thematic review

Role of ABCG1 and other ABCG family members in lipid metabolism

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Abstract **The molecular cloning and identification of mutations in ATP-binding cassette transporters in hereditary diseases have greatly expanded our knowledge of the normal physiology of intracellular lipid transport processes. In addition to the well-known ATP-binding cassette transporter A1 (ABCA1) molecule, ABC transporters belonging to the ABCG (White) subfamily (ABCG1, ABCG5, and ABCG8) have been shown to be critically involved in the regulation of lipid-trafficking mechanisms in macrophages, hepatocytes, and intestinal mucosa cells. ABCG1, the product of a sterol-induced gene, participates in cholesterol and phospholipid efflux. The ABCG5 and ABCG8 transporters, defective in β-sitosterolemia, are also now considered interesting targets in the control and influence of total body sterol homeostasis. In this review, advances referring to the regulation and function of ABCG half-size transporters are summarized and discussed. In addition, new implications for the transcriptional control, as well as the intracellular routing and localization, of these proteins are presented.** —Schmitz. G., T. Langmann, and S. Heimerl. **Role of ABCG1 and other ABCG family members in lipid metabolism.** ***J. Lipid Res.*** **2001.** 42: **1513–1520.**

Supplementary key words adaptor proteins • *Drosophila* ABC transporters • gene regulation • macrophage • β-sitosterolemia • vesicular transport

ABCG (WHITE) SUBFAMILY OF ATP-BINDING CASSETTE TRANSPORTERS

According to the well-established ATP-binding cassette (ABC) transporter homepage (http://nutrigene.4t.com/humanabc.htm), the total number of human ABC proteins now finally comprises 51 different genes. The ABCG or White subfamily with its five fully characterized human members consists of half-size ABC proteins that are intended to dimerize to form active membrane transporters (**Fig. 1**). Among the half-size molecules ABCG proteins have a peculiar domain organization characterized by a nucleotide-binding domain (ATP-binding cassette) at the N terminus followed by six transmembrane-spanning domains (1). The founding member of this group, ABCG1, was independently described by Chen et al. (2) and Croop et al. (3) as the human homolog of the *Drosophila white* gene, and its genomic organization including the promoter region has been described (4, 5). Various transcripts of ABCG1 have been detected in different cells arising from alternative splicing events or the use of different transcription initiation sites (5). Interestingly, the 12-amino acid linker region between the ATP-binding cassette and the transmembrane region is also subject to alternative splicing generating two major protein forms of ABCG1 (Fig. 1) (2). Earlier indications linked ABCG1 with the congenital recessive deafness (DFNB10) syndrome, using its chromosomal localization on chromosome 21q22.3 (6). However, a more recent report (7) has excluded ABCG1 along with five other known genes as candidates for DFNB10. Also, conflicting data exist concerning whether the G2457A polymorphism in the 3′ untranslated region of ABCG1 mRNA is associated with mood and panic disorders and related to suicidal behavior (8, 9). The second well-known member of the ABCG subfamily, ABCG2 (Fig. 1), has been identified by different approaches and is known as placenta-specific ABC (10), breast cancer resistance protein (11), and mitoxantrone resistance-associated protein (12). The protein has been shown to be amplified and overexpressed in human cancer cells and is capable of mediating drug resistance even in the absence of the classic multidrug resistance (MDR) proteins MDR1 and MRP1 (12–14). Most interestingly, new evidence of the function of ABCG2 as a direct drug efflux pump is provided by data localizing the bulk of ABCG2 protein to the plasma membrane, with a minor fraction found within intracellular membranes (15). It was only a short time ago when two other ABCG transporters, ABCG5 and ABCG8, had been identified and linked to the human disease β-sitosterolemia by two independent approaches (16, 17). In addition to these five fully cloned ABCG

Abbreviations: ABCG1, ATP-binding cassette transporter G1; acLDL, acetylated low density lipoprotein; AP, adaptor protein; apoE, apolipoprotein E; HDL_3, high density lipoprotein class 3; HPS, Hermansky-Pudlak syndrome; LXR, liver X receptor; MDR, multidrug resistance protein; RXR, retinoid X receptor; Usp, ultraspiracle.

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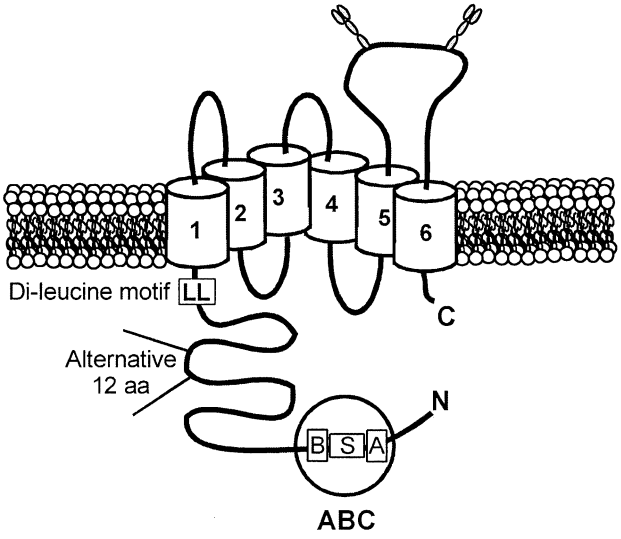

Gene	Synonyms	Chrom.	Disease	Tissue	Substrates	Induction by Sterols	Influence on Sterol Absorption
ABCG1	ABC8, White	21q22.3	-	Ubiquitous	Sterols, Lipids	⇑⇑	?
ABCG2	ABCP, BCRP1, MXR1,	4q22	MDR	Placenta, Breast, Liver	Chemoth. Drugs	-	-
ABCG3	-	8p12	-	-	-	-	-
ABCG4	White 2	11q23	-	-	-	-	-
ABCG5	White 3, Sterolin 1	2p21	β-Sitoste-rolemia	Small Intestine, Liver	Sterols	⇑⇑	⇓⇓
ABCG6	-	7	-	-	-	-	-
ABCG7	-	15	-	-	-	-	-
ABCG8	White 4, Sterolin 2	2p21	β-Sitoste-rolemia	Small Intestine, Liver	Sterols	⇑⇑	⇓⇓

Fig. 1. The ABCG (White) subfamily. Top: Domain organization of ABCG1, the prototypic member of ABCG subfamily half-size transporters. A large intracellular domain containing the ATP-binding cassette (ABC) with its Walker A (A), Walker B (B), and signature motif (S) is followed by six transmembrane segments. The location of an alternative dodecapeptide resulting from alternative splicing is indicated. Bottom: Compendium of ABCG subfamily members

proteins, four other family members, namely, ABCG3, ABCG4, ABCG6, and ABCG7 (Fig. 1), have been identified by expressed sequence tag database search analysis (10, 18) but await further analysis. In summary, the complete subgroup of ABCG transporters now comprises eight proteins.

REGULATION AND FUNCTION OF ABCG1 (ABC8, HUMAN WHITE)

After its cloning in 1996 (2), it took 4 years until ABCG1 attracted great attention because of its striking similarities to ABCA1 in the expression pattern in monocytic cells. Using a differential display approach along with a complete survey of what was known about ABC transporters, our laboratory was able to identify ABCG1 as a target gene involved in macrophage lipid homeostasis (19). Like ABCA1, the first ABC transporter shown to be regulated in macrophages (20), ABCG1 is upregulated during the differentiation process of monocytes into mature macrophages and is strongly induced by foam cell conversion of these macrophages under sterol-loading conditions using acetylated LDL (acLDL). Conversely, cholesterol-unloading conditions achieved by further incubation with HDL class 3 (HDL_3) as the cholesterol acceptor results in the suppression of ABCG1 mRNA and protein expression (19). In the meantime, these results

have been confirmed by other groups as well (21, 22). The observed upregulation of ABCG1 is not restricted to acLDL but is also operative when using other types of modified LDL, such as oxidized LDL (23) or enzymatically modified LDL (E-LDL) (G. Schmitz, T. Langmann, and S. Heimerl, unpublished observation), but not with free cholesterol or native LDL (21). Of special interest is the finding that ABCG1 regulation by lipids occurs exclusively in human or murine monomyeloid cells, such as primary human macrophages (19), THP-1 cells (23), RAW246.7 cells, peritoneal macrophages (21), and foam cells of atherosclerotic lesions (22). The sterol-sensitive induction seen in these cells is independent of proinflammatory stimuli and the oxidative state of the cell as treatment with tumor necrosis factor α or lipopolysaccharide has no impact on ABCG1 mRNA expression (21). In addition to lipoprotein-derived lipids, some oxysterols and retinoid X receptor (RXR)-specific ligands upregulate ABCG1 expression via the liver X receptor (LXR)/RXR pathway. Evidence of a significant role of these nuclear receptors in ABCG1 induction comes from two different types of experiments. First, macrophages devoid of LXRα and LXRβ fail to upregulate ABCG1 mRNA on oxysterol treatment (21), and second, retroviral expression of LXRα in RAW246.7 cells facilitates the induction of ABCG1 in response to LXR and LXR ligands (24). However, these findings pose several questions. The first question is how these bioactive oxysterols are generated and whether they are of physiological significance. One hypothesis implies that oxysterols are constituents of modified LDL, whereas a second explanation argues that sterols are oxidized intracellularly after their uptake via scavenger receptors. Clearly, these different possibilities remain to be explored. The second important question involves the transcriptional regulation of the ABCG1 gene by LXR/RXR. A first characterization of the ABCG1 promoter demonstrated its functionality and elucidated the minimal promoter region required for liver- and macrophage-specific expression of the gene (4). Although these regulatory regions and a more recently identified alternative promoter (5) contain putative LXR-responsive elements, no significant induction by oxysterols even in combination with overexpression of LXR could be achieved in promoter assays (T. Langmann, unpublished observations). In addition to these activating, sterol-regulated pathways, an independent inhibitory mechanism involving the transcriptional repressor zinc finger transcription factor 202 has been described (25). This factor regulates a number of genes involved in general lipid metabolism (26) and in particular has been shown to bind the apolipoprotein E (apoE), ABCA1, and ABCG1 promoters and thereby to modulate cellular lipid efflux (25).

Although the remarkable regulation of ABCG1 gene expression by cellular lipid components revealed its importance in macrophage lipid metabolism, direct evidence of a functional role in lipid trafficking came from an antisense strategy to block ABCG1 expression (19). Specific antisense oligonucleotides that had no effect on ABCA1 levels caused a 32% and a 25% reduction in macrophage cholesterol and phospholipid efflux, respectively, thereby directly linking ABCG1 with cellular lipid trafficking. Because the same ABCG1 antisense oligonucleotides also lead to a significant inhibition of apoE secretion, the pathways involving ABCG1 seem to be at least in part distinct from acceptor-mediated lipid efflux (27). Also, the residual phospholipid and cholesterol efflux present in cells from patients with Tangier disease along with a compensatory upregulation of ABCG1 in these cells (22) further supports a function of ABCG1 in intracellular mobilization of lipid stores. First steps to elucidate the localization of ABCG1 showed that the protein is predominantly localized in intracellular compartments mainly associated with the endoplasmic reticulum (ER) and Golgi membranes (19, 24). The small fraction of ABCG1 surface staining detected in immunocytochemical analysis is presumably due to unspecific binding of polyclonal ABCG1 antibodies to the macrophage receptor (22), as an ABCG1-green fluorescent protein fusion protein is absent from the plasma membrane (24). There is still a lack of knowledge regarding the question concerning whether ABCG1 functions as a heterodimer or homodimer. Both forms are conceivable for ABCG1 because both cases have been described within the subfamily, for example, ABCG2 acts as homodimer, whereas ABCG5 and ABCG8 most likely cooperate as heterodimers.

NEW PERSPECTIVES FOR ABCG1: LESSONS FROM *DROSOPHILA* PROTEINS

To develop new ideas about the function of ABCG1 we combined the limited information available about the mammalian system with data concerning the well-characterized ABC transporters from *Drosophila*. A striking similarity between human ABCG1 and *Drosophila* homolog E23 is their transcriptional regulation by nuclear hormone receptors (28). As depicted in **Fig. 2A**, the metamorphosis-regulating steroid hormone 20-hydroxyecdysone binds its receptor (EcR), which heterodimerizes with Ultraspiracle (Usp, product of the *Drosophila* RXR gene) and after nuclear localization activates E23 gene transcription. The findings that ectopic expression of E23 protein suppressed ecdysone-induced larval development revealed that E23 reduces the effective concentration of ecdysone within the cell, most likely by mediating its export and inactivation. They also suggest that E23 acts as a homodimer; however, no data are available concerning whether E23 is located only in intracellular membranes or also on the plasma membrane. The question concerning the cellular localization of half-size ABC transporters has been addressed for the *Drosophila* White and Scarlet proteins. These ABC transporters are involved in the formation of eye color pigments and it has been generally assumed that they function in the plasma membrane to facilitate the uptake of pigment precursors. However, in an elegant study using immunogold labeling and electron microscopy, Mackenzie et al. (29) found the White and Scarlet proteins in intracellular membranes surrounding the pigment granules in pigment cells (Fig. 2B) and not in the plasma membrane.

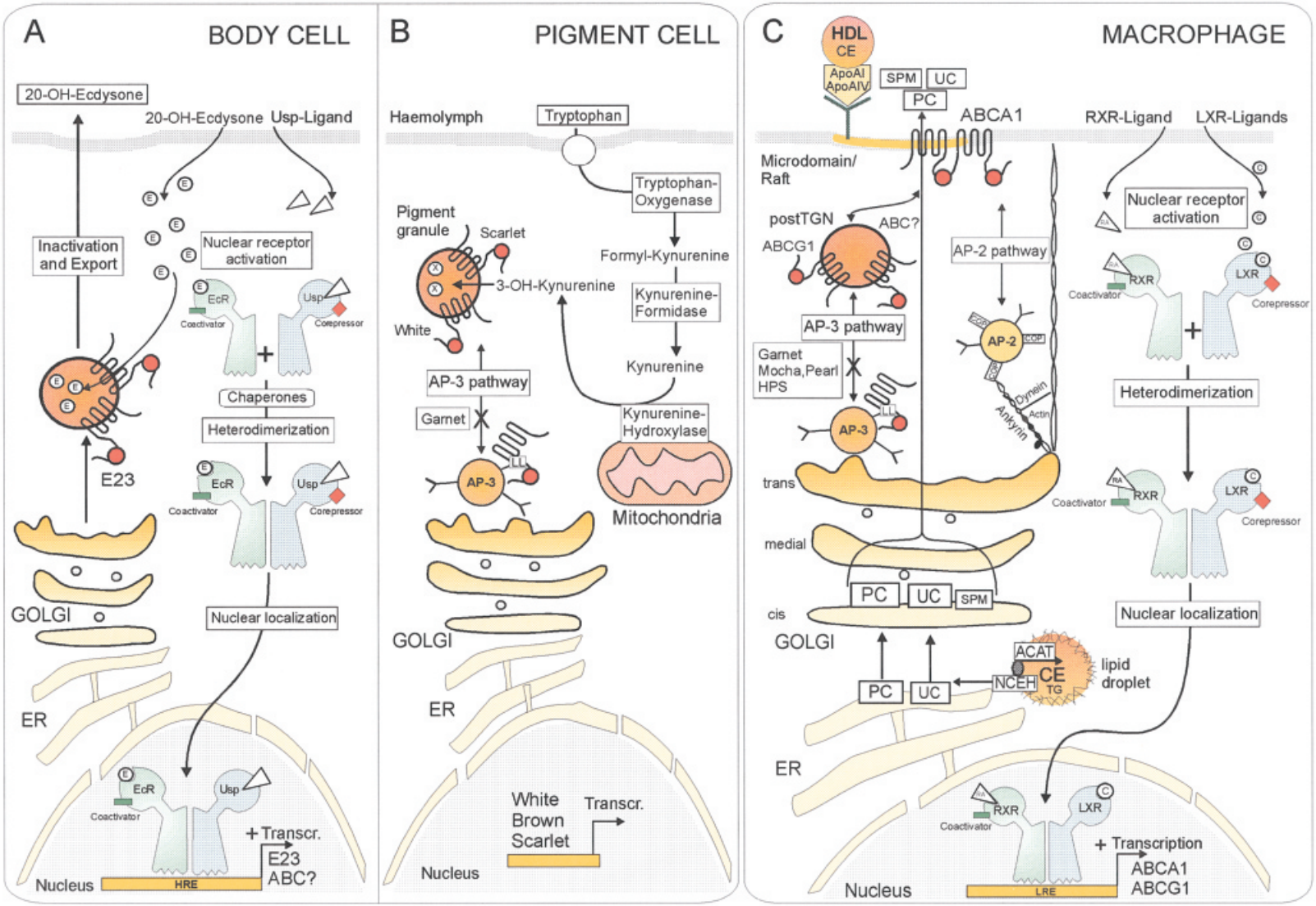

Fig. 2. Regulation, localization, and function of *Drosophila* and human ABCG proteins. A: 20-Hydroxyecdysone (20-OH-ecdysone)-induced expression of the *Drosophila* E23 ABC transporter via EcR/Usp-mediated transcriptional activation. Upregulation of E23 leads to a reduction of intracellular 20-hydroxyecdysone, most likely by inactivation or export mechanisms. B: *Drosophila* White and Scarlet proteins are located within pigment granule membranes to facilitate the uptake of the pigment precursor 3-hydroxykynurenine. These ABC transporters contain dileucine motifs, which are required for the AP-3-mediated intracellular targeting to specialized compartments such as granule vesicles. C: Model for the role of human ABCG1 in cellular lipid trafficking in macrophages. Uptake of sterols causes LXR/RXR-dependent transcriptional activation of the ABCG1 and ABCA1 genes. ABCA1, which resides in the plasma membrane, is targeted via AP-2-controlled mechanisms, whereas ABCG1 is routed to the *trans*-Golgi network compartment, presumably by dileucine-based AP-3-mediated vesicular transport. ABCG1 may function either as a homodimer or together with a second half-size transporter to translocate lipids to the plasma membrane, where ABCA1 facilitates further efflux from specialized membrane microdomains. Abbreviations not defined in text: C, cholesterol; COP, coatomer protein; E, Ecdysone; HRE, hormone response element; NCEH, neutral cholesteryl ester hydrolase; PC, phosphatidylcholine; RA, retinoic acid; SPM, sphingomyelin; TGN, *trans*-Golgi network; UC, unesterified cholesterol.

This suggests that White and Scarlet are not primarily involved in the cellular uptake of tryptophan from the hemolymph but, rather, in transport of the metabolic intermediate 3-hydroxykynurenine from the cytoplasm into the pigment granules (Fig. 2B). Interestingly, the *garnet* gene product, which also modulates eye color biogenesis of *Drosophila*, shows strong homology with the δ subunit of the human adaptor protein 3 (AP-3) complex (30, 31), which is mainly associated with the *trans*-Golgi network and other peripheral membranes (31). The adaptor complex is involved in intracellular targeting of proteins to specialized vesicular compartments and these proteins often harbor dileucine motifs, which are recognized by the AP-3 complex (32, 33). In addition to *garnet* in *Drosophila*, mutations in the subunits of AP-3 also produce the mouse coat color mutations Mocha and Pearl (34) and are responsible for the defect in a subset of patients with Hermansky-Pudlak syndrome (HPS) (35). HPS consists of a group of several genetically different disorders involving proteins required for the formation, trafficking, and fusion of intracellular vesicles (36). A highly informative alignment of White, Brown, and Scarlet from *Drosophila* (29) and human ABCG proteins (**Table 1**) based on a CLUSTAL analysis reveals a conserved region near the first membrane domain containing a putative dileucine motif. This finding raises the possibility that ABCG1 and other family members may interact with AP-3 in order to reach their subcellular compartment. From the data available for human ABCG1 and based on the regulation (Fig. 2A) and localization (Fig. 2B) of homologous *Drosophila* ABC transporters, we propose a new model for the role of ABCG1 in the cellular trafficking of lipids in macrophages (Fig. 2C). According to this model, uptake of sterols (LXR ligands) and concomitant activation of the nuclear receptor pathway via LXR/RXR causes transcriptional induction of ABCG1 and ABCA1. After its synthesis ABCG1 is located to specialized intracellular compartments associated with the *trans*-Golgi network via targeting mechanisms involving the AP-3 complex and a dileucine signaling motif in the N-terminal region of the ABC transporter. Within these compartments ABCG1 may function as either a homodimer or a heterodimer with an as yet identified ABCG family member in order to facilitate the translocation of phospholipids and cholesterol to the plasma membrane where ABCA1-facilitated efflux mechanisms are active in association with specialized lipid microdomains. Alternatively, ABCG1 could participate in ABCA1-independent efflux pathways, for example, via apoE or passive diffusion mechanisms.

TABLE 1. Alignment of putative dileucine signals in human and Drosophila ABCG proteins

Protein	Dileucine Signal	Accession Number
ABCG1	NH_2--IMRDS **VL** TH--- 3aa-TM	P45844
ABCG2	NH_2--RSFKN **LL** GN--- 4aa-TM	Q9UNQ0
ABCG4	NH_2--ILRDT **VL** TH--- 3aa-TM	-
ABCG5	NH_2--SKLGV **LL** RR---15aa-TM	AAG53099
ABCG8	NH_2--QQFTT **LI** RR---13aa-TM	AAG40004
dWhite	NH_2--VLKEP **LL** VK--- 3aa-TM	P10090
dBrown	NH_2--IYQVY **LL** MV---17aa-TM	P12428
dScarlet	NH_2--RASLT **LL** RD--- 8aa-TM	P45843

CLUSTAL analysis of human and *Drosophila* ABCG proteins displaying conserved dileucine signal motifs. The putative dileucine motifs are in boldface and boxed. The distance to the first transmembrane (TM) domain is given by numbers. SwissProt accession numbers are listed except for ABCG4, the peptide sequence of which was derived from nucleotide database analysis and subsequent translation.

ROLE OF ABCG5 AND ABCG8 IN DIETARY STEROL ABSORPTION

In addition to the above-described lipid efflux pathways operative in macrophages, two other members of the ABCG subfamily, namely ABCG5 and ABCG8 (Fig. 1), have been implicated in the efflux of dietary sterols from intestinal epithelial cells back into the gut lumen and from the liver to the bile duct (**Fig. 3**). Sterols in a normal Western diet usually consist of cholesterol (250–500 mg) and noncholesterol sterols (200–400 mg), mainly plant sterols such as sitosterol and also sterols from fish. In healthy individuals approximately 50–60% of the cholesterol is absorbed and retained, whereas the retention of noncholesterol sterols is less than 1% (37, 38). These subtle mechanisms are disrupted in β-sitosterolemia, also known as phytosterolemia or shellfish sterolemia, a rare autosomal recessive disorder first described by Bhattacharyya and Connor in 1974 (39). The disease is characterized by enhanced trapping of cholesterol and other sterols, including plant and shellfish sterols, within the intestinal cells and the inability to concentrate these sterols in the bile. As a consequence affected individuals have strongly increased plasma levels of plant sterols, for example, β-sitosterol, campesterol, stigmasterol, avenosterol, and 5α-saturated stanols, whereas total sterol levels remain normal or are just moderately elevated (40, 41). Another biochemical feature of β-sitosterolemia is reduced cholesterol synthesis due to a lack of HMG-CoA reductase (41). Despite the almost normal total plasma sterol levels, the disease shares several clinical characteristics with homozygous familial hypercholesterolemia. Patients suffer from tendon and tuberous xanthomas at an early age, premature development of atherosclerosis, and coronary artery disease. In some cases hemolytic episodes, hypersplenism, platelet abnormalities, arthralgias, and arthritis have been described (42).

In 1998 Patel et al. (43) managed to localize the β-sitosterolemia locus to chromosome 2p21 and fine mapping narrowed the location of the gene to within a 2-cM region between markers D2S2294 and Afm210ex9 (44). Using a combination of positional cloning and genome database survey, Lee et al. (17) identified ABCG5, which was mutated in nine unrelated β-sitosterolemia patients. Almost at the same time, Berge et al. (16) used a microarray analysis to search for LXR-regulated genes and identified ABCG5. Because ABC transporters are often found in clusters the group screened nearby regions and found a second new member of the ABCG subfamily, ABCG8, which displayed 61% sequence similarity and was also mu-

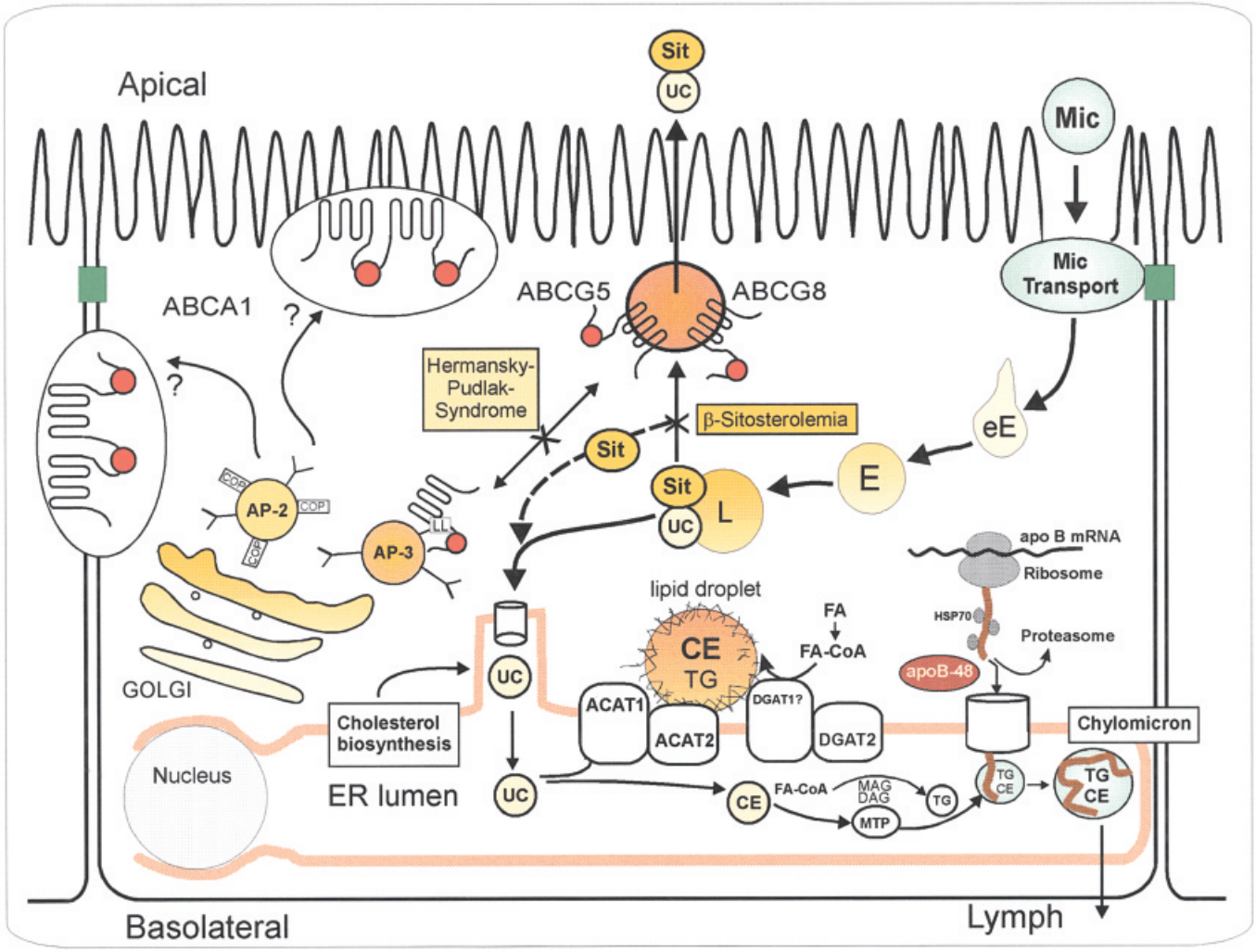

Fig. 3. Role of ABCG and ABCA proteins in intestinal sterol metabolism. ABCG5, ABCG8, and ABCA1 are sterol-induced members of the ABC transporter family. ABCG5 and ABCG8, which are mutated in sitosterolemia, form a heterodimer to mediate the export of absorbed plant sterols and cholesterol into the gut lumen. In contrast, ABCA1 expression and function are required for the uptake of sterols into intestinal epithelial cells. Implications for the intracellular location and vesicular trafficking of these proteins are presented. Abbreviations not defined in text: CE, cholesteryl ester; DAG, diacylglyceride; DGAT, acyl CoA:diacylglycerol transferase; HSP70, heat shock protein 70; L, lysosome; MAG, monoacylglyceride; Mic, micelle; MTP, microsomal transfer protein; Sit, sitosterol.

tated in sitosterolemia patients. The fact that the translational start sites of both ABC transporter genes are separated by only 374 bp and are arranged in a head-to-head orientation led to the assumption that ABCG5 and ABCG8 have a bidirectional promoter and share common regulatory elements (16); however, no functional promoter data have been provided so far. The highest expression level of both transporters is found in liver and intestine and high cholesterol diet feeding in mice induced the expression of both genes (16). These findings together with the observed clinical and biochemical features of β-sitosterolemia patients suggest that ABCG5 and ABCG8 play an important role in reducing intestinal absorption and promote biliary excretion of sterols. Until today several mutations and a number of polymorphisms have been identified in ABCG5 and ABCG8 (16, 17, 45). Interestingly, sequence analysis of both genes showed that the majority of patients analyzed were homozygous for a single mutation and that the total number of different mutations is low (45). This strongly suggests that sitosterolemia has its origin in a limited number of founder individuals. Another striking finding is that mutations in β-sitosterolemia patients occur exclusively either in ABCG5 or ABCG8, but never in both genes together [see refs. (16) and (17); and S. Heimerl, T. Langmann, U. Beil, K. Von Bergmann, H. Kather, M. Dean, and G. Schmitz, unpublished observations]. The coordinate regulation of both genes and the finding that mutations in either gene cause β-sitosterolemia strongly suggest that the ABCG5 and ABCG8 proteins form a functional heterodimer. As depicted in Fig. 3, dietary sterols including cholesterol and plant sterols, which enter the intestinal epithelial cells via micellar transport, are released along the lysosomal route. β-Sitosterol and other plant sterols are directly transported back to the gut lumen by the heterodimeric ABCG5-ABCG8 complex by means of a sort of kickback mechanism, which may also efflux cholesterol, thereby regulating total sterol absorption. The retained sterols are routed along the ACAT

pathway in the ER and either stored as cholesteryl esters in lipid droplets or alternatively packed into chylomicrons for further transport back to the liver (Fig. 3). In the liver alternative processes are conceivable. The sterols are either transported to peripheral tissues by VLDL and LDL particles or converted to bile acids. Also, a direct track into the bile duct for excretion exists, possibly mediated by ABCG5 and ABCG8. In addition to ABCG5 and ABCG8, other ABC transporters including ABCG1 and ABCA1 may also participate in intestinal sterol absorption mechanisms. Although the intracellular localization of ABCA1 has not been demonstrated so far, data from ABCA1$^{-/-}$ mice strongly suggest that ABCA1 is involved in the absorption of cholesterol and in the uptake of lipophilic vitamins (46, 47). In this respect, it will be of special interest to determine in which membrane compartment, the apical or the basolateral part of intestinal epithelial cells, the ABCA1 molecule is located.

CONCLUSIONS

Although our knowledge of the regulation of lipid-transporting human ABC transporters has grown substantially, the molecular mechanisms controlling the intracellular localization, substrate specificity, and functional activity of these proteins are still poorly characterized. By means of a detailed comparison of human ABCG proteins with homologs from various other species and within the ABCG subgroup, we have identified striking similarities in the regulation, intracellular routing, and function of these molecules, even though the nature of the translocated substrates may be different. We propose that further analysis of the conserved proteins presented in this review could significantly help to improve our understanding of the complex network of ABC transporters in transmembrane lipid transport and its relation to human disease.

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