The ABCs of sterol transport[1]

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Abstract Mammalian cells have developed various responses to minimize accumulation of unesterified cholesterol, as the latter can result in cell toxicity and death [reviewed in this edition by Björkhem (Björkhem, I. 2009. Are side-chain oxidized oxysterols regulators also in vivo? J. Lipid Res. In press)]. These responses include esterification to sequester excess sterol in intracellular lipid droplets, repression of both cholesterol synthesis and LDL receptor expression (thus reducing endocytosis of LDL), and induction of a panoply of genes that promote sterol efflux and affect lipid metabolism. The nuclear receptor liver-X-receptor (LXR) functions as a cellular "sterol sensor" and plays a critical role in these latter transcriptional changes [reviewed in this edition by Glass (Shibata, N., and Glass C, K. 2009. Regulation of macrophage function in inflammation and atherosclerosis. *J. Lipid Res.* In press)]. Activation of LXR by either endogenous oxysterols or synthetic agonists induces the expression of many genes, including those encoding ATP-binding cassette (ABC) transporters ABCA1, ABCG1, ABCG5, and ABCG8. As discussed below, these four proteins function to promote sterol efflux from cells.—Baldán, Á., D. D. Bojanic, and P. A. Edwards. **The ABCs of sterol transport. *J. Lipid Res.* 2009.** 50: **S80–S85.**

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The ABC transporters comprise a large (>250) family of transmembrane proteins that are present across all phyla (as reviewed in Ref. 1). The ABC motif spans ~120 amino acids, which contain three small noncontiguous conserved domains termed Walker A, C-loop or signature motif, and Walker B. The C-loop distinguishes ABC transporters from other ATP-binding proteins. The members of this family have been categorized into seven groups (ABC-A through ABC-G), but traditionally they were referred to as "half transporters" or "full transporters;" the former contain six transmembrane domains and one ABC motif, and the latter are a tandem repetition of the 6 + 1 structure. These proteins facilitate the transport of a wide array of substrates, including phospholipids, sterols, bile acids, peptides, and various drugs, across membrane bilayers by a process requiring the binding and hydrolysis of ATP.

ABCA1

The importance of ABCA1 was realized in 1999, 5 years after its original cloning, when it was shown that patients with Tangier disease have mutations in both copies of the gene (as reviewed in Ref. 2). These patients have little or no plasma HDL and accumulate cholesterol esters and lipids in reticuloendothelial cells. Patients with familial hypoalphalipoproteinemia are heterozygotes with one ABCA1 mutant allele and also exhibit abnormally low HDL levels (2). Intriguingly, in spite of the reduced HDL levels, these latter patients do *not* have an increased risk for cardiovascular disease (3). It is now well accepted that: *i*) ABCA1 is expressed in multiple cells and tissues (**Fig. 1**); *ii*) the gene is highly induced following activation of LXR; *iii*) ABCA1 protein localizes to both the plasma membrane and intracellular vesicles; and *iv*) ABCA1 promotes the efflux of phospholipids and cholesterol to lipid-poor apoproteins, such as apoA1, so as to generate preβ HDL (2). Besides being controlled by intracellular oxysterol levels through LXR, ABCA1 expression has also been reported to be modulated by intracellular cAMP levels, PKC-dependent phosphorylation, hormone-sensitive lipase activity, peroxisome proliferator-activated receptors, and certain fatty acids (4).

HDL, lipid efflux, and ABCA1

The exact molecular mechanism involved in the ABCA1-dependent lipid mobilization has yet to be established. Although apoA1 has been shown to bind to ABCA1 in the plasma membrane, it is unclear whether this is necessary for ABCA1-mediated lipid efflux (2). Indeed, there is con-

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[1] We dedicate this paper to Roger Davis (1945–2008), a true friend and long-time scientific colleague.

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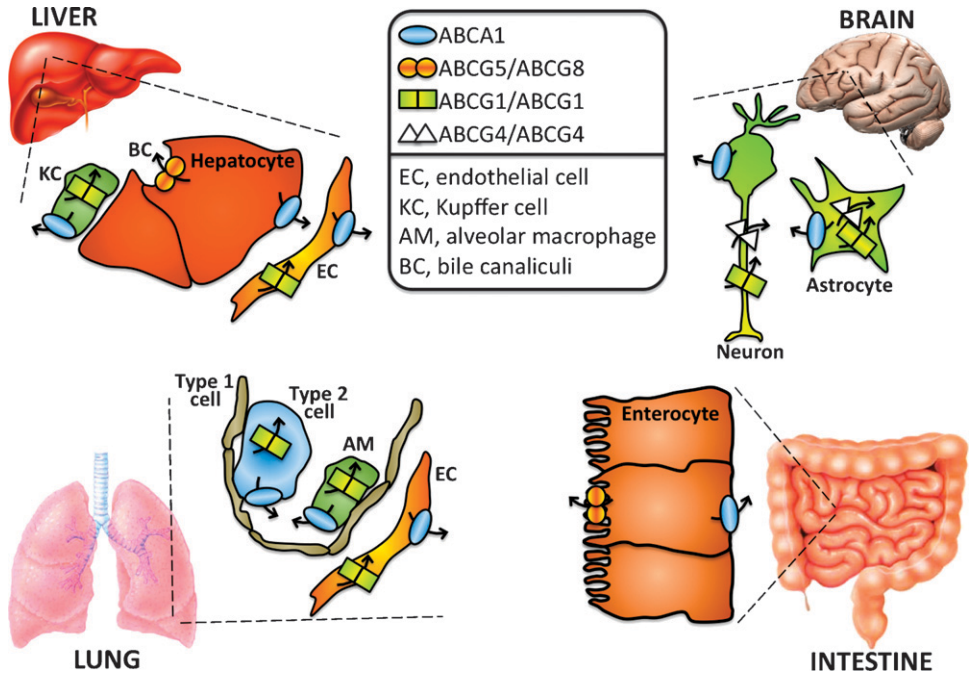

Fig. 1. ABC transporters control sterol homeostasis in a variety of tissues. Hepatic and intestinal ABCA1 play an essential role in HDL biogenesis. This transporter is also found in multiple cells where it is thought to control intracellular sterol levels. ABCG5/ABCG8 limit sterol absorption in the intestine and facilitate sterol efflux from hepatocytes into the bile. ABCG1 controls intracellular sterol levels in monocytic cells, such as Kupffer cells or alveolar macrophages. It is also highly expressed in endothelial cells, kidney epithelial cells, and the central nervous system (CNS). ABCG4 expression is highly restricted to the brain and eye, where it shares a similar function with ABCG1; whether or not ABCG1 and ABCG4 form heterodimers in these tissues remains to be elucidated.

siderable disagreement as to the physiological substrate of ABCA1: it is still not clear if ABCA1 directly transports both phospholipids *and* cholesterol to lipid-poor apoA1, or whether it effluxes phospholipids to apoA1, with cholesterol being transferred subsequently via an ABCA1-independent pathway (5). It has been proposed that ABCG1 may be important in the "secondary" cholesterol efflux process and thus function synergistically with ABCA1 (see section on ABCG1) (4). Evidence has also been presented to support a model in which ABCA1 increases cholesterol levels in the outer leaflet of the plasma membrane, thus promoting the formation of membrane buds or protrusions to which apoA1 binds before dissociating with bound lipid (6). Yet another model involves retroendocytosis of apoA1, intracellular lipidation via ABCA1, and then resecretion (4). However, a recent study raised doubts about the importance of the latter pathway (7). Regardless of the exact mechanism, the net result is that hepatic and intestinal ABCA1 contribute ~80% and ~20%, respectively, to HDL biogenesis (8, 9). Accordingly, HDL levels of *Abca1*$^{-/-}$ mice are decreased >90% (10, 11).

Macrophage ABCA1 and atherosclerosis

Consistent with a role for ABCA1 in effluxing intracellular sterols, *Abca1*$^{-/-}$ mice show evidence of cholesterol accumulation in a variety of macrophage-rich tissues including the lung, spleen, lymph nodes, thymus, and skin (10, 11). These data suggest that ABCA1 is important for macrophages to regulate sterol homeostasis. Indeed, *Abca1*$^{-/-}$ macrophages show increased free and esterified cholesterol, and enhanced inflammatory responses (12).

Despite the increase in "foamy" macrophages in several tissues, there is no corresponding increase in atherosclerosis in *Abca1*$^{-/-}$ (10, 11), *Abca1*$^{-/-}$*Ldlr*$^{-/-}$, or *Abca1*$^{-/-}$ *ApoE*$^{-/-}$ (13) mice. To explore the specific role of macrophage ABCA1 in atherosclerosis, investigators utilized bone marrow transplant studies using atherosclerotic-susceptible hyperlipidemic recipient mice; these studies showed that atherosclerotic lesions were increased ~60% or reduced ~30% when donor cells were derived from either *Abca1*$^{-/-}$ mice or transgenic ABCA1 mice, respectively (13, 14). These studies demonstrate that loss of ABCA1 from macrophages results in sterol accumulation within these cells, while plasma lipoprotein levels are unchanged.

ABCA1 and Alzheimer's disease

In addition to its role in controlling plasma HDL and macrophage sterol levels, ABCA1 also affects lipoprotein levels in the central nervous system (CNS). The cerebrospinal fluid of *Abca1*$^{-/-}$ mice contains very low levels of an abnormally poorly lipidated ApoE (see Ref. (15) and references therein). Given the association between apoE and Alzheimer's disease (AD), it was proposed that ABCA1 might impact amyloid protein deposition by modulating

the lipidation and clearance of apoE. In agreement with this proposal, deposition of brain amyloid protein in amyloid precursor protein transgenic mice was increased or decreased following either deletion or overexpression of ABCA1, respectively (15). These results suggest that ABCA1 plays a critical role in amyloidogenesis, and, consequently, it has been proposed that pharmacological intervention in the ABCA1/apoE/amyloid protein axis might prove useful in the control of AD (15).

ABCG1

Mammalian ABCG1 was identified in the mid 1990s, and shown to encode a protein of 74 kDa that has 33% amino acid identity with the *Drosophila* transporter *White* (16). Subsequent studies demonstrated that ABCG1 mRNA levels were highly induced following sterol loading of cells and/or activation of the nuclear receptor LXR (as reviewed in Ref. 17) in multiple tissues (Fig. 1). The cellular localization of ABCG1 remains to be clearly established, as it has been reported to reside in either intracellular compartments or the plasma membrane (18–20).

ABCG1 and sterol homeostasis

The initial studies demonstrated that ABCG1 could mediate the efflux of intracellular cholesterol to different exogenous lipid acceptors such as HDL, LDL, small phospholipid vesicles, apoA1-phospholipid complexes and even cyclodextrin, but not to lipid-poor apoproteins (as reviewed in Ref. 17). In contrast to ABCA1, ABCG1 does not affect phospholipid efflux. A two-step process has been proposed in which ABCA1 initially promotes lipidation of lipid-poor apo-proteins, and ABCG1 subsequently facilitates a further enrichment with cholesterol (as reviewed in Ref. 4). However, the physiological relevance of this model is unclear as, in contrast to *Abca1*$^{-/-}$ mice (10, 11), plasma lipoprotein levels are unchanged in *Abcg1*$^{-/-}$ mice (21).

Although ABCG1 is expressed at high levels in many cell types and tissues, the major phenotype of *Abcg1*$^{-/-}$ mice is an age-dependent pulmonary lipidosis that involves deposition of free and esterified cholesterol in alveolar macrophages, increased surfactant levels, and enlarged type 2 cells containing abnormal lamellar bodies (21, 22). This lipidosis is accelerated when *Abcg1*$^{-/-}$ mice are fed a high-fat, high-cholesterol diet (21–23), suggesting that dietary lipids contribute to the sterol imbalance in the lungs. We have suggested that the lung phenotype noted in *Abcg1*$^{-/-}$ mice may result from the continual cellular uptake of cholesterol-containing surfactant coupled with a defect in ABCG1-dependent sterol efflux (22). Clearly the increased expression of *Abca1* in the lungs of these mice (22) is insufficient to compensate for the loss of ABCG1.

Besides the overt lipid phenotype in the lungs, gene expression was also altered in the brains of *Abcg1*$^{-/-}$ mice: *Abca1* was increased while *Srebp-2* and *Srebp-2* target genes involved in cholesterol biosynthesis were decreased (19, 24). Conversely, overexpression of ABCG1 led to increased expression of *Srebp-2* and its targets (19, 24). These changes suggest that the brains of *Abcg1*$^{-/-}$ mice contain increased levels of oxysterols that activate LXR and repress SREBP-2 maturation and activity. Together, these results support the proposal that the function of ABCG1 is to regulate the intracellular mobilization of sterols.

ABCG1 and atherosclerosis

The observation that cholesterol ester droplets accumulate in pulmonary *Abcg1*$^{-/-}$ macrophages (21–23) suggested that loss of ABCG1 might be associated with an increased atherosclerotic burden due to increased numbers of lipid-loaded macrophages in the artery wall. However, *Abcg1*$^{-/-}$ mice are not hyperlipidemic, and thus do not develop atherosclerosis even on a Western diet (21). To circumvent this problem bone-marrow-transplant studies were performed using donor cells from *Abcg1*$^{-/-}$ or wild-type mice and recipient hyperlipidemic *Ldlr*$^{-/-}$ mice (25–27). Two studies reported a significant decrease (20–50%) in lesion size in mice transplanted with *Abcg1*$^{-/-}$ cells (26, 27) that was attributed to either accelerated apoptosis of *Abcg1*$^{-/-}$ macrophages (26), or to increased secretion of athero-protective apoE by the *Abcg1*$^{-/-}$ macrophages (27). The relative importance of apoptosis versus apoE secretion in providing lesion protection in this model remains to be determined. In contrast, a third paper reported a "moderately significant" increase in lesion size in the *Ldlr*$^{-/-}$ mice receiving bone marrow from *Abcg1*$^{-/-}$ mice (25). The reasons for these variable results remain obscure.

Based on the proposed role of ABCG1 in facilitating cholesterol efflux from macrophages, it seemed likely that transgenic ABCG1 mice would be protected from diet-induced atherosclerosis. Thus, the finding that overexpression of ABCG1 in *Ldlr*$^{-/-}$ (28) or *ApoE*$^{-/-}$ (29) mice either moderately increased or had no effect on lesion size, respectively, is perplexing. Additional studies will be necessary to determine the role of ABCG1 in vivo.

Recently, *Abca1*$^{-/-}$*Abcg1*$^{-/-}$ mice were generated to better understand the functional relationship between these two lipid transporters (30, 31). These double knock-out mice exhibited massive neutral lipid deposition in multiple tissues (30, 31). Due to the fact that these mice are not hyperlipidemic, the investigators again turned to bone marrow transplantation into *Ldlr*$^{-/-}$ mice. Contradictory results were reported as lesions from mice receiving donor *Abca1*$^{-/-}$*Abcg1*$^{-/-}$ cells were either smaller (30) or larger (31), as compared with those receiving *Abca1*$^{-/-}$ donor cells. Perhaps these conflicting results arise because of different genetic backgrounds of the double knock-out mice? Nevertheless, and in light of these inconsistencies, the role of ABCG1 in lesion development remains controversial.

ABCG1 and apoptosis

It has been proposed that increased (oxy)sterols in *Abcg1*$^{-/-}$ macrophages promote apoptosis (26, 32). Support for this idea comes from studies showing that *i*) the lungs of *Abcg1*$^{-/-}$ mice contain increased numbers of apoptotic cells (33, 34); *ii*) *Abcg1*$^{-/-}$ cells contain high levels of oxysterols (32); *iii*) addition of ox-LDL, 7-ketocholesterol or

7β-hydroxycholesterol to *Abcg1*$^{-/-}$ primary macrophages increased apoptosis, as compared with wild-type cells (26, 31, 32); and *iv*) overexpression of ABCG1 in cultured cells promotes the efflux of cholesterol and specific oxysterols (32). At the current time, the mechanism by which these sterols promote apoptosis is unknown.

ABCG1 and inflammation

Recent reports indicate that the lungs of *Abcg1*$^{-/-}$ mice show signs of chronic inflammation that include accumulation of activated macrophages, lymphocytic infiltration, elevated levels of cytokines and cytokine receptors, and chitinase-like crystals (33, 34). Peritoneal macrophages from *Abcg1*$^{-/-}$ mice also exhibit increased cytokine expression (26, 31). These changes are likely a response to the altered levels of intracellular (oxy)sterols in the *Abcg1*$^{-/-}$ cells.

ABCG1 and diabetes

Macrophages obtained from *db/db* and *KK*^{ay} diabetic mice, and from patients with type 2 diabetes, were reported to express very low levels of ABCG1, contain excess cholesteryl esters, and exhibit impaired efflux of cholesterol to HDL or serum (35, 36). Based on these observations, it was proposed that down-regulation of ABCG1 in macrophages could promote the formation of foam cells in the diabetic vasculature.

ABCG1 and cell proliferation

Recently, Bensinger et al. (37) demonstrated that activation and subsequent proliferation of lymphocyte T cells led to a rapid induction of the oxysterol-metabolizing enzyme SULT2B1, suppression of ABCG1 and ABCA1, and induction of SREBP target genes. T-cell proliferation was greatly attenuated when wild-type or *Abca1*$^{-/-}$ cells, but not *Abcg1*$^{-/-}$ cells, were treated with a potent LXR agonist. These data suggest that lymphocytic proliferation is dependent on *i*) the metabolism/inactivation of oxysterol ligands for LXR by SULT2B1, and *ii*) the specific repression of ABCG1 (37). According to this proposal, specific intracellular sterols, as yet to be identified, function to signal cell division.

ABCG4

ABCG4 is highly expressed in the brain and the eye (38) (Fig. 1). Like ABCG1, overexpression of ABCG4 in cultured cells stimulates the efflux of cellular cholesterol to HDL but not to lipid-poor apoA1 (39). However, in contrast to ABCG1, recent studies have shown that ABCG4 expression is highly restricted to astrocytes and neurons of the CNS, is absent from macrophages, and the gene is unresponsive to LXR activation (19, 40). Interestingly, epitope-tagged ABCG4 and ABCG1 colocalize in intracellular vesicles of astrocytes and neurons where they appear to function in the transfer of endogenous sterols away from the endoplasmic reticulum (19).

Based on in situ hybridization and immunostaining of tissues, it was reported that ABCG4 is highly expressed in microglial cells that were closely located to senile plaques in the brains of patients with AD (41). These authors suggested that ABCG4 may suppress the development or progression of AD through increased apoE lipidation and attenuated neurotoxicity of apoE (41). To date, our analysis of the brains of *Abcg4*$^{-/-}$ mice have failed to detect evidence of pathological changes (unpublished data). However, it is possible that crossing *Abcg4*$^{-/-}$ mice with models of AD may provide additional insight into the role of ABCG4 in the CNS.

Recently, *Abcg1*$^{-/-}$*Abcg4*$^{-/-}$ mice were generated, and the brains of these mice shown to contain elevated levels of desmosterol, lathosterol, and lanosterol (intermediates in the cholesterol biosynthetic pathway), as well as 27-hydroxycholesterol (40). In addition, combined deficiency of these two transporters resulted in impaired efflux of desmosterol and cholesterol to HDL and the accumulation of these sterols in primary astrocytes (40). It was suggested that ABCG4 and ABCG1 share overlapping functions in astrocytes by facilitating the efflux of cholesterol and desmosterol to HDL-like lipoproteins. Future studies are likely to determine the precise role of ABCG4 and ABCG1 in lipid homeostasis in the CNS and in neurological disease.

ABCG5 AND ABCG8

The accumulation of a number of plant sterols, including β-sitosterol, in the plasma of two sisters was initially reported in 1974 and the disease termed β-sitosterolemia (as reviewed in Ref. 42). Patients with this disease exhibit significant increases in plant sterols in both plasma and tissues, and may present with various other abnormalities including deposition of sterols in the skin (xanthomas) and coronary arteries, the latter increasing the risk of premature atherosclerosis (42). This rare autosomal recessive disorder results from mutations in either ABCG5 or ABCG8 (43). Surprisingly, these genes are arranged head-to-head, separated by approximately 140 bp, and are coordinately expressed and induced following activation of LXR (43). ABCG5 and ABCG8 are half transporters that form obligate heterodimers that localize to *i*) apical membranes of intestinal enterocytes, where they function to limit absorption of plant and shellfish sterols; and *ii*) canalicular membranes of hepatocytes, promoting biliary excretion of sterols (44, 45) (Fig. 1).

The lipid abnormalities associated with β-sitosterolemia are reproduced in *Abcg5*$^{-/-}$ or *Abcg8*$^{-/-}$ mice, thus providing an excellent model system for study of this disease (44). Conversely, transgenic mice showed enhanced secretion of biliary cholesterol and reduced absorption of dietary sterols, providing additional support for the crucial role of these proteins in mammals (45).

In a series of studies Hobbs, Cohen, et al. (see Ref. (46) and references therein) demonstrated that ABCG5 and ABCG8 proteins are glycosylated and that heterodimerization is required for mobilization to the cell surface. Mutation studies identified the Walker A and Walker B motifs of ABCG5 and the Signature motif of ABCG8 as being impor-

tant for sterol transport, whereas mutations of Walker A or B motifs of ABCG8 or the Signature motif of ABCG5 had a small effect (46). Studies using recombinant protein or endogenous ABCG5/ABCG8 purified from mouse livers showed that the heterodimer transported both cholesterol and sitosterol (46), consistent with the accumulation of both sterols in *Abcg5*$^{-/-}$ and *Abcg8*$^{-/-}$ mice and patients with sitosterolemia. The finding that ATP hydrolysis by ABCG5/ABCG8 was stimulated in vitro by various androgen hormones and analogs, but not by cholesterol or sitosterol (47), is perplexing as loss of ABCG5/ABCG8 is not linked to changes in androgens in vivo.

Consistent with the proposed role of these transporters in sterol homeostasis, atherosclerotic lesions were smaller in *Ldlr*$^{-/-}$ mice overexpressing ABCG5/ABCG8, compared with *Ldlr*$^{-/-}$ control animals (48). In contrast, liver-specific transgenic mice had reduced lesions only when fed Ezetimibe (a synthetic NPC1L1 inhibitor) suggesting that intestinal sterol absorption compensates for the athero-protective effects of hepatic ABCG5/ABCG8 (49). This hypothesis is supported by the recent finding that loss of NPC1L1 reverts the sitosterolemic phenotype of *Abcg5*$^{-/-}$ *Abcg8*$^{-/-}$ mice (50). Collectively, these studies illustrate how intestinal and hepatic ABCG5/ABCG8 expression controls different aspects of sterol homeostasis.

CONCLUSION

Multiple genes and pathways are involved in maintaining adequate intracellular sterol levels. These genes include, but are not limited to, the ABC transporters discussed above and other membrane transporters such as NPC1L1, NPC1, NPC2, LDLR, and SR-B1, plus the transcription factors SREBP-2 and LXR. Functional mutations and/or altered expression of these proteins result in abnormal sterol homeostasis that can potentially lead to systemic effects that include hyperlipidemia and atherosclerosis. Future studies will help us better understand the precise mechanisms by which these many proteins orchestrate cellular sterol homeostasis.

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