

Genetics and molecular biology: The ABC of cholesterol efflux and high-density lipoprotein formation Stefan Lorkowski^{a,b} and Paul Cullen^c

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Abbreviations

ABC	ATP-binding cassette
apoA-I	apolipoprotein A-I
HDL	high-density lipoprotein

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The efflux of free cholesterol from peripheral cells is a major factor regulating the lipid content, size, fractional catabolic rate and plasma level of high-density lipoproteins (HDLs). Cellular cholesterol efflux is the first step in reverse cholesterol transport, the process whereby HDL transports cholesterol from peripheral tissues to the liver. Free cholesterol is taken up by small lipid-poor apolipoprotein A-I (apoA-I) particles, which are secreted by the liver and are efficient acceptors of cell membrane lipids. The process of cellular cholesterol efflux is mediated by the ATP-binding cassette (ABC) transporter ABCA1. Mutations in the ABCA1 gene prevent cholesterol efflux, leading accumulation of sterols in macrophages and to generation of small lipid-poor apoA-I particles that are rapidly cleared from the plasma. Heterozygous carriers exhibit hypoalphalipoproteinemia; homozygotes or compound heterozygotes exhibit Tangier disease, which is characterized by severe decreases in the size, lipidation, and level of HDL and by hypercatabolism of apoA-I.

The mechanism of ABCA1/apoA-I-mediated cholesterol efflux is unclear and the nature of the molecular interaction between cholesterol acceptors and ABCA1 a subject of controversy. In a recent paper, Gaus *et al.* [1] provide data supporting a model for apoA-I-mediated cholesterol efflux that involves two discrete interactions with microdomains of the plasma membrane. First, apoA-I binds to lipid rafts. This stimulates an initial fast efflux of a small membrane cholesterol pool originating from these rafts. The interaction of apoA-I with lipid rafts permits initiation of cholesterol efflux from larger and more slowly released pools. The bulk of cholesterol efflux, including that derived from

cholesterol ester hydrolysis, occurs by this slower pathway. The authors suggest that this second pathway is the ABCA1-dependent process and involves interactions between apoA-I and non-raft domains of the plasma membrane.

Two models have been hypothesized to describe the mechanism of ABCA1-mediated cholesterol efflux. In the ‘action at a distance’ model, ABCA1 is postulated to act by translocating phospholipids to the outer leaflet of the plasma membrane. It is proposed that apoA-I binds to phospholipid-enriched sites and extracts both phospholipids and cholesterol in a process that requires no direct interaction between apoA-I and ABCA1. By contrast, the ‘direct association’ model proposes that ABCA1 acts as a receptor to which apoA-I binds directly. This direct interaction is thought to stimulate the cholesterol-efflux activity of ABCA1, resulting in the transfer of cholesterol and phospholipids to the acceptor apolipoprotein. The studies recently presented by Fitzgerald *et al.* [2*] and Chroni *et al.* [3*] provide support for the latter hypothesis by showing that amphipathic apolipoproteins form the high-affinity molecular complexes that are required for cholesterol efflux and that direct associations occur between apoA-I helices and ABCA1. However, additional experiments will be necessary to further explore the two-step model of cholesterol efflux and to decipher the role of ABCA1 and apoA-I in this process.

Not only ABCA1, but also ABCG1 and ABCG4 are up-regulated during foam cell formation and down-regulated during lipid release [4–6]. This regulation is mediated by the liver X receptor/retinoid X receptor (LXR/RXR) pathway [5,7], which is important in lipid metabolism and cellular lipid export. Although ABCA1 has been identified as the key regulator of cholesterol efflux, the expression of ABCG1 increases much more than that of ABCA1 during cholesterol or oxysterol loading of human macrophages [5]. Phylogenetic studies also offer clues that, like ABCA1, ABCG1 and ABCG4 are involved in cellular cholesterol and lipid metabolism (G. Fuellen, M. Spitzer, P. Cullen, S. Lorkowski, unpublished observations). First experimental hints of the physiological role of ABCG1 were given in a study by Klucken *et al.* which showed that inhibition of ABCG1 results in a decrease in the efflux of cholesterol and phosphatidylcholine [8]. The details of how ABCG1 is involved in this process are still not known.

New insight into the role of ABCG1 and ABCG4 may now derive from a study performed by Wang *et al.* [9]. This group, led by Alan Tall at Columbia University in New York City, showed that cellular overexpression of ABCG1 or ABCG4 results in an increase in cholesterol efflux to HDL-2 and HDL-3, whereas overexpression of the remaining ABCG proteins had no effect. The ability of ABCG1 and ABCG4 to stimulate cholesterol efflux to acceptor molecules was not limited to HDL particles. Efflux to low-density lipoprotein and cyclodextrin was also increased, whereas none of the ABCG proteins was able to stimulate cholesterol efflux to lipid-poor apoA-I. In addition, the authors showed that no direct interaction between HDL and ABCG1 or ABCG4 occurs. Although the results of this study are limited by concerns relating to cell viability and to the conformation and cellular localization of the overexpressed ABCG protein, they nevertheless provide evidence that both ABCG1 and ABCG4 are in some way involved in cellular cholesterol efflux. Interestingly, in a mouse model, adenovirus-mediated overexpression of ABCG1 in the liver lowered HDL while increasing biliary cholesterol secretion [10,11]. The physiological relevance of these experiments is, however, uncertain. In the liver, ABCG1 is expressed mainly in Kupffer cells [12], whereas adenovirus expression occurs mainly in hepatocytes [9].

Numerous epidemiological and clinical studies have clearly shown that HDL is a major atheroprotective factor, but that the mechanisms underlying this effect are not clear. At least some of the atheroprotective role of HDL is likely to be due to its role in reverse cholesterol transport. Nonetheless, several so-called pleiotropic effects of HDL have been described that enforce the anti-atherogenic character of this particle. The recent work by Nofer *et al.* provides evidence that HDL-associated lysophospholipids mediate the vasodilatory effect of HDL via Akt-mediated activation of endothelial nitric oxide synthase both *in vitro* and in isolated mouse and rat aortae [13••]. The authors also showed that HDL and the lysophospholipids also induce vasodilation in rats *in vivo*. In addition, the lysophospholipid receptor S1P3 was identified as an integral component of HDL- and lysophospholipid-mediated vasodilation. This mechanism may contribute to the

vasoactive effect of HDL and probably represents a novel aspect of its anti-atherogenic function.

Thus, despite the advances of recent years, much remains to be learned about how ABCA1 and HDL cooperate to remove excess cholesterol from cells and about why high levels of HDL cholesterol are associated with reduced atherosclerotic risk. Watch this space.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
 - of outstanding interest
- 1 Gaus K, Kritharides L, Schmitz G, *et al.* Apolipoprotein A-1 interaction with plasma membrane lipid rafts controls cholesterol export from macrophages. *FASEB J* 2004; 18:574–576.
 - 2 Fitzgerald ML, Morris AL, Chroni A, *et al.* ABCA1 and amphipathic apolipoproteins form high-affinity molecular complexes required for cholesterol efflux. *J Lipid Res* 2004; 45:287–294.
 - 3 Chroni A, Liu T, Fitzgerald ML, *et al.* Cross-linking and lipid efflux properties of apoA-I mutants suggest direct association between apoA-I helices and ABCA1. *Biochemistry* 2004; 43:2126–2139.
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 - 9 Wang N, Lan D, Chen W, *et al.* ATP-binding cassette transporters G1 and G4 mediate cellular cholesterol efflux to high-density lipoproteins. *Proc Natl Acad Sci USA* 2004; 101:9774–9779.
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 - 11 Ito T. Physiological function of ABCG1. *Drug News Perspect* 2003; 16:490–492.
 - 12 Hoekstra M, Kruijt JK, Van Eck M, Van Berkel TJ. Specific gene expression of ATP-binding cassette transporters and nuclear hormone receptors in rat liver parenchymal, endothelial, and Kupffer cells. *J Biol Chem* 2003; 278:25448–25453.
 - 13 Nofer JR, van der Giet M, Tolle M, *et al.* HDL induces NO-dependent vasorelaxation via the lysophospholipid receptor S1P3. *J Clin Invest* 2004; 113:569–581.

Recommended reading

Abbott A. Ageing: growing old gracefully. *Nature* 2004; 428:116–118.

• Interesting feature presenting news on research projects that aim to identify the genetic and environmental factors that allow some people to remain healthy and active into their eighties, nineties and beyond. It is perhaps not a great surprise that gene products involved in cholesterol metabolism seem to play an important role in longevity in humans.

Chroni A, Liu T, Fitzgerald ML, *et al.* Cross-linking and lipid efflux properties of apoA-I mutants suggest direct association between apoA-I helices and ABCA1. *Biochemistry* 2004; 43:2126–2139.

This study is a follow-up of Fitzgerald *et al.* [2*] and is a more detailed analysis of the interaction between ABCA1 and apoA-I.

Fitzgerald ML, Morris AL, Chroni A, *et al.* ABCA1 and amphipathic apolipoproteins form high-affinity molecular complexes required for cholesterol efflux. *J Lipid Res* 2004; 45:287–294.

This study provides data supporting the direct association model of ABCA1-mediated cholesterol efflux to apoA-I.

Nofer JR, Herminghaus G, Brodde M, *et al.* Impaired platelet activation in familial high density lipoprotein (HDL)-deficiency (tanger disease). *J Biol Chem* 2004; 279:34032–34037.

• Interesting article showing the importance of ABCA1 in human platelets for normal δ -granule maturation and release. The observations presented support the hypothesis that the presence of reduced dense bodies and giant granules in Tangier platelets is a consequence of defective ABCA1-mediated regulation of endosomal sorting and distorted δ -granule maturation.

Nofer JR, van der Giet M, Tolle M, *et al.* HDL induces NO-dependent vasorelaxation via the lysophospholipid receptor S1P3. *J Clin Invest* 2004; 113:569–581.

This study identifies a new mechanism by which HDL may contribute to vasodilation. This probably represents a novel aspect of the anti-atherogenic function of HDL.

Palmer AM, Murphy N, Graham A. Triglyceride-rich lipoproteins inhibit cholesterol efflux to apolipoprotein (apo) A1 from human macrophage foam cells. *Atherosclerosis* 2004; 173:27–38.

This study demonstrates that triglyceride-rich lipoproteins increase the lipid content of cholesterol-loaded macrophages, while compromising the subsequent cholesterol efflux to lipid-poor apoA-I. This effect may contribute to the generation of macrophage foam cells *in vivo* and explains, at least in part, the accelerated atherogenesis seen in individuals with type II diabetes.

Rye KA, Barter PJ. Formation and metabolism of prebeta-migrating, lipid-poor apolipoprotein A-I. *Arterioscler Thromb Vasc Biol* 2004; 24:421–428.

Helpful and interesting review describing the current knowledge regarding the formation, metabolism, and regulation of monomolecular, lipid-free/lipid-poor apoA-I in plasma.

Sugimoto K, Tsujita M, Wu CA, *et al.* An inhibitor of acylCoA: cholesterol acyltransferase increases expression of ATP-binding cassette transporter A1 and thereby enhances the ApoA-I-mediated release of cholesterol from macrophages. *Biochim Biophys Acta* 2004; 1636:69–76.

• The authors showed that acylCoA:cholesterol acyltransferase inhibition increases the release of cholesterol from cholesterol-loaded macrophages by increasing expression of ABCA1, putatively through shifting cholesterol distribution from the esterified to the free compartments. However, it remains unclear if this effect is just caused by an increased formation of oxysterols due to the increased amounts of free cholesterol.

Yancey PG, Kawashiri MA, Moore R, *et al.* In vivo modulation of HDL phospholipid has opposing effects on SR-BI- and ABCA1-mediated cholesterol efflux. *J Lipid Res* 2004; 45:337–346.

The data provided in this study give rise to an interesting model explaining the reciprocal effects of in-vivo modulation of serum phospholipid on receptor-mediated cellular cholesterol efflux mediated either by ABCA1 or scavenger receptor class BI.