CMLS, Cell. Mol. Life Sci. 61 (2004) 2343–2360
1420-682X/04/182343-18
DOI 10.1007/s00018-004-4087-y

CMLS Cellular and Molecular Life Sciences

Review

New insight on the molecular mechanisms of high-density lipoprotein cellular interactions

L. O. Martinez, S. Jacquet, F. Tercé, X. Collet, B. Perret and R. Barbaras*

Institut Féderatif de Recherche Claude de Preval, IFR 30, Institut National de la Santé et de la Recherche Médicale, Unité 563, Centre de Physiopathologie de Toulouse-Purpan, Département Lipoprotéines et Médiateurs Lipidiques, Toulouse cedex (France), Fax: +33 561 77 94 01, e-mail: ronald.barbaras@toulouse.inserm.fr

Received 29 January 2004; received after revision 10 May 2004; accepted 28 May 2004

Abstract. High-density lipoprotein (HDL) cholesterol is an independent negative risk factor for coronary artery disease and thus represents today the only protective factor against atherosclerosis. The protective effect of HDL is mostly attributed to its central function in reverse cholesterol transport (RCT), a process whereby excess cell cholesterol is taken up and processed in HDL particles, and is later delivered to the liver for further metabolism and bile excretion. This process relies on specific interactions between HDL particles and cells, both peripheral (cholesterol efflux) and hepatic (cholesterol disposal) cells, and on the maturation of HDL particles within the vascular compartment. The plasma level of HDL cholesterol will thus result also from the complex interplay with cellular partners. Among them, some contribute to HDL formation – for instance ATP binding cassette AI protein – while others are mostly involved in HDL catabolism, the scavenger receptor-class B type I or the recently described membrane-bound ATP synthase/hydrolase. The last decade has seen major breakthroughs in the identification and elucidation of the role of cellular partners of HDL metabolism, and in their transcriptional regulations, opening up new perspectives in the modulation of HDL cholesterol.

Key words. Lipoprotein; Atherosclerosis; HDL; lipoprotein receptors; ABCA1 protein; scavenger receptors; F_1F_0-ATPsynthase; HDL cholesterol.

Introduction

Several large prospective studies have established that high-density lipoprotein (HDL) cholesterol is an independent negative risk factor for coronary artery disease and thus represents today a major protective factor against atherosclerosis. For instance, a decrease of only 10 mg/l in HDL cholesterol is associated with a rise in the cardiovascular risk of 2% for women and of 3% for men. Moreover, low HDL cholesterol is often part of a set of metabolic alterations including hypertriglyceridemia, accumulation of small and dense low-density lipoprotein (LDL), central obesity and insulin resistance, which define the so-called metabolic syndrome. Altogether, these alterations have additive effects on the cardiovascular risk.

HDLs exert pleiotropic effects, displaying anti-inflammatory, anti-thrombotic and anti-oxidant properties. For instance, HDLs are potential carriers of enzymatic proteins, such as paraoxonase [1] and platelet-activating factor (PAF)-acetyl hydrolase [2], which can hydrolyze phospholipid peroxides [3] and cholesteryl ester peroxides [4]. In this respect, low levels of paraoxonase have been measured in a population at high risk for coronary artery disease [5, 6]. Also, HDLs can directly antagonize the effects of oxidized LDL on endothelial and smooth muscle vascular cells. Indeed, HDLs were shown to decrease the expression of adhesion molecules, of mono-

* Corresponding author.

cyte recruitment factors and of prothrombotic molecules while promoting the synthesis of the vasodilator nitric oxide and prostacyclin [7].

However, besides these various beneficial effects, the protective effect of HDLs is mostly attributed to their central functions in reverse cholesterol transport (RCT), a process whereby excess cell cholesterol is taken up and processed in HDL particles, and is later delivered to the liver for further metabolism and bile excretion. This process relies on specific interactions between HDL particles and cells, both peripheral (cholesterol efflux) and hepatic (cholesterol disposal) cells, and on the maturation of HDL particles within the vascular compartment. Indeed, plasma HDLs undergo complex remodeling upon action of enzymatic and lipid transfer proteins, which enable the handling and esterification of large amounts of cell-derived cholesterol, resulting in the formation of large HDL particles further cleared by the liver. The plasma level of HDL cholesterol will thus result from the complex interplay of those molecular partners. Among them, some contribute to HDL formation – for instance lipoprotein lipase or ATP binding cassette A1 protein (ABCA1) – while others are mostly involved in HDL catabolism, such as hepatic lipase, cholesteryl ester transfer protein (CETP), scavenger receptor-class B type I (SR-BI) or the recently described membrane-bound ATP synthase/hydrolase. The last decade has seen major breakthroughs in the identification and elucidation on the role of cellular partners of HDL metabolism and in their transcriptional regulations, opening up new perspectives in the modulation of HDL cholesterol. Genetic variants of these proteins have also been reported, and new ones will probably be discovered, which might partly account for the interindividual variability of HDL levels.

Nevertheless, cell surface-binding proteins for HDL on different cell types are key partners in the regulation of cholesterol metabolism. The mechanisms of interactions and the cellular partners are still fairly unknown but represent a major goal in the fight against cardiovascular diseases.

This review will focus on the major partners of hepatic cholesterol uptake and peripheral cell cholesterol efflux, analyzing the molecular and cellular mechanisms involved in HDL metabolism at these two steps of reverse cholesterol transport.

HDL and hepatocytes

Research on the cellular partners involved in the interactions between hepatic cells and HDL has been challenged for many years. A number of proteins have been identified as putative HDL binding proteins, but the lack of physiological effects induced by those proteins has prevented their classification as true receptors. One of the main difficulties is the reported characteristics of HDL binding on cells, which showed poor affinities and a broad range of specificities, conditions that might lead to the identification of nonspecific proteins (see for review the excellent paper by N. Fidge [8]). An interesting approach to circumvent these problems was realized by M. Krieger's lab, which used LDL to screen a complementary DNA (cDNA) library expressed in LDL receptor-deficient CHO cells to identify a new protein able to bind LDL, SR-BI, which also proved able to bind HDL [9]. Generations of SR-BI transgenic mice in the same lab further demonstrated the major importance of SR-BI in the metabolism of HDL cholesterol ester, at least in rodents. But so far the importance of the human homolog of SR-BI [10] in HDL cholesterol ester metabolism in humans, where the major cholesterol carriers are classically in the range of the LDL, still needs to be demonstrated.

SR-BI and selective cholesterol ester uptake

The interest of this protein is evidenced by the number of recent excellent reviews available concerning it [11–13]. SR-BI is a ubiquitous receptor with a high level of expression in steroidogenic tissues and the liver [9, 10, 14]. One of the most interesting observations is its implication in selective cholesterol ester (CE) uptake by the liver. This process, originally described in rats by Glass and colleagues [15], had never been supported by molecular data until the characterization of SR-BI. This pathway is defined as a high-capacity system in which cells internalize more cholesteryl esters than apolipoprotein components of the HDL particle [15]. This is in contrast with the classical LDL (apo B/E) receptor pathway, where the entire particle is internalized through clathrin vesicles for degradation and recycling. In the present review, we will focus mainly on the role of SR-BI in the CE selective uptake. The molecular mechanisms of SR-BI will be further detailed below.

How does CE selective uptake work? Basically, SR-BI mediates a bidirectional exchange of lipids, mainly promoting transfer of CE and free cholesterol [14, 16] from HDL and LDL [16] to the cells, and an efflux of FC from cell plasma membranes to lipoproteins [17] and non-lipoprotein acceptors [18]. It is noteworthy that SR-BI can also transfer phosphatidylcholine, sphingomyelin and, in lesser proportion, triglycerides (TGs) to HDL [19] and LDL. To adequately function, the selective CE uptake must circumvent two main problems. First, the lipoprotein particle, which binds to SR-BI, is thermodynamically stable, and thus the equilibrium between apolipoproteins and lipids must be disturbed to trigger lipid uptake. So any modification of the lipoprotein particles that modulate this steady state will modify CE selective uptake. For instance, an increase in the TG/CE ratio decreases CE uptake in contrast to lipoprotein lipase (LPL) or hepatic lipase (HL)-mediated HDL-TG hydrolysis, which increases

CE uptake [19, 20]. Second, plasma membranes have a limited capacity to incorporate CE. In adrenocortical cells (Y1-BS1) for instance, CE uptake has a low activation energy, meaning that this uptake occurs in a lipid fluid environment [21]. Incorporation of CE molecules in plasma membranes occurs down a concentration gradient, but the membranes cannot accommodate more than 3 mol% of CE [22]. This low capacity of membranes to accommodate CE raises the hypothesis that SR-BI can form or open a hydrophobic channel between a few SR-BI molecules, prior to shuttling CE to intracellular non-lysosomal-specific compartments. Historically CE selective uptake has been considered as a non-endocytosis mechanism [22, 23], mainly because chloroquine and monensin inhibitors of clathrin-coated pit endocytosis did not affect CE uptake, in contrast to apoA-I (the main apolipoprotein in HDL) uptake. However, this kind of experimental approach must be considered cautiously, since it cannot inhibit the very early events that might occur in CE uptake, such as the accumulation of ligand/receptor complexes in early endosomes [24]. One very interesting alternative pathway is the intracellular uptake of holo-HDL followed by resecretion of a modified particle. This retro-endocytosis process was first described in macrophages [25], followed by CaCo-2 human intestinal cells and murine [26], rat [27] and human hepatocytes [28]. This mechanism was supported by our observations that HDL_3 was highly internalized via a clathrin-vesicle pathway [29] in human hepatocytes. Moreover, fluorescent labeling of HDL proteins (Alexa), and incorporation of Dil for phospholipids (PLs) and bodipy-labeled CE into the same HDL particle have shown in mouse hepatocytes a colocalization of the labeling with SR-BI in intracellular compartments: basolateral membranes that subapical compartment and that juxtanuclear endosomal recycling compartment (ERC) [30]. Surprisingly, in hepatocytes, the retro-endocytosis pathway does not seem to depend on clathrin vesicles or caveolae, the two known receptor-dependent endocytosis pathways, as suggested by the use of dynamin and caveolin-1 dominant negative mutants [26]. Indeed, while SR-BI colocalizes at least partially with caveolin-1 in many tissues and cells [31, 32], caveolin expression is not required for SR-BI activity in monocytes, macrophages or human adrenal (NCI-H295R) cell lines which do not express caveolin-1 but present CE selective uptake [33, 34]. In HepG2 cells, where no caveolin-1 is present, the overexpression of this protein increases the selective uptake of CE-HDL, but not of CE-LDL. In hepatocytes, the level of caveolin is low and does not seem to participate in CE-selective uptake as suggested by the use of dominant negative mutants [26]. Thus, caveolin-1 is not strictly required for SR-BI activity but, when present, modulates CE-selective uptake.

The relative contribution of the retro-endocytosis pathway versus CE-selective uptake needs to be established. It could be very difficult to measure, since in both cases CE uptake was quantified. The main difference is in the processing of the HDL particle. One alternative for measuring the contribution of both pathways in integrated systems (i.e. animal models) could be to block any endocytosis of the HDL particle, using for instance, an in situ approach such as perfused rodent liver [35].

However, cells devoid of SR-BI are capable of CE-selective uptake. Accordingly, the LDL-related protein receptor (LRP receptor), an endocytotic receptor, can take up CE-HDL in human primary adipocytes or cell lines (SW872) [36]. Furthermore, the LRP receptor interacts with HDL via LPL, acting as a cross-linker in this process. LPL also participates in SR-BI-independent uptake of CE-LDL in HEK293 cells [37], and of HDL in HEK293 and BHK [38]. This effect is independent of the catalytic activity of LPL and requires proteoglycans onto which LPL can bind.

In rat liver, selective CE uptake was estimated to be about 2 for the CE/protein ratio (as compared to 7 for adrenal cells), meaning that the HDL protein contribution in the RCT could be nonnegligible [39]. Thus, retro-endocytosis could be an alternative pathway for taking into account both the lipid and protein contributions in the HDL clearance by the liver. Indeed, the liver remains the main organ for the uptake (and synthesis) of apoA-I as compared to kidney, for instance [39]. Nevertheless, it was recently shown that cubilin, an intrinsic factor/vitamin B12 receptor in kidney, could bind apoA-I [40] and thus contribute to the clearance of apoA-I by this tissue. Recently, it was demonstrated that the kidney was able to clear lipid-free apoA-I and small HDL particles from the circulation [41], probably through apoA-I binding to cubilin, which now needs to be demonstrated. However, the contribution of the HDL clearance by the kidney in reverse cholesterol transport still remains to be established.

The limitations of the explanation of 'pure' CE-selective uptake regarding HDL metabolism in hepatocytes, with, for instance, the description of both HDL lipids and HDL proteins present inside different compartments in liver cells, have raised the question of the existence of more 'classical' (clathrin-dependent?) pathways, which could help to explain the RCT process.

ATP synthase, a new concept in HDL endocytosis

Binding sites for HDL, or apolipoprotein (apoA-I), have been identified in liver plasma membranes [42] and human hepatoma cells [43]. Both a high-affinity (10^{-9} M) binding site and another component of lower affinity (10^{-7} M) were evidenced, the latter possibly reflecting weaker protein-protein and/or protein-lipid interactions. Interestingly, lipid-free apoA-I (named free apoA-I) binds only to the high-affinity sites and thus constitutes a selective ligand to study this receptor.

We have previously shown that HDLs are internalized in HepG2 cells via the formation of clathrin-coated vesicles, following engagement of the low-affinity binding sites [29]. We have further observed that binding and endocytosis of HDL were modulated by metabolic events that affect HDL structure and distribution. Indeed, in the liver, large-sized HDL particles, enriched in triglycerides (triglyceride-rich HDL2 or TG-HDL2), are preferential substrates for hepatic lipase, acting at the endothelial surface of sinusoid capillaries and leading to the formation of a triglyceride and phospholipid-poor 'remnant HDL' [44]. TG-HDL2 displayed only low-affinity binding whereas the post-lipolysis remnant HDL could bind to both low- and high-affinity sites. Moreover, the remnant-HDL were internalized faster and in higher amounts than their parent TG-HDL2, suggesting that engagement of high-affinity receptors might stimulate HDL-endocytosis occurring through low-affinity binding sites [44].

We recently achieved the purification and characterization of apoA-I high-affinity binding sites on hepatocytes which we identified as the β-chain of human ATP synthase, a major protein complex of mitochondria inner membrane, involved in ATP synthesis. Mitochondrial ATP synthase has two major domains, F_1 and F_0 [45]. F_1 is a peripheral membrane protein complex which consists of five different subunits (among them, the β-chain), containing binding sites for ATP and ADP, including the catalytic site for ATP synthesis. F_1 is held to the membrane through its interaction with Fo, an integral membrane protein complex in mammalian mitochondria that contains a transmembrane channel through which protons can cross the membrane [46]. The synthesis of ATP requires an electrochemical proton gradient across the inner mitochondrial membrane. The collapse or absence (for instance when the F_1 complex is present alone) of the electrochemical proton gradient induces a switch in enzymatic activity from ATP synthesis to ATP hydrolysis. In this case, the catalytic domain of ATP synthase, present mainly in the β-subunits, catalyzes the hydrolysis of ATP to ADP and phosphate, an activity that is regulated in mitochondria by a natural inhibitor protein, IF1 [47–49].

The surprising finding of the presence of the β-chain of ATP synthase in hepatocyte plasma membrane preparations led us to demonstrate that it was also present on the cell surface of intact hepatocytes, as previously reported for lymphocytes [50] or human endothelial cells [51–53]. In the later case, cell surface ATP synthase was acting as a receptor for different ligands, such as angiostatin, suggesting implication of this membrane-associated ATP synthase in angiogenesis. Furthermore, Beisiegel and Mahley [54, 55] had earlier identified the α- and β-chains of ATP synthase as receptors for apolipoprotein E-enriched HDL, but they did not demonstrate the presence of this protein on the cell surface, nor they did propose a role for it. The presence of the β-chain, probably associated with its counterpart, the α-chain, was demonstrated on the cell surface of $HepG_2$, IHHs (immortalized human hepatocytes) or primary human hepatocytes, but not on epithelial cells such as the CHO cell line. This observation suggests that the presence at the cell surface of this protein is more dependent on the cell type than on the tumorigenic status of the cells, as suggested by Das et al. [50].

In our model, the presence on the cell surface of hepatocytes of both the α- and β-chains of ATP synthase strongly suggests that the entire F1-ATPase might be present. Moreover, we showed a cell surface hydrolysis activity of ATP to ADP. Interestingly, the IF_1 protein, which inhibits only the ATP hydrolysis activity of the F_1-ATPase, induced a decrease in the ADP present in the cell medium, demonstrating that the ATP hydrolysis measured is dependent on F_1-ATPase. The presence of extracellular ATP, physiologically or as secreted in the culture medium, is well documented [56]. Also, different ATP and ADP hydrolysis activities have been described at the cell surface, and some phosphatases, such as members of the ecto-ATPase family, have been identified [57]. However, the almost complete absence of AMP generated in the time course of our experiments allowed us to exclude implication of this ecto-ATPase. Finally, ADP generation was strongly stimulated by adding free apoA-I, a process again inhibited by the IF_1 protein. This demonstrates the regulation of ATP synthase hydrolysis activity by the binding of apoA-I.

HDL endocytosis could occur by two different pathways in hepatocytes: one is dependent on internalization via SR-BI and represents a selective transcytosis of lipoprotein cholesterol, which could explain the selective sorting of cholesterol to the bile canaliculus; the other one is independent of SR-BI and could represent the HDL protein catabolic pathway, mediated by an unknown receptor [26]. Thus, endocytosis of HDL is the primary event for both pathways. Based on our previous demonstration that HDLs were internalized in $HepG_2$ cells through low-affinity binding [29], and on the use of specific ligands for high- and low-affinity sites (free-apo AI or TG-HDL2 respectively), we have shown stimulation of holo-TG-HDL_2 internalization (both the protein and the cholesteryl ester moieties), through the binding of apoA-I to high-affinity binding sites, i.e. the β-chain of ATP synthase. Thus stimulation of high affinity could be an upstream event in the HDL endocytosis pathway. Moreover, ADP, but not ATP, was able to stimulate the internalization of TG-HDL_2 at a level comparable to the effect of free apoA-I, and not only the stimulation, but also the basal internalization was regulated by the presence of extracellular ADP. This specific ADP-dependent effect suggests that stimulation of HDL internalization is under the control of purinergic receptors such as P2X or P2Y type receptors. Which receptor exactly is involved in the HDL endocytosis process remains to be determined.

This effect of ADP was found highly specific for HDL endocytosis, as demonstrated by the absence of any effect on LDL endocytosis through its LDL receptor (as a typical cargo receptor), or of EGF receptor endocytosis (as a tyrosine kinase-type receptor).

Finally, the implication of the F_1-ATPase activity in HDL internalization, as demonstrated by inhibition of TG-HDL_2 internalization in the presence of IF_1 protein was demonstrated. This observation, associated with inhibition of ATP hydrolysis by IF_1, again argues in favor of the role of the ADP produced by the ATP hydrolysis activity of ATP synthase in the HDL endocytosis process. This clearly demonstrates that free apoA-I and IF_1 have the same cellular target, i.e. membrane-associated F_1-ATPase.

HDL and peripheral cells

The term 'peripheral cells' is commonly used in cholesterol metabolism in reference to non-hepatic cells. It is a convenient term that includes all cells that cannot metabolize cholesterol (with the exception of steroidogenic cells). Cholesterol has an essential function in membranes of peripheral cells, where it modulates fluidity and maintains the barrier between cells and environment. Many peripheral cells modulate their membrane cholesterol content by well-characterized feedback systems that control the rate of cholesterol biosynthesis and uptake by the LDL receptor [58]. In most of the cells, this system is sufficient to prevent excess cholesterol accumulation. However, some cells, such as macrophages, can also acquire cholesterol by a phagocytotic pathway that is not feedback regulated by cholesterol. These cells must either store this excess of cholesterol as cholesteryl ester or secrete it. Excess cholesterol secretion, also called cholesterol efflux, is therefore a key event in the regulation of cellular cholesterol content, particularly in macrophages. When cholesterol efflux is disregulated, macrophages accumulate excess of cholesterol and become foam cells. This process of foam cell formation is critical for the development of both early- and late-stage atherosclerotic lesions [59]. Since there is no significant release of CE from peripheral cells, cholesterol efflux can be defined as the movement of unesterified, or 'free' cholesterol (FC) molecules from the cell to an extracellular acceptor [60]. More than 30 years ago it was been established that cholesterol molecules can be removed from cells by exposing them to serum or more specifically to the HDL fraction of serum [61]. Therefore, according to Glomset's original hypothesis [62], cholesterol efflux, in which excess cellular cholesterol is released from cells and transferred to HDL particles, appeared to be the first step of reverse cholesterol transport. In subsequent years, a major research effort was focused on elucidating the molecular mechanisms involved in the efflux of cellular cholesterol to the HDL particles. We are just beginning to understand these mechanisms in which specific molecular interactions of HDL or its apolipoproteins with peripheral tissues is essential to promote cholesterol efflux. These insights arise both from studies on SR-BI and recent studies of Tangier disease and its defective gene product *ABCA1*.

Thus, three distinct pathways of cellular cholesterol efflux involving HDL and its apolipoproteins can be described: passive diffusion, SR-BI-facilitated efflux and ABCA1-mediated active efflux.

HDL and cellular cholesterol passive diffusion

The first mechanism involves passive diffusion of cholesterol to the HDL particle, a mechanism also called diffusion-mediated cell cholesterol. Free cholesterol molecules spontaneously desorb from the plasma membrane, diffuse through the aqueous phase and subsequently incorporate into HDL particles. In this mechanism, cholesterol diffusion can be mediated by a wide variety of 'acceptors', not only HDL but also other plasma components including LDL, albumin [63], globulins [64], artificial lipid microparticles, such as phospholipid vesicles, and other specific molecules with a high affinity for cholesterol, such as cyclodextrins [65]. It is believed that during this reaction, it is the cholesterol from plasma membrane compartments that is mainly mobilized rather than cholesterol from intracellular compartments [66]. The net cholesterol efflux is regulated by the gradient of free cholesterol contents between the acceptor and cell membrane compartments and by the acceptor ability for cholesterol adsorption and desorption. Consequently, the reaction is mostly bidirectional and is driven by the phospholipid content of lipoprotein acceptors [67], but does not involve any specific factor such as HDL binding to cell [68] or intracellular signaling pathways. Therefore, the cholesterol diffusion pathway is entirely independent of HDL. However, HDL particles are believed to be an excellent physiological acceptor involved in passive diffusion because of the ability of the HDL particle to esterify its cholesterol through the action of one of its associated enzymes, the lecithin:cholesterol acyltransferase (LCAT). In this case, the net release of cellular free cholesterol would be driven by cholesterol esterification in HDL by LCAT. Different studies have tried to validate this hypothesis, but results seem to depend on the cell cholesterol loading conditions. Several laboratories have evidenced that LCAT promotes net cell cholesterol efflux not by increasing the outflow but by reducing the inflow of HDL cholesterol to the cell, due to a decrease in the available unesterified cholesterol in HDL [69, 70]. In contrast, Czarnecka and colleagues showed that LCAT-mediated cholesterol esterification enhanced direct cholesterol out-

flow from cholesterol pre-loaded cells [71], supporting the idea that active cholesterol esterification by LCAT of the HDL particle plays a physiological role in the passive diffusion of cell cholesterol from peripheral cells.

SR-BI-facilitated aqueous diffusion of cholesterol to HDL

SR-BI is a multi-ligand receptor that interacts with a broad range of acceptors, including HDL, LDL, oxidized LDL, acetylated LDL, maleylated BSA and anionic phospholipids [72, 73]. We have already described above how SR-BI mediates the in vivo selective uptake of LDL and HDL lipids into mice hepatocytes and steroidogenic cells [73, 74] and also facilitates in vitro FC efflux from CHO and COS-7 transfected cells to HDL [17, 75]. Thus, SR-BI expression in peripheral tissues may facilitate uptake of FC by HDL particles. Supporting this idea, the rates of FC efflux from a panel of cultured cell types correlated well with SR-BI expression levels [18, 65]. In addition, SR-BI can also facilitate FC efflux to LDL and other non-lipoprotein acceptors such as phospholipid vesicles [18, 65]. Therefore, SR-BI mediates a bidirectional exchange of FC via a concentration gradient between cell plasma membrane and a wide variety of phospholipid-containing acceptors, in a way that looks like a 'facilitated aqueous diffusion'. Different groups have investigated the mechanism by which SR-BI facilitates cellular cholesterol efflux, and these studies can be used to formulate a broadly based model, even if the detailed mechanism remains unclear.

SR-BI binding

Since the identification of SR-BI as an HDL-binding protein [9], an impressive number of independent studies have reported HDL binding to SR-BI. However, SR-BI binding domains and HDL components involved in the binding to SR-BI are still under investigation and will be discussed in this section.

Many attempts to identify the SR-BI binding domain to the HDL particles have been made. Results based on the analysis of SR-BI chimeric receptors and SR-BI mutants suggest that distinct extracellular receptor domains may be involved in SR-BI binding to HDL [76, 77]. Gu and colleagues, using SR-BI mutants, have reported that Gln-402 and Gln-418 close to the C-terminal transmembrane domain of SR-BI are critical for HDL receptor activity [78, 79]. However, in these experiments, the loss of HDL binding ability may be due to structural disruptions in SR-BI induced by the mutations.

Another point to be discussed is the role of the different components in HDL particles (lipids and apolipoproteins) in interactions with SR-BI. Our group reported that SR-BI antibodies that compete up to 70% of the HDL binding to hepatocytes at 4°C failed to block lipid-free ^{125}I-apoA-I binding [80], suggesting that lipid-free apoA-I is not an efficient SR-BI ligand. Many studies have clearly indicated that HDL apoA-I, apoA-II, apoC-III and apoE isoforms in phospholipid-complexed forms can mediate binding to SR-BI and also inhibit HDL binding to SR-BI up to 85% [81–83]. These studies show that lipid-free apoA-I is not an effective competitor of ^{125}I-HDL binding. Depending on experimental conditions, lipid-free apoA-I only reduces the binding of ^{125}I-HDL by 38% [81], 25% [82] and 45% [83], suggesting that phospholipid association with apoA-I is required for binding to SR-BI. Although, some studies have previously reported a low-affinity association of lipid-free apoA-I to SR-BI and have achieved chemical cross-linking between SR-BI and lipid-free apoA-I [83,84]. We never observed any significant specific lipid-free apoA-I binding to SR-BI at 4°C on transfected CHO cells [unpublished observations]. Our observation is consistent with studies by Zannis and colleagues reporting that they could not establish an SR-BI-dependent binding of lipid-free apoA-I despite numerous direct binding experiments [82]. An explanation for these discrepancies could be related to the noteworthy ability of apoA-I to associate with cellular phospholipids. Such association of apoA-I with phospholipids could be enhanced if the binding experiment is performed at 37°C. It would have been informative in the previous binding studies [83, 84] to perform the Scatchard plot [85] of the apoA-I binding to detect any apo AI lipidation. Indeed, according to studies from Mendel et al. [86], ligand heterogeneity such as lipidation state may cause a curvilinear concave-downward Scatchard plot. However, in most cases, Mendel and colleagues experiments showed that ligand heterogeneity does not cause recognizable deviations from linearity in the Scatchard plot and therefore, cannot be distinguished from homogeneous ligand. The elegant studies by Williams and colleagues demonstrated an interaction between SR-BI and the class A amphipathic α-helices [84]. These studies strongly suggest that SR-BI interacts with HDL via the amphipathic α-helical repeat units of apoA-I, which may therefore explain the interaction of SR-BI with a wide variety of apolipoproteins via a specific secondary structure, the class A amphipathic α-helix, which is a common structural motif in the apolipoproteins of HDL. These motifs bound and cross-linked to SR-BI only when complexed with dimyristoylphosphatidylcholine (DMPC), which again suggests that phospholipids play a significant role probably by maintaining the amphipathic α-helix in a conformation able to bind SR-BI. Consistent with this idea, apoA-I complexed with the longer chain of palmitytoleoylphosphatidylcholine (POPC) is less effective than apoA-I complexed with DMPC [83], and differences in apoA-I conformation in different-sized reconstituted HDL particles (rHDL) markedly influence apoA-I recognition by SR-BI [87].

SR-BI binding and efflux

The correlation between weak or no binding of lipid-free apoA-I to SR-BI and its inability to promote FC efflux via SR-BI suggest that binding is required for cholesterol efflux. Studies performed by Krieger's group have tried to assess this concept. Gu and colleagues demonstrated that an SR-BI antibody that blocked HDL binding also decreased FC efflux [78]. However, in those experiments, it could not be ruled out that immunglobulin G (IgG) may exert a steric hindrance that therefore would inhibit SR-BI function. In order to minimize the steric hindrance effect of the IgG, it would have been interesting to perform the same experiment using the smaller Fab fragments of the SR-BI antibody. Gu and colleagues also showed that a mutant SR-BI that lost most of the ability to bind HDL but preserved its ability to bind LDL displayed decreased efflux to HDL but not to LDL [78]. Altogether, these studies suggest that there is an efficient component of SR-BI-mediated FC efflux that is linked to binding of acceptor to SR-BI. However, other studies have shown that HDL binding to SR-BI was not enough to ensure efficient FC efflux. CD36, another scavenger receptor from the same family as SR-BI which has a higher affinity for HDL than SR-BI, failed to facilitate FC efflux to HDL [75, 88]. SR-BIs ability to promote lipid efflux to apolipoprotein-free phospholipid vesicles that do not bind SR-BI, such as phosphatidylcholine (PC) or sphingomyelin liposomes, confirms that the major stimulation of FC efflux occurs independently of acceptor binding to SR-BI. On the other hand, phospholipid content, and particularly PC, of the HDL particles is a critical factor in the mechanism of SR-BI-mediated cholesterol efflux. Studies have reported that enrichment of HDL with PC increases SR-BI-mediated efflux, whereas depletion of HDL-PC by treatment with phospholipase-A2 decreases SR-BI-mediated FC efflux [89]. Furthermore, the presence of phospholipids in the acceptor is totally required since no cholesterol efflux to lipid-free apoA-I occurs via SR-BI. Altogether, these results has led to the idea that SR-BI does not have to interact directly with an extracellular acceptor of FC in order to promote cholesterol efflux.

SR-BI function

Nevertheless, to assess a physiological relevance of HDL binding to SR-BI, different groups have proposed that SR-BI functions in a two-step process where HDL binding to SR-BI (step 1) is coupled to the flux of FC (step 2) [21, 79, 83]. Thus, the quantitative contribution to FC efflux of HDL binding to SR-BI has recently been under intense investigation. Zannis and colleagues have observed that SR-BI-facilitated efflux to discoidal HDL particles, composed of apoA-I molecules with two different double point mutations, was dramatically reduced, but there was no decrease in binding affinity to SR-BI compared to particles containing wild-type apoA-I [90]. Efflux was restored to the HDL particles containing the mutant apoA-I molecules when SR-BI was also mutated. These observations led the authors to propose that the formation of an efficient complex between HDL and SR-BI, in which the lipoprotein and the receptor must either be precisely aligned or have the capacity to undergo appropriate conformational changes, is required for efficient SR-BI-facilitated cholesterol efflux. Using discoidal rHDLs that have different sizes with the same CE/phospholipid ratio, but different apoA-I conformations, Thuahnai and colleagues demonstrated that the level of FC efflux was dependent on the number of rHDL particles bound to SR-BI at low ligand concentrations, but once the receptor was saturated, the FC efflux became independent of rHDL binding to SR-BI [91]. Thus, despite saturation of binding sites on SR-BI, FC efflux continues to increase. This finding is consistent with an elegant study published in 1988, a long time before SR-BI was identified as an HDL receptor [92]. In this study, Mendel and colleagues observed that apoE-free HDL_3 binding to human fibroblasts and HDL_3-mediated cholesterol efflux from these cells did not saturate at similar HDL_3 concentrations which indicated dissociation between HDL-mediated cholesterol efflux and specific HDL binding. Thus, contrary to first expectations, HDL binding to cell surface SR-BI may enhance FC efflux via SR-BI at low ligand concentrations [78, 91], but is independent of SR-BI in a more physiological situation, i.e. when the receptor is saturated. The mechanism by which SR-BI could promote FC efflux independently of HDL binding is still under investigation.

SR-BI cholesterol efflux and plasma membrane domains

It has been shown that SR-BI expression increases the pool of cholesterol efflux to cyclodextrin acceptors and also increases the pool of cholesterol accessible to cholesterol oxidase [75, 93]. These data suggest that SR-BI could induce redistribution of plasma membrane cholesterol in domains favorable to cholesterol release. These events occur in the absence of HDL, indicating that these effects on plasma membrane cholesterol are due to SR-BI and not to HDL interaction with SR-BI. Very little is known about the plasma membrane domains of SR-BI-facilitated FC flux. In cultured cells, SR-BI has been found to colocalize within plasma membrane caveolae [31, 94, 95], which are membrane domains rich in cholesterol and sphingomyelin (SPM) [96] characterized by the presence of particular protein cav-1 (cav-1). However whether or not caveolin-1 expression affects SR-BI-facilitated FC efflux to HDL particles is still controversial [97–101]. It is likely that cav-1 alone could stimulate cholesterol efflux independently of SR-BI by enhancing cholesterol transfer to cholesterol-rich domains of plasma membrane [101, 102]. However, it is difficult to assess the physiological role of cav-1 on cholesterol efflux since

experiments have mostly been performed in systems overexpressing cav-1. Nevertheless, these observations suggest that SR-BI might physiologically operate in the same way as cav-1, by generating specific plasma membrane domains where cholesterol trafficking between cells and HDL could be enhanced. Accordingly, Peng and colleagues showed in different cell types that SR-BI-facilitated cholesterol trafficking between cells and HDL occurs primarily in clusters of SR-BI on microvillar extensions of the plasma membrane, but independently of caveolae or raft domains [102]. These observations are consistent with previous studies in steroidogenic cells that localized SR-BI predominantly on cell surface microvillar channels that are filled with HDL [103]. SR-BI expression in insect cells elicits the formation of double membrane structures similar to the microvillar channels of steroidogenic cells [104]. Furthermore, comparison of adrenal gland ultrastructure in wild-type and SR-BI knockout mice has shown that SR-BI is absolutely required for the formation of microvillar channels [105]. Altogether these data strongly suggest that SR-BI is preferentially associated with specific membrane domains and may play a role in the organization of such domains. The nature of these domains is unknown but distinct from classical membrane rafts rich in detergent-resistant saturated phospholipids [102]. In conclusion, it is likely that the ability of SR-BI to stimulate cholesterol efflux may reflect the reorganization of membrane cholesterol domains, fluider than rafts or caveolae, and therefore facilitating FC flux between plasma membrane and HDL. Other parameters such as HDL-apolipoprotein composition, conformation and binding to SR-BI are not required but may enhance this process. This would explain why HDL is the more physiological particle involved in SR-BI-facilitated cholesterol efflux.

ABCA1-mediated lipid efflux

The concept of apolipoprotein-mediated lipid release from cells was initiated more than 20 years ago by the observation that HDL could stimulate hydrolysis and secretion of cytoplasmic cholesterol esters in macrophages at a higher rate than the one expected from the cholesterol esterification rate in HDL [64]. Oram and colleagues [106, 107] identified specific HDL and apoA-I binding sites without internalization at the cell surface of cultured fibroblasts and macrophages. Subsequently, the same group observed that HDL binding was upregulated by cholesterol loading of cells [108, 109] and showed that interaction of HDL apolipoproteins with cholesterol-loaded cells stimulated translocation of free cholesterol away from esterifying enzymes to sites accessible to apolipoproteins [110]. Thus, in contrast to the aqueous diffusion and SR-BI-facilitated FC efflux, the identified mechanism seemed to promote a unidirectional FC flux to HDL-apolipoprotein acceptors and mobilize the intracellular cholesterol pool. Unfortunately, identification of the physiological HDL-binding proteins, other than SR-BI, was not conclusive. A new approach for this issue has been initiated by Hara and colleagues [111, 112], who observed that helical lipid-free apolipoproteins of HDL such as apoA-I, A-II and E were able to induce phospholipid and cholesterol release from cells to generate nascent HDL particles with physical and biochemical characteristics of pre-β-HDL. In humans, the average plasma concentration of free apoA-I and lipid poor pre β-HDL has been estimated to be 60–120 μg/ml [113–115], indicating that lipid-free apoA-I might be a physiological acceptor involved in phospholipid and cholesterol efflux from cells. This process of lipid-free apolipoprotein-mediated lipid efflux was an active temperature-sensitive cellular process, mobilizing intracellular cholesteryl esters pools [112, 116], therefore confirming a mechanism distinct from passive aqueous diffusion or SR-BI-facilitated FC efflux. Synthetic peptides with an amphipathic α-helix structure were also able to promote lipid efflux to an extent similar to lipid-free apoA-I, suggesting that such structures, which are found in repeat motifs in helical apolipoproteins, are necessary and sufficient for the process [112, 117]. Helical apolipoprotein binding and mediated lipid efflux were abolished by proteolytic treatment of cells, which strongly suggested that a membrane 'receptor' is involved in this process [118]. Subsequently, Smith and colleagues [119] found that treatment of mouse macrophage RAW cells with 8-bromo-cAMP (cAMP) stimulated cholesterol efflux to apoA-I and apoE. They proposed a model in which cAMP induces expression of a membrane receptor for apolipoproteins that transfers both cholesterol and phospholipids to apolipoproteins acceptor, resulting in net cholesterol efflux from cells. This hypothesis has been supported by the identification in 1999 of a 200-kDa plasma protein induced by cAMP and that binds apoA-I [120]. However, on the basis of physicochemical properties of the amphipathic helix [121], an alternative mechanism has been suggested, consisting of the insertion of the helical apolipoprotein into the membrane in the absence of specific receptors. In this mechanism, apoA-I might spontaneously insert into the membrane bilayer, acting as a protein detergent and leading, by membrane microsolubilization, to phospholipids and cholesterol efflux [122].

ABCA1 and efflux

A breakthrough in this area has been the discovery by different groups that the apoA-I-mediated specific phospholipids and cholesterol efflux from the cell is critically dependent on the function of ABCA1, a member of the ABC transporter family of proteins, which is defective in Tangier disease [123–126]. This major discovery led to the idea that the ABCA1 transporter could be an apoA-I

receptor. The binding of apoA-I to fibroblasts from Tangier patients has been reported to be abnormal in some studies [127–129] but not in others [130]. Heterogeneity of ABCA1 mutations and therefore the different biochemical bases for Tangier disease could explain these different findings. This is consistent with the recent identification from Tangier disease patients of an ABCA1 mutation (W590S) characterized by a moderate increase in apoA-I binding but defective lipid efflux, whereas other mutations (Q597R, C1477R and S1506L) failed to interact with apoA-I and to promote lipid efflux [131].

ABCA1 and apoA-I interactions

To elucidate the molecular interaction between apoA-I and ABCA1 at the cell surface, a large number of studies have been performed. Early studies have confirmed that ABCA1 was the cAMP-inductible apolipoprotein receptor [132], and a direct molecular interaction between ABCA1 and apoA-I at the cell surface has been proposed on the basis of chemical cross-linking experiments [132, 133]. Moreover, ABCA1 expression was directly correlated both with lipid efflux and with an increase in lipid-free apoA-I binding, but not with any increase in HDL binding [133]. The ability of lipid-free apoA-I to bind ABCA1 has been confirmed by many independent studies performed on different cell types [131, 134–139]. These studies allowed the determination of apoA-I binding parameters to ABCA1 (table 1). As expected, the binding capacity (Bmax) for apoA-I increased upon ABCA1 overexpression by agonists such as 8-Br-cAMP [134] or 9cisRA/22R-OH [139] and decreased upon ABCA1 inhibition by the sulfonylurea derivative glybenclamide [136]. Variations in the Bmax for apoA-I were observed according to cell type, which probably reflected different levels of ABCA1 protein expression between cell lines [140]. However, the high variability in binding affinity (K_d) observed in the different experiments (table 1) is somewhat surprising. Indeed, the presence of more ABCA1 in the plasma membrane should not change binding affinity of apoA-I to ABCA1. However, the numerous binding experiments on ABCA1 (table 1), have mainly been performed without Scatchard representations [85], and reports of kinetic assays (association and dissociation experiments) have been poorly documented. These data are, however, crucial to predict whether ABCA1 might function as a significant receptor for apoA-I [141]. For example, the calculated Kd obtained by different approaches, such as kinetics and Scatchard experiments, should be comparable in order to conclude to a classical protein-protein interaction excluding any lattice effects [80, 142]. In a recent study performed on HEK 293 transfected cells, Fitzgerald and colleagues [143] reported that cell association measurements of ^{125}I-apoA-I binding had a nearly 20-fold lower affinity than that measured for the specific cross-linking of apoA-I to ABCA1 (K_d = 3.836 µg/ml vs. K_d = 0.196 µg/ml, respectively). Moreover, the authors observed that cross-linking experiments were saturable (0.75 µg/ml apoA-I), while association experiments did not clearly saturate at the highest apoA-I concentration tested (20 µg/ml apoA-I). This discrepancy between data obtained from cross-linking and association experiments might reflect an interaction that differs from the classic protein-protein interaction. ApoA-I affinity to ABCA1 is significantly increased at 37 °C (table1), apoA-I dissociation from ABCA1 is almost completely inhibited at 4 °C or 15 °C [139] and no cross-linking apoA-I/ABCA1 complex has been identified when the initial apoA-I cell binding assay is carried out at 4 °C [143]. These results suggest that temperature might alter plasma membrane conformation, affecting apoA-I cell association, or alternatively, that apoA-I binding to ABCA1 might be controlled by an energy-dependent process, such as ABCA1 ATPase activity. This proposition is consistent with two independent studies that showed that ATPase-deficient forms of ABCA1 failed to elicit a specific cell association of apoA-I [135, 136]. One hypothesis is that the ATPase activity could induce conformational change in ABCA1, which would increase the affinity of the transporter for apoA-I. This hypothesis is compatible with other models of ABC transporters such as the bacterial lipid A transporter, MsbA, and the multidrug transporter P-glycoprotein, in which the conformational changes in the transporter induced by ATPase activity seem to regulate substrate interactions [144, 145]. The strict energy dependence of apoA-I binding to ABCA1, together with the binding affinity (K_d) variability and the fact that ABCA1 has been shown to bind other helical apolipoproteins such as apoA-II, apoA-IV, apoC-I, apoC-II, apoC-III, apoE and also amphipathic helical motifs [143, 146], challenged the classical view of a sequence-based interaction.

Separation between flippase activity and apoA-I binding

The idea that apoA-I binding may not involve a direct molecular interaction between ABCA1 and apoA-I was first developed by Chimini and colleagues [135]. They observed that ABCA1 expression at the cell surface was not linearly correlated with apoA-I binding. They found that the lateral mobility of ABCA1 tagged with green fluorescent protein in membranes was consistent with the molecular interaction of apoA-I with rapidly diffusing lipids rather than with a membrane-anchored receptor [135]. Considering that ABCA1 promotes the transbilayer redistribution of phosphatidylserine (PS) within the plasma membrane [147], the authors proposed that ABCA1 might favor the docking of apoA-I at the cell surface by providing a distinctive spatial arrangement of PS in the exofacial membrane leaflet. However, Smith and colleagues [148] reported that although ABCA1 ex-

Table 1. ABCA1 binding parameters.

Cell type	Binding (0/4 °C)	Association (37 °C)	Ref
HEK293 cells transfected by ABCA1	chemical cross-link and ^{125}I-apoA-I binding histogram incubation time: 2 h for ^{125}I-apoA-I = 1 μg/ml, B_{value}: ~ 3 ng/mg cell protein	incubation time: 1.5 h for ^{125}I-apoA-I = 1 μg/ml, B_{value}: ~ 12 ng/mg cell protein and ~ 0 ng/mg cell protein upon glybenclamide treatment (1 mM)	[133, 136]
HEK293 cells transfected by ABCA1	chemical cross-link and ^{125}I-apoA-I binding incubation time: 1 h ^{125}I-apoA-I = 1 μg/ml no interaction observed between apoA-I and ABCA1	chemical cross-link: incubation time: 1 h ^{125}I-apoA-I dose response 0.1 to 6 μg/ml K_d: 0.196 ± 0.05 μg/ml (7.0 ± 1.9 nM) B_{max} ~ 4.5 ng/mg cell protein half-time dissociation ($t_{1/2}$): 25 min ^{125}I-apoA-I binding: incubation time: 1 h ^{125}I-apoA-I dose response 0.1 to 20 μg/ml K_d: 3.836 μg/ml (137 nM), no binding saturation.	[131, 143]
Hela cells transfected by ABCA1	incubation time: 1 h ^{125}I-apoA-I dose response 0.1 to 1.2 μg/ml K_d = 0.60 μg/ml B_{max} from Scatchard: 7.2 ng/mg cell protein		[146]
Baby hamster kidney (BHK) cells transfected by ABCA1	incubation time: 2 h for ^{125}I-apoA-I = 1 μg/ml, B_{value}: ~ 20 ng/mg cell protein		[138]
Murine macrophages RAW264	incubation time: 1 h for ^{125}I-apoA-I = 3 μg/ml, B_{value}: ~ 2 ng/mg cell protein and ~ 4.5 ng/mg cell protein upon 16 h with 8-Br-cAMP treatment (0.3 mM)	incubation time: 3 h for ^{125}I-apoA-I = 3 μg/ml, B_{value}: ~ 7.5 ng/mg cell protein and ~ 40 ng/mg cell protein upon 16 h with 8-Br-cAMP treatment (0.3 mM)	[134]
Cultured human skin fibroblasts (HSF) induced with 9cisRA and 22R-OH	incubation time: 2 h ^{125}I-apoA-I: 10 μg/ml no binding saturation K_d ~ 0.08 μg/ml	incubation time: 2 h ^{125}I-apoA-I dose response 2.5 to 20 μg/ml K_d: 0.65 μg/ml B_{max}: 0.1 ng/mg cell protein half-time association ($t_{1/2}$) ~ 30 min half-time dissociation ($t_{1/2}$) ~ 80 min	[139]
Human fibroblasts	incubation time: 2 h ^{125}I-apoA-I dose response 0.1 to 100 μg/ml K_d: 19 ± 4 μg/ml (700 nM) B_{max}: 300 ± 100 ng/mg cell protein B_{max}: 700 ± 100 ng/mg cell protein upon FC loading		[151]
Different cell lines	Incubation Time: 2h for ^{125}I-apoA-I ~ 0.1 μg/ml no activated (either by 8-Br-cAMP, cholesterol loading or 22R-OH/9cisRA)		[137]
THP-1 (human differentiated macrophages)	B_{value}: ~ 10 ng/mg cell protein (~ 400 fmol/mg cell protein)		
J774 (mouse macrophages)	B_{value}: ~ 25 ng/mg cell protein (~ 900 fmol/mg cell protein)		
U-937 (human differentiated macrophages)	B_{value}: 3 ng/mg cell protein (100 fmol/mg cell protein)		
human skin fibroblasts	B_{value}: 0.6 ng/mg cell protein (20 fmol/mg cell protein)		

pression increased PS levels on the exofacial leaflet, the increased PS was not sufficient to mediate cellular apoA-I binding and lipid efflux because annexin V, a PS-binding protein, did not compete with ABCA1-mediated lipid efflux to apoA-I [136, 148]. Although this result suggested a less direct apoA-I/PS interaction, it is also possible that annexin V and apoA-I bind to noncompeting sites. An elegant study by Genest and colleagues [139] demonstrated that treatment of intact human fibroblasts with either phosphatidycholine-specific phospholipase C or sphingomyelinase affected neither apoA-I/ABCA1 cross-linking nor apoA-I association at 37 °C. Mendez and colleagues [149] also documented that cholesterol and sphingomyelin-rich membrane rafts did not provide lipids for efflux promoted by apolipoproteins through the ABCA1-mediated lipid secretory pathway and that ABCA1 is not associated with these domains. Altogether, these results suggested that the physical interaction between apoA-I and ABCA1 does not depend on either the flippase activity of the transporter or on the membrane PL composition. This idea is consistent with a subsequent study by Chimini and colleagues [150] providing evidence that a mutant form of ABCA1 (W590S) that avidly binds apoA-I but fails to promote cholesterol efflux [131] does not stimulate the translocation of phosphatidylserine to the outer leaflet of the plasma membrane. This result ruled out the initial idea that ABCA1 might favor the association of apoA-I by providing more PS in the exofacial leaflet of the plasma membrane [135] but established that ABCA1 flippase activity and apoA-I association are separated events which are both required to mediate lipid efflux to apoA-I.

Binding and efflux are separated but concomitant events

The high difference between the K_d for apoA-I cellular binding and the rate constant K_m for cholesterol efflux ($K_d = 19 \pm 4$ vs. $K_m = 0.7 \pm 0.2$ μg of apoA-I/ml) measured on human fibroblasts [151] indicates that maximal binding of apoA-I is not required to achieve the maximal rate of removal of FC by apoA-I. Furthermore, the fact that the W590S mutant defective in cholesterol efflux was able to release apoA-I in a normal manner as compared to the wild-type ABCA1 (t1/2 dissociation 30 vs. 25 min, respectively) also revealed that cellular release of apoA-I and transfer of PL and cholesterol are separable events, and thus are probably distinct steps in the efflux mechanism [143]. These results confirm that apoA-I interaction is not by itself sufficient to drive lipid loading of apolipoproteins, which also requires the membrane destabilization characterized by the outward PS flip. A recent study from Vaughan and Oram [138] showed that ABCA1 overexpression in baby hamster kidney cells resulted in a redistribution of membrane cholesterol to cell-surface domains accessible to treatment with enzyme cholesterol oxidase, independently of apolipoprotein interactions. Thus, it is likely that the redistribution of membrane PS and cholesterol induced by ABCA1 activity, when combined with the tethering of apoA-I at the cell surface, might enable phospholipids and cholesterol efflux to apoA-I. Whether ABCA1 directly promotes both phospholipids and cholesterol efflux to apoA-I, or mediates only phospholipid efflux to apoA-I with a subsequent release of cholesterol by the apoA-I/phospholipid complex in an ABCA1-independent fashion, is still not clear and is under investigation [136, 152, 153].

New insights into the controversy concerning the interaction between apoA-I and ABCA1 has been provided by a recent study from Panagotopulos and colleagues [154] using apoA-I truncation mutants. The authors noted a strong positive correlation between cholesterol efflux and the lipid-binding characteristics of apoA-I when mutations were made in helix 10 (deletion of residues 221–243). These findings led to a composite model to explain the interaction between apoA-I and ABCA1: helix 10 of apoA-I tethers the lipid free apolipoprotein to the ABCA1-generated lipid domain and then diffuses within the plane of the membrane until it comes in contact with ABCA1. This model could explain the variability in the calculated binding affinity (Kd) values and the reduced Kd at a temperature below one which allows apolipoprotein to diffuse in the membrane (table 1). However, the data do not reveal whether apoA-I/lipid binding precedes the interaction of apoA-I with ABCA1 or the other way around.

As a matter of conclusion

The ability of both SR-BI and ABCA1 to bind a large number of ligands challenged the classical view of a sequence-based interaction. The inverse ability of ABCA1 and SR-BI to respectively bind apoA-I and HDL may have implications for their physiological roles in vivo. For example, the remodeling of lipoproteins by serum enzymes and lipid transfer proteins may influence the relative efficiency of ABCA1 and SR-BI cellular efflux, with ABCA1-mediated efflux being enhanced by dissociation of apolipoproteins from lipoprotein particles [155]. ABCA1 is clearly involved in the production of HDL and therefore in the elimination of excess cholesterol from cells. The physiological contribution of passive aqueous diffusion and SR-BI-facilitated diffusion in cellular cholesterol efflux to HDL particles remain to be established, because both pathways promote a bidirectional movement of cholesterol between HDL and plasma membrane. Furthermore, the idea that passive aqueous diffusion assimilates the plasma membrane as a homogeneous lipid bilayer and recent discovery have denied this textbook view by demonstrating that the plasma membrane could

contain organized lipid domains. The contribution of SR-BI to cholesterol efflux may depend on the tissue and cell type. So, SR-BI could favor CE selective uptake from lipoproteins in hepatocytes, in contrast to peripheral tissues where SR-BI promotes a major efflux of FC to lipoproteins. However, macrophages, in which the accumulation of cholesterol is critical for the development of atherosclerosis, poorly expressed SR-BI [17, 156]. Moreover, there is no difference in cholesterol efflux to HDL between wild-type and SR-BI knockout macrophages [157]. Altogether, these results suggest that the major mechanism for cholesterol efflux from macrophages is non-SR-BI dependent, but is probably due to ABCA1 or passive diffusion.

After the discovery of the ABCA1 function in apolipoprotein-mediated lipid release, the idea emerged that nascent HDL particles produced from the ABCA1-mediated cholesterol efflux to apoA-I might serve as an acceptor for SR-BI facilitated cholesterol efflux. However, contrary to this hypothesis, Tall and colleagues [156] found that in SR-BI-transfected RAW cells, ABCA1-mediated cholesterol efflux was inhibited by SR-BI. This work suggest that, at least in macrophages, SR-BI could promote cellular re-uptake of cholesterol actively effluxed by ABCA1 and also question the physiological role of SR-BI in macrophages cholesterol efflux.

Physiopathologic relevance

To determine the role of SR-BI in humans, the human *SRB1* gene has been characterized and its genetic variations investigated [158]. In a Caucasian population, three variants at exons 1, 8 and intron 5, with allele frequencies > 0.1, have been identified; exon 1 variant (G-A) was associated with anti-atherogenic lipid profiles, i.e. increased C-HDL and lower C-LDL values, in men [158]. Further studies on the influence of the allele distribution of the exon 1 variant on lipoprotein profile during fasting diets have shown a slight but significant effect on C-LDL as opposed to the unchanged C-HDL content [159]. In contrast, in populations with plant stanol consumption, the exon 1 allele distribution tended to present lower C-HDL and C-LDL concentrations for heterozygous as compared to homozygous polymorphisms [160]. In the context of the Framingham study, the SR-BI exon 1 genotype was significantly associated with type 2 diabetes, indicating that diabetic subjects with the less common allele have lower lipid concentrations (i. e. C-LDL) [161]. The exon 8 variant (C-T) was associated with lower LDL-C concentrations as compared with homozygous normal allele carriers, and, more recently, with an increase in C-HDL. It has been reported that women carriers of the intron 5 variant (C-T) presented a higher body mass index than those women homozygous for the normal allele [162]. The associations found between those mutations and both LDL and C-HDL suggest that SR-BI may play a role in the metabolism of both lipoprotein classes in humans. Interestingly, in a genetic analysis of familial hypercholesterolemia (FH) within a Japanese population, the author described a minor effect of SR-BI polymorphisms (among other polymorphisms) on the lipid and lipoprotein profile [163]. Finally, two new mutations in the SR-BI gene promoter region have been described in a Chinese population with a direct influence on the transcriptional activity of the gene in HepG2 cells [164].

Deleterious mutations in the *ABCA1* gene have been found to be the cause of Tangier disease, which is characterized by an almost complete absence of serum cholesterol HDL. [125,165,166]. Mutations in the *ABCA1* gene have also been found to be the cause of familial hypoalphalipoproteinemia (150–350 mg/l C-HDL) [167]. Many different mutations have already been described. However, when the prevalence of seven different mutations was evaluated among subjects with low C-HDL taken from the general population, the frequency was zero for all these mutations [168]. This was indirectly confirmed by scanning genes using anonymous markers, which has not shown a significant linkage between C-HDL and the region (9q22-31) where the *ABCA1* gene has been mapped [169–171].

Altogether, we could speculate that the genetic influence on the C-HDL levels might result from the combined contributions of polymorphisms and rare mutations, present for instance in the *ABCA1* gene as well as SR-BI and numerous other genes coding for proteins involved in HDL metabolism, such as CETP, LPL, HL, apolipoproteins and so on.

What are the actual roles of ABCA1, SR-BI or of the ectopic ATP synthase in human HDL metabolism? ABCA1 is clearly involved in Tangier disease. Human SR-BI has retained mainly the cellular futures of its rodent homolog, but its role in the metabolism of human lipoproteins, which is considerably different from rodents, needs further investigation. It is likely that SR-BI exhibits a broader spectrum of functions than its involvement in cholesterol metabolism. The ectopic ATP synthase may open a new field of approaches to understand the interaction of HDL with human hepatocytes. Nevertheless, we have to keep in mind that all the proteins described above are part of a very complex process, cholesterol metabolism, in which each participant plays an important role, but in coordination with the others, like the instruments in a symphonic orchestra. Consequently, the future of therapeutic interventions involving HDL will need to take into account the modulation of different targets at the same time. For instance, one therapeutic approach could be to stimulate cholesterol efflux from peripheral cells by increasing ABCA1 expression (perhaps using the LXR or RXR activators; [172]), and to simultaneously stimulate

CE-HDL uptake by the liver through positive modulation of SR-BI and/or cell surface ATP-synthase pathways to increase CE uptake. Even more, blocking intestinal reabsorption of the bile acids and cholesterol could also be beneficial. As an illustration, the recent clinical use of Ezetimibe as an intestinal cholesterol absorption blocking agent associated with a very low level of statins (thus with fewer potential side effects of the statins) to decrease the level of LDL cholesterol may open the way to a new strategic therapeutic approach, better adapted specifically to the case of more complicated HDL, as compare with the LDL metabolism pathway.

1 Mackness M. I., Mackness B. and Durrington P. N. (2002) Paraoxonase and coronary heart disease. Atheroscler. Suppl. **3:** 49-55
2 Kamido H., Kuksis A., Marai L. and Myher J. J. (1995) Lipid ester-bound aldehydes among copper-catalyzed peroxidation products of human plasma lipoproteins. J. Lipid. Res. **36:** 1876–1886
3 Mertens A. and Holvoet P. (2001) Oxidized LDL and HDL: antagonists in atherothrombosis. FASEB J. **15:** 2073–2084
4 Aviram M., Hardak E., Vaya J., Mahmood S., Milo S., Hoffman A. et al. (2000) Human serum paraoxonases (PON1) Q and R selectively decrease lipid peroxides in human coronary and carotid atherosclerotic lesions: PON1 esterase and peroxidase-like activities. Circulation **101:** 2510–2517
5 Robertson K. S., Hawe E., Miller G. J., Talmud P. J. and Humphries S. E. (2003) Human paraoxonase gene cluster polymorphisms as predictors of coronary heart disease risk in the prospective Northwick Park Heart Study II. Biochim. Biophys. Acta **20:** 203–212
6 Mackness B., Durrington P., McElduff P., Yarnell J., Azam N., Watt M. et al. (2003) Low paraoxonase activity predicts coronary events in the Caerphilly Prospective Study. Circulation **107:** 2775–2779
7 O'Connell B. J. and Genest J. Jr (2001) High-density lipoproteins and endothelial function. Circulation **104:** 1978–1983.
8 Fidge N. H. (1999) High density lipoprotein receptors, binding proteins and ligands. J. Lipid. Res. **40:** 187–201
9 Acton S. L., Scherer P. E., Lodish H. F. and Krieger M. (1994) Expression cloning of SR-BI, a CD36-related class B scavenger receptor. J. Biol. Chem. **269:** 21003–21009
10 Calvo D. and Vega M. A. (1993) Identification, primary structure and distribution of CLA-1, a novel member of the CD36/LIMPII gene family. J. Biol. Chem. **268:** 18929–18935
11 Rhainds D. and Brissette L. (2004) The role of scavenger receptor class B type I (SR-BI) in lipid trafficking. Defining the rules for lipid traders. Int. J. Biochem. Cell Biol. **36:** 39–77
12 Azhar S. and Reaven E. (2002) Scavenger receptor class BI and selective cholesteryl ester uptake: partners in the regulation of steroidogenesis. Mol. Cell. Endocrinol. **195:** 1–26
13 Silver D. L. and Tall A. R. (2001) The cellular biology of scavenger receptor class B type I. Curr. Opin. Lipidol. **12:** 497–504
14 Acton S., Rigotti A., Landschulz K. T., Xu S., Hobbs H. H. and Krieger M. (1996) Identification of scavenger receptor SR-BI as a high density lipoprotein receptor. Science **271:** 518–520
15 Glass C., Pittman R. C., Weinstein D. B. and Steinberg D. (1983) Dissociation of tissue uptake of cholesterol ester from that of apoprotein A-I of rat plasma high density lipoprotein: selective delivery of cholesterol ester to liver, adrenal, and gonad. Proc. Natl. Acad. Sci. USA **80:** 5435–5439
16 Stangl H., Cao G., Wyne K. L. and Hobbs H. H. (1998) Scavenger receptor, class B, type I-dependent stimulation of cholesterol esterification by high density lipoproteins, low density lipoproteins and nonlipoprotein cholesterol. J. Biol. Chem. **273:** 31002–31008
17 Ji Y., Jian B., Wang N., Sun Y., Moya M. L., Phillips M. C. et al. (1997) Scavenger receptor BI promotes high density lipoprotein-mediated cellular cholesterol efflux. J. Biol. Chem. **272:** 20982–20985
18 Jian B., de la Llera-Moya M., Ji Y., Wang N., Phillips M. C., Swaney J. B. et al. (1998) Scavenger receptor class B type I as a mediator of cellular cholesterol efflux to lipoproteins and phospholipid acceptors. J. Biol. Chem. **273:** 5599–5606
19 Greene D. J., Skeggs J. W. and Morton R. E. (2001) Elevated triglyceride content diminishes the capacity of high density lipoprotein to deliver cholesteryl esters via the scavenger receptor class B type I (SR-BI). J. Biol. Chem. **276:** 4804–4811
20 Collet X., Tall A. R., Serajuddin H., Guendouzi K., Royer L., Oliveira H. et al. (1999) Remodelling of HDL by CETP in vivo and by CETP and hepatic lipase in vitro results in enhanced uptake of HDL CE by cells expressing scavenger receptor B-I. J. Lipid. Res. **40:** 1185–1193
21 Rodrigueza W. V., Thuahnai S. T., Temel R. E., Lund-Katz S., Phillips M. C. and Williams D. L. (1999) Mechanism of scavenger receptor class B type I-mediated selective uptake of cholesteryl esters from high density lipoprotein to adrenal cells. J. Biol. Chem. **274:** 20344–20350
22 Knecht T. P. and Pittman R. C. (1989) A plasma membrane pool of cholesteryl esters that may mediate the selective uptake of cholesteryl esters from high-density lipoproteins. Biochim. Biophys. Acta **1002:** 365–375
23 Rinninger F., Jaeckle S. and Pittman R. C. (1993) A pool of reversibly cell-associated cholesteryl esters involved in the selective uptake of cholesteryl esters from high-density lipoproteins by Hep G2 hepatoma cells. Biochim. Biophys. Acta **1166:** 275–283
24 Rhainds D., Falstrault L., Tremblay C. and Brissette L. (1999) Uptake and fate of class B scavenger receptor ligands in HepG2 cells. Eur. J. Biochem. **261:** 227–235
25 Schmitz G., Robenek H., Lohmann U. and Assmann G. (1985) Interaction of high density lipoproteins with cholesteryl ester-laden macrophages: biochemical and morphological characterization of cell surface receptor binding, endocytosis and resecretion of high density lipoproteins by macrophages. EMBO J. **4:** 613–622
26 Silver D. L., Nan W., Xiao X. and Tall A. R. (2001) HDL particle uptake mediated by SR-BI results in selective sorting of HDL cholesterol from protein and polarized cholesterol secretion. J. Biol. Chem. **276:** 25287–25293
27 DeLamatre J. G., Sarphie T. G., Archibold R. C. and Hornick C. A. (1990) Metabolism of apoE-free high density lipoproteins in rat hepatoma cells: evidence for a retroendocytic pathway. J. Lipid Res. **31:** 191–202
28 Kambouris A. M., Roach P. D., Calvert G. D. and Nestel P. J. (1990) Retroendocytosis of high density lipoproteins by the human hepatoma cell line, HepG2. Arteriosclerosis **10:** 582–590
29 Garcia A., Barbaras R., Collet X., Bogyo A., Chap H. and Perret B. (1996) High density lipoprotein3 (HDL3) receptor-dependent endocytosis pathway in a human hepatoma cell line (HepG2). Biochemistry **35:** 13064–13070
30 Wustner D., Mondal M., Huang A. and Maxfield F. R. (2003) Different transport routes for high density lipoprotein (HDL) and its associated free sterol in polarized hepatic cells. J. Lipid Res. **16:** 16
31 Babitt J., Trigatti B., Rigotti A., Smart E. J., Anderson R. G., Xu S. et al. (1997) Murine SR-BI, a high density lipoprotein receptor that mediates selective lipid uptake, is N-glycosylated and fatty acylated and colocalizes with plasma membrane caveolae. J. Biol. Chem. **272:** 13242–13249

32 Matveev S., Uittenbogaard A., van Der Westhuyzen D. and Smart E. J. (2001) Caveolin-1 negatively regulates SR-BI mediated selective uptake of high-density lipoprotein-derived cholesteryl ester. Eur. J. Biochem./FEBS **268:** 5609–5616
33 Graf G. A., Connell P. M., van der Westhuyzen D. R. and Smart E. J. (1999) The class B, type I scavenger receptor promotes the selective uptake of high density lipoprotein cholesterol ethers into caveolae. J. Biol. Chem. **274:** 12043–12048
34 Briand O., Lestavel S., Pilon A., Torpier G., Fruchart J. C. and Clavey V. (2003) SR-BI does not require raft/caveola localisation for cholesteryl ester selective uptake in the human adrenal cell line NCI-H295R. Biochim. Biophys. Acta **20:** 42–50
35 Martinez L. O., Jacquet S., Esteve J. P., Rolland C., Cabezon E., Champagne E. et al. (2003) Ectopic beta-chain of ATP synthase is an apolipoprotein A-I receptor in hepatic HDL endocytosis. Nature **421:** 75–79
36 Vassiliou G., Benoist F., Lau P., Kavaslar G. N. and McPherson R. (2001) The low density lipoprotein receptor-related protein contributes to selective uptake of high density lipoprotein cholesteryl esters by SW872 liposarcoma cells and primary human adipocytes. J. Biol. Chem. **276:** 48823–48830
37 Seo T., Al-Haideri M., Treskova E., Worgall T. S., Kako Y., Goldberg I. J. et al. (2000) Lipoprotein lipase-mediated selective uptake from low density lipoprotein requires cell surface proteoglycans and is independent of scavenger receptor class B type 1. J. Biol. Chem. **275:** 30355–30362
38 Rinninger F., Brundert M., Brosch I., Donarski N., Budzinski R. M. and Greten H. (2001) Lipoprotein lipase mediates an increase in selective uptake of HDL-associated cholesteryl esters by cells in culture independent of scavenger receptor BI. J. Lipid Res. **42:** 1740–1751
39 Glass C., Pittman R. C., Given M. and Steinberg M. (1985) Uptake of high-density lipoprotein-associated apoprotein A-I and cholesterol esters by 16 tissues of the rat in vivo and by adrenal cells and hepatocytes in vitro. J. Biol. Chem. **260:** 744–750
40 Kozyraki, R., Fyfe, J., Kristiansen, M., Gerdes, C., Jacobsen, C., Cui, S. et al. (1999) The intrinsic factor-vitamin B12 receptor, cubilin, is a high-affinity apolipoprotein A-I receptor facilitating endocytosis of high-density lipoprotein. Nat. Med. **5:** 656–661
41 Lee J. Y., Lanningham-Foster L., Boudyguina E. Y., Smith T. L., Young E. R., Colvin P. L. et al. (2004) Prebeta high density lipoprotein has two metabolic fates in human apolipoprotein A-I transgenic mice. J. Lipid Res. **45:** 716–728
42 Morrison J. R., McPherson G. A. and Fidge N. H. (1992) Evidence for two sites on rat liver plasma membranes which interact with high density lipoprotein3. J. Biol. Chem. 267: 13205–13209
43 Barbaras R., Collet X., Chap H. and Perret B. (1994) Specific binding of free apolipoprotein A-I to a high-affinity binding site on HepG2 cells: characterization of two high-density lipoprotein sites. Biochemistry **33:** 2335–2340
44 Guendouzi K., Collet X., Perret B., Chap H. and Barbaras R. (1998) Remnant high density lipoprotein2 particles produced by hepatic lipase display high-affinity binding and increased endocytosis into a human hepatoma cell line (HEPG2). Biochemistry **37:** 14974–14980
45 Boyer P. D. (1997) The ATP synthase: a splendid molecular machine. Annu. Rev. Biochem. **66:** 717–749
46 Stock D., Leslie A. G. and Walker J. E. (1999) Molecular architecture of the rotary motor in ATP synthase. Science **286:** 1700–1705
47 Harris D. A. and Das A. M. (1991) Control of mitochondrial ATP synthesis in the heart. Biochem. J. 280: 561–573
48 Walker J. E. (1994) The regulation of catalysis in ATP synthase. Curr. Opin. Struct. Biol. **4:** 912–918
49 Cabezon E., Runswick M. J., Leslie A. G. and Walker J. E. (2001) The structure of bovine IF(1), the regulatory subunit of mitochondrial F-ATPase. EMBO J. **20:** 6990–6996
50 Das B., Mondragon M. O., Sadeghian M., Hatcher V. B. and Norin A. J. (1994) A novel ligand in lymphocyte-mediated cytotoxicity: expression of the beta subunit of H+ transporting ATP synthase on the surface of tumor cell lines. J. Exp. Med. **180:** 273–281
51 Moser, T. L., Stack, M. S., Asplin, I., Enghild, J. J., Hojrup, P., Everitt, L. et al. (1999) Angiostatin binds ATP synthase on the surface of human endothelial cells. Proc. Natl. Acad. Sci. USA **96:** 2811–2816
52 Moser T. L., Kenan D. J., Ashley T. A., Roy J. A., Goodman M. D., Misra U. K. et al.. (2001) Endothelial cell surface F1-F0 ATP synthase is active in ATP synthesis and is inhibited by angiostatin. Proc. Natl. Acad. Sci. USA **98:** 6656–6661
53 Chang S. Y., Park S. G., Kim S. and Kang C. Y. (2001) Interaction of the C-terminal domain of p43 and the alpha subunit of ATP synthase: its functional implication in endothelial cell proliferation. J. Biol. Chem. **277:** 8388–8394
54 Beisiegel, U., Weber, W., Havinga, J. R., Ihrke, G., Hui, D. Y., Wernette-Hammond, M. E. et al. (1988) Apolipoprotein E-binding proteins isolated from dog and human liver. Arteriosclerosis **8:** 288–297
55 Mahley R. W., Hui D. Y., Innerarity T. L. and Beisiegel U. (1989) Chylomicron remnant metabolism. Role of hepatic lipoprotein receptors in mediating uptake. Arteriosclerosis **9:** I14–118
56 Ralevic V. and Burnstock G. (1998) Receptors for purines and pyrimidines. Pharmacol. Rev. **50:** 413–492
57 Plesner L. (1995) Ecto-ATPases: identities and functions. Int. Rev. Cytol. **158**: 141–214
58 Brown M. S. and Goldstein J. L. (1986) A receptor-mediated pathway for cholesterol homeostasis. Science **232:** 34–47
59 Small D. M., Bond M. G., Waugh D., Prack M. and Sawyer J. K. (1984) Physicochemical and histological changes in the arterial wall of nonhuman primates during progression and regression of atherosclerosis. J. Clin. Invest. **73:** 1590–1605
60 Johnson W. J., Mahlberg F. H., Rothblat G. H. and Phillips M. C. (1991) Cholesterol transport between cells and high-density lipoproteins. Biochim. Biophys. Acta **1085:** 273–298
61 Bailey J. M. (1965) Lipid metabolism in cultured cells. Iv. Serum alpha globulins and cellular cholesterol exchange. Exp. Cell Res. **37:** 175–182
62 Glomset J. A. (1973) The metabolic role of lecithin: cholesterol acyltransferase: perspectives from pathology. Adv. Lipid Res. **11:** 1–65
63 Zhao Y. and Marcel Y. L. (1996) Serum albumin is a significant intermediate in cholesterol transfer between cells and lipoproteins. Biochemistry **35:** 7174–7180
64 Ho Y. K., Brown M. S. and Goldstein J. L. (1980) Hydrolysis and excretion of cytoplasmic cholesteryl esters by macrophages: stimulation by high density lipoprotein and other agents. J. Lipid Res. **21:** 391–398
65 Rothblat G. H., de la Llera-Moya M., Atger V., Kellner-Weibel G., Williams D. L. and Phillips M. C. (1999) Cell cholesterol efflux: integration of old and new observations provides new insights. J. Lipid Res. **40:** 781–796
66 Haynes M. P., Phillips M. C. and Rothblat G. H. (2000) Efflux of cholesterol from different cellular pools. Biochemistry **39:** 4508–4517
67 Phillips M. C., Johnson W. J. and Rothblat G. H. (1987) Mechanisms and consequences of cellular cholesterol exchange and transfer. Biochim. Biophys. Acta **906:** 223–276
68 Karlin J. B., Johnson W. J., Benedict C. R., Chacko G. K., Phillips M. C. and Rothblat G. H. (1987) Cholesterol flux between cells and high density lipoprotein. Lack of relationship to specific binding of the lipoprotein to the cell surface. J. Biol. Chem. **262:** 12557–12564
69 Fielding C. J. and Fielding P. E. (1981) Evidence for a lipoprotein carrier in human plasma catalyzing sterol efflux from cultured fibroblasts and its relationship to lecithin:cholesterol acyltransferase. Proc. Natl. Acad. Sci. USA **78:** 3911–3914

70 Kilsdonk E. P., Dorsman A. N. and van Tol A. (1993) Effect of LCAT on HDL-mediated cholesterol efflux from loaded EA.hy 926 cells. Int. J. Biochem. **25:** 219–221

71 Czarnecka H. and Yokoyama S. (1996) Regulation of cellular cholesterol efflux by lecithin:cholesterol acyltransferase reaction through nonspecific lipid exchange. J. Biol. Chem. 271: 2023–2028

72 Rigotti A., Acton S. L. and Krieger M. (1995) The class B scavenger receptors SR-BI and CD36 are receptors for anionic phospholipids. J. Biol. Chem. **270:** 16221–16224

73 Acton S., Rigotti A., Landschulz K. T., Xu S., Hobbs H. H. and Krieger M. (1996) Identification of scavenger receptor SR-BI as a high density lipoprotein receptor. Science **271:** 518–520

74 Rigotti A., Trigatti B. L., Penman M., Rayburn H., Herz J. and Krieger M. (1997) A targeted mutation in the murine gene encoding the high density lipoprotein (HDL) receptor scavenger receptor class B type I reveals its key role in HDL metabolism. Proc. Natl. Acad. Sci. USA **94:** 12610–12615

75 de la Llera-Moya M., Rothblat G. H., Connelly M. A., Kellner-Weibel G., Sakr S. W., Phillips M. C. and Williams D. L. (1999) Scavenger receptor BI (SR-BI) mediates free cholesterol flux independently of HDL tethering to the cell surface. J. Lipid Res. **40:** 575–580

76 Connelly M. A., Klein S. M., Azhar S., Abumrad N. A. and Williams D. L. (1999) Comparison of class B scavenger receptors, CD36 and scavenger receptor BI (SR-BI), shows that both receptors mediate high density lipoprotein- cholesteryl ester selective uptake but SR-BI exhibits a unique enhancement of cholesteryl ester uptake [In Process Citation]. J. Biol. Chem. **274:** 41–47

77 Connelly M. A., de la Llera-Moya M., Monzo P., Yancey P. G., Drazul D., Stoudt G. et al. (2001) Analysis of chimeric receptors shows that multiple distinct functional activities of scavenger receptor, class B, type I (SR-BI), are localized to the extracellular receptor domain. Biochemistry **40:** 5249–5259

78 Gu X., Kozarsky K. and Krieger M. (2000) Scavenger receptor class B, type I-mediated [3H]cholesterol efflux to high and low density lipoproteins is dependent on lipoprotein binding to the receptor. J. Biol. Chem. **275:** 29993–30001

79 Gu X., Lawrence R. and Krieger M. (2000) Dissociation of the high density lipoprotein and low density lipoprotein binding activities of murine scavenger receptor class B type I (mSR-BI) using retrovirus library-based activity dissection. J. Biol. Chem. **275:** 9120–9130

80 Martinez L. O., Georgeaud V., Rolland C., Collet X., Tercé F., Perret B. et al. (2000) Characterization of two high-density lipoprotein binding sites on porcine hepatocyte plasma membranes: contribution of scavenger receptor class B type I (SR-BI) to the low-affinity component. Biochemistry **39:** 1076–1082

81 Xu S., Laccotripe M., Huang X., Rigotti A., Zannis V. I. and Krieger M. (1997) Apolipoproteins of HDL can directly mediate binding to the scavenger receptor SR-BI, an HDL receptor that mediates selective lipid uptake. J. Lipid Res. **38:** 1289–1298

82 Liadaki K. N., Liu T., Xu S., Ishida B. Y., Duchateaux P. N., Krieger J. P. et al. (2000) Binding of high density lipoprotein (HDL) and discoidal reconstituted HDL to the HDL receptor scavenger receptor class B type I. Effect of lipid association and APOA-I mutations on receptor binding. J. Biol. Chem. **275:** 21262–21271

83 Thuahnai S. T., Lund-Katz S., Anantharamaiah G. M., Williams D. L. and Phillips M. C. (2003) A quantitative analysis of apolipoprotein binding to SR-BI: multiple binding sites for lipid-free and lipid-associated apolipoproteins. J. Lipid Res. **44:** 1132–1142

84 Williams, D. L., de La Llera-Moya, M., Thuahnai, S. T., Lund-Katz, S., Connelly, M. A., Azhar, S. et al. (2000) Binding and cross-linking studies show that scavenger receptor BI interacts with multiple sites in apolipoprotein A-I and identify the class A amphipathic alpha-helix as a recognition motif. J. Biol. Chem. **275:** 18897–18904

85 Scatchard G. (1949) Ann. N. Y. Acad. Sci. **51:** 660–672

86 Mendel C. M., Licko V. and Kane J. P. (1985) The effect of ligand heterogeneity on the Scatchard plot. Particular relevance to lipoprotein binding analysis. J. Biol. Chem. **260:** 3451–3455

87 de Beer M. C., Durbin D. M., Cai L., Jonas A., de Beer F. C. 260: van der Westhuyzen D. R. (2001) Apolipoprotein A-I conformation markedly influences HDL interaction with scavenger receptor BI. J. Lipid Res. **42:** 309–313

88 de Villiers W. J., Cai L., Webb N. R., de Beer M. C., van der Westhuyzen D. R. and de Beer F. C. (2001) CD36 does not play a direct role in HDL or LDL metabolism. J. Lipid Res. **42:** 1231–1238

89 Yancey P. G., de la Llera-Moya M., Swarnakar S., Monzo P., Klein S. M., Connelly M. A. et al. (2000) High density lipoprotein phospholipid composition is a major determinant of the bi-directional flux and net movement of cellular free cholesterol mediated by scavenger receptor BI. J. Biol. Chem. **275:** 36596–36604

90 Li X., Kan H. Y., Lavrentiadou S., Krieger M. and Zannis V. (2002) Reconstituted discoidal ApoE-phospholipid particles are ligands for the scavenger receptor BI. The amino-terminal 1-165 domain of ApoE suffices for receptor binding. J. Biol. Chem. **277:** 21149–21157

91 Thuahnai S. T., Lund-Katz S., Dhanasekaran P., De La Llera-Moya M., Connelly M. A., Williams D. L. et al. (2004) SR-BI-mediated cholesteryl ester selective uptake and efflux of unesterified cholesterol: Influence of HDL size and structure. J. Biol. Chem.

92 Mendel C. M. and Kunitake S. T. (1988) Cell-surface binding sites for high density lipoproteins do not mediate efflux of cholesterol from human fibroblasts in tissue culture. J. Lipid Res. **29:** 1171–1178

93 Kellner-Weibel, G., de La Llera-Moya, M., Connelly, M. A., Stoudt, G., Christian, A. E., Haynes, M. P. et al. (2000) Expression of scavenger receptor BI in COS-7 cells alters cholesterol content and distribution. Biochemistry **39:** 221–229

94 Graf G. A., Connell P. M., van der Westhuyzen D. R. and Smart E. J. (1999) The class B, type I scavenger receptor promotes the selective uptake of high density lipoprotein cholesterol ethers into caveolae. J. Biol. Chem. **274:** 12043–12048

95 Graf G. A., Matveev S. V. and Smart E. J. (1999) Class B scavenger receptors, caveolae and cholesterol homeostasis. Trends Cardiovasc. Med. **9:** 221–225

96 Kurzchalia T. V. and Parton R. G. (1999) Membrane microdomains and caveolae. Curr. Opin. Cell Biol. **11:** 424–431

97 Matveev S., Uittenbogaard A., van Der Westhuyzen D. and Smart E. J. (2001) Caveolin-1 negatively regulates SR-BI mediated selective uptake of high-density lipoprotein-derived cholesteryl ester. Eur. J. Biochem. **268:** 5609–5616

98 Frank P. G., Pedraza A., Cohen D. E. and Lisanti M. P. (2001) Adenovirus-mediated expression of caveolin-1 in mouse liver increases plasma high-density lipoprotein levels. Biochemistry **40:** 10892–10900

99 Frank P. G., Marcel Y. L., Connelly M. A., Lublin D. M., Franklin V., Williams D. L. et al. (2002) Stabilization of caveolin-1 by cellular cholesterol and scavenger receptor class B type I. Biochemistry **41:** 11931–11940

100 Wang L., Connelly M. A., Ostermeyer A. G., Chen H. H., Williams D. L. and Brown D. A. (2003) Caveolin-1 does not affect SR-BI-mediated cholesterol efflux or selective uptake of cholesteryl ester in two cell lines. J. Lipid Res. **44:** 807–815

101 Fu Y., Hoang A., Escher G., Parton R. G., Krozowski Z. and Sviridov D. (2004) Expression of caveolin-1 enhances cholesterol efflux in hepatic cells. J. Biol. Chem.

102 Peng Y., Akmentin W., Connelly M. A., Lund-Katz S., Phillips M. C. and Williams D. L. (2004) Scavenger receptor BI (SR-BI) clustered on microvillar extensions suggests that this plasma membrane domain is a way station for cholesterol trafficking between cells and high-density lipoprotein. Mol. Biol. Cell **15:** 384–396

103 Reaven E., Nomoto A., Leers-Sucheta S., Temel R., Williams D. L. and Azhar S. (1998) Expression and microvillar localization of scavenger receptor, class B, type I (a high density lipoprotein receptor) in luteinized and hormone-desensitized rat ovarian models. Endocrinology **139:** 2847–2856

104 Reaven E., Leers-Sucheta S., Nomoto A. and Azhar S. (2001) Expression of scavenger receptor class B type 1 (SR-BI) promotes microvillar channel formation and selective cholesteryl ester transport in a heterologous reconstituted system. Proc. Natl. Acad. Sci. USA **98:** 1613–1618

105 Williams D. L., Wong J. S. and Hamilton R. L. (2002) SR-BI is required for microvillar channel formation and the localization of HDL particles to the surface of adrenocortical cells in vivo. J. Lipid Res. **43:** 544–549

106 Biesbroeck R., Oram J. F., Albers J. J. and Bierman E. L. (1983) Specific high-affinity binding of high density lipoproteins to cultured human skin fibroblasts and arterial smooth muscle cells. J. Clin. Invest. **71:** 525–539

107 Oram J. F., Johnson C. J. and Brown T. A. (1987) Interaction of high density lipoprotein with its receptor on cultured fibroblasts and macrophages. Evidence for reversible binding at the cell surface without internalization. J. Biol. Chem. 262: 2405–2410

108 Oram J. F., Brinton E. A. and Bierman E. L. (1983) Regulation of high density lipoprotein receptor activity in cultured human skin fibroblasts and human arterial smooth muscle cells. J. Clin. Invest. **72:** 1611–1621

109 McKnight G. L., Reasoner J., Gilbert T., Sundquist K. O., Hokland B., McKernan P. A. et al. (1992) Cloning and expression of a cellular high density lipoprotein-binding protein that is up-regulated by cholesterol loading of cells. J. Biol. Chem. **267:** 12131–12141

110 Oram J. F., Mendez A. J., Slotte J. P. and Johnson T. F. (1991) High density lipoprotein apolipoproteins mediate removal of sterol from intracellular pools but not from plasma membranes of cholesterol-loaded fibroblasts. Arterioscler. Thromb. **11:** 403–414

111 Hara H. and Yokoyama S. (1991) Interaction of free apolipoproteins with macrophages. Formation of high density lipoprotein-like lipoproteins and reduction of cellular cholesterol. J. Biol. Chem. **266:** 3080–3086

112 Hara H., Komaba A. and Yokoyama S. (1992) Alpha-helical requirements for free apolipoproteins to generate HDL and to induce cellular lipid efflux. Lipids **27:** 302–304

113 Ishida B. Y., Frolich J. and Fielding C. J. (1987) Prebeta-migrating high density lipoprotein: quantitation in normal and hyperlipidemic plasma by solid phase radioimmunoassay following electrophoretic transfer. J. Lipid Res. **28:** 778–786

114 Bovet P., Darioli R., Essinger A., Golay A., Sigwart U. and Kappenberger L. (1989) Phospholipids and other lipids in angiographically assessed coronary artery disease. Atherosclerosis **80:** 41–47

115 Asztalos B. F. and Roheim P. S. (1995) Presence and formation of 'free apolipoprotein A-I-like' particles in human plasma. Arterioscler. Thromb. Vasc. Biol. **15:** 1419–1423

116 Mendez A. J. (1997) Cholesterol efflux mediated by apolipoproteins is an active cellular process distinct from efflux mediated by passive diffusion. J. Lipid Res. **38:** 1807–1821

117 Mendez A. J., Anantharamaiah G. M., Segrest J. P. and Oram J. F. (1994) Synthetic amphipathic helical peptides that mimic apolipoprotein A-I in clearing cellular cholesterol. J. Clin. Invest. **94:** 1698–1705

118 Li Q., Czarnecka H. and Yokoyama S. (1995) Involvement of a cellular surface factor(s) in lipid-free apolipoprotein-mediated cellular cholesterol efflux. Biochim. Biophys. Acta **1259:** 227–234

119 Smith J. D., Miyata M., Ginsberg M., Grigaux C., Shmookler E. and Plump A. S. (1996) Cyclic AMP induces apolipoprotein E binding activity and promotes cholesterol efflux from a macrophage cell line to apolipoprotein acceptors. J. Biol. Chem. **271:** 30647–30655

120 Sakr S. W., Williams D. L., Stoudt G. W., Phillips M. C. and Rothblat G. H. (1999) Induction of cellular cholesterol efflux to lipid-free apolipoprotein A- I by cAMP [In Process Citation]. Biochim. Biophys. Acta **1438:** 85–98

121 Vogt T. C. and Bechinger B. (1999) The interactions of histidine-containing amphipathic helical peptide antibiotics with lipid bilayers. The effects of charges and pH. J. Biol. Chem. **274:** 29115–29121

122 Gillotte K. L., Zaiou M., Lund-Katz S., Anantharamaiah G. M., Holvoet P., Dhoest A. et al. (1999) Apolipoprotein-mediated plasma membrane microsolubilization. Role of lipid affinity and membrane penetration in the efflux of cellular cholesterol and phospholipid. J. Biol. Chem. **274:** 2021–2028

123 Lawn R. M., Wade D. P., Garvin M. R., Wang X., Schwartz K., Porter, J. G. et al. (1999) The Tangier disease gene product ABC1 controls the cellular apolipoprotein-mediated lipid removal pathway. J. Clin. Invest. **104:** R25–31

124 Bodzioch, M., Orso, E., Klucken, J., Langmann, T., Bottcher, A., Diederich, W. et al. (1999) The gene encoding ATP-binding cassette transporter 1 is mutated in Tangier disease. Nat. Genet. **22:** 347–351

125 Rust S., Rosier M., Funke H., Real J., Amoura Z., Piette J. C. et al. (1999) Tangier disease is caused by mutations in the gene encoding ATP-binding cassette transporter 1. Nat. Genet. **22:** 352–355

126 Brooks-Wilson A., Marcil M., Clee S. M., Zhang L. H., Roomp K., van Dam M. et al. (1999) Mutations in ABC1 in Tangier disease and familial high-density lipoprotein deficiency. Nat. Genet. **22:** 336–345

127 Francis G. A., Knopp R. H. and Oram J. F. (1995) Defective removal of cellular cholesterol and phospholipids by apolipoprotein A-I in Tangier Disease. J. Clin. Invest. **96:** 78–87

128 von Eckardstein A., Chirazi A., Schuler-Luttmann S., Walter M., Kastelein J. J., Geisel J. et al. (1998) Plasma and fibroblasts of Tangier disease patients are disturbed in transferring phospholipids onto apolipoprotein A-I. J. Lipid. Res. **39:** 987–998

129 Oram J. F., Mendez A. J., Lymp J., Kavanagh T. J. and Halbert C. L. (1999) Reduction in apolipoprotein-mediated removal of cellular lipids by immortalization of human fibroblasts and its reversion by cAMP: lack of effect with Tangier disease cells. J. Lipid Res. **40:** 1769–1781

130 Remaley A. T., Schumacher U. K., Stonik J. A., Farsi B. D., Nazih H. and Brewer H. B. Jr (1997) Decreased reverse cholesterol transport from Tangier disease fibroblasts. Acceptor specificity and effect of brefeldin on lipid efflux. Arterioscler. Thromb. Vasc. Biol. **17:** 1813–1821

131 Fitzgerald M. L., Morris A. L., Rhee J. S., Andersson L. P., Mendez A. J. and Freeman M. W. (2002) Naturally occurring mutations in the largest extracellular loops of ABCA1 can disrupt its direct interaction with apolipoprotein A-I. J. Biol. Chem. **277:** 33178–33187

132 Oram J. F., Lawn R. M., Garvin M. R. and Wade D. P. (2000) ABCA1 is the cAMP-inducible apolipoprotein receptor that mediates cholesterol secretion from macrophages. J. Biol. Chem. **275:** 34508–34511

133 Wang N., Silver D. L., Costet P. and Tall A. R. (2000) Specific binding of ApoA-I, enhanced cholesterol efflux, and altered plasma membrane morphology in cells expressing ABC1. J. Biol. Chem. **275:** 33053–33058

134 Takahashi Y. and Smith J. D. (1999) Cholesterol efflux to apolipoprotein AI involves endocytosis and resecretion in a calcium-dependent pathway. Proc. Natl. Acad. Sci. USA **96:** 11358–11363

135 Chambenoit O., Hamon Y., Marguet D., Rigneault H., Rosseneu M. and Chimini G. (2001) Specific docking of apolipoprotein A-I at the cell surface requires a functional ABCA1 transporter. J. Biol. Chem. **276:** 9955–9960

136 Wang N., Silver D. L., Thiele C. and Tall A. R. (2001) ATP-binding cassette transporter A1 (ABCA1) functions as a cholesterol efflux regulatory protein. J. Biol. Chem. **276:** 23742– 23747

137 Burgess J. W., Kiss R. S., Zheng H., Zachariah S. and Marcel Y. L. (2002) Trypsin-sensitive and lipid-containing sites of the macrophage extracellular matrix bind apolipoprotein A-I and participate in ABCA1-dependent cholesterol efflux. J. Biol. Chem. **277:** 31318–31326

138 Vaughan A. M. and Oram J. F. (2003) ABCA1 redistributes membrane cholesterol independent of apolipoprotein interactions. J. Lipid Res. **44:** 1373–1380

139 Denis M., Haidar B., Marcil M., Bouvier M., Krimbou L. and Genest J. Jr (2004) Molecular and cellular physiology of apolipoprotein A-I lipidation by the ATP-binding cassette transporter A1 (ABCA1). J. Biol. Chem. **279:** 7384–7394

140 Denis M., Bissonnette R., Haidar B., Krimbou L., Bouvier M. and Genest J. (2003) Expression, regulation and activity of ABCA1 in human cell lines. Mol. Genet. Metab. **78:** 265–274

141 Bylund D. B. (1980) In: Receptor Binding Techniques. Analysis of receptor binding data, pp. 70–99, Cincinnati-Society for Neuroscience

142 Barbaras R., Collet X., Chap H. and Perret B. (1994) Specific binding of free apolipoprotein A-I to a high-affinity binding site on HepG2 cells: characterization of two high-density lipoprotein sites. Biochemistry **33:** 2335–2340

143 Fitzgerald M. L., Morris A. L., Chroni A., Mendez A. J., Zannis V. I. and Freeman M. W. (2004) ABCA1 and amphipathic apolipoproteins form high affinity molecular complexes required for cholesterol efflux. J. Lipid Res. **45:** 287–294

144 Chang G. and Roth C. B. (2001) Structure of MsbA from *E. coli*: a homolog of the multidrug resistance ATP binding cassette (ABC) transporters. Science **293:** 1793–1800

145 Higgins C. F. and Linton K. J. (2001) Structural biology. The xyz of ABC transporters. Science **293:** 1782–1784

146 Remaley A. T., Stonik J. A., Demosky S. J., Neufeld E. B., Bocharov A. V., Vishnyakova T. G. et al. (2001) Apolipoprotein specificity for lipid efflux by the human ABCAI transporter. Biochem. Biophys. Res. Commun. **280:** 818–823

147 Hamon Y., Broccardo C., Chambenoit O., Luciani M. F., Toti F., Chaslin S. et al. (2000) ABC1 promotes engulfment of apoptotic cells and transbilayer redistribution of phosphatidylserine. Nat. Cell Biol. **2:** 399–406

148 Smith J. D., Waelde C., Horwitz A. and Zheng P. (2002) Evaluation of the role of phosphatidylserine translocase activity in ABCA1-mediated lipid efflux. J. Biol. Chem. **277:** 17797–17803

149 Mendez A. J., Lin G., Wade D. P., Lawn R. M. and Oram J. F. (2001) Membrane lipid domains distinct from cholesterol/sphingomyelin-rich rafts are involved in the ABCA1-mediated lipid secretory pathway. J. Biol. Chem. **276:** 3158–3166

150 Rigot V., Hamon Y., Chambenoit O., Alibert M., Duverger N. and Chimini G. (2002) Distinct sites on ABCA1 control distinct steps required for cellular release of phospholipids. J. Lipid Res. **43:** 2077–2086

151 Gillotte-Taylor K., Nickel M., Johnson W. J., Francone O. L., Holvoet P., Lund-Katz S. et al. (2002) Effects of enrichment of fibroblasts with unesterified cholesterol on the efflux of cellular lipids to apolipoprotein A-I. J. Biol. Chem. **277:** 11811–11820

152 Wang N., Lan D., Gerbod-Giannone M., Linsel-Nitschke P., Jehle A. W., Chen W. et al. (2003) ATP-binding cassette transporter A7 (ABCA7) binds apolipoprotein A-I and mediates cellular phospholipid but not cholesterol efflux. J. Biol. Chem. **278:** 42906–42912

153 Smith J. D., Le Goff W., Settle M., Brubaker G., Waelde C., Horwitz A. et al. (2004) ABCA1 mediates concurrent cholesterol and phospholipid efflux to apoAI. J. Lipid Res. **45:** 635–644

154 Panagotopulos S. E., Witting S. R., Horace E. M., Hui D. Y., Maiorano J. N. and Davidson W. S. (2002) The role of apolipoprotein A-I helix 10 in apolipoprotein-mediated cholesterol efflux via the ATP-binding cassette transporter ABCA1. J. Biol. Chem. **277:** 39477–39484

155 Yancey P. G., Kawashiri M. A., Moore R., Glick J. M., Williams D. L., Connelly M. A. et al. (2003) In vivo modulation of HDL phospholipid has opposing effects on SR-BI- and ABCA1-mediated cholesterol efflux. J. Lipid Res. **45:** 337–346

156 Chen W., Silver D. L., Smith J. D. and Tall A. R. (2000) Scavenger receptor-BI inhibits ATP-binding cassette transporter 1-mediated cholesterol efflux in macrophages [In Process Citation]. J. Biol. Chem. **275:** 30794–30800

157 Zhang W., Yancey P. G., Su Y. R., Babaev V. R., Zhang Y., Fazio S. et al. (2003) Inactivation of macrophage scavenger receptor class B type I promotes atherosclerotic lesion development in apolipoprotein E-deficient mice. Circulation **108:** 2258–2263

158 Acton S., Osgood D., Donoghue M., Corella D., Pocovi M., Cenarro A. et al. (1999) Association of polymorphisms at the SR-BI gene locus with plasma lipid levels and body mass index in a white population. Arterioscler. Thromb. Vasc. Biol. **19:** 1734–1743

159 Perez-Martinez P., Ordovas J. M., Lopez-Miranda J., Gomez P., Marin C., Moreno J. et al. (2003) Polymorphism exon 1 variant at the locus of the scavenger receptor class B type I gene: influence on plasma LDL cholesterol in healthy subjects during the consumption of diets with different fat contents. Am. J. Clin. Nutr. **77:** 809–813

160 Plat J. and Mensink R. P. (2002) Relationship of genetic variation in genes encoding apolipoprotein A-IV, scavenger receptor BI, HMG-CoA reductase, CETP and apolipoprotein E with cholesterol metabolism and the response to plant stanol ester consumption. Eur. J. Clin. Invest. **32:** 242–250

161 Osgood D., Corella D., Demissie S., Cupples L. A., Wilson P. W., Meigs J. B. et al. (2003) Genetic variation at the scavenger receptor class B type I gene locus determines plasma lipoprotein concentrations and particle size and interacts with type 2 diabetes: the framingham study. J. Clin. Endocrinol. Metab. **88:** 2869–2879

162 Osgood-McWeeney D., Galluzzi J. R. and Ordovas J. M. (2000) Allelic discrimination for single nucleotide polymorphisms in the human scavenger receptor class B type 1 gene locus using fluorescent probes. Clin. Chem. **46:** 118–119

163 Yu W., Nohara A., Higashikata T., Lu H., Inazu A. and Mabuchi H. (2002) Molecular genetic analysis of familial hypercholesterolemia: spectrum and regional difference of LDL receptor gene mutations in Japanese population. Atherosclerosis **165:** 335–342

164 Hsu L. A., Ko Y. L., Wu S., Teng M. S., Peng T. Y., Chen C. F. et al. (2003) Association Between a Novel 11-Base Pair Deletion Mutation in the Promoter Region of the scavenger receptor class B type I gene and plasma HDL cholesterol levels in Taiwanese chinese. Arterioscler. Thromb. Vasc. Biol. **23:** 1869–1874

165 Brooks-Wilson A., Marcil M., Clee S. M., Zhang L. H., Roomp K., van Dam M. et al. (1999) Mutations in ABC1 in Tangier disease and familial high-density lipoprotein deficiency. Nat. Genet. **22:** 336–345

166 Bodzioch M., Orso E., Klucken J., Langmann T., Bottcher A., Diederich W. et al. (1999) The gene encoding ATP-binding cassette transporter 1 is mutated in Tangier disease [see comments]. Nat. Genet. 22: 347–351

167 Marcil M., Brooks-Wilson A., Clee S. M., Roomp K., Zhang L. H., Yu L. et al. (1999) Mutations in the ABC1 gene in familial HDL deficiency with defective cholesterol efflux. Lancet **354:** 1341–1346
168 Woll P. S., Hanson N. Q. and Tsai M. Y. (2003) Absence of ABCA1 mutations in individuals with low serum HDL-cholesterol. Clin. Chem. **49:** 521–522
169 Luciani M. F., Denizot F., Savary S., Mattei M. G. and Chimini G. (1994) Cloning of two novel ABC transporters mapping on human chromosome 9. Genomics **21:** 150–159
170 Almasy L., Hixson J. E., Rainwater D. L., Cole S., Williams J. T., Mahaney M. C. et al. (1999) Human pedigree-based quantitative-trait-locus mapping: localization of two genes influencing HDL-cholesterol metabolism. Am. J. Hum. Genet. **64:** 1686–1693
171 Arya R., Duggirala R., Almasy L., Rainwater D. L., Mahaney M. C., Cole S. et al. (2002) Linkage of high-density lipoprotein-cholesterol concentrations to a locus on chromosome 9p in Mexican Americans. Nat. Genet. **30:** 102–105
172 Repa J. J., Turley S. D., Lobaccaro J. A., Medina J., Li L., Lustig K. et al. (2000) Regulation of absorption and ABC1-mediated efflux of cholesterol by RXR heterodimers [see comments]. Science **289:** 1524–1529