ATP-Binding Cassette Transporter A1: A Cell Cholesterol Exporter That Protects Against Cardiovascular Disease

JOHN F. ORAM AND JAY W. HEINECKE

Department of Medicine, University of Washington, Seattle, Washington

I. INTRODUCTION

Cholesterol plays several structural and metabolic roles that are vital to human biology. Although cholesterol distributes along the entire plasma membrane of cells, where it modulates fluidity, it concentrates in specialized sphingolipid-rich domains called rafts and caveolae (8). These contain a variety of signaling molecules that de-
pend on a well-maintained cholesterol content for normal activity. In addition, cholesterol is a substrate for steroid production and covalently links to a protein involved in limb development (158). Too much cholesterol in cells, however, can have pathological consequences. This is particularly true for cells of the artery wall, where accumulation of cholesterol initiates atherosclerotic cardiovascular disease (CVD) (95). The body therefore relies on a complex homeostatic network to modulate the availability of cholesterol for tissues. This network operates on both the cellular level and within the plasma compartment.

Approximately two-thirds of the cholesterol in human plasma is carried in a class of lipoprotein particles called low-density lipoprotein (LDL). LDL provides a source of cholesterol for steroidogenesis and cellular membranes. This occurs through the interaction of LDL with a cell-surface receptor that mediates internalization and degradation of the lipoprotein particles (27). The hepatic LDL receptor is responsible for clearing most of the LDL cholesterol from the plasma.

Cells other than those in steroidogenic tissues and the liver cannot metabolize cholesterol. Instead, they modulate their membrane cholesterol content by a feedback system that controls the rate of cholesterol biosynthesis and uptake by the LDL receptor (26). With most cell types this system is sufficient to provide cells with enough cholesterol for membrane integrity and function without overloading them. Some cells, particularly macrophages, can ingest cholesterol by endocytotic and phagocytotic pathways that are not feedback regulated by cholesterol (213). These cells must either store this excess cholesterol as esters or secrete it.

High-density lipoprotein (HDL), which carries about one-third of the cholesterol in human plasma, is involved in the removal of excess cholesterol from cells. HDL is a multifunctional and heterogeneous class of particles that transports a variety of lipids and lipophilic molecules between tissues and other lipoproteins. One of the major functions of HDL is to transport cholesterol from peripheral tissues to the liver for elimination in the bile (69, 90, 210). This process, called reverse cholesterol transport, is widely believed to account for much of the inverse relationship between plasma HDL levels and CVD revealed by population studies.

HDL components can remove cellular cholesterol by multiple mechanisms (210, 237). HDL phospholipids absorb cholesterol that diffuses from the plasma membrane into the aqueous phase, a passive process that is facilitated by the interaction of HDL particles with scavenger receptor B1 (143). Four cell membrane transporters have been identified that mediate cholesterol efflux from cells to HDL components by metabolically active pathways. All four belong to a superfamily of ATP-binding cassette transporters (ABCs). ABCA1 mediates the transport of cellular cholesterol, phospholipids, and other metabolites to HDL proteins (apolipoproteins) that are associated with no or very little lipid (204, 287). ABCA1 is highly expressed in the liver and tissue macrophages (149, 296). ABCA7, a close homolog of ABCA1, selectively transports phospholipids to lipid-depleted apolipoproteins (1, 116, 133, 140, 164, 285). It is highly expressed in myelolymphatic tissues, lung, adrenal, and brain. ABCG1 and ABCG4 mediate cholesterol transport from cells to HDL particles and are highly expressed in tissue macrophages and brain cells, respectively (120, 188, 202, 244, 284).

ABCA1 has been more extensively characterized than the other three ABC lipid transporters. Numerous studies of cultured cells, human HDL deficiencies, and animal models have shown that ABCA1 is a major determinant of plasma HDL levels and a potent atheroprotective factor (4, 131, 204, 255, 287). This transporter has therefore become an important new therapeutic target for drug development designed for clearing cholesterol from arterial macrophages and preventing CVD. This review focuses on the biology and pathophysiology of ABCA1.

II. CELL BIOLOGY

A. Structure

ABC transporters are the largest membrane transporter family, with members in all phyla (54). ABCs are grouped into seven subclasses labeled ABCA through ABCG. Of the 49 ABCs in humans, 13 are in the ABCA subclass (54). Mutations in ABC genes cause a variety of diseases, including cystic fibrosis, Startgart’s macular degeneration, and disturbances in lipid and lipoprotein metabolism. All ABC transporters utilize ATP to generate the energy needed to transport metabolites across membranes. Structurally, ABCs fall into two groups: 1) whole transporters having two similar structural units joined covalently; and 2) half transporters of single structural units that form active heterodimers or homodimers (54). ABCA1 and ABCA7 are whole transporters, whereas ABCG1 and ABCG4 are homodimeric half transporters.

ABCA1 is a 2,261-amino-acid integral membrane protein that comprises two halves of similar structure (71). Each half has a transmembrane domain containing six helices and a nucleotide binding domain (NBD) containing two conserved peptide motifs known as Walker A and Walker B, which are present in many proteins that utilize ATP, and a Walker C signature unique to ABC transporters (Fig. 1) (54). ABCA1 is predicted to have an NH2 terminus oriented into the cytosol and two large extracellular loops that are highly glycosylated and linked by one or more cysteine bonds (Fig. 1) (31, 54).

B. Function

ABCA1 mediates the transport of cholesterol, phospholipids, and other lipophilic molecules across cellular
membranes, where they are removed from cells by lipid-poor HDL apolipoproteins (204). Its homology with other better-characterized ABC transporters suggests that ABCA1 forms a channel in the membrane that promotes flipping of lipids from the inner to outer membrane leaflet by an ATPase-dependent process (203, 204). ABCA1 localizes to the plasma membrane and intracellular compartments (193, 194), where it could potentially facilitate transport of lipids to either cell surface-bound or internalized apolipoproteins.

ABCA1 appears to target specific membrane domains for lipid secretion. These are likely to be regions that are sensitive to accumulation of cholesterol and other lipophilic compounds. This same source of cholesterol feeds into intracellular compartments that are the preferred substrate for the esterifying enzyme acyl CoA: cholesterol acyltransferase (ACAT) (176, 304). Thus ABCA1 removes cholesterol that would otherwise accumulate as cytosolic cholesteryl ester lipid droplets. One possibility for the link between ABCA1 and ACAT is that both proteins function to protect cells from incorporating too much free cholesterol into the endoplasmic reticulum where it may disrupt the peptide biosynthetic machinery. When cholesterol esterification is blocked in sterol-laden macrophages, the potentially cytotoxic free cholesterol that accumulates is a preferred substrate for the ABCA1 pathway (136).

These observations imply that ABCA1 associates with cholesterol-rich membrane lipid domains. The properties of these domains are unknown, but they appear to be separate from sphingolipid-rich rafts and caveolae (177). A fraction of ABCA1, however, may associate with membrane rafts that are selectively solubilized by the detergent Lubrol and are relatively enriched with cholesterol and phosphatidylcholine (60). Immunogold electron microscopy showed that apolipoproteins interact with diffuse structures protruding from the plasma membrane (163). It is likely that these structures are formed by the lipid transport activity of ABCA1, as has been shown for another ABC phospholipid transporter (52).

Two models have been proposed to account for the ability of ABCA1 to target specific lipid domains (Fig. 2). The exocytosis model implies that excess intracellular cholesterol is packaged into transport vesicles or rafts, perhaps in the Golgi apparatus, which translocate to domains in the plasma membrane containing ABCA1 (205). In support of this model are results showing that induction of ABCA1 in the absence of apolipoproteins increases the appearance of cholesterol on the cell surface (276). The retroendocytosis model suggests that ABCA1- and apolipoprotein-containing vesicles endocytose to intracellular lipid deposits, where ABCA1 pumps lipids into the vesicle lumen for release by exocytosis (242, 261). Consistent with this mechanism are studies showing that ABCA1 recycles rapidly between the plasma membrane and late endosomal/lysosomal compartments, that these compartments accumulate cholesterol in cells with dysfunctional ABCA1, and that ABCA1-containing intracellular vesicles also contain apolipoproteins (193, 194). It is still unclear, however, which model represents the dominant pathway for ABCA1-dependent lipid efflux.

C. Substrates

ABCA1 appears to mediate the transport of diverse types of molecules. In addition to cholesterol and phospholipids, ABCA1 has been reported to promote secretion of α-tocopherol (208), apoE (279), and interleukin-1β (101, 313). ABCA1-mediated secretion of α-tocopherol mimics that of cholesterol (208), suggesting similar transport mechanisms for these substrates.

ABCA1 can promote phospholipid efflux from cells even when membranes are depleted of cholesterol (276,
286), consistent with phospholipids being the primary substrate for ABCA1. Analysis of lipids removed by apolipoproteins implicates phosphatidylcholine as the major phospholipid substrate (70, 166). There is evidence that ABCA1 translocates phosphatidylserine to the exofacial side of the plasma membrane (34, 233, 309), which was proposed to account for ABCA1-mediated cellular apolipoprotein binding and cholesterol efflux (34). Another study, however, suggests that the increased phosphatidylserine translocation associated with ABCA1 expression is too small to account for the enhanced apolipoprotein binding and cholesterol efflux (257). Moreover, there is no selective enrichment of phosphatidylserine in the phospholipids removed by apolipoproteins (70, 166), implicating little interaction with phosphatidylserine-rich domains. Nevertheless, there is evidence that ABCA1 promotes engulfment of apoptotic cells by macrophages (100) and generates microparticles that bleb from plasma membranes (49, 166), two processes that may require phosphatidylserine surfacing.

The broad substrate specificity of ABCA1 is consistent with targeting specific lipid domains for removal from the cell. It is possible that ABCA1 can simultaneously transport several molecules, provided they are associated with phosphatidylcholine. These could include cholesterol and other lipophiles (e.g., α-tocopherol or peptides) that accumulate in membrane domains accessible to ABCA1. Although ABCA1 substrates may be diverse, the most physiologically relevant of these are likely to be cholesterol and phospholipids, because overloading cells with cholesterol induces ABCA1 expression (see below) and phospholipids are required cofactors for cholesterol transport.

D. Lipid Acceptors

HDL apolipoproteins selectively interact with plasma membrane lipid domains that are formed by the action of ABCA1 (86, 87, 166). This interaction is specific for apolipoproteins that contain no or very little lipids. This was evident from studies showing that purified HDL apolipoproteins promote cholesterol and phospholipid efflux from cells exclusively by this pathway (75, 226). In contrast, lipidated apolipoproteins, such as mature HDL particles, promote cholesterol efflux by multiple mechanisms involving passive diffusion, interaction with SR-B1, and the transport activities of ABCG1, ABCG4, and ABCA1. Whether or not an HDL particle has activity for the ABCA1 pathway probably depends on its ability to act as a donor of lipid-free apolipoproteins that dissociate from the particles (178, 201).

The ABCA1 pathway has broad specificity for multiple HDL apolipoproteins, including apolipoproteins A1, AII, E, CI, CII, CIII, and AIV (226). These apolipoproteins contain 11–22 amino acid repeats of amphipathic α-helices (249). In this type of helix, the charged amino acids align along one face of the long axis while the hydrophobic residues align along the other face.

The amphipathic α-helices in HDL apolipoproteins fall into two major subclasses based on the distribution of charged amino acids (Fig. 3). Class A helices, the most common subclass, tend to cluster their positively charged amino acids along the polar-nonpolar boundaries and the negatively charged amino acids along the center of the polar region (249). Type Y amphipathic α-helices have a similar distribution pattern, except they have a positive charge disrupting the cluster of negative charges on the polar face (249). Type Y helices have a higher lipid

---

**Fig. 2.** Models for ABCA1-mediated cellular lipid trafficking and secretion. Exocytosis: free cholesterol (FC) derived from hydrolysis of esterified cholesterol (EC) lipid droplets is assembled with phospholipids (PL), perhaps in the Golgi apparatus, to generate vesicles that translocate to plasma membrane domains containing ABCA1 (U-shaped structure). Lipids are transported across the plasma membrane where they interact with apolipoproteins (squiggles) and are removed from the cell, forming nascent HDL particles (disks). Retroendocytosis: apolipoproteins interact with ABCA1 or partner proteins and are endocytosed with ABCA1 to intracellular deposits of C and PL. The lipids are transported into the vesicle lumen where they interact with apolipoproteins, and the lipid-apolipoprotein complex is released from cells after the vesicle fuses with the plasma membrane.
affinity than type A helices (86). Because of these novel distributions of charged residues, apolipoproteins associate with lipid surfaces but can freely exchange between surfaces through the aqueous environment. These properties also allow lipid free apolipoproteins to assemble phospholipids and free cholesterol to generate nascent HDL particles.

The most abundant apolipoprotein in HDL is apoA-I, which comprises 70% of the total HDL protein content. ApoA-I contains eight 22-amino acid and two 11-amino acid tandem amphipathic \( \alpha \)-helical domains (Fig. 3). Studies of synthetic peptides corresponding to each of these helices showed that helices 1 and 10 have the greatest affinity for phospholipids (86, 277). These peptides, however, failed to mediate ABCA1-dependent cholesterol efflux unless they were covalently linked to the 11-mer helix 9 (189). This linkage produced a linear array of acidic amino acids along the polar face of the helices, suggesting that this may be an important apoA-I structural determinant for lipid removal by the ABCA1 pathway (189). The importance of the COOH-terminal helices in this process is supported by studies showing that truncation mutants of apoA-I lacking the 10th helix were unable to remove cholesterol from cells by the ABCA1 pathway (215).

A synthetic 18-amino acid peptide that is an analog of class A amphipathic \( \alpha \)-helices (Fig. 3, top left) can mimic apoA-I in removing cholesterol and phospholipids by the ABCA1 pathway. A dimer of this peptide is more efficacious (176, 306), suggesting cooperativity between tandem helices. These studies imply that the amphipathic \( \alpha \)-helix is the major structural motif required for removing ABCA1-transported lipids. Interestingly, the \( d \)-isomer of the 18-mer \( \alpha \)-helix is just as active as the \( l \)-isomer (9, 227), suggesting that there are no stereoactive requirements for these peptides to interact with ABCA1 lipid transport.

The broad specificity for amphipathic \( \alpha \)-helices implies that proteins other than apolipoproteins containing this structural motif could remove cellular lipids by the ABCA1 pathway. Indeed, it was shown that phospholipid transfer protein (PLTP), which contains amphipathic \( \alpha \)-helices, can interact with ABCA1 and remove cellular cholesterol and phospholipids (209). Because of its low lipid binding capacity, PLTP tends to transfer these lipids to HDL rather than generate new lipoprotein particles. PLTP plays important and diverse roles in lipoprotein metabolism, including transferring phospholipids between lipoproteins, remodeling HDL to generate lipopoor particles, and facilitating the production of triglyceride-rich apoB-containing particles in the liver (5, 123, 125, 126, 271). At physiological levels, PLTP may help overcome some of its atherogenic effects by transferring excess cholesterol from macrophages to HDL. Because of its low lipid binding, however, high concentrations of PLTP may actually block lipid removal from cells by interfering with apolipoprotein interactions. These observations may partially explain why both deleting and hyperexpressing PLTP in mice increases atherosclerosis (126, 273).

Serum amyloid A (SAA) also removes cellular lipids by the ABCA1 pathway (258). SAA is an acute-phase protein that is induced over 1,000-fold during inflammation (110). SAA mostly associates with HDL in the plasma. It contains two tandem amphipathic \( \alpha \)-helices that differ in amino acid charge distribution from those in apolipoproteins. Although the function of SAA is unknown, one activity may be to remove excess lipids through its interactions with ABCA1. This may become necessary at sites of inflammation where macrophages ingest cholesterol-rich membranes from apoptotic and necrotic cells.
E. Apolipoprotein Binding

Covalent cross-linking studies revealed that apolipoproteins bind directly to ABCA1 with saturability and high affinity ($K_d < 10^{-7}$ M) (45, 72). This binding is temperature sensitive and readily reversible. The apolipoprotein binding sites on ABCA1 have a broad specificity for multiple HDL apolipoproteins, including apos A-I, A-II, C-I, C-II, and C-III (72). These sites also recognize the 18-mer synthetic amphipathic $\alpha$-helix (72) and PLTP (209). Thus the broad specificity of the ABCA1 binding sites closely mirrors the ABCA1-dependent lipid transport activity of the acceptors. These binding sites have not yet been identified, but their loose specificity for amphipathic $\alpha$-helices suggests that they may be rich in hydrophobic amino acids.

Mutational analyses have uncovered a dissociation between the lipid transport activity of ABCA1 and its apolipoprotein binding activity. Nearly all missense mutations in ABCA1 that impair lipid efflux to apolipoproteins also impair apolipoprotein binding to ABCA1 (73). One substitution mutation (W590S), however, severely reduces apolipoprotein-mediated lipid efflux without having much effect on apolipoprotein binding (73). These results imply that apolipoprotein binding to ABCA1 is essential but not sufficient for removing lipids. As discussed below, the intrinsic lipid transport activity of ABCA1 and its apolipoprotein binding activity can also be regulated independently. It is therefore likely that these two activities depend on different properties of ABCA1.

F. Lipid Transport Model

Although the structure of ABCA1 has not been characterized, electron microscopy and X-ray crystallography of other ABC transporters have generated molecular models that may apply to ABCA1. The two most studied ABC structures belong to mammalian P-glycoprotein (234, 235), which extrudes a variety of lipophilic compounds from cells, and bacterial/pathogenic MsbA (35, 36), which translocates lipids from the inner to outer membrane leaflets. Despite some disagreement about mechanisms involved, the consensus of these analyses suggests that the two symmetrical transmembrane bundles come together to form a chamber that scans the inner leaflet of the membrane for substrates, incorporates them into the chamber, and flips them to the outer leaflet for extrusion from the cell. This involves a series of conformational changes in the ABC protein that is probably driven by the NBD domains (Fig. 4) (35, 235).

These structural studies suggest the following model for the ABCA1 pathway. Excess cellular cholesterol along with phospholipids accumulate within domains of the cytosolic leaflet of the plasma membrane or intracellular vesicle membranes (Fig. 4, top left). This cholesterol is not accessible to apolipoproteins and therefore must be translocated to the cell surface or into the vesicle lumen for removal. These lipid domains may assemble in the vicinity of ABCA1 molecules, or ABCA1 may migrate to these domains after they are formed. Other lipophilic molecules may also accumulate in these domains.

The MsbA model predicts that the transmembrane chamber of ABCA1 is initially open at the bottom (Fig. 4, top left) (35, 36). Lipids in the inner membrane leaflet are laterally transported into the chamber by a process that is facilitated by high-affinity phospholipid binding sites. This phospholipid recognition induces ATP binding to the NBDs, which promotes their dimerization and thus closes the chamber at the membrane outer leaflet (step B). Lipids are extruded from the chamber into cholesterol-rich domains on the cell surface, where they are removed by apolipoproteins to form nascent HDL particles (step C). The structure of the ABCA1 chamber reverts back to its substrate uptake conformation after ADP dissociates from the NBDs (step D).
the chamber (Fig. 4, step A). The interactions of phospholipid polar head groups with charged amino acids in the chamber flips the trapped lipids to the outer leaflet. The hydrolysis of ATP by the NBDs forms an ADP-bound intermediate that changes the conformation of the transmembrane domains, opening the chamber at the membrane outer leaflet (Fig. 4, step B). Because of a decreased affinity for phospholipid binding, lipids are extruded from the chamber into cholesterol-rich domains on the cell surface, where they are removed by apolipoproteins to form nascent HDL particles (Fig. 4, step C). The structure of the ABCA1 chamber reverts back to its substrate uptake conformation after ADP dissociates from the NBDs (Fig. 4, step D). Although this is a plausible model for ABCA1 function, more direct evidence is needed to confirm that it actually applies to this transporter.

The removal of lipids by apolipoproteins appears to involve a two-step process, whereby apolipoproteins first bind to ABCA1 and then solubilize the ABCA1-transported lipids (Fig. 4, step C) (45). Apolipoprotein binding to ABCA1 is not required for lipid flippase activity, as ABCA1 translocates cholesterol to the cell surface in the absence of apolipoproteins (276). This binding may serve to target apolipoproteins to the lipid domains formed by ABCA1. It might also promote extrusion of lipids from the open chamber by dissociating phospholipids from their binding sites.

There is evidence that ABCA1 forms oligomers in both intracellular membranes and the plasma membrane and that the homotetramer is the major functional unit (55). It was postulated that the interaction of apolipoproteins with each of these units may be responsible for generation of nascent HDL particles containing four or more apolipoprotein molecules per particle. Binding of apolipoproteins to ABCA1 does not appear to be required for its oligomerization. The oligomeric structure of functional ABCA1 is consistent with what has been observed for other ABC transporters (225, 236).

III. REGULATION

A. Expression

1. Transcription

As expected for a transporter that mediates secretion of excess cellular cholesterol, transcription of ABCA1 is markedly induced by overloading cells with cholesterol. This induction occurs exclusively through activation of the nuclear receptors liver X receptor (LXRα and/or LXRβ) and retinoid X receptor (RXR) (51, 247). LXR and RXR form obligate heterodimers that preferentially bind to response elements within the ABCA1 gene promoter and the first intron. LXRαs and RXRs bind to and are activated by oxysterols and retinoic acid, respectively (230). Binding of either one or both ligands can activate transcription. Treatment of cells with either an oxysterol or 9-cis-retinoic acid induces ABCA1, but their combined treatment has marked synergistic effects (51, 247). The LXRα gene promoter in human macrophages contains a LXR response element (146, 297), indicating that LXRα can autoregulate its own expression. This would serve to amplify the effects of oxysterols on the ABCA1 lipid efflux pathway.

Because uptake of nonoxidized cholesterol by cells increases ABCA1 expression, cholesterol must be converted to oxysterols before inducing ABCA1. The most potent LXR ligands contain a single stereoselective oxygen on the side chain that functions as a hydrogen bond acceptor (121). It is believed that 22-hydroxycholesterol, 24-hydroxycholesterol, and 24,25-epoxycholesterol are the major naturally occurring liver LXR ligands (121). Most oxysterols are generated by cytochrome P-450 enzymes that are particularly prevalent in the liver and play a role in bile acid metabolism. One of these enzymes, sterol 27-hydroxylase (Cyp27), is broadly distributed in various tissues and cell types, including macrophages, suggesting that 27-hydroxycholesterol is the major LXR ligand in macrophages and other peripheral cells (80). Consistent with this idea is the accelerated atherosclerosis that is associated with cerebrotendinous xanthomatosis, a rare inherited disease characterized by a lack of Cyp27 (124). Recent studies with mouse models lacking or overexpressing Cyp27, however, suggest that 27-hydroxycholesterol has little impact on whole body cholesterol homeostasis (175).

Lipid metabolites other than sterols can modulate ABCA1 expression by the LXR system (Table 1). Polyunsaturated fatty acids act as antagonists to oxysterol binding to response elements in the LXRα gene (214), potentially interfering with induction of ABCA1 by sterols (272). Geranylgeranyl pyrophosphate (GGPP), a product of the mevalonate pathway that isoprenylates proteins, was shown to suppress LXR-induced ABCA1 synthesis by two mechanisms: as an antagonist of the interaction of LXR with its nuclear coactivator SRC-1 and as an activator of Rho GTP-binding proteins (82). This second mechanism might alter sterol trafficking in cells, reducing their availability as ligands for LXR. Activators of peroxisome proliferator activating nuclear receptors (PPARs) also enhance ABCA1 transcription in some cells. PPARγ activators stimulate cholesterol efflux from cells by activating transcription of the LXRα gene, which in turn induces ABCA1 transcription (37, 41). Thyroid hormone receptor was reported to suppress ABCA1 transcription by forming a complex with RXR that competes for LXR/RXR binding to its response elements (115).

ABCA1 transcription is also regulated by factors independent of LXR/RXR (Table 1). Membrane-permeable
The rapid protein turnover is largely due to mRNA and protein degradation at a fast rate (half-life of 4–2 h) (290). The rapid protein turnover is largely due to mRNA and protein degradation at a fast rate (half-life of 4–2 h) (290). When ABCA1 inducers are removed in the absence of apolipoproteins, ABCA1 mRNA and protein are degraded at a fast rate (half-life of 1–2 h) (290). The rapid protein turnover is largely due to the sequence at amino acids 1283–1306 in the first intracellular loop that is enriched in proline-glutamate-serine-threonine (PEST motif) (283). Phosphorylation of T1286 and T1305 in this motif (Fig. 5) promotes ABCA1 proteolysis by an unknown member of the calpain protease family (173, 283). There are several metabolic factors that modulate the rate of ABCA1 protein degradation by either this calpain system or other processes. A) Apolipoproteins. The interaction of apolipoproteins with ABCA1-expressing cells dramatically reduces the rate of ABCA1 degradation (9, 283). This acts as a feedback mechanism to increase ABCA1 levels when acceptors for lipid transport are available. Studies have uncovered two possible mechanisms that might contribute to this protein stabilization. First, the cellular interactions of apolipoproteins interfere with the phosphorylation of the PEST motif, reducing calpain-mediated proteolysis of ABCA1 (173, 283, 287). Second, the removal of membrane sphingomyelin by apolipoproteins activates phosphatidylincholine phospholipase C, which signals phosphorylation of ABCA1 at a site that stabilizes the protein (305). It is unknown if either or both of these mechanisms require apolipoprotein binding to ABCA1.

<table>
<thead>
<tr>
<th>Effector</th>
<th>Mediator</th>
<th>ABCA1</th>
<th>Mechanism</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterols</td>
<td>LXRα, LXRβ</td>
<td>+</td>
<td>+ ABCA1 transcription</td>
<td>51, 245</td>
</tr>
<tr>
<td>Retinoids</td>
<td>RXR</td>
<td>+</td>
<td>+ ABCA1 transcription</td>
<td>51, 245</td>
</tr>
<tr>
<td>Polyunsaturated fatty acids</td>
<td>LXRα, LXRβ</td>
<td>−</td>
<td>Antagonizes sterols</td>
<td>212, 270</td>
</tr>
<tr>
<td>GeranylgeranylPP</td>
<td>gPP, Rho</td>
<td>−</td>
<td>− LXR activity</td>
<td>82</td>
</tr>
<tr>
<td>Thiazolidinediones</td>
<td>PPARγ</td>
<td>+</td>
<td>+ LXR transcription</td>
<td>37, 41</td>
</tr>
<tr>
<td>Thyroid hormone</td>
<td>Hormone receptor</td>
<td>−</td>
<td>− ABCA1 transcription</td>
<td>23, 204</td>
</tr>
<tr>
<td>Verapamil</td>
<td>Calcium²</td>
<td>+</td>
<td>+ ABCA1 transcription</td>
<td>258</td>
</tr>
<tr>
<td>?</td>
<td>ZNF202</td>
<td>−</td>
<td>− ABCA1 transcription</td>
<td>221</td>
</tr>
<tr>
<td>?</td>
<td>Sp1</td>
<td>+</td>
<td>+ ABCA1 transcription</td>
<td>146</td>
</tr>
<tr>
<td>?</td>
<td>Sp3</td>
<td>−</td>
<td>Antagonizes Sp1</td>
<td>146</td>
</tr>
<tr>
<td>?</td>
<td>USF1/USF2/Fra2</td>
<td>−</td>
<td>− ABCA1 transcription</td>
<td>306</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>?</td>
<td>+</td>
<td>+ ABCA1 transcription</td>
<td>214</td>
</tr>
<tr>
<td>TGF-β</td>
<td>?</td>
<td>−</td>
<td>Antagonizes IFN-γ</td>
<td>10, 214</td>
</tr>
<tr>
<td>Oncostatin M</td>
<td>?</td>
<td>+</td>
<td>+ ABCA1 transcription</td>
<td>146</td>
</tr>
<tr>
<td>EC sterol depletion</td>
<td>SREBP 2</td>
<td>−</td>
<td>− ABCA1 transcription</td>
<td>307</td>
</tr>
</tbody>
</table>

IFN, interferon; TGF, transforming growth factor; LXR, liver X receptor; RXR, retinoid X receptor; PPAR, peroxisome proliferator activating nuclear receptor.

The only posttranscriptional processes so far identified that affect ABCA1 expression involve modulation of ABCA1 protein stability (Table 1). When ABCA1 inducers are removed in the absence of apolipoproteins, ABCA1 mRNA and protein are degraded at a fast rate (half-life of 1–2 h) (290). The rapid protein turnover is largely due to the sequence at amino acids 1283–1306 in the first intracellular loop that is enriched in proline-glutamate-serine-threonine (PEST motif) (283). Phosphorylation of T1286 and T1305 in this motif (Fig. 5) promotes ABCA1 proteolysis by an unknown member of the calpain protease family (173, 283). There are several metabolic factors that modulate the rate of ABCA1 protein degradation by either this calpain system or other processes.

A) Apolipoproteins. The interaction of apolipoproteins with ABCA1-expressing cells dramatically reduces the rate of ABCA1 degradation (9, 283). This acts as a feedback mechanism to increase ABCA1 levels when acceptors for lipid transport are available. Studies have uncovered two possible mechanisms that might contribute to this protein stabilization. First, the cellular interactions of apolipoproteins interfere with the phosphorylation of the PEST motif, reducing calpain-mediated proteolysis of ABCA1 (173, 283, 287). Second, the removal of membrane sphingomyelin by apolipoproteins activates phosphatidylincholine phospholipase C, which signals phosphorylation of ABCA1 at a site that stabilizes the protein (305). It is unknown if either or both of these mechanisms require apolipoprotein binding to ABCA1.

B) Free fatty acids. Unsaturated free fatty acids directly destabilize ABCA1 protein in cultured cells (289, 290). When ABCA1 is induced by LXR-independent mechanisms, mono-, di-, and polyunsaturated fatty acids in-
crease the rate of ABCA1 protein degradation (290). In contrast, saturated fatty acids have no effect on ABCA1 turnover in these cells. However, if ABCA1 is induced by LXR ligands, the saturated fatty acids palmitate and stearate also destabilize ABCA1 (289). This is because one of the other genes induced by LXR encodes an enzyme called stearoyl-CoA desaturase, which converts these two saturated fatty acids to their monounsaturated derivatives, palmitoleate and oleate (289). Thus, in most cholesterol-loaded cells in vivo, where ABCA1 is induced by LXR-activating sterols, it is likely that exposure to both saturated and unsaturated fatty acids impair the ABCA1 cholesterol secretion pathway by reducing ABCA1 levels.

The effects of fatty acids on ABCA1 stability appear to be mediated by a signaling pathway involving activation of phospholipase D2 and phosphorylation of ABCA1 (291). The biologic reason for this cross-regulation by different lipid classes is unclear, but it is possible that suppressing ABCA1-mediated lipid secretion by unsaturated fatty acids plays a physiological role in cellular lipid homeostasis, perhaps to retain excess cholesterol as a reservoir for membrane synthesis. This regulation, however, may have pathological consequences. Type 2 diabetes and the metabolic syndrome are characterized by elevated fatty acids, low plasma HDL levels, and prevalent CVD (19, 88). Palmitate and oleate, the two most common fatty acids, destabilize ABCA1 over a fatty acid-to-albumin molar ratio within the range observed for subjects with these disorders (289, 290). It is possible that impaired ABCA1-mediated cholesterol secretion from cells contributes to the low plasma HDL levels and enhanced atherosclerosis in these subjects.

○ FREE CHOLESTEROL. Loading cultured macrophages with cholesterol in the presence of a drug that inhibits cholesterol esterification induces apoptosis, which is triggered by the accumulation of free cholesterol in the endoplasmic reticulum (67). This condition also destabilizes ABCA1 protein, possibly by enhancing degradation by proteasomes (66). Thus an impaired ability of ABCA1 to remove lipids from macrophages may potentiate the damaging effects of free cholesterol. Such a mechanism could contribute to progression of atherosclerosis, as macrophages progressively accumulate large amounts of free cholesterol in advanced lesions.

D) REACTIVE CARBONYLS. Treatment of cultured macrophages with the reactive carbonyl species glyoxal and glycoaldehyde acutely and severely impair the ABCA1 pathway, presumably by directly damaging ABCA1 protein (220). These carbonyls are generated during glucose metabolism and protein glycoxidation and form advanced glycated end products (AGEs) on tissue proteins (15, 28, 89, 267, 300). AGEs accumulate during aging, and their formation is enhanced by diabetes. In addition to glycoxidation, activated phagocytes can generate glycoaldehyde from amino acids using myeloperoxidase (7). Thus the inflammation that underlies multiple disorders including diabetes is likely to produce carbonyl stress in tissues and impair the ABCA1 pathway. This could be another mechanism that contributes to the increased CVD associated with these metabolic disorders.

3. Protein trafficking

Modulation of ABCA1 trafficking could have important influences on ABCA1 protein levels and function (Table 1). ABCA1 recycles rapidly between the plasma membrane and late endosomal/lysosomal compartments (193, 194), which may play a role in both lipid transport
and protein processing. ABCA1 is selectively expressed on the basolateral membranes of cultured intestinal cells (185, 200), gallbladder epithelial cells (151), brain capillary endothelial cells (218), alveolar type II cells (312), and hepatocytes (192), indicating the presence of factors that target ABCA1 to specific membranes in polarized cells. There is emerging evidence that the interaction of proteins with the COOH-terminal domain may be involved in directing intracellular trafficking of ABCA1.

The COOH terminus of ABCA1 contains a ESTV motif that conforms to the consensus sequence for binding to PDZ domain-containing proteins (74), a diverse group of proteins that form multimeric complexes that often mediate cytoskeleton interactions and protein trafficking (139). Yeast two-hybrid screens identified three PDZ proteins (α1- and β2-syntrophin and Lin7) that interact with the COOH-terminal domain of ABCA1 (29, 184). Overexpressing α-syntrophin in cells stabilized ABCA1 and enhanced cholesterol efflux (184), suggesting that its interaction with ABCA1 is important for optimum function. When a mutant for ABCA1 lacking the PDZ binding domain was modestly overexpressed in cells, cholesterol efflux was less than half that seen with cells expressing a similar amount of wild-type ABCA1 (74). However, when cells were forced to express high levels of this mutant, there was no impairment of cholesterol efflux. Thus it appears that interactions of PDZ proteins with ABCA1 have modest effects on its function but are not essential for activity. The role of these interactions in trafficking of ABCA1 to membranes in polarized cells has not been explored in detail.

Intracellular protein trafficking often involves the fusion of transport vesicles, which is mediated by a group of vesicle membrane receptors called SNAREs, including the syntaxin family of proteins. Screening macrophages for syntaxins that are increased in response to cholesterol loading led to the identity of syntaxin 13 as an ABCA1 binding protein (14). Silencing of syntaxin 13 with siRNA reduced ABCA1 protein levels and lipid efflux to HDL and apoA-I without affecting ABCA1 mRNA levels (14). Thus syntaxin 13 appears to stabilize ABCA1 protein, probably by modulating its vesicular trafficking. In addition to endosomes, macrophage phagosomes also appear to contain syntaxin 13, raising the possibility that phagocytic vesicles play a role in processing ABCA1.

Several compounds have been described that appear to affect ABCA1 levels or activity through their effects on ABCA1 trafficking. Ceramide was shown to enhance cholesterol efflux to apolipoproteins by increasing the cell surface content of ABCA1 (298). In contrast, cyclosporin A was reported to inhibit ABCA1-dependent lipid efflux by trapping ABCA1 on the cell surface where it lost activity (156). These studies show that agents that alter the trafficking patterns of ABCA1 can have significant effects on its cellular content and activity.

B. Activity

1. Signaling pathways

The lipid transport and apolipoprotein binding activities of ABCA1 are regulated by several signaling pathways that have no or little effect on ABCA1 expression (Table 2). Most of these pathways affect ABCA1 by direct phosphorylation. Several protein kinases have been described that modulate ABCA1 lipid transport and/or apolipoprotein binding activity. These signaling pathways can either increase or decrease different aspects of ABCA1 activity and thus coordinate its overall function.

A) PROTEIN KINASE A. Activation of protein kinase A (PKA) by cAMP is required for optimum lipid transport activity of ABCA1 (248, 264). Mutational studies and in vitro kinase assays showed that this occurs by PKA-mediated phosphorylation of serines 1042 and 2054 at 10.220.33.1 on June 22, 2017 http://physrev.physiology.org/ Downloaded from

<table>
<thead>
<tr>
<th>Table 2. Regulation of ABCA1 Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Effector</strong></td>
</tr>
<tr>
<td>Apolipoproteins ?</td>
</tr>
<tr>
<td>Cytokines ?</td>
</tr>
<tr>
<td>Apolipoproteins</td>
</tr>
<tr>
<td>Apolipoproteins</td>
</tr>
<tr>
<td>Partner proteins ?</td>
</tr>
<tr>
<td>?</td>
</tr>
<tr>
<td>?</td>
</tr>
<tr>
<td>Substrate trafficking Cholesterol</td>
</tr>
<tr>
<td>Apolipoproteins</td>
</tr>
<tr>
<td>?</td>
</tr>
</tbody>
</table>
activated in the NBDs (Fig. 5) (248). Mutation of serine 1042 alone did not affect ABCA1 activity, suggesting that phosphorylation of serine in NBD-2 has the greatest impact. Suppressing this phosphorylation severely reduced lipid transport to apolipoproteins without altering apolipoprotein binding to ABCA1-expressing cells or to ABCA1 (248, 264). Thus the PKA-mediated phosphorylation of ABCA1 appears to be necessary for optimum intrinsic lipid transport activity but not for apolipoprotein binding activity.

Although PKA-mediated phosphorylation of ABCA1 is required for activity, the importance of this signaling pathway as a physiological modulator of ABCA1 is unclear. Some studies have shown that incubating cells with cAMP analogs had no stimulatory effect on ABCA1 activity or phosphorylation, even though PKA inhibitors could reduce these processes, consistent with the idea that the intracellular cAMP content is already saturating for its effects on ABCA1 (207, 264). Other studies, however, have shown that increasing the cellular cAMP content enhances ABCA1 activity in association with increased ABCA1 phosphorylation (97, 98). Moreover, the interaction of apoA-I with ABCA1-expressing cells was reported to increase the cellular cAMP content and ABCA1 phosphorylation (89). The reason for these discrepancies is unknown, but it may reflect difference in cell lines and culture conditions. In support of this idea is a study showing that immortalizing primary fibroblasts increased the responsiveness of the ABCA1 pathway to a cAMP analog (207).

b) Protein Kinase CK2. Protein kinase 2 or CK2 is a serine/threonine kinase that plays a role in multiple processes related to cell survival and transformation (165, 243). Studies using recombinant peptides spanning the intracellular domains of ABCA1 showed that CK2 phosphorylates ABCA1 at threonines 1242 and 1243 and serine 1255, which are downstream from NBD-1 (Fig. 5) (233). Site-directed mutagenesis of these domains prevented CK2-mediated ABCA1 phosphorylation and enhanced lipid flipping, apolipoprotein binding to cells, and apolipoprotein-mediated lipid efflux (233). Thus CK2 acts as a down-regulator of ABCA1 lipid transport and apolipoprotein binding activities. Interestingly, CK2-mediated phosphorylation of a chicken homeoprotein called Engrailed 2 inhibits its secretion from cells (170). Although transported in vesicles and secreted, Engrailed 2 lacks a classic signal sequence. A similar secretion mechanism has been described for interleukin-1β, a reported substrate for ABCA1 (101, 313). These observations are consistent with an attenuating role of CK2 in ABCA1-dependent secretion of substrates that are important for normal cell function and viability.

c) Janus Kinase 2. Janus kinase 2 (JAK2) is a tyrosine kinase that is activated by over half of the cytokine/hematopoietin superfamilly of receptors (137). Activation of JAK2 is the initiating step in downstream signaling for most of these receptors. JAK2 also plays a role in modulating ABCA1 activity. A JAK2-specific inhibitor reduces apoA-I-mediated lipid transport and apoA-I binding to ABCA1, and mutant cells lacking JAK2 have a severely impaired ABCA1 pathway (264). In contrast to PKA, which regulates the lipid transport but not apolipoprotein binding activity of ABCA1, JAK2 is required for apolipoprotein binding to ABCA1 but has little effect on the intrinsic cholesterol flipase activity (264). These studies show that the lipid transport and apolipoprotein binding activities of ABCA1 can be regulated independently by different signaling processes.

The interaction of apoA-I with ABCA1-expressing cells acutely stimulates autophosphorylation of JAK2 (264), generating the active JAK2 that phosphorylates its target proteins. This suggests that apolipoproteins potentiate their own interactions with ABCA1 by activating JAK2, which in turn increases the apolipoprotein binding to ABCA1 required for lipid removal. It is unknown if the initiating step in this process is mediated by binding of apolipoproteins to ABCA1 or to other proteins, or whether the JAK2-targeted protein is ABCA1 or a partner protein.

d) Protein Kinase C. In addition to the possibility that phosphorylation of ABCA1 by protein kinase C (PKC) helps stabilize the protein, there is circumstantial evidence that PKC may play a role in modulating the lipid transport activity of ABCA1. Before the discovery of ABCA1, several studies showed that inhibition or activation of PKCs respectively decreased or increased cholesterol efflux from cells to HDL or purified apoA-I (160, 179, 266, 282), implicating PKCs as modulators of ABCA1 activity. More recent evidence suggested that activation of phospholipase C and D may be critical for apoA-I-mediated cellular cholesterol efflux (99, 281). However, this also appeared to be the case in cells with defective ABCA1 (99), suggesting that it may not directly involve this transporter. Moreover, short-term treatment of ABCA1-transfected cells with a broad-spectrum PKC inhibitor was shown to have no effect on apoA-I-mediated cholesterol or phospholipid efflux (264). It is possible that PKCs do not directly activate ABCA1 but modulate trafficking of lipid substrates to ABCA1 (see below).

2. Partner proteins

There is growing evidence that ABCA1 activity is modulated by the interaction of a diverse group of proteins (Table 2). ABCA1 was coimmunoprecipitated with Cdc42 (270), a member of the RhoGTPase family that forms complexes with proteins that control the cytoskeletal architecture and vesicular transport (262). This interaction may cause the changes in cell morphology associated with under- or overexpressing ABCA1 (57, 270). Moreover, forced expression of Cdc42 was shown to en-
hance apoA-I-mediated cholesterol efflux, whereas expression of dominant negative Cdc42 impaired this efflux, consistent with a role of Cdc42 in modulating ABCA1 function (108, 197).

Immunoprecipitation studies also identified Fas-associated death domain protein (FADD) as an ABCA1 interacting protein (30). Blocking this interaction impaired apolipoprotein-mediated phospholipid efflux. The biological significance of the FADD/ABCA1 association is unclear, as FADD is mainly involved in death receptor-induced apoptosis. These findings raise the possibility that ABCA1 has an antiapoptotic function, perhaps related to its ability to extrude lipophilic molecules from cells.

It is likely that additional proteins will be discovered that interact with ABCA1 and modulate activity. The COOH-terminal region of ABCA1 contains a highly conserved VFVNFA motif that is required for activity (74). Mutations in this motif have no effect on ABCA1 trafficking but severely impair its apoA-I interactions and lipid efflux. Peptide competition experiments suggest that this motif promotes the interaction of ABCA1 with an unknown cellular protein that is a component of the active complex (74).

3. Substrate trafficking

The activity of the ABCA1 pathway is influenced by factors that control intracellular trafficking of lipids (Table 2). Cholesterol, phospholipids, and other lipophiles are likely to be transported between cellular compartments in vesicular membranes. ADP-ribosylation factors (ARFs) and ARF-like proteins (ARLs) are Ras-related small GTPases that control the vesicle budding involved in vesicular transport pathways. One of these ARLs (ARL7) was shown to be induced by cholesterol loading of macrophages and by LXR/RXR agonists (65). Immunofluorescent studies suggested that ARL plays a role in vesicular transport between a perinuclear compartment and the plasma membrane. Expression of native and dominant-active ARL7 stimulated cholesterol efflux from cells by the ABCA1 pathway (65). These observations suggest that ARL7 is involved in trafficking of cholesterol from intracellular compartments to plasma membrane domains containing ABCA1, although they do not exclude the possibility that these ARL7-containing vesicles target ABCA1 to lipid domains.

 Trafficking of lipid substrates to ABCA1 may also be mediated by signaling processes elicited by the interaction of apolipoproteins with ABCA1-expressing cells. The ability of apolipoproteins to remove cholesterol selectively from sites of cholesterol esterification has been reported to involve activation of PKC by apolipoproteins (304).

Disorders that affect intracellular cholesterol trafficking have an impact on the ABCA1 pathway. Niemann-Pick type C (NPC) disease is a neurodegenerative disorder characterized by impaired intracellular lipid trafficking and accumulation of free cholesterol in late endosomes/lysosomes (84, 144, 195, 222). This is due to mutations in the gene for NPC1, which mediates transport of lipoprotein-derived cholesterol from these intracellular compartments to the Golgi apparatus, endoplasmic reticulum, and plasma membrane. Cells lacking NPC1 have below normal levels of ABCA1 expression and activity (42), consistent with an impaired transport of intracellular cholesterol to sites that regulate ABCA1 transcription and to membrane domains targeted for efflux. This impaired ABCA1 pathway may explain why patients with NPC have low plasma HDL levels and an overaccumulation of cellular cholesterol (42).

IV. IN VIVO FUNCTIONS

ABCA1 is widely expressed throughout animal tissues where it may have multiple and diverse functions. In humans, ABCA1 mRNA was reported to be most abundant in liver, placenta, small intestine, and lung (138). In the baboon, ABCA1 mRNA was shown to be abundant in tissues containing inflammatory cells and lymphocytes, but it was also detected in noninflammatory cells in the kidney, medulla, testis, and brain (149). In mice, ABCA1 mRNA was reported to be most abundant in liver, kidney, adrenal, heart, bladder, testis, and brain (296). Interestingly, measurements of ABCA1 protein levels in tissues showed discordance with mRNA abundance, in that some tissues with high mRNA levels (kidney, heart, bladder, and brain) had relatively low protein levels (296). These observations are consistent with the possibility that posttranscriptional regulation plays a major role in tissue expression of ABCA1.

Tissue culture studies predict that macrophage-rich tissues would have relatively high levels of ABCA1. As scavenger cells, macrophages ingest modified lipoproteins and damaged cell membranes and thus can accumulate large amounts of cholesterol, an inducer of ABCA1. Indeed, ABCA1 mRNA levels were shown to be higher in cholesterol-loaded cultured human macrophages than in any human tissues (138). ABCA1 is also highly expressed in the liver, where it is upregulated when mice are fed a high-cholesterol diet (296). It is therefore predictable that hepatic ABCA1 would make an important contribution to whole body lipoprotein metabolism. Current knowledge about the physiological role of ABCA1 in vivo is based largely on studies of human genetic disorders, mouse genetic models, and cultured tissue-specific cells.
A. HDL Production

1. Genetic variations

Mutations in ABCA1 can cause an autosomal recessive genetic disorder called Tangier disease, which is characterized by very low levels of plasma HDL and deposition of sterols in tissue macrophages (11). Tangier disease was first identified in the 1960s as an HDL deficiency syndrome affecting families from Tangier Island in the Chesapeake Bay, United States of America (77). It was discovered in the mid 1990s that the ability of purified apoA-I to remove cholesterol and phospholipids from these cells was severely impaired (75). In 1999, four laboratories independently identified the defective Tangier disease gene as ABCA1 (21, 24, 150, 239).

Over 70 mutations in ABCA1 have been identified in subjects with low plasma HDL levels, nearly one-third of which are missense mutations (48, 78, 255). Although these mutations occur throughout the gene, they tend to cluster in the extracellular loops, the NBD domains, and the COOH-terminal region.

The functional impact of a small subset of the missense mutations has been studied in cell culture. When expressed in cells, most of these mutants appear in the plasma membrane but have severely impaired lipid transport and apolipoprotein binding activities (73). Some studies have suggested that ABCA1 proteins with substitution mutations Q597R and R587W in the first extracellular loop do not localize to the plasma membrane (232, 263), but other studies have contradicted these findings (73, 150). Missense mutations in the second extracellular loop and in the ninth membrane-spanning domain were reported to prevent ABCA1 trafficking to the plasma membrane (6). Only one mutant (W590S) has been described that has near-normal apolipoprotein binding activity but defective lipid transport (73).

The initial loss-of-function mutations in ABCA1 were discovered in case reports and family studies. More recently, rare mutations were identified by screening ABCA1 from subjects with low plasma HDL levels for single nucleotide polymorphisms (SNPs) (48, 78, 255). Most of the identified rare alleles (mutations) were absent in populations of subjects with high HDL levels (48, 78), implying that they play a causative role in lowering HDL levels. Some mutations, however, were found to be more frequent in subjects with the highest HDL levels, consistent with an enhancement of the ABCA1 pathway. On the basis of mutation frequency in those with the lowest 1% of HDL cholesterol, it was estimated that as many as 10% of the HDL-deficient individuals in the general population are heterozygous for ABCA1 mutations (78). This is likely to be an underestimate, as these studies may have missed critical intronic or regulatory mutations. Moreover, these reports did not examine allelic frequencies in subjects with moderately reduced HDL levels, which is more typical of Tangier disease heterozygotes (255). Taken together, these observations are consistent with ABCA1 being a major determinant of plasma HDL levels in humans.

The role of ABCA1 as a modulator of HDL levels and size was supported by two independent reports that measured cholesterol efflux from cells cultured from Tangier disease heterozygotes. Although these patients as a group have approximately half normal plasma HDL levels, there is a wide variation among patients (255). The type of ABCA1 mutation may have some influence on the relative activity of the ABCA1 pathway, since truncation mutants may form dominant-negative interactions with the wild-type allele (255). This range in activity allowed for comparisons of apoA-I-mediated cholesterol efflux from skin fibroblasts cultured from these patients with their plasma HDL levels and particle size. Both studies showed a significant correlation between ABCA1 activity in their cultured skin fibroblasts and the level and size of plasma HDL particles (25, 47). Thus, at least among this subject group, the intrinsic activity of the ABCA1 pathway is a major determinant of plasma HDL cholesterol levels.

SNP analyses have also identified over 20 common polymorphisms (>1% allelic frequency) in the coding, promoter, and 5’-UTR regions of ABCA1 (48, 78, 255). Several of these are associated with either low or high plasma HDL levels. The most studied of these SNPs is the R219K variant, with the K allele being associated with higher levels of HDL (255). V771M and V825I SNPs have also been reported to be associated with increased HDL levels, whereas R1587K is associated with low levels of HDL (78, 255). Although the frequencies vary between groups, all of these more common SNPs were present in individuals having both high and low HDL levels, suggesting that they do not have a strong phenotypic effect.

2. Animal models

Studies of animal models have provided additional evidence that ABCA1 is a major determinant of plasma HDL levels. The only naturally occurring animal model of Tangier disease is the Wisconsin Hypoalpha Mutant (WHAM) chicken. ABCA1 in these chickens has a missense mutation near the NH2 terminus that produces a defective protein (13). Like Tangier patients, the WHAM chicken hypercatabolizes apoA-I and accumulates cholesteryl esters in tissues, particularly in hepatic parenchymal and intestinal epithelial cells. Targeted disruption of the Abca1 gene in mice produces a phenotype similar to that of human Tangier disease (4, 174, 211), including an HDL deficiency and accumulation of sterols in some tissues. Conversely, overexpressing ABCA1 in mice elevates plasma HDL levels (131, 256, 294).
Several lines of evidence suggest that the liver ABCA1 pathway in mice is responsible for generating most of the plasma HDL. Selective hyperexpression of ABCA1 in the liver using adenovirus transgenes markedly increases plasma HDL levels (294). Bone marrow transplantation showed that the ABCA1 pathway in peripheral macrophages in mice makes only a minor contribution to HDL formation (96). Another study showed that targeted disruption of liver ABCA1 in chow-fed mice reduced plasma HDL levels by 80% (153). These observations imply that the major cause of the HDL deficiency in Tangier patients and ABCA1 knockout mice is an impaired liver ABCA1 pathway.

B. Reverse Cholesterol Transport

It is widely believed that a major function of HDL is to transport cholesterol from peripheral cells to the liver for elimination in the bile (90). The studies described above suggest that ABCA1 plays a major role in this reverse cholesterol transport pathway. The presumed major precursor for this pathway is lipid-poor apoA-I (69, 210), which is initially synthesized and secreted by the liver (Fig. 6). Most of this apoA-I may immediately interact with liver ABCA1, but some may circulate to the periphery where it interacts with ABCA1 on cholesterol-loaded cells, particularly macrophages. It is more likely, however, that most of the lipid-poor apoA-I in peripheral tissues is generated from the surface of mature HDL particles that are transported there (Fig. 6, dotted arrow) (240). The ABCA1-bound apoA-I rapidly acquires free cholesterol and phospholipids, becoming partially lipidated. Most of these nascent particles then mature into spherical HDL particles (Fig. 6), which deliver their cholesteryl esters to the liver for secretion in the bile after binding to SR-B1, the HDL receptor that selectively transfers cholesteryl esters into hepatocytes. These cholesteryl esters can also be delivered to the liver after transfer to other lipoproteins, such as LDL (Fig. 6).

A general function of ABCA1 in reverse cholesterol transport might be to channel tissue cholesterol into the hepatic SR-B1 pathway for elimination from the body. There is evidence that hepatic SR-B1 acts to target HDL cholesterol for biliary secretion. In polarized cells, SR-B1 selectively sorts HDL protein and cholesterol across the basolateral and apical membranes, respectively (254). This polarity in hepatocytes would transport HDL cholesterol into the bile while regenerating apolipoproteins in the plasma. The major sources of cholesterol entering the reverse cholesterol transport pathway via peripheral ABCA1 would likely be cell debris and modified lipoproteins taken up by tissue macrophages. The major sources of cholesterol secreted by ABCA1 from the liver are probably dietary and lipoprotein cholesterol delivered to the liver by chylomicron and LDL receptors. This liver-processed sterol may need to be repackaged into HDL particles for efficient biliary secretion.

In support of this concept are studies showing that HDL, not LDL, is the major source of cholesterol used for bile acid production (246) and that overexpressing SR-B1 in mice increases transport of lipoprotein cholesterol into the bile (254). It has been reported, however, that biliary cholesterol secretion was unimpaired in mice lacking ABCA1 (94), suggesting that either ABCA1 is unnecessary for this process or that other hepatic mechanisms can compensate for the absence of ABCA1 and low HDL levels in these animals. Additional studies are needed to define the role of hepatic ABCA1 in lipoprotein metabolism.

C. Tissue-Specific Functions

1. Brain

The brain is the most sterol-rich organ in the body, containing ~25% of the total body cholesterol (58). Most
of the brain cholesterol is synthesized locally, with only a small amount transported from the circulation across the blood-brain barrier (58). Flux of cholesterol out of the brain occurs largely after conversion to the more polar 24S-hydroxycholesterol, which passively traverses the blood-brain barrier (20). ABCA1 is highly expressed in glial and neuronal cells of the central nervous system, particularly in Purkinje cells and pyramidal cortical neurons (142, 149, 296). There is emerging evidence that ABCA1 plays an important role in maintaining brain cholesterol homeostasis and protecting against disease.

The major ABCA1-interacting apolipoprotein in the brain is apoE, which is synthesized locally by glial cells (169). ABCA1 is highly induced in cultured astrocytes, neuronal cells, and microglial cells by LXR/RXR ligands, where it promotes cholesterol and phospholipid efflux to lipid-free apolipoproteins, including apoE (81, 142, 259). Interestingly, glial expression of ABCA1 appears to modulate synthesis and secretion of apoE. Glial cells cultured from mice lacking ABCA1 secreted smaller lipoprotein particles with a markedly reduced cholesterol and apoE content compared with cells from ABCA1-expressing animals (109, 280). The cortex and cerebral spinal fluid of ABCA1-deficient mice have a much lower level of apoE than those expressing ABCA1 (280). These findings suggest that glial ABCA1 has the dual function of promoting lipid efflux and generating apolipoprotein lipid acceptors for lipoprotein particle formation.

ABCA1 is also expressed in cultured brain capillary endothelial cells (218), where it may play a role in sterol transport across the blood-brain barrier. These cells synthesize and secrete apoA-I, which can serve as the acceptor for the lipids extruded by ABCA1 (218). With these cells, however, ABCA1 is localized to the basolateral membrane and apoA-I is secreted in this direction, consistent with generation of lipoprotein particles in the brain parenchymal fluid (218). ABCA1 is highly induced in brain capillary endothelial and glial cells by 24S-hydroxycholesterol through the LXR/RXR system (218). This is consistent with the possibility of a feedback mechanism, whereby 24S-hydroxycholesterol-induced ABCA1 generates lipoprotein carriers that transport this oxysterol to the blood-brain barrier for diffusion into the circulation (218). Capillary endothelial ABCA1 may also facilitate transport to the brain of circulating ABCA1 substrates that are important for neuronal function, such as \( \alpha \)-tocopherol.

Clinical and cell culture studies have implicated ABCA1 as being involved in the etiology of late-onset Alzheimer’s disease, a neurodegenerative disorder affecting memory and cognition in the elderly. Two studies have reported that genetic variations of ABCA1 modify risk for Alzheimer’s disease (134, 301), whereas another case-control study found no influence of ABCA1 polymorphisms on this disease (161).

It is believed that accumulation in the brain of plaques enriched in \( \beta \)-amyloid (A\( \beta \)) peptides causes Alzheimer’s disease. A\( \beta \) peptides are small 39- to 42-kDa secreted cleavage products of a cellular amyloid precursor protein (APP). A\( \beta \) production is positively related to the cholesterol content of neuronal cells, suggesting that brain cholesterol may be a risk factor for Alzheimer’s disease (293). In support of this idea are clinical studies showing that cholesterol-lowering drugs (statins) reduce the prevalence of this disease (128, 302). Cell culture studies have generated conflicting results about the influence of ABCA1 on A\( \beta \) production, with one study showing that increased ABCA1 expression increased generation of A\( \beta \) in cultured neuronal cells (81) and two others showing the opposite effect (142, 259). Although the reason for this discrepancy is unknown, it may reflect the possibility that ABCA1 can modulate A\( \beta \) production either directly or as a secondary response to cholesterol depletion. Additional studies are needed to confirm a role for ABCA1 in A\( \beta \) metabolism and Alzheimer’s disease.

2. Intestines

Early studies suggested that ABCA1 in intestinal enterocytes could play a role in promoting resecretion of sterols back into the intestinal lumen across the apical membrane and thus reduce absorption of dietary cholesterol. This was largely based on the observation that LXR/RXR agonists inhibit absorption of dietary cholesterol in mice in association with an induction of intestinal ABCA1 (141, 231). Since then, two other intestinal LXR-inducible ABC transporters (ABCG5 and ABCG8) were discovered. It now appears that most of the LXR-modulated resecretion of dietary cholesterol in the intestine is mediated by heterodimers of ABCG5 and ABCG8 (17, 93, 154, 167). Studies of cultured intestinal-derived Caco-2 cells showed that ABCA1 is localized to the basolateral membrane, as is the case with other polarized cells (68, 118, 200). A lack of ABCA1 in the WHAM chicken did not impede LXR agonist-induced reduction in dietary cholesterol absorption, but it did suppress cholesterol secretion from the basolateral side of the intestines (183). Intestinal cells also synthesize apoA-I and secrete it from the basolateral side (118). It is therefore likely that intestinal ABCA1 functions to generate HDL particles that transport dietary cholesterol to the liver.

3. Gallbladder

Bile is the major route of cholesterol secretion from the body. The concentration of bile during the interdigestive phase in the gallbladder can supersaturate the bile with cholesterol, exposing the apical side of gallbladder epithelial cells to high concentrations of cholesterol and leading to gallstone formation and cholesterolosis. As with liver and intestinal cells, gallbladder epithelial cells
express ABCA1 in their basolateral membranes (151). Interestingly, addition of apoA-I to either the apical or basolateral sides of these cultured cells stimulates ABCA1-mediated cholesterol efflux to the basolateral side (151). This may be because apoA-I induces the synthesis and basolateral secretion of apoE (152). These cells also synthesize and secrete apoA-I into the basolateral media in response to LXR/RXR ligands (152). These observations suggest that ABCA1 in gallbladder epithelial cells functions to reduce biliary cholesterol content and thus protects against gallstone formation.

4. Lung

Disruption of the ABCA1 gene in mice leads to an accumulation of lipids within lung alveolar type II cells, consistent with ABCA1 playing an important role in lipid homeostasis in the lung (174). ABCA1 in cultured type II cells is induced by LXR/RXR ligands and promotes phospholipid efflux from the basolateral side (22, 312). There is circumstantial evidence that this basolateral phospholipid export may function to modulate surfactant secretion. Surfactant is secreted from the apical side of alveolar type II cells into the alveolus, where it is essential for normal breathing. The major component of surfactant is phosphatidylcholine containing saturated fatty acid side chains. Stimulation of phospholipid synthesis in cultured murine alveolar cells was reported to induce ABCA1 transcription by an LXR-independent mechanism (312). Because the phospholipids secreted by the ABCA1 pathway were enriched with unsaturated fatty acids, these results suggested that ABCA1 might increase the relative saturated phospholipid content of alveolar cells, thus providing the appropriate phospholipids for producing surfactant. Such a mechanism, however, appears to have little consequences for normal lung physiology in humans, as there is no evidence that ABCA1-impaired individuals have breathing difficulties.

5. Testis

ABCA1 is highly expressed in the testis (296), suggesting that it plays a role in modulating testicular lipid transport. ABCA1 is enriched in the Sertoli cells of the seminiferous tubule, which phagocytose residual bodies and apoptotic sperm cells. ABCA1-deficient mice accumulate lipid droplets in Sertoli cells (250), implying that ABCA1 promotes removal of excess cellular lipids derived from phagocytosed membranes. Cultured Sertoli cells lacking ABCA1 fail to transport cholesterol to apolipoproteins (250). The pathological consequences of impaired ABCA1 in these cells are unclear. Although ABCA1-deficient male mice have lower spermatogenesis and fertility than wild-type mice (250), this may be due to the absence of plasma HDL, which is a major supplier of cholesterol for steroidogenesis by Leydig cells. Moreover, there is no evidence for reduced fertility in human male subjects lacking a functional ABCA1.

6. Kidney

Dyslipidemia accelerates the progression of renal disease, and cholesterol-lowering drugs can retard this effect (2, 135). Lipid-laden foam cell are frequently present in glomeruli of subjects with chronic renal disease, suggesting that cholesterol homeostasis in these cells is disrupted. Several forms of renal injury promote accumulation of cholesterol within the renal cortex, and this can induce ABCA1 in proximal tubular cells (129). Cultured glomerular mesangial cells have been shown to express LXR-inducible ABCA1, which promotes cholesterol efflux from these cells to apoA-I (303). These results suggest that ABCA1 may function in the kidney to maintain normal cholesterol homeostasis and protect against hyperlipidemic renal disease. One study of ABCA1-deficient mice showed the presence of glomerulonephritis, but this appeared to be due to deposition of immune complexes in the kidney (43). There have been no published reports of renal disease in human subjects with dysfunctional ABCA1.

V. ABCA1 AND CARDIOVASCULAR DISEASE

Numerous population studies have shown an inverse relationship between plasma HDL levels and CVD risk, implying that HDL protects against atherosclerosis. Although there are multiple mechanisms by which HDL can be atheroprotective, it is clear that the relative activity of ABCA1 plays a major role. Genetic and cell biology studies suggest that low plasma HDL levels in many individuals reflect an impaired ABCA1 pathway, which would also promote accumulation of cholesterol in tissue macrophages. Accumulation of sterol-laden macrophage “foam cells” in the artery wall is an early event in formation of atherosclerotic lesions. Thus both the relative activity of the cellular ABCA1 pathway and the availability of functional apolipoproteins in the artery wall would be expected to have a major impact on atherogenesis. These assumptions were borne out by studies of human subjects with genetic HDL deficiencies and of genetically modified mice.

A. ABCA1 Activity

Tangier disease homozygotes (or compound heterozygotes) over the age of 30 have a sixfold higher than normal incidence of CVD (251), which applies equally to both men and women. This moderately high risk for atherosclerosis is not as dramatic as one would expect for individuals with a virtual absence of HDL, a well-known
atheroprotective lipoprotein. Their low levels of LDL may partially protect these Tangier disease subjects from atherogenesis. Studies of Tangier disease heterozygotes, who tend to have more normal levels of LDL, showed a significant inverse correlation between ABCA1 activity in their cultured skin fibroblasts and the prevalence and severity of CVD (47). Dysfunctional ABCA1 mutations were shown to be associated with greater intima-media thickness of the carotid arteries (273). In general, premature CVD is associated with ABCA1 mutations that impair its function (48, 78, 255).

Polymorphisms in ABCA1 are associated with either increased or decreased CVD (255). Interestingly, many of these variants show an altered severity of atherosclerosis without changes in plasma lipid levels.

Mouse model studies have also provided support for the atheroprotective effects of ABCA1. One report showed that overexpression of human ABCA1 in transgenic C57Bl/6 mice significantly reduced diet-induced atherosclerosis (132). This was accompanied by a favorable change in lipoprotein profile. Conflicting data were reported, however, for apoE null mice overexpressing ABCA1, where one study showed a decrease in atherosclerosis (256) while another showed an actual increase (132). Studies with ABCA1 null mice have been difficult to interpret because, as with Tangier disease patients, the absence of a functional Abca1 gene also lowers plasma levels of atherogenic lipoproteins (3). This is probably due to the impact ABCA1 has on liver and intestinal lipoprotein metabolism. However, reciprocal bone marrow transplantation studies using wild-type and Abca1 null mice have shown that ABCA1 expressed selectively in macrophages has a significant influence on atherogenesis (3, 274). These studies underscore the importance of macrophage ABCA1 as a target for therapeutic interventions for treating CVD.

B. ABCA1-Interacting Apolipoproteins

1. Apolipoprotein supply

The ABCA1 pathway must rely on an adequate supply of lipid acceptors for optimal activity. Cell culture studies showed that these are lipid-poor apolipoproteins (210). The ABCA1-mediated lipid efflux from cultured cells saturates at an apolipoprotein concentration of 10^{-5} M, <1% of the total plasma concentration of apolipoproteins. Thus only trace amounts of lipid-poor apolipoproteins would be required to maximally stimulate this pathway in vivo, as long as they can be continually regenerated. Studies of HDL particle composition have shown that 5–8% of the plasma apoA-I is associated with no or very little lipid (12). The concentration of lipid-poor apoA-I is even higher in interstitial fluids where it would have direct contact with ABCA1. Lipid-poor apoE (111) and apoA-IV (61) have also been identified in plasma. It would therefore appear that the steady-state supply of lipid-poor apolipoproteins would be in excess of that required to maximally stimulate the cellular ABCA1 pathway in most cases. Nevertheless, factors that influence the availability and activity of lipid-poor apolipoproteins in the artery wall may modulate the atheroprotective effects of ABCA1.

A severe deficiency of apoA-I in both humans and mice tends to increase CVD, but this is not true in all cases (278). This inconsistency may reflect the apolipoprotein redundancy in cholesterol mobilization or the need for other atherogenic factors to be present for apolipoproteins to manifest their protective effects. It is noteworthy that apoA-I null alleles are associated with CVD in the homozygous state when no evidence of disease exists in heterozygotes (278), consistent with the concept that only trace quantities of apolipoproteins are required to saturate the ABCA1 pathway. Overexpression of apoA-I in transgenic mice and rabbits increased HDL levels and reduced CVD (62, 238, 265). These findings conform to the possibility that the arterial supply of lipid-poor apolipoproteins can be limiting for the ABCA1 pathway. It is also possible that atheroprotective effects of HDL independent of ABCA1 account for these observations.

Factors that promote regeneration of lipid-poor apolipoproteins from mature HDL particles may also play an important role in providing lipid acceptors for the ABCA1 pathway. It is possible that distinct subpopulations of HDL particles are the major sources of apoA-I that dissociates from the particle surface. These could be large apoA-I-containing particles lacking apoA-II that appear in the HDL2 subfraction (38). This idea is supported by studies showing that these particles have a larger fraction of exchangeable apoA-I (39) and are correlated better with protection against CVD (38, 79) than are apoA-II-containing HDL particles. A reduced availability of lipid-poor apoA-I may contribute to the increased formation of atherosclerotic lesions in transgenic mice overexpressing apoA-II (292). Remodeling of HDL particles may also generate lipid-poor apolipoproteins (Fig. 6). Plasma cholesterol ester and phospholipid transfer proteins, hepatic lipase, and scavenger receptor B1 can all produce lipid-poor apoA-I from HDL. In human and mouse models, variations in expression of these molecules can have either pro- or anti-atherogenic effects (114, 117, 122, 127), suggesting a complex interaction with other factors. The relative ability to generate lipid-poor apolipoproteins may contribute to this complexity.

A poorly understood area of apolipoprotein metabolism relates to factors that modulate apolipoprotein availability in the atherosclerotic lesions. Although HDL apolipoproteins have been shown to accumulate in lesions, it is unknown what fraction of these is lipid deficient. Apolipoproteins bind to matrix proteins (33), which could also affect their efficacy in interacting with ABCA1. This
possibility is supported by a study showing that apoA-I bound to matrix established by cultured macrophages is more active in removing cellular cholesterol than apoA-I in solution (32). Bone marrow transplantation studies have shown that overexpression of apoA-I (171) or apoE (16) in macrophages reduces CVD in mice. Because apoE is synthesized and secreted by macrophages, it is likely to have an autocrine/paracrine effect on the ABCA1 pathway in lesion cells. ApoE has the additional ability of mobilizing cholesterol from macrophages by ABCA1-independent mechanism during its synthesis and secretion (112). Macrophages may secrete other molecules that remodel HDL, such as hepatic lipase (91) or phospholipid transfer protein (PLTP) (145, 199).

2. Dysfunctional apolipoproteins

Modification of apolipoprotein structure could impair its ability to interact with ABCA1 and mobilize excess cholesterol from arterial macrophages. In support of this possibility is a study showing that most of the apoA-I in HDL isolated from atherosclerotic lesions migrates as a larger than normal molecular weight species on electrophoretic gels (18), consistent with extensive structural modifications of HDL apolipoproteins within these lesions. Although apolipoproteins could be modified by multiple processes, it is likely that oxidation reactions play a major role.

Many lines of evidence support the hypothesis that oxidation converts LDL into an atherogenic form in animal models of hypercholesterolemia (104, 105, 299). Oxidized LDL has also been detected in human atherosclerotic lesions (104, 105, 299), raising the possibility that oxidative modifications of lipoproteins may be clinically significant. Moreover, chlorinated or nitrated apoA-I has been found in HDL isolated from human blood and atherosclerotic tissue, indicating that HDL is a target for oxidation in vivo (18, 221, 310). In vitro, chlorination but not nitration markedly impairs the ability of apoA-I to promote cholesterol efflux from artery wall cells by the ABCA1 pathway (18, 253, 310).

A) CHLORINATION AND NITRATION OF HDL. One oxidative pathway in the human artery wall involves myeloperoxidase, a phagocyte heme protein that colocalizes with arterial macrophages (53). Myeloperoxidase uses \( \text{H}_2\text{O}_2 \) and chloride to generate hypochlorous acid (HOCl), a powerful oxidant (113). The importance of this reaction is underscored by the presence of enzymatically active myeloperoxidase in human atherosclerotic lesions (53). In vitro studies demonstrate that myeloperoxidase or reagent HOCl converts tyrosine to 3-chlorotyrosine, a stable product (59, 102). Studies of model systems (59, 102) and myeloperoxidase-deficient mice (85) have demonstrated that 3-chlorotyrosine is a molecular fingerprint that implicates the myeloperoxidase pathway in oxidative damage.

Levels of 3-chlorotyrosine in HDL isolated from human atherosclerotic lesions were sixfold higher than those in HDL circulating in human blood (18, 310). They were eightfold higher in HDL isolated from plasma of subjects with coronary artery disease than in HDL from plasma of healthy subjects (18, 310). These findings support the hypothesis that HOCl derived from myeloperoxidase contributes to HDL oxidation in the artery wall.

Another oxidative pathway involves nitric oxide (nitrogen monoxide, NO), which is generated by vascular wall cells (182). NO reacts rapidly with superoxide to form peroxynitrite (ONOO\(^-\)), a reactive nitrogen species (119). ONOO\(^-\) reacts in vitro with tyrosine residues to yield nitrotyrosine, a stable product (119). NO can also autoxidize to nitrite (NO\(_2\)), and plasma levels of NO\(_2\) rise markedly during acute and chronic inflammation (182). Because NO\(_2\) is a substrate for myeloperoxidase and other peroxidases (63, 64), it might be used to nitrate tyrosine in vivo. Indeed, myeloperoxidase uses hydrogen peroxide (\( \text{H}_2\text{O}_2 \)) and NO\(_2\) to generate reactive nitrogen species that nitrate tyrosine residues in animal models of inflammation, and this pathway is partially inhibited in mice deficient in myeloperoxidase (85).

HDL is nitrated on tyrosine residues in vivo (221, 310). Levels of 3-nitrotyrosine were fivefold higher in HDL isolated from atherosclerotic tissue than in circulating HDL, and plasma HDL from humans with established coronary artery disease contained twice as much 3-nitrotyrosine as that from healthy subjects (221, 310). There was also a strong relationship between levels of 3-nitrotyrosine and 3-chlorotyrosine in plasma HDL, suggesting that myeloperoxidase is the major pathway for nitrating HDL that appears in the circulation (221). These observations suggest that elevated levels of 3-chlorotyrosine and 3-nitrotyrosine in circulating HDL might represent a novel marker for clinically significant atherosclerosis.

A key question is whether chlorination or nitration of HDL in the artery wall promotes the development of atherosclerotic plaque. In vitro studies indicate that Tyr-192 of apoA-I is the single major target for chlorination by the myeloperoxidase-\( \text{H}_2\text{O}_2 \)-chloride system or HOCl (18, 253, 311) and for nitration by ONOO\(^-\) (253, 311). The ability of apoA-I to promote ABCA1-dependent cholesterol efflux was dramatically impaired when Tyr-192 was chlorinated (18, 253). In contrast, nitration of Tyr-192 had little impact on this biological function (253, 310). These observations indicate that Tyr-192, an aromatic amino acid, is the major target for oxidation when apoA-I is exposed to reactive chlorine and nitrogen species and that chlorination and nitration exert different effects on the ability of the modified apolipoprotein to promote ABCA1-dependent cholesterol efflux. It is important to note that these studies do not prove that chlorination of Tyr-192 is responsible for impairing apoA-I function be-
because other amino acids are also modified when apoA-I is exposed to oxidizing intermediates (253, 311).

Several lines of evidence suggest that myeloperoxidase-mediated oxidation of apoA-I may be sufficient to impair ABCA1-dependent cholesterol efflux from macrophage foam cells in the human artery wall (18, 221, 253). The level of tyrosine chlorination in lesion HDL ranged from 100 to 300 μmol 3-chlorotyrosine/mol Tyr (18), suggesting that ~1 in every 800 apoA-I molecules was chlorinated. It is likely, however, that apolipoprotein oxidation is more prevalent in the immediate pericellular environment than in the overall extracellular compartment. Thus epitopes for 3-nitrotyrosine colocalize with myeloperoxidase and macrophages in human atherosclerotic tissue (221). Because nitrogen dioxide radical, the source of 3-nitrotyrosine, is a short-lived species, this finding implies that proteins in close proximity to macrophages are prime targets for oxidative damage. Moreover, apoA-I is poorly nitrated by both myeloperoxidase and ONOO⁻ when it is associated with HDL (253), raising the possibility that lipid-poor apoA-I, the biologically active ligand for ABCA1, is the major target for nitration in the artery wall. Thus oxidation of apoA-I appears to be physiologically important, lipid-poor apoA-I may be the primary target for oxidation, and microenvironments depleted of antioxidants might enable oxidation to occur. One such environment surrounds tissue macrophages, which colocalize with myeloperoxidase in the artery wall and generate high local concentrations of oxidants.

B) TYROSYLATION OF HDL. HDL can also be modified by tyrosylation by exposing particles to tyrosyl radicals generated by a peroxidase. There is evidence that this form of oxidatively modified lipoprotein might actually retard the formation of atherosclerotic lesions (168). Hypercholesterolemic mice deficient in apolipoprotein E were protected from atherosclerosis more effectively when they were injected intraperitoneally with tyrosylated mouse HDL than when they were treated similarly with native mouse HDL (168). Because myeloperoxidase produces tyrosyl radical in vitro, this apparently paradoxical finding may be relevant to vascular wall biology (106, 107). These studies are also intriguing because they suggest that not all forms of oxidized lipoprotein are atherogenic.

The mechanism that allows tyrosylated HDL to protect hyperlipidemic mice from atherosclerosis more effectively than HDL is poorly understood. However, tyrosylated HDL is more effective than native HDL at removing cholesterol from lipid-laden fibroblasts and macrophages in vitro by a process that appears to involve interacting with ABCA1 (76). Consistent with this possibility, production of plasma HDL levels appeared to be higher in the mice treated with tyrosylated HDL than in those treated with native HDL (168). These findings suggest that tyrosylated HDL promotes cholesterol efflux from macrophages and other tissues more effectively than HDL in atherosclerotic mice. It would be of interest to determine whether similarly modified species of HDL exist in vivo.

Cross-linked heterodimers of apoA-I and apoA-II in tyrosylated HDL appear to be responsible for the enhanced reverse cholesterol transport (288). ApoA-I cross-linked to apoA-II represented a minor fraction (~14%) of the total apolipoproteins in the tyrosylated HDL used in the studies of atherosclerotic mice (168). If these heterodimers are responsible for the atheroprotective effects of tyrosylated HDL, relatively low concentrations of such cross-linked apolipoproteins (or synthetic peptides) might be sufficient to strongly affect atherogenesis. These heterodimers act more like lipid-free apolipoproteins, perhaps because conformational changes in apoA-I expose more amphipathic α-helices to ABCA1. It will be of interest to determine whether tyrosylated HDL interacts with ABCA1 and to investigate the underlying mechanisms for enhanced cholesterol transport by this pathway.

C) OTHER OXIDATIVE PATHWAYS. Unmodified HDL protects LDL from oxidative modification by pathways that require metal ions (219), and HDL that has been oxidized by copper loses its ability to remove cholesterol from cultured cells (186, 241). These observations suggest that metal-catalyzed HDL oxidation is detrimental and should promote cardiovascular disease. However, the physiological significance of lipoprotein oxidation catalyzed by metal ions is not yet established (105, 155). Several other mechanisms, including ability of HDL to inhibit LDL oxidation, reduce lipid hydroperoxides, and transport oxidized lipids to the liver for excretion, may also be cardioprotective (44, 83, 275). Methionine and phenylalanine residues in apoA-I are oxidized by reactive intermediates, but it is unclear if this process affects the ability of apolipoprotein to remove cholesterol from cells (172, 217).

VI. ABCA1 AS A THERAPEUTIC TARGET

The proven atheroprotective activity of ABCA1 has made this transporter an important new therapeutic target for treating CVD, and multiple programs have been initiated by the pharmaceutical industry to develop agonists for this pathway. These programs are based on the assumption that an increased ABCA1 activity in arterial macrophages would prevent foam-cell atherosclerotic lesion formation and that an increased liver ABCA1 activity would elevate plasma HDL levels and thus enhance the diverse atheroprotective functions of this lipoprotein subclass. The activity of the ABCA1 pathway can be modulated at multiple levels, providing an array of potential therapeutic targets for enhancing this pathway. While ABCA1 transcription has received the most attention, other posttranscriptional processes may prove to be important targets under special circumstances.
A. Transcriptional Regulation

1. LXR agonists

Because of the robust induction of ABCA1 by sterols, most of the therapeutic approaches for enhancing ABCA1 activity have focused on generating LXR agonists that induce ABCA1 transcription, and several of these have been developed and characterized. An additional advantage of these agonists is that they induce other proteins involved in several steps along the reverse cholesterol transport pathway: ABCG1 (244), which mediates cholesterol efflux to HDL particles (284); apoE (147), which promotes cholesterol efflux from macrophages by both ABCA1-independent and -dependent mechanisms (112); and liver and intestinal ABCG5 and ABCG8 (228), which promote excretion of liver and dietary cholesterol into the bile. Thus LXR agonists have the potential for activating multiple processes that coordinate excretion of excess cholesterol from the body. Two nonsteroidal synthetic LXR agonists (TO901317 and GW39695) have been shown to increase plasma HDL levels and reduce atherosclerosis in mouse models (130, 180, 268).

A limitation to first generation LXR agonists is that they increase hepatic fatty acid synthesis and esterification and thus generate fatty livers and hypertriglyceridemia when administered to animals (229, 245). LXR agonists induce fatty acid synthase and a transcriptional factor named sterol regulatory binding element protein-1c (SREBP-1c) that activates genes for several enzymes in the fatty acid biosynthetic pathway (212). Moreover, SREBP-1c induces stearoyl-CoA desaturase, which converts saturated fatty acids to unsaturated fatty acids that destabilize ABCA1 protein (198). One study showed that GW3965 can be administered to mice at a concentration that elevates plasma HDL levels without causing hypertriglyceridemia (180), although this agonist activates SREBP-1c at high concentrations in vitro (224). A synthetic oxysterol ligand for the LXR receptor was shown to stimulate ABCA1 synthesis with only a limited ability to increase hepatic SREBP-1c (224), indicating that divergent gene regulation by LXR is possible. A major challenge is to produce potent synthetic LXR agonists that selectively induce ABCA1 and other cholesterol transporters.

2. RXR agonists

Agonists for RXR, the heterodimeric partner for LXR, also stimulate ABCA1 transcription in cultured cells (51, 247). Administration of an LXR agonist to atherosclerotic mice was shown to reduce atherosclerosis (46). The limitation for this class of agonists is that RXR forms active heterodimers with several other nuclear receptors that control many different metabolic pathways. LXRs have therefore been the preferred target for modulating ABCA1 expression. It was reported, however, that retinoic acid receptor activators can induce ABCA1 without affecting other LXR-targeted genes (50), raising the possibility of selective drug therapy through the RXR system.

3. PPARγ agonists

The nuclear receptor PPARγ regulates transcription of multiple genes involved in cell differentiation and lipid metabolism. PPARγ is also the receptor for thiazolidinediones (TZDs) (157), a class of drugs developed for treating insulin resistance and type 2 diabetes. Cholesterol loading of macrophages induces PPARγ (187), which has several effects on cholesterol trafficking. PPARγ directly stimulates transcription of a scavenger receptor (CD36) that promotes uptake of oxidized lipoproteins (269). PPARγ also stimulates cholesterol efflux from cells by increasing ABCA1 expression, an effect that is secondary to inducing LXRα (37, 41). In mice, PPARγ activators lessen atherosclerosis (37, 159), suggesting that they have a favorable effect on macrophage cholesterol balance. PPARγ activators therefore have the potential of both improving insulin sensitivity and reducing the atherosclerosis that is often associated with diabetes.

B. Posttranscriptional Regulation

Another potential limitation in therapeutic targeting of ABCA1 transcription is that ABCA1 protein levels and activity are highly regulated by posttranscriptional processes. The discordance between ABCA1 mRNA and protein levels among mouse tissues underscores the physiological significance of posttranscriptional regulation (296). For example, heart and kidney were shown to express relatively high levels of ABCA1 mRNA but were among tissues with the lowest ABCA1 protein content (296). It is therefore likely that a variety of diverse cellular and extracellular factors dramatically influence the cell content and activity of ABCA1 protein. Cellular factors would include proteases, signaling enzymes, and partner proteins, and extracellular factors would include metabolites (e.g., fatty acids and reactive carbonyls), cytokines, and hormones. These complex processes have received little attention as therapeutic targets. In many cases, however, the best approach would be treating the underlying metabolic disorders that generate or modify these factors.

C. Apolipoprotein Supply

Therapeutic strategies to increase ABCA1 activity would only be effective if there were an adequate supply of functional apolipoproteins in the artery wall. The striking observation that most of the apoA-I in HDL isolated from atherosclerosis lesions is structurally modified (18)
is consistent with the possibility that the lesion environment damages apolipoproteins, impairing their ability to remove cellular cholesterol from macrophages by the ABCA1 pathway. In this case, effective CVD therapy would need to be directed at increasing the arterial supply of functional ABCA1 ligands or at blocking apolipoprotein-damaging reactions.

There are multiple biochemical processes that control the production, degradation, and regeneration of lipid-poor apolipoproteins (see Fig. 6), and each one is a potential therapeutic target for elevating ABCA1 ligands. It has also been demonstrated that infusion of HDL apolipoproteins into animals and humans can retard or reverse atherosclerosis. Infusion of apoA-I into cholesterol-fed rabbits was shown to significantly retard atherosclerosis (181). As discussed above, infusion of tyrosylated HDL into atherosclerosis-susceptible mice reduced atherogenesis to a greater extent than infusion of normal HDL (168). Phospholipid vesicles containing a naturally-occurring mutant form of apoA-I (R173C), called apoA-I (Milano), reduced atherosclerosis when infused into animal models (40, 252) or human subjects (196). ApoA-I (Milano) was identified in family members from rural Italy who had very low levels of HDL but unusual longevity, implying that their HDL is cardioprotective. A clinical trial showed that weekly intravenous injections of apoA-I (Milano)-phospholipid vesicles into human subjects with established CVD reduced atheroma volume by 4.2% after only 5 wk (196). It is unknown, however, if injections of native apoA-I would have had similar effects. The atheroprotective mechanisms of infused apolipoproteins have not been established, but it is possible that ABCA1 interactions play a role.

One promising therapeutic approach involves administering amphiphatic α-helical peptide mimetics of apolipoproteins. A mimetic peptide containing 18 d-amino acids (D-4F) is absorbed orally into the bloodstream of mice where it has a low rate of turnover (190). Administration of this peptide in the drinking water was shown to markedly reduce atherosclerosis in mouse models (190). These effects were associated with an increase in the anti-inflammatory properties of HDL particles and an enhanced cholesterol efflux from macrophages (191), suggesting that D-4F protects against atherosclerosis by several mechanisms, including possible interactions with ABCA1. The potential advantage of these peptides over full-length apolipoproteins is that they can be given orally and are long-lived.

Prevention of oxidative damage to apolipoproteins in the artery wall could also be an important therapeutic approach for enhancing the ABCA1 pathway. Lipid-soluble antioxidants have been shown to inhibit atherogenesis in hypercholesterolemic animal models (56), which may stem from their ability to inhibit oxidation of both LDL and HDL. These studies raised the possibility that dietary antioxidants, such as β-carotene and vitamins C and E, could have therapeutic benefits for treating CVD. Large randomized trials, however, have failed to show a consistent ability of antioxidant vitamins, either alone or in combination, to reduce clinical coronary end points (92, 103). Design of effective atheroprotective antioxidants will require more knowledge about the specific oxidation pathways involved in damaging apolipoproteins and their functional consequences.

Another strategy for protecting apolipoproteins from damage would be to administer apolipoproteins or peptides that are resistant to oxidation. Amino acid residues in apoA-I that are targeted for oxidative modification could be mutagenized to residues resistant to these modifications. It should also be possible to engineer small apolipoprotein mimetic peptides that are resistant to oxidation.

VII. CONCLUSIONS

Studies of human HDL deficiencies, transgenic mice, and cultured cells have shown that ABCA1 is the major exporter of cellular cholesterol and phospholipids to HDL apolipoproteins and that this activity is essential for formation of HDL particles in vivo. ABCA1 may also play a role in transporting a variety of other compounds, including vitamins and peptides. There is also evidence that ABCA1 is involved in processes other than modulating lipid homeostasis, such as promoting macrophage engulfment of apoptotic cells and generating microparticles that bleb from cell membranes. ABCA1 expression and activity are highly regulated by transcriptional and posttranscriptional mechanisms. Although transcriptional induction by sterols conforms to its role as a cholesterol exporter, the biological relevance of other regulatory processes is still unclear.

The HDL-elevating and atheroprotective functions of ABCA1 have made this transporter an important new therapeutic target for preventing and even reversing atherosclerotic CVD. First-generation protocols have focused on development of LXR agonists that stimulate transcription of ABCA1 and other genes involved in cholesterol transport. The potential benefits of these agents have been overshadowed by their broad specificity for lipogenic genes. Several studies, however, have offered proof-in-principle that drugs selective for cholesterol transport genes can be developed in the near future.

There are several lines of evidence that challenge the therapeutic benefits of targeting ABCA1 transcription. First, ABCA1 transcription in cells overloaded with cholesterol may already be maximally induced and thus resistant to further induction by agonists. Second, results showing a strong discordance between ABCA1 mRNA and protein levels in mouse tissues implicate posttrans-
scriptional regulation as having a dominant influence on ABCA1 expression. Third, factors that are elevated in individuals with metabolic syndrome and diabetes, such as fatty acids and glycoxidation products, destabilize ABCA1 in cultured macrophages, raising the possibility that damaged ABCA1 contributes to the increased CVD that is prevalent in these common disorders. This might limit the effectiveness of ABCA1 transcriptional activators for treating these affected individuals. It will be important to determine if factors associated with the metabolic syndrome and diabetes actually destabilize ABCA1 in vivo.

A relatively neglected area of research concerns the atherogenic effects of structural modifications of ABCA1-interacting apolipoproteins. Studies have shown HDL can be oxidatively modified in humans. Myeloperoxidase-catalyzed chlorination and nitration may be physiologically relevant pathways, because elevated levels of 3-chlorotyrosine and 3-nitrotyrosine have been detected in HDL isolated from blood of subjects with CVD and from atherosclerotic tissue. Chlorination and perhaps nitration of apoA-I might promote atherosclerosis by impairing its ability to interact with ABCA1. These observations raise the possibility that damaged apolipoproteins could contribute to the enhanced atherogenesis associated with common inflammatory disorders. It will be important to establish the molecular basis for the altered ability of oxidatively modified apoA-I to interact with ABCA1 and to determine whether such alterations play a role in the pathogenesis of CVD.

In summary, these observations indicate that the ABCA1 pathway is highly complex and far from being completely understood. Additional studies are needed to identify the molecular players in the ABCA1-dependent lipid transport pathway, to determine the array of substrates targeted by this transporter, to establish the biological functions of ABCA1 in different cell types, and to characterize the diverse processes that regulate ABCA1 expression and activity. These studies will not only provide insight into the role of ABCA1 in health and disease, but will uncover novel therapeutic targets for treating these diseases.

ACKNOWLEDGMENTS
Address for reprint requests and other correspondence: J. F. Oram, Dept. of Medicine, Box 556426, Univ. of Washington, Seattle, WA 98195-6426 (E-mail: joram@u.washington.edu).

GRANTS
The authors’ work described in this review was supported by National Institutes of Health Grants HL-18645, HL-75340, HL-55362, and DK-02456.

REFERENCES


Kim E, Niethammer M, Rothschild A, Jan YN, and Sheng M.


Leevendal TR, Rasmussen JE, Hsu FF, Mueller DM, Pen
tathur S, and Heinecke JW. Mass spectrometric quantification of markers for protein oxidation by tyrosyl radical, copper, and hydro


Lehmann JM, Moore LB, Smith-Oliver TA, Will


Lin G and Oram JF. Apolipoprotein binding to protruding mem


Kruth JS, Comly ME, Butler JD, Vanier MT, Fink JK, Weng


Krieger M. Charting the fate of the “good cholesterol”: identifica

Krut HS, Comly ME, Butler JD, Manier W, Fink JK, Weng


development of atherosclerosis in apolipoprotein E-deficient mice.


Downloaded from http://physrev.physiology.org/ on 2010-03-01 13:45:16 "Physiol Rev" • VOL 85 • OCTOBER 2005 • www.prv.org


