ATP-binding cassette transporter A1: regulation of cholesterol efflux

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Abstract
The ATP-binding cassette transporter A1 (ABCA1) is involved in the regulation of cholesterol efflux from cells. Mutations in ABCA1 give rise to familial high-density lipoprotein (HDL) deficiency and Tangier disease, which is characterized by very low levels of HDL in plasma and cholesteryl ester accumulation in tonsils and other reticuloendothelial cells. The mechanism of action of ABCA1 is still unclear, but requires the transfer of phospholipid and cholesterol to apolipoprotein A1 bound by or close to the transporter. An important factor in the regulation of ABCA1 is cholesterol itself, which provides oxysterol ligands for liver X receptors that stimulate ABCA1 transcription. ABCA1-deficient mice show increased cholesterol absorption, suggesting that ABCA1 could also help to transport dietary cholesterol back out of intestinal absorptive cells into the lumen. Thus ABCA1 is intimately connected to various aspects of the regulation of whole-body cholesterol metabolism and probably plays an important role in protecting against the development of cardiovascular disease.

Introduction to cholesterol efflux
In the 5 years since the ATP-binding cassette transporter A1 (ABCA1) was identified as the defective gene in Tangier disease, there has been a wealth of experimentation into its structure and function. However, as yet, no definitive picture has emerged. Results obtained with different systems have often proved incompatible and there is a general lack of indisputable hard evidence, particularly relating to the mechanism of action. Indeed, there is little direct evidence that any ABC transporter actually transports any substrate! So, this brief article is largely subjective, giving a broad overview of the mechanisms that seem most plausible at this time. More detailed information can be found in a series of reviews edited by Tall [1].

It has long been known that high-density lipoprotein (HDL) is atheroprotective. There is an inverse relationship between plasma HDL concentration and the development of coronary heart disease (CHD), and HDL has been shown to be an antioxidant, with anti-inflammatory and anti-platelet activity. The demonstration that HDL could act as an acceptor for cholesterol released from cells in vitro gave rise to the concept of reverse cholesterol transport, by which cholesterol from peripheral tissues is transferred to HDL and transported to the liver for elimination. HDL comprise a heterogeneous set of particles in which the protein and lipid components are in a constant state of exchange and flux, both within HDL and between HDL and chylomicron remnants. Thus, it has been difficult to determine the precise site of HDL synthesis, although the general sequence of events is well established. Most of the apolipoprotein (apo) components are synthesized and secreted by the liver. The main protein component, apoA1, acts as an acceptor for phospholipid and cholesterol from the cell membrane and other membranes to form discoidal pre-β-HDL particles, which are then acted upon by lecithin:cholesterol acyltransferase in the circulation and pick up further cholesterol and protein components to form mature spherical HDL with cholesteryl esters in the core. The initial transfer of cholesterol and phospholipid to apoA1 could be achieved in a number of ways. It could occur by aqueous diffusion down a concentration gradient from the cell surface to apoA1, but this is slow (half-life of 1–10 h) and could be limited by the difficulty of transferring the lipid to the outer leaflet of the membrane. The HDL receptor SR-B1 (scavenger receptor class B type 1) has been shown to be bi-directional and so could act to accelerate the diffusion mechanism, but it requires phospholipid in the acceptor particles. However, studies with macrophages suggested that there could be an alternative mechanism, since cholesterol-enriched cells were shown to release cholesterol, accompanied by phospholipid, to lipid-free acceptor proteins, such as apoA1 and apoE.

Discovery of ABCA1
The elucidation of the alternative mechanism for regulating cholesterol efflux from cells came from the study of a rare condition found originally in an isolated community on Tangier Island in Chesapeake Bay, MD, U.S.A. These patients present with large orange tonsils, peripheral neuropathies, hepatosplenomegaly and CHD [2]. Homozygous subjects
are characterized by a virtual absence of plasma HDL and apoA1, and an accumulation of cholesteryl esters in reticuloendothelial cells. The apoA1 gene is normal in these patients, but apoA1 in plasma is degraded very rapidly. Surprisingly, efflux of cholesterol from cultured cells to HDLs as acceptors is not impaired, but efflux to lipid-free apoA1 is markedly reduced [3]. In 1999, four groups using a variety of strategies identified ABCA1 as the defective gene in Tangier disease patients, and proposed that the protein controls the transfer of both cholesterol and phospholipid to apoA1, the initial step in HDL synthesis. If ABCA1 did not function, apoA1 remained lipid-free and was removed rapidly by the kidneys, preventing the formation of HDLs. It was also shown to be defective in familial HDL deficiency. ABCA1 has 12 transmembrane domains with two large extracellular loops, a cytoplasmic loop containing a hydrophobic region and two cytoplasmic nucleotide-binding domains. It is a member of the ABC transporter family, of which there are 49 members in humans. They are involved in transporting a variety of small molecules, and defects can lead to diseases as diverse as cystic fibrosis, familial intrahepatic cholestasis and retinitis pigmentosum. However, they all have a common feature, in that they use ATP as an energy source to regulate the transport of lipids or other metabolites across cell membranes. Many, if not all, achieve this by flipping molecules from the inner to the outer leaflet of the membrane.

**ABCA1 transgenic mice**

About 50 mutations in the ABCA1 gene have been reported in patients with Tangier disease or HDL deficiency. Unexpectedly, there is no consistent association with CHD. This has been studied further using transgenic mice. Overexpression of ABCA1 in liver and macrophages produced an increase in pre-β-HDL and also in mature HDL [4,5] through a decrease in the rate of degradation [4]. There was a 65% decrease in aortic atherosclerosis when mice were maintained on an atherogenic diet [6]. However, when expressed on an apoE-knockout background, the results were inconsistent, with both an increase [6] and a decrease [5] in atherosclerosis reported. Study of ABCA1-knockout mice has not helped to resolve this issue. The knockout animals showed the expected absence of HDL, but there was not the expected increase in atherosclerosis [7]. Tangier disease patients show a decrease in plasma low-density lipoprotein (LDL) concentration, which was also seen in the ABCA1-knockout mice. Again, the results from overexpression were inconsistent and there was no obvious effect on overall body cholesterol flux that could explain any of these effects [8]. Experiments in which ABCA1 was selectively inactivated in macrophages using bone-marrow transplantation may provide a clue to the inconsistencies. Knocking-out ABCA1 in macrophages of both apoE-null and LDL receptor-null mice significantly enhanced the development of atherosclerosis [9]. Thus, it seems that ABCA1-mediated cholesterol efflux can reduce atherosclerosis, but that this benefit can be overcome by other pro-atherogenic effects.

The selective inactivation of ABCA1 in macrophages had little or no effect on the plasma concentration of HDL [10], indicating that macrophage cholesterol, although important for atherosclerosis, does not provide a significant proportion of that in HDL. The bulk of the pre-β-HDL is probably formed in the liver. This can be deduced from the observations that ABCA1 is highly expressed in the liver, that the protein is present on the basolateral surface of hepatocytes [11] and that increased hepatic cholesterol concentrations have been observed in a Tangier disease patient [12]. More direct evidence comes from mice in which there has been specific overexpression of ABCA1 in the liver [13]. These animals show a significant increase in mature lipidated HDL levels in plasma. There was no net decrease in liver cholesterol content, but a compensatory increase in the expression of 3-hydroxy-3-methylglutaryl-CoA reductase and LDL receptors to make up for the loss. Thus, hepatic ABCA1-mediated cholesterol efflux contributes to the generation of mature HDL and the regulation of liver cholesterol levels. It provides a further pathway for the removal of cholesterol from the liver and allows a two-way traffic of HDL cholesterol to and from the peripheral tissues.

**Mechanism of ABCA1 action**

There has been considerable controversy over the mechanism of action of ABCA1, particularly in relation to the binding of acceptor molecules. There is no doubt that ABCA1-mediated lipid efflux requires an acceptor apolipoprotein containing an amphipathic helix, such as apoA1, apoAII or apoE. It is also known that ABCA1 activity affects membrane morphology [14], probably by flipping phospholipid to the outer leaflet, and that an active ATPase is essential for apoA1 binding to the cell surface [15]. This has led to the idea that apoA1 binds to a region of the membrane disturbed by ABCA1, a conclusion supported by the different lateral mobilities of ABCA1 and apoA1 on the cell surface [15]. On the other hand, there is also evidence for a direct interaction between ABCA1 and the acceptor. ApoA1 cross-links to ABCA1 [16], and natural mutations in the extracellular loops that abolish cholesterol efflux also abolish cross-linking [17]. Furthermore, a specific mutation in the first loop (W590S) reduced efflux without affecting apoA1 binding, indicating that acceptor binding is necessary, but not sufficient, for cholesterol efflux. A recent study suggests that both interpretations may be valid [18]. In this it was shown that the terminal helix 10 of apoA1 was necessary for binding and that there was a correlation between cholesterol efflux and apoA1 binding. Thus a sequence can be envisaged in which ATP-dependent ABCA1 activity disturbs the membrane and allows apoA1 to bind through helix 10. It then diffuses until it directly interacts with ABCA1, when the productive complex lipitates the apoA1 to form nascent HDL discs.

The way in which the lipids are transferred to the acceptors has also been the subject of controversy. A lack of stoichiometry between phospholipid and cholesterol transfer led to the proposal that ABCA1 transported only phospholipid.
to form apoA1–phospholipid complexes, which then picked up cholestero from cholesterol-rich domains of the membrane. More recently, this two-step mechanism has given way to the idea of microsolvulization, whereby lipid-poor apoA1 simultaneously removes phospholipid and cholesterol from cell surface lipid domains in whatever proportions they are present. This implies that ABCA1 transports both phospholipid and cholesterol, and would confer a greater degree of specificity. Finally, and intuitively more plausible, the lipids could be directly transferred to the tethered acceptor. The precise means of transfer will probably not be defined until the way in which the transporter acts has been elucidated.

There is no direct information on the mechanism by which ABCA1 translocates lipid across the membrane. Nevertheless, some ideas can be gained by comparison with other ABC transporters such as p-glycoprotein (the product of the multidrug resistance gene) and analogous Escherichia coli transporters. Single-particle imaging of p-glycoprotein established some time ago that the molecule forms a 12-member ring of transmembrane helices perpendicular to the plane of the membrane and, apparently, closed at the cytoplasmic side [19]. Somehow, binding and hydrolysis of ATP flips the substrate from the inner leaflet of the membrane either directly to the outer leaflet, or into the outer medium from which it transfers to an acceptor or back into the outer leaflet. More detailed structures of E. coli transporters showed bundles of transmembrane helices either touching at the outer surface [20] or touching through their cytoplasmic domains [21]. This gave rise to the proposed tilting mechanism of action, in which the helices bind the substrate when open at the cytoplasmic side and then release it to the outside when the V-shaped structure is inverted. These structures have been criticised as being inconsistent with cross-linking data and forming an unstable nucleotide-binding domain interface [22]. An alternative mechanism has been proposed in which the helices do not move perpendicular to the plane of the membrane, but spin round their long axes parallel to the membrane, so that a substrate bound on the membrane side of the helical ring would be transferred to the inside and released. This is thermodynamically easier, does not require a vectorial transfer of the binding site across the membrane and can accommodate multiple binding sites. Evidence supporting this mechanism was obtained by using non-metabolizable compounds to trap the ATPase cycle at different stages and determining structure and substrate affinity [23,24]. Binding of ATP produces marked conformational changes in the transmembrane domains and a reduction in affinity, which are reversed on hydrolysis of the ATP and release of ADP and phosphate.

ABCA1 is not restricted to the cell surface. It shuttles between the surface and early and late endosomes [25], suggesting that it could help to recycle apoA1 and transport cholesterol to the cell surface for export. There is also evidence that ABCA1 could influence the traffic of vesicles from the Golgi, possibly by affecting vesiculation through its phospholipid flippase activity [26].

### Regulation of ABCA1 expression

Cholesterol efflux is stimulated when macrophages are loaded with cholesterol, and various studies have now shown that the ABCA1 gene is tightly regulated by the cholesterol status of the cell. This effect of cholesterol was shown to be mediated by stimulation of gene transcription by the liver X receptor (LXR), a nuclear hormone receptor that is activated by oxysterol ligands [27]. The human ABCA1 gene has two promoters, both of which contain elements that bind LXR [28]. However, neither contain obvious response elements that could account for the well-known stimulation of cholesterol efflux by cAMP, suggesting that there could be more remote enhancers.

ABCA1 activity can also be greatly influenced by post-translational processes. Thus, unsaturated fatty acids and cholesterol can increase ABCA1 degradation, although the pathway involved is not known [29]. It has been shown recently that ABCA1 contains so-called PEST (Pro-Glu-Ser-Thr) sequences required for proteolysis through the calpain pathway. Binding of apoA1 greatly stabilized ABCA1 by inhibiting calpain-mediated degradation [30].

### ABCA1 and cholesterol absorption

LXR binds as a heterodimer with retinoid X receptor (RXR). Feeding mice with rexinoids, which are ligands for RXR, markedly increased intestinal ABCA1 mRNA content and abolished net absorption of cholesterol [31]. This suggested that ABCA1 could, at least to some extent, regulate cholesterol absorption by exporting cholesterol out of absorptive cells and back into the intestinal lumen. Again, results with ABCA1-knockout mice were inconsistent, with one study showing a 60% increase in cholesterol absorption [7] and another showing no effect on sterol excretion [8]. We have addressed this problem by studying the effects of fibrate and cholesterol on intestinal ABCA1 mRNA levels and cholesterol absorption in mice [32]. Fibrates are pharmacological activators of the transcription factor peroxisome proliferator-activated receptor-α (PPARα), which also has RXR as a heterodimeric partner. Fibrate feeding greatly increased the ABCA1 mRNA content of the intestine. Cholesterol also produced a marked increase in intestinal ABCA1 mRNA levels, but the effects of fibrate and cholesterol were not additive, indicating that the both acted through the same saturable mechanism involving LXR. ABCA1 protein levels in the intestine increased correspondingly. Importantly, there was a highly significant inverse correlation between intestinal ABCA1 mRNA content and cholesterol absorption, with points from fibrate and cholesterol feeding falling on the same line. This effect on cholesterol absorption was reflected in a reduction, by fibrate, of diet-derived cholesterol in liver and plasma. Fibrate and cholesterol both increased mRNA for another LXR-regulated gene, SREBP-1c (sterol-regulatory-element-binding protein 1c), in the intestine, but had no effect on the amount of mRNA for LXR itself. Thus, the effects of PPARα on ABCA1 transcription must be mediated by the availability of ligands for LXR, possibly oxysterols.
produced by cytochrome P450 enzymes that are sensitive to PPARα.

Expression of ABCG5/G8, the heterodimeric plant sterol transporter, can also be regulated by LXR [33], although since its exact specificity and site of action is unclear it is difficult to predict the contribution that it could make to the observed effects. Similarly, there could be effects on the unknown targets of the absorption inhibitor Ezetimibe, which does not seem to act through either ABCA1 or ABCG5/G8 [34]. Nevertheless, our results provide evidence for a physiological role for ABCA1 in regulating cholesterol absorption, and indicate that PPARα can influence the process by modulating ABCA1 activity by a mechanism that involves LXR. Activation of PPARα by ω − 3 fatty acids could provide an explanation for the reduced uptake of cholesterol of subjects on a diet rich in fish oil.

Summary

It is now established that ABCA1 is of great importance in regulating the efflux of cholesterol from cells. The precise mechanism by which it acts and the physiological agents that affect its activity are still unclear. However, there is evidence that it is involved not only in removing cholesterol from peripheral tissues but also in influencing the production of HDL by the liver and the rate of cholesterol absorption by the gut. Thus, pharmacological control of ABCA1 activity could prove beneficial by reducing the lipid content of arterial and other macrophage foam cells and by regulating the concentration of cholesterol in plasma.

References


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