Expression and Regulation of Multiple Murine ATP-binding Cassette Transporter G1 mRNAs/Isoforms That Stimulate Cellular Cholesterol Efflux to High Density Lipoprotein

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The murine Abcg1 gene is reported to consist of 15 exons that encode a single mRNA (herein referred to as Abcg1-a) and protein. We now demonstrate that (i) the murine gene contains two additional coding exons downstream of exon 1, (ii) transcription involves the use of multiple promoters, and (iii) the RNA undergoes alternative splicing reactions. As a result, three mRNAs are expressed that encode three putative protein isoforms that differ at their amino terminus. ABCG1 transcripts are induced in vivo in multiple tissues in response to the liver X receptor ligand T0901317. Identification and characterization of four liver X receptor response elements in the intron downstream of exon 2 provides a mechanism by which this induction occurs. Importantly, cholesterol efflux to high density lipoprotein was stimulated following transfection of Hek293 cells with plasmids encoding individual ABCG1 isoforms. In situ hybridization studies in embryonic day 11.5–15.5 mouse embryos revealed strong expression of ABCG1 transcripts in the olfactory epithelium, hind brain, eye, and dorsal root ganglia. The relatively high levels of expression in neuronal tissues and the eye suggest that ABCG1-dependent cholesterol efflux may be critical for normal neuronal function in addition to its role in macrophages.

There are more than 373 members in the ATP-binding cassette (ABC) superfamily of transmembrane transporters that are expressed in species as diverse as plants, yeast, Drosophila, and mammals (1). To date, 52 members of this superfamily have been identified in mammals (2, 3). ABC transporters have been subdivided into full and half transporters; full transporters contain two ATP-binding domains and two multitransmembrane domains, each typically containing six transmembrane α-helices. In contrast, half transporters contain one ATP-binding cassette and one transmembrane domain and must dimerize to form a functional pump (4–6). Binding and hydrolysis of ATP by these membrane-bound pumps provides the energy to transport small hydrophobic substrates from the cytoplasm either to the outside of the cell or into intracellular compartments such as the endoplasmic reticulum, peroxisomes, or mitochondria (5, 7). The importance of these proteins is illustrated by the fact that numerous human diseases are caused by mutations in specific members of the ABC transporter superfamily (2, 3, 8, 9).

ABC transporters are further subdivided into seven groups, ABCA through ABCG. Members of the ABCG subtype (ABCG1, ABCG2, ABCG3, ABCG4, ABCG5, ABCG8, and Drosophila white, brown, and scarlet) are unique in that the ATP-binding cassette of these half transporters is at the amino terminus and the transmembrane domains are at the carboxyl terminus of the protein. In the original studies the cDNAs corresponding to ABCG1 were referred to as either ABC8 or/and white (10, 11). Both the murine and human cDNAs encode proteins of ~67 kDa that have 34% amino acid identity with the Drosophila white protein (10, 11). Drosophila white dimerizes with either scarlet or brown to form heterodimers (white/scarlet or white/brown, respectively) that transport precursors of the ommochromes and pteridines into the pigment granule of the fly eye (12–15). However, no mammalian homologues of Drosophila brown or scarlet that might function as the heterodimeric partner of ABCG1 have been identified. The mammalian ABCG4 protein has 94% amino acid identity with ABCG1, consistent with gene duplication (16, 17). However, ABCG4 expression is reported to be limited to the brain and eye (17) and this would preclude the formation of obligate ABCG1/ABCG4 functional heterodimers in many tissues.

In an attempt to determine the function for mammalian ABCG1, Klucken et al. (18) treated human macrophages with antisense oligonucleotides to ABCG1. This treatment resulted in decreased expression of a protein of 110 kDa and decreased efflux of cholesterol and phospholipids to HDL3 (18). Surprisingly, the antisense oligonucleotides also decreased apoE secretion from macrophages (19).

The mammalian ABCG1 cDNA was originally identified from studies using degenerate PCR and RNA from either a murine macrophage cell line (10), a murine pre-B cell library (11), or a human Jurkat T-cell line (11) or after using exon-trapping and a human chromosome 21 cDNA library (20). These murine and human cDNAs had 97 and 87% identity at the amino acid and nucleic acid levels, respectively. In general, high expression of ABCG1 mRNA was noted in spleen, lung, thymus, and brain, whereas expression in kidney, liver, and heart was low or undetectable (10, 11). Subsequent studies...
demonstrated that murine ABCG1 mRNA levels were highly induced when macrophages were either incubated with LXR agonists or were converted to cholesterol ester-loaded foam cells (18, 21, 22).

Recently, we and others identified multiple human ABCG1 transcripts that are produced as a result of transcriptional initiation at different exons and alternative mRNA splicing (23–25). Each of the resulting mRNAs has a unique 5′ sequence upstream of exons 11–23 (20, 25, 26). Each unique upstream sequence contains an in-frame ATG, resulting in the synthesis of multiple putative protein isoforms that contain variable amino termini of 56–203 amino acids and a conserved 583 amino acids at their carboxyl termini (24, 25).2 It is not known whether such variations at the amino terminus affect protein localization, dimerization, or function.

In contrast to the multiple human isoforms, only a single murine ABCG1 cDNA has been identified to date. The LXR response element (LXRE) (27) that is presumed to be necessary for the activation of the murine gene by LXR/RXR has yet to be identified.

In the current study we report on the identification of new exons and novel mRNA sequences that encode three ABCG1 protein isoforms. In addition, we identify multiple LXREs within an intron of the Abcg1 gene. Promoter-reporter studies suggest that these LXREs are necessary and sufficient to activate the gene in numerous tissues in response to LXR ligands. In situ hybridization studies show that ABCG1 is expressed at relatively high levels in the olfactory bulb epithelium, hind brain, hippocampus, choroid plexus, eye, and dorsal root ganglia of developing embryos and/or adults. Finally, we show that each ABCG1 isoform stimulates the efflux of cholesterol to HDL. These data suggest that ABCG1 may play a key role in regulating lipid efflux from neurons and epithelial cells in addition to macrophages.

MATERIALS AND METHODS

Reagents—Mammalian expression vectors for LXRα and RXRα (pCMX-LXRα, pCMX-RXRα) were gifts from Dr. Ron Evans (Salk Institute, La Jolla, CA). RXRα polyclonal antibody (D20, sc-553X) was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Synthetic LXR ligand T0901317 (27) was purchased from Cayman Chemical (Ann Arbor, MI), and synthetic RXR ligand LG100153 (28) was a gift from Dr. Richard Heyman (Ligand Pharmaceuticals, La Jolla, CA). Human HDLα was obtained from Intracel. Total HDL (density 1.063–1.21 g/ml) was obtained by ultracentrifugation of human plasma.

Animals—C57BL/6 mice were purchased from Jackson Laboratories (Bar Harbor, ME) and maintained on standard rodent chow or chow supplemented with 0.05% T0901317. This corresponds to (Bar Harbor, ME) and maintained on standard rodent chow or chow supplemented with 0.05% T0901317. This corresponds to

Regulation of Murine ABCG1

HeK293 cells were grown in Dulbecco’s modified Eagle’s medium + 10% fetal bovine serum and plated in 24-well plates (0.3 × 10^6 cells/ml) and allowed to adhere overnight. We used LipofectAMINE 2000 (Invitrogen) to transiently transfected triplicate wells of HeK293 cells with the empty pCMX vector (1 µg/well) or the pCMX vector containing a cDNA encoding a specific ABCG1 isoform. Analysis of cells transfected with plasmids encoding green fluorescent protein and pCMX indicated that the transfection efficiency was >70%.

Cholesterol Efflux—Following transfection of HeK293 cells with the indicated plasmids, the cells were washed twice with phosphate-buffered saline and incubated for 24 h in media (Dulbecco’s modified Eagle’s medium + 0.2% bovine serum albumin) supplemented with an acyl-CoA cholesterol O-acyltransferase inhibitor (58-035, 2 µg/ml) (32), [3H]cholesterol (1 µCi/ml), and where indicated, T0901317 (1 µM) and LG100153 (100 nM). The cells were washed twice with phosphate-buffered saline and then incubated for an additional 4 h in media A and were then incubated with T0901317 and LG100153. After this equilibration period, the cells were washed twice more with phosphate-buffered saline and then incubated for an additional 4 h in media A or in media A supplemented with either HDLα (50 µg/ml) or total HDL (50 µg/ml). Media were removed and centrifuged at 14,000 × g for 2 min, and the radioactivity in the supernatant determined as described (33). Isopropanol was added to each well to solubilize cell-associated radioactivity. After centrifugation, aliquots were removed and the radioactivity determined (33). Cholesterol efflux was determined by dividing the radioactive content of the media by the sum of the radioactivity in the cells and media. Efflux was linear for >8 h.

5′-RACE—5′-RACE was performed using cDNA from Sure-RACE™ Multi-Tissue RACE panels (OriGene Technologies, Rockville, MD) according to the manufacturer’s protocol. A adaptor containing sequences complimentary to two primers, ADP1 and ADP2, was attached to the 5′ end of the cDNAs. First round PCR was performed with a gene specific primer (GSP1) corresponding to exon 4 of the mouse gene and ADP1 following under the conditions: 94 °C for 3 min, 10 cycles of 94 °C for 30 s, 68 °C for 30 s, 72 °C for 2 min, and 15 cycles of 94 °C for 30 s, 62 °C for 30 s, 72 °C for 2 min, then extension at 72 °C for 6 min. The first round PCR products were diluted 10-fold, and an aliquot was used as template for second round PCR with nested primers ADP1 and ADP2 following under the conditions: 94 °C for 3 min, 5 cycles of 94 °C for 30 s, 65 °C for 30 s, 72 °C for 2 min, 5 cycles of 94 °C for 30 s, 62 °C for 30 s, 72 °C for 2 min, 25 cycles of 94 °C for 30 s, 60 °C for 30 s, 72 °C for 2 min, extension at 72 °C for 6 min. The PCR products were separated on agarose gels and recovered fragments were cloned into pcR2.1-TOPO vector (Invitrogen) for sequencing.

RNA Isolation and Real Time Quantitative PCR—Total RNA was isolated using TRIzol Reagent (Invitrogen). Real time PCR was performed as described (35). The nucleotide sequences of the primers and probes are given in Table I. Probes were modified at the 5′ end with 6-carboxyfluorescein and at the 3′ end with 6-carboxytetramethylrhodamine (Integrated DNA Technology, Coralville, IA). EMA—EMSA—EMSA were performed as described (29). LXRα and RXRα were synthesized in vitro using the T7-coupled reticulocyte system (Promega, Madison, WI) (23–25).

In Situ Hybridization of Embryos—In situ hybridization was performed as described (37). Briefly, a 905-bp fragment, corresponding to 725 bp of the 3′-UTR and 180 bp of the open reading frame of the murine ABCG1 cDNA, was subcloned into pCI-Neo (Promega) to generate pCI-Neo-mABC1G1. The linearized plasmid was transfected with either T7 or T3 RNA polymerase using the (digoxigenin)-UTP labeling kit (Roche Applied Science), to generate sense or antisense digoxigenin-labeled cRNA probes. For whole mount in situ hybridization, embryos were dissected at embryonic day 11.5 and fixed overnight in 4% paraformaldehyde in phosphate-buffered saline at 4 °C. For in situ hybridization of embryo sections, embryos were dissected at embryonic day 12.5 or embryonic day 15.5, embedded in optimal cutting temperature (OCT) compound (10:24% polyvinyl alcohol, 4:26% w/v polyethylene glycol) (VWR, West Chester, PA), and stored at −20 °C.

2 M. A. Kennedy and P. A. Edwards, unpublished data.
Twenty-μm cryosections (or the whole mount embryos) were hybridized with the indicated probe for 18 h at 68 °C prior to addition of alkaline phosphatase-conjugated anti-digoxigenin. The sections then incubated in BM-Purple (Roche Applied Science) for 48 h to allow for color development.

RESULTS

Identification of ABCG1 Transcripts That Encode Novel Isoforms—A single murine ABCG1 cDNA was originally reported by Savary et al. and Croop et al. (10, 11). For comparative purposes, we refer to this transcript, corresponding to exons 1 and 2–15, as ABCG1-a (Fig. 1). To determine whether additional murine ABCG1 transcripts are produced, we used 5′-RACE to analyze cDNAs derived from multiple tissues. The approach, described in detail under “Materials and Methods,” utilized cDNAs that contained a unique adapter at the 5′ end. These cDNAs were used as templates for PCR reactions, utilizing primers corresponding to the 5′ linker and to exon 4. Nested PCR reactions were subsequently performed utilizing internal primers corresponding to the linker and exon 4, and the resulting DNA products were cloned and sequenced. Many clones contained sequences with 100% identity to exons 1, 2, 3, and 4 and thus presumably corresponded to transcript ABCG1-a (data not shown). However, one cDNA, derived from the spleen library, contained a novel 220-bp sequence upstream of exon 2. Comparison of the novel sequence with genomic DNA databases indicated that all 220 bps were present in two new exons (exons 1A and 1B in Fig. 1A) that were localized 3′ of exon 1. The novel transcript, containing sequences corresponding to exons 1A + 1B + 2–15, is referred to as ABCG1-b (Fig. 1B). Subsequently, we utilized spleen RNA as a template to generate full-length cDNA using primers to exon 15 and either exon 1 or exon 1A. As expected, this approach resulted in the generation of cDNAs corresponding to ABCG1-a and ABCG1-b (Fig. 1B). Surprisingly, a third cDNA was isolated that corresponded to a transcript containing exon 1A fused to exons 2–15 (ABCG1-c in Fig. 1B). The 5′ sequences of these three transcripts are shown in Fig. 1C.

Previous studies (10, 11) identified an in-frame methionine in exon 1 of the ABCG1-a transcript (Fig. 1, B and C). Translation initiated at this methionine would produce a protein containing 666 amino acids (Fig. 1B). In contrast, ABCG1-b and ABCG1-c lack exon 1, but contain an in-frame methionine in exon 1A; translation initiated at this methionine would generate proteins containing either 714 or 682 amino acids, respectively (Fig. 1B). Thus, proteins derived from translation of the three mRNAs are predicted to contain an identical carboxyl terminus of 652 amino acids (encoded by exons 2–15) but to differ at their amino termini (Fig. 1, B and C).

Comparison of Murine and Human ABCG1 Genomic Organization—We performed comparative studies of murine and human genomic DNA to identify conserved exonic sequences. These studies utilized VISTA and various exon predicting programs (including MZEF, GENIE, GRAIL 1, GRAIL2, GRAIL 1A, FGENE, and GENESCAN). The results indicate a very high degree of conservation between the nucleotide and amino acid sequences of exons 1 and 2–15 of the murine gene and exons 5, 7, and 11–23 of the human gene (Fig. 2). However, nucleotide sequences corresponding to the novel murine exons 1A and 1B were not identified in human genomic or EST data bases. Nonetheless, we probed human RNA blots with probes corresponding to murine exons 1A and 1B and performed reverse transcription-PCR at low stringency using pooled human cDNAs and primers corresponding to murine exons 1A and 1B. In both cases we failed to identify human transcripts that contained sequences corresponding to murine exons 1A or 1B (data not shown). Complementary approaches that involved Northern blot assays, and reverse transcription-PCR failed to identify human transcripts that corresponded to human exons 1–4, 6, 8, 9 and 10 (data not shown).

The 17 murine exons span 58 kb of genomic DNA located on chromosome 17 between nucleotides 29772527 and 29831370 (Ensemble data base). The intron-exon boundaries of the murine Abcg1 gene are shown in Table II. All intron/exon boundaries display canonical GT/AT sequences (38).

Expression and Regulation of Different Murine ABCG1 Transcripts in Vivo—Previous studies have shown that ABCG1 expression is induced when isolated peritoneal macrophages are treated with T0901317, the LXR ligand (33). To investigate the tissue expression and regulation of ABCG1 transcripts in vivo we fed mice normal chow or chow supplemented with T0901317 (50 mg/kilogram body weight/day) for 10 days prior to RNA isolation. Real time quantitative PCR was then used to determine the expression and regulation of all ABCG1 transcripts (ABCG1-a, -b, and -c) that contain the identical 3′-UTR or the individual transcripts ABCG1-a or ABCG1-b (Fig. 3). To date, we have been unable to generate a specific probe for ABCG1-c.

The data of Fig. 3A show that treatment with T0901317
induced ABCG1 transcripts in all tissues examined with the exception of the brain. T0901317-treated murine peritoneal macrophages served as a positive control (Fig. 3). The ABCG1-a transcript showed a very similar pattern of tissue expression and induction in response to T0901317 (Fig. 3, compare B with A). In contrast, the data from real time quantitative PCR indicated that the ABCG1-b mRNA was restricted to the lungs and isolated peritoneal macrophages (Fig. 3C). However, ABCG1-b mRNA levels were induced following treatment with the LXR ligand T0901317 (Fig. 3C). Nonetheless, very low levels of ABCG1-b and ABCG1-c mRNAs must be expressed in a number of murine tissues because (i) the ABCG1-b transcript was originally identified in cDNA derived from the spleen (see "Materials and Methods"), and (ii) nested reverse transcrip-
determine the pattern of expression of murine ABCG1 mRNA from four individual mice, and the values are mean ± S.E. *n, p < 0.01 by Student's t test.

### Expression of ABCG1 Transcripts during Development—To determine the pattern of expression of murine ABCG1 mRNA in different tissues and cell types during development, we performed in situ hybridization studies. These studies utilized a 905-bp digoxigenin-labeled probe corresponding to the 3'-UTR of murine ABCG1 mRNA to identify all ABCG1 transcripts. Fig. 4 shows the results of whole mount in situ hybridization studies; the ABCG1 antisense probe identified transcripts in the hind brain, brachial arches, forebrain, nasal processes, dorsal root ganglia, and limb buds of embryonic day 11.5 embryos taken from chow-fed mothers (Fig. 4, compare A and B). The sense probe gave no detectable signal (Fig. 4C).

In an attempt to induce the fetal expression of ABCG1, pregnant mice were fed a diet supplemented with T0901317 for 2, 7, or 10 days and then isolated RNA from embryonic day 12.5 and embryonic day 15.5 embryos (Fig. 5). Northern blot assays indicate that administration of T0901317 induced ABCG1 levels in the kidneys of the pregnant mice and rapidly induced both ABCG1 and ABCA1 mRNA levels in embryonic day 12.5 and embryonic day 15.5 embryos (Fig. 5). Based on these results, we propose that the synthetic LXR agonist crosses the placenta and is delivered to the developing embryo. In situ hybridization is not a quantitative technique, and the lack of a discernable effect of T0901317 on signal intensity (Fig. 4) in all tissues is thus not surprising. Interestingly, no embryos were present at embryonic day 12.5 when T0901317 was administered to mice (n = 4) 1 day after identification of a vaginal plug (data not shown). We suspect that under these conditions, T0901317 prevents fetal development.

The data of Fig. 5 also show that ABCG1 is expressed in the adult murine eye and is induced in response to dietary T0901317. Because the Drosophila white gene, the putative homologue of mammalian ABCG1, is also expressed in the eye, this result raises the possibility that ABCG1 and white have similar functions in this organ.

The data of Figs. 3 and 4 indicate that ABCG1 transcripts are expressed in the brain. To better define the pattern of expression, we examined frozen sections from embryonic day 12.5 and embryonic day 15.5 embryos. Fig. 6A shows that high levels of ABCG1 transcripts are present in sensory tissues, including the olfactory epithelium, whisker follicles, and neuronal retina (Fig. 6A and B). Significant expression was also observed in the equatorial cells of the lens, the ventricular zone of the hind brain and choroid plexus (Fig. 6A and B), and in dorsal root ganglia (Fig. 6C). The antisense probe was specific because many tissues, including the liver and heart, gave no signal (Fig. 6C). No signal was noted when the sense probe was utilized (data not shown). Thus, with the exception of the branchial arches and limb buds, the expression of ABCG1 is
predominantly neural in midgestation embryos. The data of Fig. 5 clearly show that ABCG1 transcripts are also expressed in the adult murine eye, and the levels are induced following inclusion of T0901317 in the diet.

Identification and Functional Characterization of LXREs That Regulate Expression of the Murine ABCG1 Gene—The experiments described above together with published data (21, 22, 39, 40), indicate that murine Abcg1 gene expression is induced in response to ligands for LXR. To identify potential LXREs that are required for such gene activation, we used the FINDPATTERNS algorithm in the GCG suite of analysis software to analyze 120 kb of genomic DNA encompassing the murine gene. This approach identified seven putative LXREs that spanned −34 kb of genomic DNA (Fig. 7A, LXRE-1 to -7).

Fig. 7B shows the results of EMSAs that utilized radiolabeled probes corresponding to each putative murine ABCG1 LXRE. A positive control (hLXRE-1) taken from the human Abcg1 gene (25) was included in each EMSA. The data demonstrate that both LXRE-3 and LXRE-6 form prominent shifted complexes with recombinant LXR/RXR, whereas LXRE-4 and LXRE-7 form a relatively weak shifted complex (Fig. 7B). In the presence of LXR and RXR, these four LXREs were also supershifted in the presence of antibody to RXR (data not shown). No shifted complex was observed when the EMSA was performed with probes corresponding to LXRE-1, -2, or -5 (Fig. 7B).

To further characterize the LXREs that bound LXR/RXR we performed competition assays; a radiolabeled probe corresponding to LXRE-6 was incubated in the presence of LXR and RXR and in the absence (lanes 1 and 15) or presence of increasing concentrations of LXRE-3, -4, -6, -7, or mutant LXRE-6 (Fig. 7C). The competition studies demonstrate that LXRE-6, -3, and -7 have higher affinities for LXR/RXR than LXRE-4. In addition, the failure of the mutant LXRE-6 to function as a competitor in the EMSA illustrates that the binding of LXR/RXR to the DNA is specific (Fig. 7C).

The data derived from EMSAs suggest that LXRE-3, -4, -6, and -7 may activate transcription of the Abcg1 gene. To test this hypothesis, two copies of either LXRE-3 or LXRE-6 were linked to a minimal TK promoter-reporter gene. Each reporter construct was transiently transfected into HepG2 cells in the presence or absence of plasmids encoding LXR and RXR, and the cells were then incubated for 24 h in the presence or absence of ligands for LXR (T0901317) and/or RXR (LG100153). The data of Fig. 8 show that reporter genes under the control of two copies of either LXRE-3 or LXRE-6 were highly induced when the cells were treated with T0901317 and/or LG100153. We hypothesize that these LXREs are involved in the activation of the Abcg1 gene. In other studies, cells were transfected with a luciferase reporter gene under the control of 5 kb of genomic DNA upstream of exon 1 and the first 50 bp of exon 1. This reporter gene was active. However, because no additional activation was observed when the cells were cotransfected with plasmids encoding LXR and RXR and then incubated in the presence of T0901317 (data not shown),
we conclude that the 5-kb proximal promoter upstream of exon 1 does not contain an LXRE.

All ABCG1 Isoforms Stimulate Cellular Cholesterol Efflux to HDL—To determine whether individual ABCG1 isoforms affect the efflux of lipid to exogenous acceptors, we performed the experiments shown in Fig. 9. Hek293 cells were transiently transfected with an empty plasmid or a plasmid expressing a specific ABCG1 isoform and then incubated in the presence of 

\[ ^{3}H \text{cholesterol} \] for 24 h. The efflux of radiolabeled cholesterol to media or media supplemented with either HDL3 or total HDL was then determined during a subsequent 4-h period.

The data show that transient transfection of ABCG1-a significantly increased cholesterol efflux to HDL3 or total HDL (Fig. 9, A and B). The data also demonstrate that each ABCG1 isoform was equally effective in stimulating cholesterol efflux to HDL in both untreated cells and in cells that had been pretreated with LXR/RXR ligands to activate endogenous LXR response to LXR agonists. However, the findings that these LXREs are located 9,771–18,312 bp downstream of exon 1 make it difficult to construct promoter reporter genes to directly test this proposal.

DISCUSSION

This report identifies two new exons (named exons 1A and 1B) and transcripts (ABCG1-b and ABCG1-c) that encode two putative novel murine ABCG1 isoforms (Fig. 1). Based on the fact that ABCG1 transcripts are initiated at either exon 1 or exon 1A and that these exons are separated by 3.1 kb (Fig. 1), we hypothesize that the mouse Abcg1 gene contains two distinct promoters. Previous reports demonstrated that ABCG1-a mRNA levels were induced when macrophages were treated with oxidized or acetylated LDL or ligands for LXR (18, 21, 22). We now show that the novel transcript ABCG1-b is also induced by LXR agonists, suggesting that both promoters are responsive to activated LXR. In the current study we utilized EMSAs and luciferase reporter genes to identify and characterize functional LXREs that are involved in transcription of the gene in response to LXR agonists. Surprisingly, these LXREs are located between exons 2 and 3 (Fig. 7). Because in silico analysis of 30 kb 5’ of exon 1 of the mouse gene failed to identify additional putative LXREs, we hypothesize that these four LXREs regulate the expression of all ABCG1 transcripts in response to LXR agonists. However, the findings that these LXREs are located 9,771–18,312 bp downstream of exon 1 make it difficult to construct promoter reporter genes to directly test this proposal.

The current studies indicate that each murine isoform is capable of increasing cholesterol efflux to HDL. The results are consistent with reports demonstrating that cholesterol or phospholipid efflux to HDL decreased following knock-down of ABCG1 mRNA levels after treatment of cells with either antisense oligonucleotide (18) or siRNA (41). Our data are in agreement with Wang et al. (41), who recently reported that cholesterol efflux to HDL was stimulated following overexpression of ABCG1 (ABCG1-a, using the current nomenclature). However, the current studies demonstrate that other ABCG1 isoforms also promote cholesterol efflux. The data showing that cholesterol efflux was stimulated following overexpression of single ABCG1 isoforms suggest that each isoform dimerizes to form functional homodimer transporters. Additional studies will be necessary to determine whether ABCG1 isoforms heterodimerize with other half transporters in vivo.

Three other ABC transporters, ABCG5, ABCG8, and ABCA1, are also known that are both involved in cellular sterol efflux and are activated by LXR. ABCA1, like ABCG1, is expressed in multiple tissues (42) (Fig. 3). Numerous studies have demonstrated that ABCA1 is necessary for the efflux of cellular cholesterol to lipid-poor apoproteins that include apoA1, apoE and apoC-II (43). In contrast, ABCG1 facilitates the efflux of cholesterol to HDL, rather than to lipid-poor apoproteins (18, 41) (Fig. 9). Thus, the relative importance of ABCG1 and ABCA1 in controlling cellular cholesterol efflux in vivo is likely to depend both on the relative concentration of exogenous lipid-poor apoproteins and HDL and on the relative cellular expression of these two transporters. Indeed, the finding that the relative hepatic expression of ABCG1 and ABCA1 in rat hepatocytes, Kupffer, and endothelial cells differs significantly (44) also suggests that these two transporters may have distinct roles in vivo.

In contrast to ABCG1 and ABCA1, high expression of
FIG. 7. Identification of LXREs in the murine gene. A, schematic of the murine ABCG1 gene and relative location and nucleotide sequences of seven putative LXREs. Distances (bps) relative to exon 1 are shown. The four LXREs that formed a shifted complex with recombinant LXR and RXR are marked (*). The nucleotide sequence of the six putative LXREs and their position relative to exon 1 are shown. The arrow above the sequence indicates the direction of the putative DR-4. B, electrophoretic mobility shift assays for the putative LXREs (LXRE-1 to LXRE-7). LXRα and RXRα were incubated with the indicated radiolabeled probes of similar specific activity before gel electrophoresis as described under “Materials and Methods.” The shifted DNA-protein complexes were detected by autoradiography. DNA containing the LXRE-1 from the human ABCG1 gene (25) was used as a positive control. C, a radiolabeled probe corresponding to LXRE-6 was incubated in the presence of recombinant LXRα and RXRα and in the absence or presence of 10, 50, 250, or 1,000 molar excess of the indicated unlabeled competitor DNA.
ABCG5 and ABCG8 is limited to hepatocytes and to enterocytes of the small intestine (45). The ABCG5/ABCG8 heterodimers promote the efflux of neutral sterols from hepatocytes into the bile and reduce absorption of cholesterol and plant sterols from the intestine (46, 47). However, in contrast to studies with ABCG1 and ABCA1, no exogenous acceptor has been identified to date that facilitates ABCG5/ABCG8-dependent sterol efflux. Mutations in either \textit{ABCG5} or \textit{ABCG8} result in sitosterolemia, a disease associated with altered cholesterol absorption and metabolism and accumulation of plant sterols in the plasma and tissues (48, 49). In contrast, mutations in \textit{ABCA1} (Tangier disease) result in abnormally low plasma HDL levels (50–52). The generation of \textit{Abcg1} null and transgenic mice would provide important animal models to better understand the physiological role of ABCG1 in whole body lipid homeostasis and to allow comparison of its role to those of other ABC transporters.

\textit{Drosophila} white can form two distinct heterodimers (white/brown or white/scarlet) that transport different substrates into the pigment granule of the fly eye (12). Recently Campbell and Nash demonstrated (53) that white–/– flies were resistant to certain anesthetics and proposed that \textit{Drosophila} white was also involved in controlling neuronal function in the fly brain by a process that is independent of vision. Taken together, these data suggest that \textit{Drosophila} white can dimerize with different half-transporters to alter substrate specificity in the eye and that it also has a role in neuronal signaling in the fly brain. Based on these observations with \textit{Drosophila} white and on the novel findings that show that murine ABCG1 mRNA is expressed in neural tissues, including the hind brain, dorsal root ganglia, hippocampus (54),3 retina (Figs. 4 and 6) and the epithelial cells that comprise the choroid plexus, we suggest that ABCG1 may also affect neuronal and epithelial function.

\textbf{Acknowledgment—}We thank Dr. Jim Boulter for advice on the \textit{in situ} hybridization.

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