interactions between LRH-1 and TFIID (25), antibodies to TFIID interfered with the formation of the high molecular weight complex and led to the formation of an intermediate-sized complex (Fig. 2E), suggestive of LRH-1-TFIID interactions (25) and indicating that LRH-1 may participate in recruiting the basal transcription machinery to the TATA-less ABCG5/ABCG8 promoter. Preincubation of HepG2 nuclear extracts with an anti-USF1 antibody as a negative control before probe addition had only a minor effect on complex stability, indicating that the gel shift patterns observed with the LRH-1 antibodies were attributable to specific interactions between these antibodies and the LRH-1-containing protein complexes in the HepG2 nuclear extracts.

These combined data identify LRH-1 as the factor that binds the LRH-1 binding site at nt 134–142 of the ABCG5/ABCG8 promoter and indicate that LRH-1 is present in more than one complex with DNA binding activity in the HepG2 nuclear extract.

LRH-1 overexpression and bile acid addition modulate the activity of the ABCG5 and ABCG8 promoters

To evaluate the effect of LRH-1 on ABCG5 and ABCG8 transcription, the full-length 374 bp intergenic ABCG5 and ABCG8 luciferase reporter constructs, either wild type or with mutations introduced into the LRH-1 sites, were cotransfected with either pCI-LRH or empty vector (**Fig. 3**). In Caco2 cells, LRH-1 overexpression significantly ($P < 0.02$) enhanced the activity of the wild-type but not the mutated promoter constructs for ABCG5 and ABCG8 (1.4- and 1.6-fold, respectively) (Fig. 3, left panels). LRH-1 overexpression had no effect on ABCG5/ABCG8 promoter activity in HepG2 cells (data not shown), most likely because of high endogenous levels of LRH-1 in these cells. In 293 cells, which contain little if any endogenous LRH-1 (14), LRH-1 overexpression increased ABCG5 promoter activity by 2.5-fold ($P < 0.01$) and ABCG8 promoter activity by 1.5-fold ($P < 10^{-5}$) (Fig. 3, right panels) but did not significantly increase the activity of promoter constructs containing deletions or nucleotide substitution mutations in the LRH-1 binding site (data not shown).

A distinguishing feature of LRH-1 is its downregulation by bile acids via the farnesoid X receptor (FXR)/SHP pathway (16, 26). **Figure 4A** shows that addition of the bile acid DCA decreased ABCG5 and ABCG8 promoter activity. Northern analysis confirmed that transcription of the endogenous ABCG5 and ABCG8 genes is downregulated by DCA (Fig. 4B, C). As expected by the FXR/SHP/LRH pathway, the bile acid-mediated downregulation of ABCG5/ABCG8 was accompanied by the upregulation of SHP mRNA levels (Fig. 4B, C). These findings provide evidence that the ABCG5 and ABCG8 promoters are physiological targets of LRH-1.

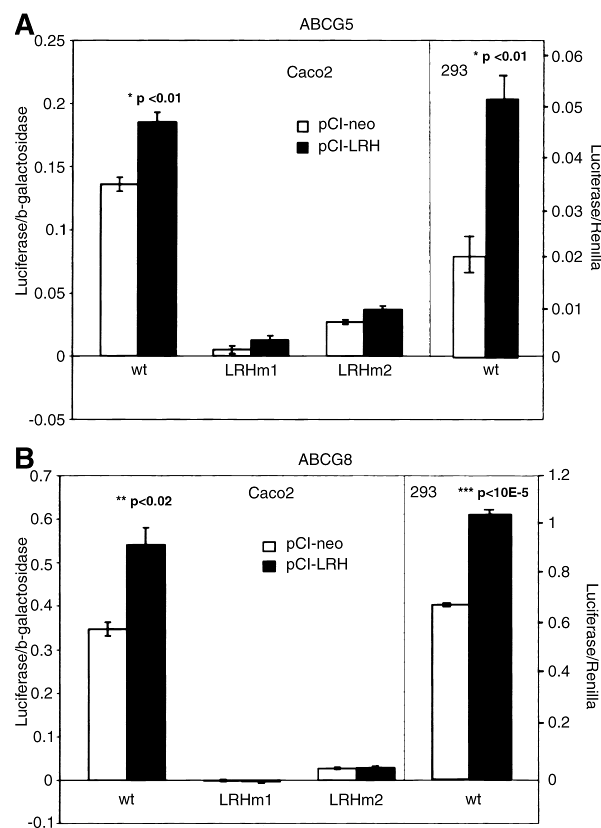

Fig. 3. LRH-1 enhances ABCG5 and ABCG8 promoter activity. Caco2 cells (left) were transfected with reporter construct [wild type (wt), LRHm1, or LRHm2] in either the ABCG5 (top panel) or ABCG8 (bottom panel) orientation. Cotransfected with the reporter plasmid was either a vector expressing LRH-1 (pCI-LRH) or empty expression vector (pCI-neo) as a control, along with pCMVβ. Transfections were performed as in Fig. 1 except that ABCG5/ABCG8 reporter plasmids, pCMVβ, and pCI-neo/pCI-LRH were decreased to 0.75, 0.05, and 0.25 μg/well, respectively. 293 cells (right) were transfected similarly but with 0.2 μg of ABCG5 or ABCG8, 0.2 μg of pCI-neo or pCI-LRH, and 0.05 μg of pRLSV40 using Fugene. Data from a representative experiment out of three independent experiments are shown for each.

DISCUSSION

This study demonstrates that LRH-1, an orphan nuclear receptor that regulates a number of genes involved in removing sterols and bile acids from liver and intestine (24, 27, 28), also regulates the activity of the intergenic ABCG5 and ABCG8 promoters. Inspection of the ABCG5/ABCG8 intergenic region revealed an LRH-1 transcription factor binding motif at position 134–142. Mutation of this site decreased the activity of the ABCG5 and ABCG8 promoters by at least 7-fold compared with the wild-type promoters, and binding of LRH-1 to this site was confirmed by EMSA using three independent LRH-1 antibodies. Overexpression of LRH-1 increased the activity of both the ABCG5 and ABCG8 promoters in Caco2 and 293 cells. Finally, the bile acid DCA downregulated the ABCG5 and ABCG8 promoters. This finding is completely consistent with the mechanism of bile acid downregulation of other

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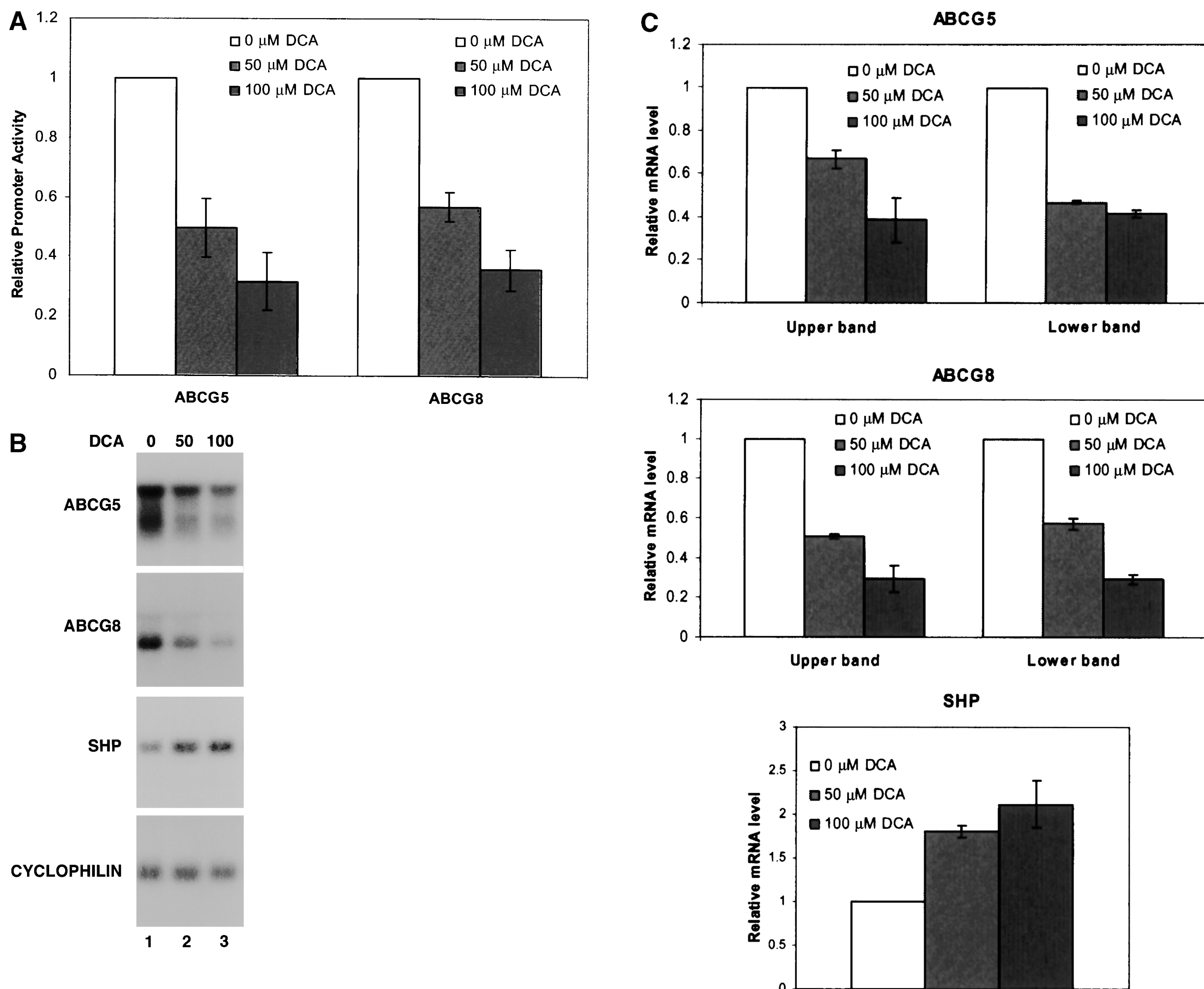

Fig. 4. A: DCA represses ABCG5 and ABCG8 promoter activity. HepG2 cells were cotransfected with ABCG5 or ABCG8 reporter constructs and pCMVβ as in Fig. 1. Twenty-four hours after transfection, DCA (0, 50, or 100 μM) was added and cells were lysed for 24 h after DCA addition. Promoter activity is expressed relative to the activity of the 0 μM DCA samples, which was defined as 1. The averages of three independent experiments are shown. B: DCA represses the transcription of the endogenous ABCG5 and ABCG8 genes. Northern blots of RNA from HepG2 cells treated with 0, 50, or 100 μM DCA for 24 h were hybridized to probes for ABCG5, ABCG8, small heterodimeric partner (SHP), or cyclophilin. A second independent experiment was performed with similar results. In both A and B, no toxicity of DCA was observed at 50 or 100 μM DCA; 150 μM DCA was toxic based on cell appearance, decreased β-galactosidase activity, and decreased cyclophilin RNA in cells (data not shown). C: Quantitation of DCA-mediated repression of ABCG5/ABCG8 mRNA. The Northern blot data from B and a second independent experiment were quantitated by densitometric scanning. For each experiment, mRNA levels for ABCG5 (top), ABCG8 (center), or SHP (bottom) normalized to cyclophilin were expressed relative to the level of the same mRNA at 0 μM DCA normalized to cyclophilin, which was defined as 1.0. Each bar represents the average value of the two independent experiments. For ABCG5 and ABCG8, both the upper band (left) and lower band (right) were quantitated. Error bars represent 1 SEM for all panels.

promoters mediated by the FXR-SHP-LRH-1 pathway (16, 26). These data demonstrate that the ABCG5/ABCG8 intergenic region contains an LRH-1 binding site that regulates the activity of both the ABCG5 and ABCG8 intergenic promoters. The addition of ABCG5 and ABCG8 to the list of genes known to be regulated by LRH-1 underscores the key role of these factors in sterol elimination.

LRH-1, also known as CPF, B1F, FTF, PHR-1, and NR5A2, was initially characterized by different laboratories as an activator of different genes, including CYP7α (14) and α1-fetoprotein (20), and was later found to be a key factor in FXR-mediated bile acid repression of CYP7α (16, 26) [reviewed in refs. (24, 27–29)]. LRH-1, which has several splice variants, is a member of the nuclear receptor family and is closely related to SF-1, another nuclear receptor that upregulates the transcription of a number of genes involved in steroidogenesis (17, 18, 30). LRH-1 has structural motifs characteristic of nuclear receptor family members: an N-terminal activation domain, a DNA binding domain, and a ligand binding dimerization/acti-

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Fig. 5. Model of LRH-1 as a transcription factor in liver and intestine that facilitates the elimination of excess cholesterol. Plus signs indicate transcriptional upregulation by LRH-1. Peripheral cells efflux excess cholesterol to poorly lipidated apolipoproteins through the ATP binding cassette (ABC) transporter ABCA1, forming HDL. SR-BI, a scavenger receptor that is transcriptionally upregulated by LRH-1 (23), transports cholesterol from HDL in the plasma to the interior of the hepatocyte. Once inside the hepatocyte, cholesterol can be converted to bile acids (BA's) by either the classic (neutral) or alternative (acidic) pathway (28, 29). LRH-1 transcriptionally upregulates the rate-limiting enzyme of the classic pathway, CYP7α, as well as the specific enzyme for cholic acid synthesis, sterol 12α-hydroxylase (CYP8B1) (48). BSEP, which is upregulated by farnesoid X receptor, transports bile acids into the bile. Cholesterol can be exported directly into the bile by ABCG5/ABCG8 localized in the apical membrane of hepatocytes. Both ABCG5 and ABCG8 are transcriptionally upregulated by LRH-1 in hepatocytes. Bile acids are taken up into the enterocyte by ASBT, which is transcriptionally regulated by LRH-1 (49). The receptor(s) that take up cholesterol from the bile as well as dietary cholesterol are unknown, but recent findings of ezetimibe inhibition of cholesterol absorption may expedite progress toward the identification of an intestinal sterol permease (50). Bile acids and LRH-1 upregulate MRP3, an ABC transporter expressed in the terminal ileum that effluxes bile acids from the basolateral surface of enterocytes into the portal vein to recycle bile acids back to the liver (51). Excess cholesterol in the enterocyte is transported into the gut lumen by ABCG5 and ABCG8, which are upregulated by LRH-1 in enterocytes. Cholesterol may also exit the enterocyte in the form of chylomicrons. Carboxyl ester lipase (CEL) hydrolyzes dietary cholesteryl esters in the presence of trihydroxylated bile salts (52). Cholesteryl ester transfer protein (CETP), which transfers cholesterol esters from HDL to apoB-containing lipoproteins in exchange for triglycerides, is transcriptionally upregulated by LRH-1, particularly in conjunction with liver X receptor α (46, 53). Cholesterol from apoB-containing lipoproteins (Lp's) can be taken up into the liver by the LDL receptor (LDLR) and LRP. Thus, LRH-1 directly stimulates a number of genes involved in eliminating excess sterols from liver and intestine. LRH-1 also upregulates genes for sterol metabolism indirectly by regulating the transcription of the transcription factors HNF4α and HNF1α (35), which are themselves master regulators of genes involved in lipid metabolism (27, 28, 54, 55). LRH-1 is itself highly regulated (24, 27, 28). ASBT, apical sodium-dependent bile salt transporter; BSEP, bile salt export pump; α-FP, α-fetoprotein; LRP, low-density lipoprotein receptor-related protein; MRP3, multidrug resistance protein 3.

vation domain that also interacts with coregulators (24, 27, 28, 31). LRH-1 has been reported to participate in the recruitment of the core transcription complex through Multibridging Factor 1 (25); our finding that an anti-TFIID antibody interferes with DNA-LRH-1 protein complex formation (Fig. 2E) is consistent with this observation and may explain the ability of LRH-1 to activate the TATA-less ABCG5/ABCG8 promoter. LRH-1, like other nuclear receptors, participates in chromatin modification pathways (14, 23, 32); indeed, the negative promoter activity observed in the LRH1 deletion mutant LRHm1 (Fig. 1) is consistent with the formation and spreading of heterochromatin by the absence of LRH-1 binding. LRH-1, like SF-1, binds as a monomer to an extended half-site with a core sequence of CAAGG, and both are "orphan" nuclear receptors whose ligands have not yet been identified. In fact, LRH-1 appears to adopt a unique conformation that may preclude the need for a ligand (33, 34). Because LRH-1, ABCG5, and ABCG8, but not SF-1, are coexpressed in liver and intestine, it is LRH-1 rather than SF-1 that activates the ABCG5 and ABCG8 promoters in liver and intestine.

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In addition to LRH-1, other transcription factors implicated in sterol metabolism have been shown to affect ABCG5/ABCG8 promoter activity. A conserved GATA site acts as a repressor for the ABCG5 but not the ABCG8 intergenic promoter (12); GATA regulates the expression of several genes involved in lipid metabolism and adipocyte differentiation. Interestingly, the mouse FTF/LRH gene is itself regulated by three GATA elements (35). Treatment with the liver X receptor (LXR) agonist T0901317 upregulates the ABCG5 and ABCG8 genes in liver and intestine of mice (1, 11, 36, 37). The LXR element(s) responsive to LXR agonists has not yet been identified but is likely to be intronic, as the ABCG5/ABCG8 374 bp intergenic region is not significantly upregulated by 22-OH-cholesterol (12). Studies in FXR$^{-/-}$ mice (38), as well as in mice treated with the FXR agonists GW4064 and chenodeoxycholic acid (11), indicate that in mice FXR activates ABCG5/ABCG8 genes, perhaps indirectly (11). ABCG5/ABCG8 mRNA levels are increased in SHP$^{-/-}$ mice (39) and decreased in liver of rats that have undergone bile duct ligation (40), a procedure that increases intrahepatic bile acid levels in mice (41). These findings are consistent with the downregulation of ABCG5/ABCG8 transcription by bile acids in vivo. We note that the LRH-1 binding site in the human ABCG5/ABCG8 intergenic region described here is not conserved in the mouse ABCG5/ABCG8 intergenic region. Interestingly, the mouse ABCG5/ABCG8 promoter contains a TATA box and binding motifs for HNF4, another bile acid-regulated nuclear receptor (28, 42), which may also mediate a novel bile acid-cytokine pathway (29, 43). Whether these species-specific differences in intergenic promoter sequences translate into differences in transcriptional regulation and whether yet other transcription factor-mediated pathways (44) may be responsible for the bile acid effects on ABCG5/ABCG8 transcription are interesting questions to pursue in the future. Other transcription factor binding sites present in the human intergenic promoter that may potentially confer sterol regulation include motifs for the progesterone receptor, estrogen receptor, and vitamin D receptor (45). Four CP2 sites and an NF-κB site are also present, and there are undoubtedly other factors required for expression in vivo. Finally, our EMSA studies indicate that HepG2 nuclear extracts have two LRH-1-containing complexes; determining whether the smaller complex contains a subset of the proteins of the larger complex or an unrelated set of proteins, and identifying the proteins present in both complexes, are also interesting topics to pursue in the future.

Whereas LRH-1 overexpression may strongly activate the transcription of some promoters without assistance from other transcription factors, in other cases, such as the CYP7α and CETP gene promoters, LRH-1 did not strongly activate by itself but instead acted as a competence factor to enhance the activity of LXR/retinoic X receptor (16, 46). A link between the LXR and LRH pathways is also suggested by the finding that the transcriptional activities of both proteins are repressed by the SHP protein (47). LRH-1 and LXR may be anticipated to operate in conjunction with each other, as LXR initiates the reverse cholesterol transport pathway in peripheral cells and the LRH-1/ABCG5/ABCG8 pathway completes this pathway by excretion of sterols from liver and intestine. Identification of the ABCG5/ABCG8 LXR element will be necessary before this question can be addressed by promoter activity assays.

A model of LRH-1 target genes and their functions leading to the elimination of cholesterol from liver and intestine is shown in **Fig. 5**. These findings identify LRH-1 as a highly regulated transcription factor with a pivotal role in maintaining cholesterol homeostasis.

In summary, we have demonstrated that the orphan nuclear receptor LRH-1 is important for the activity of the intergenic ABCG5/ABCG8 promoter. This is the first report that identifies a binding motif for a transcription factor that activates the ABCG5/ABCG8 intergenic promoter. LRH-1 appears to be a "master regulator" of a number of genes involved in the excretion of sterols from liver and intestine, a process that is anticipated to decrease the formation of atherosclerotic lesions. This transcription factor, along with other factors that may regulate the ABCG5 and ABCG8 promoters, appears to be a promising target for therapeutic intervention.

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