thematic review

Regulation and intracellular trafficking of the ABCA1 transporter

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Abstract **The discovery of the role of the ATP-binding cassette transporter A1 (ABCA1) in mediating apolipoprotein A-I-mediated efflux has led to a dramatic increase in our knowledge of the molecular mechanisms involved in cholesterol efflux and cellular metabolism. In this review, we discuss several aspects of ABCA1 regulation including *i*) transcriptional regulation, *ii*) substrate specificity and availability, *iii*) accessory proteins, *iv*) acceptor specificity and availability, and *v*) protein trafficking. The majority of studies of ABCA1 regulation to date have focused on the identification of promoter elements that determine ABCA1 gene transcription. Here we also review the potential functional role of ABCA1 in reverse cholesterol transport. Given the key role that ABCA1 plays in cholesterol homeostasis, it is likely that there are multiple mechanisms for controlling the overall transporter activity of ABCA1.**—Santamarina-Fojo, S., A. T. Remaley, E. B. Neufeld, and H. B. Brewer, Jr. **Regulation and intracellular trafficking of the ABCA1 transporter.** ***J. Lipid Res.*** **2001.** 42: **1339–1345.**

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TRANSCRIPTIONAL REGULATION OF ABCA1 GENE EXPRESSION

Regulation of ABCA1 expression

The identification of ATP-binding cassette transporter A1 (ABCA1) as the key transporter facilitating cellular cholesterol efflux initiated studies establishing that ABCA1 gene transcription is highly regulated and metabolites, including cAMP, sterols, *cis*-retinoic acid, peroxisome proliferator-activated receptor (PPAR) agonists, and interferon γ (IFN-γ), modulated ABCA1 gene expression. The following section summarizes current studies that have provided insights into ABCA1 gene transcriptional regulation.

Regulation of ABCA1 gene expression by sterols

Langmann et al. (1) first demonstrated upregulation of ABCA1 mRNA and protein levels in human monocyte-derived macrophages after incubation with acetylated LDL. This increase in ABCA1 expression was reversed by subsequent deloading of macrophages by incubation with HDL_3. Additional evidence that ABCA1 expression is induced by cholesterol loading and reduced by subsequent cholesterol removal by apolipoprotein A-I (apoA-I) incubation in human fibroblasts was provided by Lawn et al. (2), using quantitative RT-PCR. These authors observed a 17-fold increase in ABCA1 mRNA by exposing fibroblasts to serum-free medium containing cholesterol. Cell surface expression of ABCA1, as determined by cross-linking and immunoprecipitation, was also enhanced by serum deprivation and cholesterol loading of cells.

Oxysterols have also been shown to modulate ABCA1 gene expression via the liver X receptor (LXR) nuclear hormone receptor. Initial studies (3) demonstrated that incubation of mouse peritoneal macrophages with the physiological LXR ligand 22-(*R*)-hydroxycholesterol or the LXR selective agonist T0901317 led to a greater than 3-fold increase in ABCA1 mRNA. This induction was absent in peritoneal macrophages isolated from LXRα and LXRβ knockout mice. In separate experiments, Venkateswaran et al. (4) confirmed that treatment of NIH 3T3 fibroblasts or RAW264 macrophages with oxysterols led to an induction of ABCA1 mRNA. Retroviral expression of LXRα in these cells led to a 7- to 30-fold induction of ABCA1 mRNA and enhanced apoA-I-mediated cholesterol efflux. This response to oxysterols was attenuated in cells constitutively expressing dominant-negative forms of LXRα or LXRβ. Thus, ABCA1-mediated cellular cholesterol efflux was shown to be mediated at least in part by the LXR.

Regulation of ABCA1 gene expression by cAMP

cAMP also plays an important role in the regulation of ABCA1 gene expression. Lawn et al. (2) showed a 10-fold increase in ABCA1 mRNA in fibroblasts incubated with 8-Br-cAMP. Cell surface expression of ABCA1 determined by cross-linking followed by immunoprecipitation was also enhanced by 8-Br-cAMP. In separate studies, treatment of RAW264 cells with 8-Br-cAMP caused parallel increases in

Abbreviations: ABCA1, ATP-binding cassette transporter A1; GFP, green fluorescent protein; LXR, liver X receptor; PPAR, peroxisome proliferator-activated receptor.

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apoA-I-mediated cholesterol efflux, ABCA1 mRNA, and protein levels as well as incorporation of ABCA1 into the plasma membrane and binding of apoA-I to cell surface ABCA1 (5). Changes in cellular ABCA1 mRNA peaked by 24 h, and ABCA1 mRNA returned to baseline within 6 h after removal of 8-Br-cAMP. Oram et al. (5) reported that 8-Br-cAMP induced ABCA1 mRNA by enhancing transcription rather than by stabilizing ABCA1 mRNA. Cell surface biotinylation revealed a marked increase in cell surface ABCA1 24 h after incubation with 8-Br-cAMP, which decreased to basal levels within 2–6 h after removal of cAMP. Both ABCA1 mRNA and protein were unstable and rapidly degraded in the absence of inducer. Abe-Dohmae et al. (6) demonstrated that preincubation of RAW264 macrophages with dibutyl cAMP induced specific apoA-I binding and apoA-I-mediated cholesterol efflux as well as a 9- to 13-fold increase in ABCA1 mRNA within 16 h of exposure to cAMP. Finally, by using differential display and subtractive hybridization methods Takahashi et al. (7) identified the ABCA1 gene as one of the genes upregulated by treatment of RAW264 cells with cAMP. These combined cell culture studies have provided evidence that ABCA1 is the cAMP-inducible transporter that promotes cellular efflux of lipids. However, to date, the actual cAMP regulatory motif in the human ABCA1 gene promoter has not been definitively identified (8).

Regulation of ABCA1 gene expression by PPAR agonists

Chinetti et al. (9) have reported that PPARα and PPARγ agonists induce ABCA1 mRNA expression and apoA-I-mediated cholesterol efflux in normal macrophages but not in macrophages from patients with Tangier disease. LXRα mRNA was induced also by these agents. Furthermore, the addition of both PPAR and LXRα activators had an additive effect on ABCA1 expression. In parallel studies, Chawla et al. (10) demonstrated that ligand activation of PPARγ leads to induction of LXRα and enhanced macrophage expression of mouse ABCA1. This induction, as well as the basal and stimulated (rosiglitazone and GW7845) cellular cholesterol efflux, was significantly less in macrophages from PPARγ knockout mice. However, to date no functional PPAR response element has been identified in the ABCA1 promoter. These combined findings indicate that PPAR agonists may indirectly modulate ABCA1 gene expression by activation of the LXRα pathway and illustrate a complex interaction between PPARα, PPARγ, and LXRα in the cellular regulation of ABCA1 gene expression.

Regulation of ABCA1 gene expression by cytokines

IFN-γ reduces ABCA1 mRNA as well as cholesterol and phospholipid efflux to apoA-I in mouse peritoneal macrophages and foam cells (11). These authors have suggested that by decreasing cellular cholesterol efflux through pathways that include upregulation of ACAT and downregulation of ABCA1, IFN-γ may promote the conversion of macrophages to foam cells, accelerating the progression of atherosclerosis.

Cellular proliferation and ABCA1 gene expression

Cell culture conditions that suppress cell growth, such as serum deprivation, enhance ABCA1 gene expression, whereas growth of fibroblasts in serum-containing media suppresses ABCA1 expression (2). Thus it appears that ABCA1-mediated cellular lipid efflux requires cell quiescence or other states in which cellular cholesterol requirements are reduced.

STRUCTURAL AND FUNCTIONAL ANALYSIS OF THE ABCA1 PROMOTER

The cloning of human and mouse ABCA1 genes and characterization of their promoters (12–15) have led to the identification of specific motifs that modulate ABCA1 gene expression. The human ABCA1 gene promoter was first reported upstream of exon 1 (221 bp) followed by 24-kb intron 1 (**Fig. 1**). Analyses of this promoter utilizing reporter gene constructs identified important functional motifs that regulate ABCA1 gene expression (see discussion below). Cavelier et al. (16) have identified an alternative exon 1 (exon 1a), 136 bp in length and located 2,210 bp upstream of exon 2 (Fig. 1). This alternatively spliced transcript contains the entire coding sequence of human ABCA1 and is expressed in testis and liver but not macrophages. Upstream of exon 1a, an alternative promoter that contains a TATA box and CAAT sites as well as other potential binding sites for sterol-sensing nuclear receptors was identified. The importance of this alternative promoter in regulating ABCA1 gene expression in different tissues has yet to be reported.

Initial studies of the first promoter (designated promoter 1 in Fig. 1) in RAW264 cells localized a cholesterol response element within the first 990 bp upstream of the transcriptional start site of the human ABCA1 gene (13). Subsequently, a direct imperfect repeat of the nuclear receptor half-site TGACCT separated by four bases (DR4) was identified as the element responsible for the sterol LXR-dependent *trans*-activation of the human ABCA1 gene promoter (14, 15) (Fig. 1). Incubation of RAW264 cells (14, 15), human embryonal kidney 293 cells, and

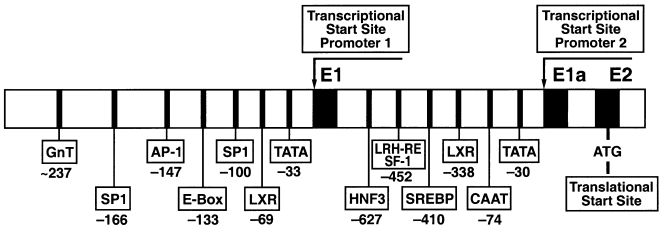

Fig. 1. The human ABCA1 gene promoter. A schematic model of the organization of the two described promoters of the human ABCA1 gene is shown. Exons 1, 1a, and 2 are indicated. The locations of motifs that may regulate ABCA1 gene expression in both promoters are illustrated. AP-1, activator protein 1; HNF-3, hepatocyte nuclear factor 3; LRH-RE, liver receptor homolog response element; SREBP, sterol regulatory element 1-binding protein.

CV-1 cells (15) with 9-*cis*-retinoic acid (CRA), 20-(*S*)-hydroxycholesterol, or 22-(*R*)-hydroxycholesterol led to an up to 9-fold induction of expression in a luciferase reporter construct. Addition of both CRA and oxysterols resulted in further gene *trans*-activation (up to 37-fold). Interestingly, mutation of this LXR motif markedly diminished induction of the ABCA1 transcription not only by oxysterols and CRA but also by cholesterol (14, 15, 17). Thus cholesterol oxidation with the formation of oxysterols may account for the observed cholesterol stimulation of ABCA1 gene expression. However, the possibility that other unidentified sterol regulatory elements are present in the human ABCA1 gene promoter has not been ruled out (18).

Two separate regions in the human ABCA1 promoter appear to play a role in repression of the human ABCA1 gene. The E-box motif located 147 bp upstream of the originally identified transcriptional start site (Fig. 1) binds the helix-loop-helix transcription factors USF1 and USF2 as well as the AP-1 motif binding protein, Fra2. These three proteins can function either as activators or repressors of gene transcription (17). Mutation or deletion of this E-box motif leads to a 2- to 3-fold induction of ABCA1 gene transcription in RAW cells and human embryonal kidney 293 cells (17), indicating a role for the E-box in ABCA1 gene regulation. Separate studies have shown that the zinc finger 202 (ZNF202) binds to the GnT repeats at bp −229 to −210 within the human ABCA1 promoter and represses ABCA1 gene expression in HepG2 and RAW cells (19). This effect requires the presence of a functional TATA box and the SCAN domain of ZNF202, which mediates selective hetero- and homodimerization. Interestingly, the gene for ZNF202 is located in 11q21-23, a chromosomal locus that influences HDL levels in humans (20).

SUBSTRATE SPECIFICITY AND AVAILABILITY

Although ABCA1 expression stimulates both cholesterol and phospholipid efflux (21), the specific substrate(s) for ABCA1 have not been definitively established. A schematic functional model for ABCA1 lipid transport (22–24) that is based on the function of multidrug resistance protein 2 (MDR2) (24), a homologous ABC transporter, is illustrated in **Fig. 2**. MDR2 has been reported to have "flippase" activity, which mediates the translocation of phospholipids from the inner leaflet of the plasma membrane to the outer leaflet (25). Cholesterol, unlike phospholipids, can spontaneously and rapidly move across lipid bilayers (26–28) and therefore is not apparently dependent on ABCA1 for translocation.

The first step of ABCA1-dependent efflux depends on the association of lipid-poor or lipid-free apolipoproteins with the cell surface (Fig. 2). The ability of apolipoproteins to insert into lipid bilayers is known to be dependent on the lipid composition of the membrane and its physical state (29). The composition of the phospholipids effluxed from cells by ABC1 is similar to the phospholipid composition of plasma membranes (30, 31) and does not

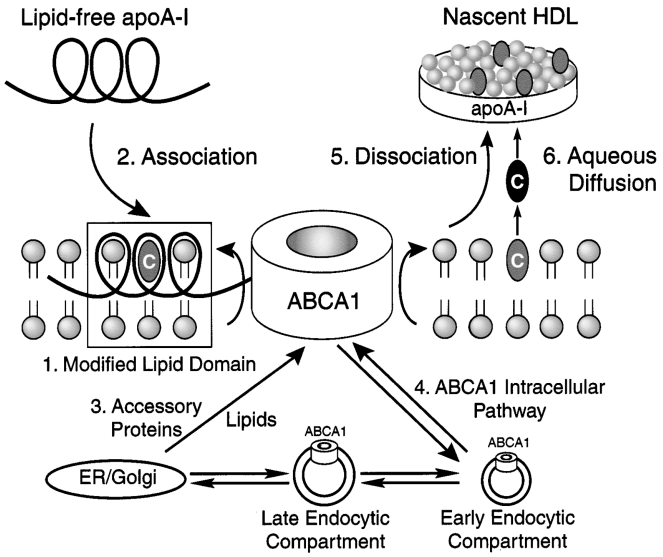

Fig. 2. Model for the mechanism of ABCA1 transport. A potential mechanism for ABCA1-mediated lipid efflux is summarized in the following steps, which are not necessarily in order: Step 1. A modified lipid domain adjacent to ABCA1 is created by the ABCA1-mediated translocation of a lipid substrate across the membrane bilayer. Step 2. Lipid-poor apolipoproteins associate either directly with ABCA1 and/or with the modified membrane domain adjacent to ABCA1. Step 3. Lipids and potential accessory proteins are transported to the plasma membrane. Step 4. Surface ABCA1 interacts with the intracellular endocytic pathway. Step 5. Lipid-bound apolipoprotein dissociates from the plasma membrane, with the simultaneous efflux of cholesterol and phospholipid. Step 6. Efflux of additional cholesterol to nascent HDL occurs by aqueous diffusion. ER, endoplasmic reticulum; C, cholesterol.

provide insight as to the substrate specificity for ABCA1-mediated efflux of cellular lipids. The translocation of specific phospholipids may be important, however, not only in determining the composition of the lipids that are removed from the cell but in modifying the plasma membrane in order to initiate the efflux process. Although phosphatidylserine represents only a minor fraction of lipids that are effluxed by ABC1 (30, 31), it was shown by staining with annexin V that ABCA1-transfected cells have increased surface expression of phosphatidylserine (22). Phosphatidylserine, in part because of its anionic nature, is known to impart several changes in the physical property of membranes and has been previously described to affect the insertion of apoA-I into lipid bilayers (32). On the basis of our current model (Fig. 2), cholesterol may be simultaneously removed with phospholipids during efflux. Depletion of cellular cholesterol with cyclodextrin before lipid efflux was shown to decrease cholesterol efflux but had no effect on phospholipid efflux (33), which supports the concept that phospholipids rather than cholesterol are the primary substrates for ABCA1 transport.

The identification of the specific substrate(s) for ABCA1 will require additional studies, but it is probable that the overall level of transport activity of ABCA1 may be modulated by the concentration and/or the availability of its substrate in the inner bilayer of the plasma membrane.

ACCESSORY PROTEINS THAT MODULATE THE ACTIVITY OF THE ABCA1 TRANSPORTER

Several ABC proteins have been reconstituted in model membranes and are able to transport their substrates in an ATP-dependent fashion (34) independent of any accessory proteins. Several prokaryotic ABC transporters, however, function in conjunction with accessory proteins. The best examples of this are the various permease transport systems that mediate the cellular uptake of exogenous ligands (35). These transport systems rely on at least two different proteins, a ligand-binding protein, which is often located in the periplasmic space, and an ABC transporter. In the case of eukaryotic ABC transporters, the sulfonyl urea receptor is also believed to function in concert with an additional protein, an ATP-sensitive potassium channel (36).

Several intracellular proteins that are involved in lipid transport have been examined to determine whether they modulate ABCA1 activity. Of particular interest is caveolin, a cholesterol-binding protein that is known to shuttle cholesterol between various cell membranes (37). Caveolin has also been previously reported to be involved in lipid efflux to HDL by aqueous diffusion (38). Increased expression of caveolin 1 in THP-1 cells after differentiation was shown to correlate with increased expression of ABCA1 and increased lipid efflux to apoA-I (39). Conversely, downregulation of caveolin 1 by treatment with antisense oligonucleotides was reported to decrease cholesterol efflux to apoA-I (39). It does not appear, however, that ABCA1 is present primarily in caveolae (40), the plasma membrane structures formed by caveolin, or that lipid that is effluxed by ABCA1 comes from caveolae (40).

Small GTP-binding proteins may also act as accessory proteins in ABCA1-mediated lipid efflux. Small GTP-binding proteins including Rho, Rac1, and Cdc42, are known to be important in vesicular trafficking and in modulating the structure of the plasma membrane (41). Tangier disease fibroblasts have been reported to have increased levels of Rho and Rac1 (42), and a decreased level of Cdc42 (43), as well as changes in the structure of the plasma membrane that may be a consequence of the altered levels of the small GTP-binding proteins (42, 43). Furthermore, transfection of cells with Cdc42 increases ABCA1-dependent efflux, whereas transfection with a dominant-negative form of Cdc42 decreases lipid efflux (43).

To date it has not been definitively established to what extent ABCA1 transporter activity is dependent on accessory proteins. Accessory proteins may provide, however, an additional mechanism for modulation of the activity of ABCA1 by altering the delivery of lipids for efflux to the plasma membrane or perhaps by modifying the structure of the plasma membrane in such a way that it affects the ability of the cell membrane to efflux lipid (Fig. 2).

APOLIPOPROTEIN ACCEPTOR SPECIFICITY AND AVAILABILITY

ApoA-I, the principal apolipoprotein on HDL, has been the lipid acceptor utilized in the majority of cellular efflux studies of ABCA1-mediated lipid transport. There are, however, many other apolipoproteins that reside on HDL. The apolipoproteins on HDL form a class of proteins designated exchangeable apolipoproteins (44) that undergo repeated cycles of dissociation and reassociation with lipoproteins. This property has been proposed (23, 24) to be important in the process of ABCA1-mediated lipid efflux, because it is the association of the lipid-poor apolipoproteins with the plasma membrane of cells that is the first and most critical step in ABCA1-mediated lipid efflux (Fig. 2). In addition to apoA-I, several of the other exchangeable apolipoproteins including apoA-II, apoC-II, apoC-III, and apoE have been shown to stimulate ABCA1-mediated lipid efflux (24). On the basis of studies using synthetic peptide mimics of apolipoproteins (45, 46), it appears that the key structural motif necessary for an apolipoprotein to function as a lipid acceptor is the presence of amphipathic helices. Amphipathic helices have been proposed to facilitate the initial interaction of the apolipoproteins with a modified lipid domain in the cell membrane created by the flippase activity of ABCA1 (Fig. 2).

The overall level of lipid efflux by ABCA1 may be limited by the quantity of exchangeable apolipoproteins that are available to serve as lipid acceptors. The synthesis and catabolism of the various exchangeable apolipoproteins are known to be modulated by several different physiologic processes (47, 48). It is likely, however, that the concentration of the lipid-poor or lipid-free apolipoprotein in the extracellular fluid, rather than the total concentration of the plasma apolipoprotein, is more relevant for determining the level of ABCA1 efflux. It has been estimated that approximately 5% of plasma apoA-I exists in the lipid-poor state (49, 50), and that the concentration of lipid-poor apoA-I in extracellular fluid is comparable to the quantity of apoA-I required in vitro to saturate ABCA1-dependent efflux (24). The mechanisms that regulate the disassociation of apolipoproteins from lipoproteins are not fully understood (51–54), but may warrant further studies because this process may represent an important, newly recognized mechanism for regulating reverse cholesterol transport by ABCA1. The lipid composition of HDL is known, for example, to affect the affinity of apoA-I for HDL (51). Increased levels of HDL triglycerides promote the dissociation of apoA-I from HDL (51). ApoA-I is also released from HDL as a consequence of the remodeling of HDL by several lipoprotein-modifying enzymes, including hepatic lipase and the phospholipid transfer protein (52–54).

LOCALIZATION AND CELLULAR TRAFFICKING OF ABCA1

Establishing the cellular localization of ABCA1 and its potential site(s) of function is critical to understanding the process of cellular lipid efflux. Initial immunocytochemical studies suggested that endogenously expressed human ABCA1 was localized on the plasma membrane (2, 55, 56). Studies using a fluorescent ABCA1-GFP (green

fluorescent protein) fusion protein established the presence of ABCA1 on the cell membrane and that the movement of ABCA1 to the cell surface was required for the transporter to function in apoA-I-dependent cellular lipid efflux (57). Furthermore, studies using stable as well as transiently expressed ABCA1-GFP fusion proteins have established that the ABCA1 transporter also resides in intracellular endocytic compartments (22, 24, 58) (Fig. 2 and **Fig. 3**). In addition to trafficking to the cell surface, trafficking of the ABCA1 transporter in endocytic compartments may also play an important role in apoA-I-mediated efflux of cellular lipids (57) (Fig. 2). Using digital video microscropy, early endosomes containing the ABCA1 transporter were observed to shuttle between the plasma membrane and intracellular endocytic compartments (57). The delivery of ABCA1 to lysosomes for degradation has also been suggested to potentially serve as a mechanism to decrease the surface expression of ABCA1, and hence, modulate apoA-I-mediated cellular lipid efflux (57).

The functional significance of the trafficking of the ABCA1 transporter along the intracellular endocytic pathway remains to be established. However, several investigators have provided evidence suggesting that ABCA1-mediated lipid efflux involves intracellular trafficking of substrate lipids (59, 60) as well as the apoA-I acceptor (61). Indeed, studies have provided evidence suggesting that extracellular apolipoproteins may be endocytosed and recycled back to the cell surface before their release from the cell (61, 62). Thus, apolipoproteins that are lipidated in an ABCA1-dependent manner may share a trafficking itinerary with the ABCA1 transporter in an intracellular endocytic compartment. Analysis has shown that the ABCA2 (63) and ABCB9 (64) transporters reside predominantly in endocytic compartments, providing further support for a potential functional role of the ABCA1 transporter in endocytic compartments.

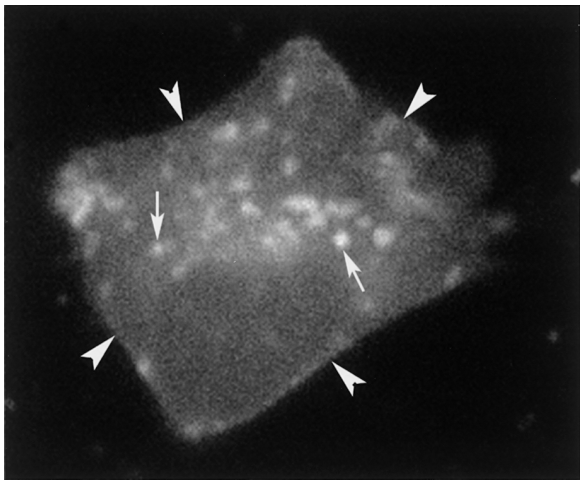

Fig. 3. Cellular localization of the ABCA1 transporter. Fluorescence microscopy of living CHO cells in culture expressing an ABCA1-GFP fusion protein reveals that the ABCA1 transporter resides on the cell surface (arrowheads) and in endocytic vesicles (arrows). ABCA1 at these sites appears to function in cellular lipid efflux (57).

SUMMARY

The functional activity of the ABCA1 transporter is coordinately regulated by a multifaceted array of physiological processes. Transcriptional regulation is mediated by two promoters, both with several structural motifs for potential responses to sterols, cAMP, and cytokines. Of particular importance in the regulation of the ABCA1 transporter is the intracellular cholesterol concentration and the LXR transcription factor, which play a pivotal role in modulating intracellular cholesterol metabolism. The precise mechanism, substrate specificity, and the role of accessory proteins in ABCA1-mediated lipid transport remain to be established. ApoA-I, as well as several other apolipoproteins that contain amphipathic helices, are effective acceptors for ABCA1-mediated lipid efflux. Of major interest was the finding that ABCA1 resides on the cell membrane and has an extensive intracellular endocytic pathway, with rapid movement of the transporter between the cell membrane and intracellular vesicles. The elucidation of the regulation of the ABCA1 transporter will provide new insights into cholesterol metabolism and the potential opportunity to develop new pharmacological agents to decrease the development and progression of atherosclerosis.

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