REVIEW

Lipid rafts: dream or reality for cholesterol transporters?

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Abstract As a key constituent of the cell membranes, cholesterol is an endogenous component of mammalian cells of primary importance, and is thus subjected to highly regulated homeostasis at the cellular level as well as at the level of the whole body. This regulation requires adapted mechanisms favoring the handling of cholesterol in aqueous compartments, as well as its transfer into or out of membranes, involving membrane proteins. A membrane exhibits functional properties largely depending on its lipid composition and on its structural organization, which very often involves cholesterol-rich microdomains. Then there is the appealing possibility that cholesterol may regulate its own transmembrane transport at a purely functional level, independently of any transcriptional regulation based on cholesterol-sensitive nuclear factors controling the expression level of lipid transport proteins. Indeed, the main cholesterol "transporters" presently believed to mediate for instance the intestinal absorption of cholesterol, that are SR-BI, NPC1L1, ABCA1, ABCG1, ABCG5/G8 and even P-glycoprotein, all present privileged functional relationships with membrane cholesterol-containing microdomains. In particular, they all more or less clearly induce membrane disorganization, supposed to facilitate cholesterol exchanges with the close aqueous medium. The actual lipid substrates handled by these transporters are not yet unambiguously determined, but they likely concern the components of membrane microdomains. Conversely, raft alterations may provide specific modulations of the transporter activities, as well as they can induce indirect effects via local perturbations of the membrane. Finally, these cholesterol transporters undergo regulated intracellular trafficking, with presumably some relationships to rafts which remain to be clarified.

Keywords Lipid rafts · Cholesterol transport · ABC proteins · SR-BI · NPC1L1 · Intestinal absorption

Abbreviations

ABC	ATP-binding cassette
ACAT	Acyl-coenzyme A:cholesterol acyltransferase
Cav1	Caveolin 1
HDL	High density lipoprotein
HMGCoA	Hydroxymethylglutaryl coenzyme A
LDL	Low density lipoprotein
LXR	Liver X receptor
(M)βCD	(Methyl)β-cyclodextrin
MDR	Multidrug resistance
MLN64	Metastatic lymph node 64 protein
NPC1(L1)	Niemann-Pick C protein 1 (-like 1)
NPC2	Niemann-Pick C protein 2
P-gp	P-glycoprotein
RND	Resistance-nodulation-division
SCP-2	Sterol carrier protein 2
SR-BI	Scavenger receptor class B type I
SSD	Sterol-sensing domain
SUV	Small unilamelar vesicles

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Introduction: cholesterol "transport" versus membrane structure and organization

Cholesterol, a key constituent of the cell membranes, is an endogenous component of mammalian cells of primary importance, and is thus subjected to highly regulated homeostasis at the cellular and the whole body levels (Ikonen 2006). Its physiological metabolism and the patho-physiological bases for its observed dysregulations are deeply relevant for pharmacological perspectives in the field of cardiovascular diseases.

Cholesterol turn-over and homeostasis in the body involve many pathways: dietary incomes, entero-hepatic cycle with biliary excretion and intestinal reabsorption, reversible fluxes in the bloodstream between liver and peripheral tissues, biosynthesis (mainly) in liver and steroidogenic tissues, and fecal elimination. The intestinal absorption via the enterocytes is the main determinant process for the global input of cholesterol in the organism (Hui and Howles 2005). In the aqueous compartments of the body, cholesterol is "transported" through its association with lipoprotein particles in the plasma and in the lymph or by inclusion in bile salt micelles in the bile and in the digestive lumen, ensuring in every cases its solubility in the biological fluids. At the cellular level in peripheral cells, cholesterol metabolism relies on uptake from LDL, efflux to HDL, neosynthesis involving HMGCoA reductase and esterification involving ACAT for storage inside the cells. Furthermore, intracellular cholesterol trafficking is a complex process involving either vesicle flux or proteolipid assemblies involving various protein partners (e.g. SCP-2, NPC2, MLN64, Cav1/cyclophilins, etc.) shuttling between organelles and the various intracellular membrane systems (Soccio and Breslow 2004). Anyway, the "limiting" step for cholesterol transfer from any aqueous compartment to another is the crossing of the cell membrane which forms the frontier between them. It is not clear whether a cholesterol molecule crossing a membrane actually mixes with the membrane lipid phase or is only transfered through the membrane without inserting. However, since a membrane exhibits functional properties largely depending on its lipid composition, especially in cholesterol, and on its structural organization which very often involves cholesterol-rich microdomains, there is the appealing possibility that cholesterol may regulate its own transmembrane transport at a purely functional level, independently of any transcriptional regulation based on cholesterol-sensitive nuclear factors controling the expression level of lipid transport proteins.

Indeed, the cell plasma membrane is currently described as composed of the juxtaposition of lateral heterogeneities, forming "membrane microdomains", relying on local lipid and protein compositions that can be distinguished from the rest of "bulk" membrane phase: this is so viewed as "more mosaic, i.e. crowdy and heterogeneous, than fluid" (Engelman 2005; Maxfield 2002). More precisely, these membrane microdomains are generally characterized by an enrichment in cholesterol and sphingolipids, leading to a lipid phase locally more ordered, more compact and thicker than the surrounding membrane, on which these so-called "lipid rafts" are floating (Pike 2004). They are also characterized by the segregation of some proteins, often presenting a GPI anchor, which can favor protein-protein interactions realizing functional regulations (Lucero and Robbins 2004). As a consequence, the lipid rafts are involved in various processes important in cell physiology such as signal transduction, protein and lipid traffic, endocytosis and even cellular entry of pathogens (Simons and Toomre 2000). However, the lipid rafts are presently under lively debates as regards to how they can be unambiguously detected. Indeed, their isolation depends on the protocol used to disrupt the plasma membrane, mostly based on their non-solubilization by a weak, generally non-ionic, detergent or by sonication in a detergent-free medium, and their microscopic observation using fluorescent probes seems to depend on the cell type (Munro 2003). As a matter of fact, these lipid rafts are still badly defined considering their structures at the molecular level, including their size, their lifetime or their dynamics (Lai 2003). To clarify our topic, and beyond the different sensibilities of the various authors cited, the term "lipid rafts" will be used here to refer to as cholesterol-enriched membrane microdomains creating lateral heterogeneities in the membrane phase; "caveolae", whose definition is also subjected to discussion, will be considered as cholesterol-enriched membrane domains containing caveolin and presenting well-discernable morphological characteristics. Nevertheless, whatever their exact definitions, these lateral heterogeneities in the cell membrane must be taken into account, since the lipid rafts are responsible for biochemical, chemico-physical and biophysical influences on local membrane processes.

In the view of these observations, we will discuss here on the functional relationships between the cholesterol-containing membrane microdomains and the various cholesterol "transporters" present at the cell membrane. We will focus mainly on those expressed at the apical and basal poles of polarized cells such as enterocytes, and proved or suspected to be more or less directly involved in intestinal cholesterol absorption, taken as illustrative of the cellular cholesterol transport processes. Within this frame, the scope of this review will more generally address important questions such as: (1) the intracellular location and traffic of these membrane proteins, (2) their possible presence in detergent-resistant membrane fractions and their functional modulation by them, (3) the effects of the cholesterol "transporters" on the membrane structure and organization,

(4) their opportunities for being regulated by protein partners (e.g. caveolin) through possible cosegregation in membrane domains.

SR-BI: a multifunction membrane "scavenger" receptor involved in cholesterol homeostasis

It is now well established that SR-BI, the scavenger receptor class B type I, is responsible for the selective (i.e. independent of whole HDL endocytosis; Acton et al. 1996) cholesteryl ester cellular uptake. This occurs in particular at the level of hepatocytes but also in steroidogenic tissues, and for bi-directional free cholesterol exchange with plasma lipoproteins in peripheral tissues, especially in macrophages (Stangl et al. 1999). Binding of the lipoprotein constitutes the first key step in this process (Liu and Krieger 2002; Thuahnai et al. 2003). Actually, SR-BI is able to bind with fair but different affinities HDL and LDL, as well as modified (acetylated or oxidized) LDL, and HDL and LDL appear not to share the same binding sites (Gu et al. 2000). The exact facilitating role of lipoprotein binding to SR-BI on cholesterol exchange with the membrane is still under debate, since the two events are reported to be correlated or not, depending on the experimental system considered (Connelly et al. 2003; de la Llera-Moya et al. 1999; Liu et al. 2002). However, cholesterol transfer is quantitatively determined by the nature of the lipoprotein particle and its lipid composition (Pownall 2006; Thuahnai et al. 2004; Yancey et al. 2000), as well as by the cholesterol cellular content (de La Llera-Moya et al. 2001). Such a property is expected for a facilitated but passive (in the sense of energy-independent) process of transport of the lipid between the lipoprotein and the membrane. In the case of cholesterol transport in the absence of lipoprotein, for example with SUV-type vesicles or mixed biliary micelles as it happens in the intestine lumen, the direct role of SR-BI is strongly suspected in-vitro (Altmann et al. 2002; Hauser et al. 1998), but not yet ascertained in-vivo although it appears involved in the digestive absorption of cholesterol along with other lipids (Altmann et al. 2002; Bietrix et al. 2006; Cai et al. 2004; Hansen et al. 2003; Levy et al. 2004; Mardones et al. 2001), and also liposoluble micronutrients such as vitamin E (Reboul et al. 2006). Anyway, the composition and the organizational structure of the membrane harboring SR-BI is obviously determining in the efficiency of the cholesterol transfer mediated by SR-BI.

As an illustration, since caveolin-1 (Cav1), a protein characterizing the caveolae involved in cellular cholesterol efflux (Simons and Ikonen 2000), has been described to directly participate to cholesterol trafficking in cells (Smart et al. 2004; Uittenbogaard et al. 1998, 2002), relationships between SR-BI and Cav1 were investigated and reported.

As a matter of fact, murine SR-BI expressed in transfected CHO cells was preferentially found in the low-density caveolin-rich plasma membrane fraction, and this was confirmed by immunofluorescence showing general colocalization of SR-BI with Cav1 in these cells, as well as in the constitutively SR-BI-expressing adrenocortical Y1-BS1 cells (Babitt et al. 1997). SR-BII, the isoform of SR-BI obtained by alternative splicing and truncated on its C-terminal part, displayed the same membrane distribution (Webb et al. 1998). In human THP-1 monocytes, SR-BI was found mainly (55–70%) in the caveolar fraction of the cells, both in undifferentiated and differentiated states (Matveev et al. 1999). Otherwise, SR-BI and Cav1 exhibited closely correlated expression levels in HEK-293T cells transfected with either one of their encoding genes; further immunolocalization in COS-7 cells cotransfected with both genes showed a cellular pattern similar to that of SR-BI-transfected cells (i.e. mainly intracellular) and not that of Cav1-transfected cells (i.e. mainly at the plasma membrane), which suggested a "stabilizing" (in the sense of dragging) role for SR-BI on Cav1 (Frank et al. 2002). Functionally, in SR-BI-transfected CHO cells, cholesterol ether (a non-hydrolyzable analogue of cholesterol ester) was rapidly and selectively uptaken from HDL in the caveolae membrane fraction, where SR-BI was mainly localized; it was also effluxed from caveolae to HDL, showing that caveolae membranes provide a reversible pool of cholesterol ether in the plasma membrane constituting a platform for exchanges with HDL (Graf et al. 1999). However, attempts on various cell types to perform chemical cross-linking or coimmunoprecipitation between SR-BI and Cav1 failed (Matveev et al. 2001), and no coimmunoprecipitation of SR-BI and Cav1 could be evidenced in duodenum detached epithelial cells or in Caco-2 cells (Levy et al. 2004). Likewise, no colocalization was observed by immunofluorescence on either stably SR-BI-transfected WI38 (human lung fibroblasts) or ACTH-treated Y1-BS1 cells (Peng et al. 2004). It has been subsequently reported that Cav1 expression was not necessary for the SR-BI activity in cholesterol handling. First, in the human adrenal cell line NCI-H295R expressing very low levels of Cav1 and devoid of any morphologically-defined caveolae, the SR-BI-mediated cholesterol ester selective uptake was normally upregulated by a forskolin treatment (Briand et al. 2003). Second, in the two cell lines HEK293 and FRT, both selective uptake of cholesterol ester and cholesterol efflux were similar when measured either in cells constitutively expressing no Cav1 or in Cav1-transfected cells (Wang et al. 2003). Finally, in mice deleted for the Cav1 gene, intestinal absorption of cholesterol was unchanged with respect to the wild-type control mice, and their treatment by ezetimibe, a drug recently discovered to inhibit intestinal cholesterol absorption, was equally efficient in both mice

types (Valasek et al. 2005). As a whole, functional relationships between SR-BI and Cav1 appear highly dependent on the cell type considered and the experimental method used for testing them; however, at the intestinal level, no connection could be evidenced.

Independently of these facultative relationships with Cav1, SR-BI was generally present, at least partially, in some cholesterol-rich membrane microdomains. In HepG2 cells, SR-BI was found in majority in the low density membrane fraction isolated on a sucrose gradient after carbonate treatment; interestingly, the unglycosylated non-mature form of SR-BI ($\approx$55 kDa instead of $\approx$82 kDa) was detected in the non-raft fractions (Rhainds et al. 2004). In primary adipocytes, SR-BI was present in the lowest density membrane sub-fraction isolated by a detergent-free protocol out of three sub-classes of rafts revealed by differences following density gradient ultracentrifugation: these "low-density rafts" contain the highest cholesterol concentration (Ortegren et al. 2006). By contrast, in the microvillar fraction of pig enterocytes treated by 1% Triton X100, SR-BI was completely extracted and found in the non-raft membranes (Hansen et al. 2003). In SR-BI-transfected Cos7 and WI38 cells, SR-BI was found in large majority in the membrane fractions solubilized by 1% Triton X-100, but also appeared to be partially present in the membrane fractions resistant to 1% Lubrol WX (Peng et al. 2004), evidencing the differences in composition of the "Triton-rafts" and the "Lubrol-rafts", consistently with what has been previously demonstrated (Schuck et al. 2003). Similarly, in NCI-H295R cells, SR-BI was mainly present in the low density membrane fraction isolated by a detergent-free protocol, whereas less than 10% was associated to the "Triton-rafts" (Briand et al. 2003).

Whatever its membrane localization with respect to the (various types of) rafts, the presence of SR-BI is however able to induce some kinds of membrane perturbations, likely revealing specific effects on the local structural organization of the plasma membrane. In COS-7 cells, SR-BI expression induced an increased size of the cell membrane cholesterol pool sensitive to metabolisation by the soluble enzyme cholesterol oxidase, whereas the related class B scavenger receptor CD36 did not (de la Llera-Moya et al. 1999); moreover, the human homologue of SR-BI, CLA-1, had the same effect as SR-BI, and this was independent of the total cholesterol cellular content (de La Llera-Moya et al. 2001); also, cholesterol extraction by 2-hydroxypropyl-β-CD revealed the induction by SR-BI of an increased size of the cholesterol pool fastly desorbed from the membrane, whereas CD36 was without any effect (Kellner-Weibel et al. 2000). In addition, SR-BI-transfection of Sf9 insect cells allowed observing an increased abundance of phosphatidylcholine with longer mono- or polyunsaturated acyl chains (Parathath et al. 2004). Otherwise, the same heterologous expression of SR-BI in insect cells promotes

the formation of double-membraned microvillar channels (Reaven et al. 2001); these specific morphological differentiations are strikingly reminiscent of those observed on endocrine cells under trophic hormonal stimulation (Reaven et al. 2000), but not in adrenal gland of SR-BI-KO mice (Williams et al. 2002), as well as in SR-BI-transfected WI38 cells (Peng et al. 2004). These modifications of plasma membrane shape are indicative of alterations in the lipid phase as regards to its local fluidity and/or its curvature, possibly linked to the modulation of intramembrane cholesterol (and possibly other lipids) distribution. It remains to determine the functional relationship of these effects with the cholesterol exchanging property of SR-BI between membranes and lipoproteins.

Reciprocally, various experimental manipulations of membrane cholesterol induced some alterations in SR-BI functioning. First, in SR-BI-transfected CHO cells, membrane cholesterol depletion by β-CD increased HDL cholesteryl ester selective uptake, while caveolae cholesterol enrichment by overexpressing Cav1 (consistent with previous data reported by the same authors (Uittenbogaard et al. 1998)) induced a decrease of cholesteryl ester uptake (Matveev et al. 2001). Second, in HepG2 cells, disruption of the lipid rafts by treatment with cholesterol oxidase or sphingomyelinase inhibited LDL-mediated cholesteryl ester selective uptake whereas it enhanced HDL$_3$-mediated cholesteryl ester selective uptake; however, intramembrane cholesterol sequestering by the polyene antibiotic filipin decreased LDL$-$, but not HDL$_3-$, mediated cholesteryl ester selective uptake; conversely, cholesterol extraction by β-CD increased HDL$_3-$, but not LDL$-$, mediated cholesteryl ester selective uptake (Rhainds et al. 2004). Third, overexpression of Cav1 induced a concommitent decrease of LDL cholesteryl ester selective uptake and an increase of HDL$_3$ cholesteryl ester selective uptake, correlated with SR-BI dimerization (Truong et al. 2006). Thus, beyond the different handling by SR-BI of the two endogenous ligands, LDL and HDL, as already described (Acton et al. 1996; Gu et al. 2000), membrane cholesterol is clearly involved in SR-BI functioning by subtle modulations involving free intramembrane cholesterol as well as cholesterol constituting the lipid rafts.

In addition, SR-BI function appears to be modulated by some cellular processes which are more or less related to the membrane microdomains. In particular, a cholesterol depletion (by an acute MβCD treatment) of SR-BI-transfected MDCK cells induced the transcytosis of SR-BI from basolateral membrane, where it was colocalized with caveolae, to apical domain by a PKA-dependent process (Burgos et al. 2004). Furthermore, intracellular trafficking of SR-BI and its surface exposure, conditioning its activity, were under the dependence of PI-3-kinase (Shetty et al. 2006). Finally, SR-BI is also described to mediate

HDL-cholesterol exchange with the cell by an endocytosis/resecretion mechanism, so-called "retro-endocytosis" (Pagler et al. 2006; Rhainds et al. 2004). The role of cholesterol-rich membrane microdomains on such cellular traffic of SR-BI is still unclear, especially in the enterocytes where it was observed by immunoelectron microscopy at the apical side in the microvilli and in plasma membrane invaginations (Levy et al. 2004). However, SR-BI appeared more associated with clathrin-coated pits than with caveolae (Hansen et al. 2003). Finally, the propency of SR-BI to dimerize or oligomerize (Reaven et al. 2004; Sahoo et al. 2007), as well as its specific interaction with its chaperone-like CLAMP/PDZK1 partner (Assanasen et al. 2005; Ikemoto et al. 2000), may provide additional modulation mechanisms involving membrane microdomains, even if their functional relevancies are still under discussion.

As a whole, SR-BI is largely involved in cholesterol metabolism and homeostasis, at variance with the other scavenger receptors, SR-A or CD36, mostly involved in the interactions with various ligands (including pathogens) and signaling processes (Krieger 2001). Indeed, although CD36 (also known as the fatty acid translocase FAT4) was partially found in CHAPS-insoluble, Cav-rich membrane fractions from adipocytes (Pohl et al. 2004), it contrasts from SR-BI since it displayed a cellular localization under the dependence of Cav1, which dragged CD36 (Frank et al. 2002), and it did not induce any change in the membrane sensitivity to cholesterol oxidase or cyclodextrin (Kellner-Weibel et al. 2000).

Thus, SR-BI appears to establish some crucial "privileged" relationships with lipid microdomains in the surrounding plasma membrane. In this way, SR-BI should be considered as coupling the destabilization of the lipid phase of the membrane to specific interactions with cholesterol donors or acceptors in the extracellular medium in order to catalyze the passive (i.e. energy-independent) exchange of cholesterol.

NPC1L1 and NPC1: two sterol "transporters" related to the bacterial multidrug transporters of the RND family

Very recently, bioinformatic technics have pointed out a novel membrane protein, called NPC1L1, as being a fair candidate for playing an important role in intestinal absorption of cholesterol, mainly on the basis of presenting a "sterol sensing domain" (SSD) in its sequence (Altmann et al. 2004). Further, this role was confirmed since the mice deleted for its gene displayed a largely reduced cholesterol and sitosterol absorption, and a resistance to diet-induced hypercholesterolemia, similarly to what was observed on wild-type mice treated with ezetimibe, the new potent drug

inhibiting intestinal cholesterol absorption (Davis et al. 2004). Moreover, ezetimibe had no effect on the NPC1L1-deleted mice (Altmann et al. 2004), and the glucuronide derivative of this molecule (its metabolized form) could be shown to specifically bind to NPC1L1 with a high affinity (Garcia-Calvo et al. 2005).

However, very few is presently known on the functioning of NPC1L1 at the molecular level. In particular, its cellular localization, initially predicted to be at the plasma membrane in enterocytes (Altmann et al. 2004), and as actually observed in transiently transfected CHO cells (Iyer et al. 2005), was subsequently reported to be largely intracellular in various cells expressing NPC1L1 either spontaneously (Davies et al. 2005; Sane et al. 2006) or after transient or stable transfection (Davies et al. 2005; Yu et al. 2006). Some light was shed by the use of fibroblasts disrupted for NPC1L1 expression (obtained from NPC1L1-null mice), that showed a marked mislocalization of Cav1 which was exclusively present at the plasma membrane where it appeared trapped, in contrast to the various intracellular structures observed in the wild-type cells; however, this "anti-caveolae effect" of the presence of NPC1L1 was not a consequence of its colocalization with Cav1 (Davies et al. 2005). Nevertheless, the SSD in NPC1L1 sequence suggests the likelihood of a specific interaction with cholesterol, hence the possibility to sense cholesterol-rich microdomains in its vicinity. This could be consistent with the relative detergent resistance of NPC1L1 evidenced by biochemical fractionation experiments using sequential detergent extraction with taurocholate and digitonin (Iyer et al. 2005). It is worth noting that the measurements of ezetimibe binding on recombinant NPC1L1 was clearly enhanced by the presence of low concentrations of taurocholate and digitonin (Garcia-Calvo et al. 2005). In this context, it was important to observe that a cell cholesterol depletion by MβCD (or to a lesser extent by the cholesterol synthesis inhibitor mevinolin) induced on NPC1L1-overexpressing hepatoma cell line a striking relocation of NPC1L1 (initially mainly intracellular) at the plasma membrane in a subdomain presenting some characteristics of an apical pole between adjacent cells (Yu et al. 2006). Such an "apical sorting" of a membrane protein induced by cholesterol manipulations is reminiscent of the reported relationship in HepG2 cells between cell cholesterol level and the sorting of various membrane proteins, which suggested the involvement of different types of cholesterol-enriched microdomains (Slimane et al. 2003). In addition, this apical expression of NPC1L1 in hepatocytes appeared functionally relevant since it was accompanied by an increased, ezetimibe-sensitive cellular cholesterol uptake (Yu et al. 2006). Moreover, this polarization is consistent with the presence of NPC1L1 in the brush border membranes of enterocytes (Iyer et al. 2005; Sane et al. 2006).

NPC1L1 has a high sequence homology with NPC1, the main protein whose mutation is responsible for Nieman-Pick type C disease: NPC1 also presents a SSD (Davies and Ioannou 2000), actually binding a photoactiveable cholesterol analogue (Ohgami et al. 2004). This should provide some guidelines about NPC1L1 functioning in the cell assuming that both proteins can share common molecular mechanisms. As a matter of fact, NPC1L1 disruption has been shown to provoke an altered intracellular trafficking of sphingolipids such as lactosylceramide as well as of cholesterol (Davies et al. 2005). This is in line with the well-described abnormal membrane trafficking in NPC1-null cells, regarding cholesterol (Cruz et al. 2000; Millard et al. 2000; Puri et al. 1999) and sphingolipids (Sagiv et al. 2006; te Vruchte et al. 2004; Zhang et al. 2001b), but also sucrose, the weakly basic drug daunorubicin, and 70 kDa-dextran, all markers of fluid phase endocytosis (Gong et al. 2006; Neufeld et al. 1999). However, NPC1 was reported to reside in intracellular membranes, within dynamic compartments related to the late endosomes (Garver et al. 2000; Ko et al. 2001; Neufeld et al. 1999; Zhang et al. 2001a). At that level, NPC1 is predominantly present in non-raft membrane fractions (Garver et al. 2000; Lusa et al. 2001), with only partial colocalization with Cav1 (Garver et al. 2000). Nevertheless, when fibroblasts or CHO cells were loaded with LDL-derived cholesterol, the fraction of detergent-resistant NPC1 increased, but it was proposed that the local cholesterol accumulation was the consequence of a defective intracellular traffic rather than of the actual working of NPC1 (Lusa et al. 2001). However, NPC1 deletion in liver homogenates from heterozygous and homozygous mice has been shown to induce an overexpression of Cav1 (Garver et al. 1997) and annexin II (Garver et al. 1999), respectively, which both participate to intracellular cholesterol trafficking (Uittenbogaard et al. 2002). Also, NPC1-deleted hepatocytes have been observed to exhibit increased raft-like microdomains, as reported by a decreased membrane fluidity and an increased resistance to detergent solubilization (Vainio et al. 2005). In addition, cholesterol depletion induced ubiquitylation (a sorting signal) of NPC1 expressed in transfected COS cells and of endogenous NPC1 in human skin fibroblasts; it also induced the association of NPC1 with SKD1, a component of the endosomal sorting complex, and these events were attributed to cholesterol recognition of the SSD in NPC1 (Ohsaki et al. 2006). Furthermore, point mutations in the NPC1 SSD induced alterations of the intracellular trafficking of LDL-derived cholesterol and of a fluorescent analogue of the sphingolipid lactosylceramide (Millard et al. 2005). Otherwise, NPC1 has been shown to present a higher global level of homology with some bacterial multidrug transporters from the RND superfamily (like AcrB or MexD) than with some other SSD-containing eukaryotic proteins (Davies et al.

2000). Indeed, NPC1 could be demonstrated to transport in a proton-dependent manner the cationic amphiphilic drug acriflavine, a substrate of AcrB along with various other lipophilic molecules, in normal fibroblasts and in NPC1-expressing *E. coli*; interestingly, a membrane transport of oleic acid could also be evidenced but not of cholesterol-oleate or of free cholesterol (Davies et al. 2000). The true relationships between NPC1 and cholesterol are thus still unclear (Ioannou 2005), and the similar question is also open for NPC1L1 (Davies and Ioannou 2006). At this stage, it can be proposed that NPC1L1 would be, actively or indirectly, involved in the vesicular traffic of cholesterol, possibly by using ionic electrochemical gradients as a driving force.

ABCA1: a full-size ABC transporter involved in lipid translocation and transfer

The superfamily of ABC proteins is composed of numerous membrane active transporters which work at the expense of ATP hydrolysis to realize the transmembrane translocation of a great variety of substrates, such as ions, sugars, aminoacids, vitamins, peptides, proteins, lipids and even drugs (Gottesman and Ambudkar 2001). They are expressed from bacteria to man, and as a rule they are formed by two transmembrane domains and two nucleotide binding domains, and they can be either monomeric or di- or tetrameric by homo- or hetero-association of such domains (Higgins 1992). In higher mammalians, ABCA1 was first considered as a phosphatidylserine translocase involved in macrophage phagocytosis (Hamon et al. 2000). Later on, ABCA1 took a novel status by being involved in Tangier disease when mutated (Remaley et al. 1999), and this suggested that ABCA1 played a role in cholesterol transfer to HDL (Young and Fielding 1999), ensuring the first steps of the physiologically important "cholesterol reverse transport" (Oliver et al. 2001). Subsequently, as the mechanism of HDL formation was progressively analyzed, ABCA1 appeared as responsible for the cellular efflux of phospholipids and cholesterol to lipid-free apoA-I or lipid-poor lipoproteins thanks to a specific interaction with apoA-I (Wang et al. 2000b). However, some controversies remain as regards the detailed molecular processes involved, in particular concerning the actual transported substrate, phospholipid or cholesterol (Vaughan and Oram 2003; Wang et al. 2001), the sequentiality for their handling, in "two steps" or "one step" (Fielding et al. 2000; Smith et al. 2004), the relationship between lipid transfer and apoA-I binding (Chambenoit et al. 2001), and whether the transfer occurs at the cell surface or inside during a "retroendocytosis" process (Cavelier et al. 2006).

Since the membrane microdomains, rafts and caveolae, were believed to mediate cholesterol export from cells in order to maintain its homeostasis (Fielding and Fielding 2000), relationships between ABCA1 and such lipid microdomains have been investigated. It then appeared the generally admitted view according to which ABCA1 is not localized in the "classical", Triton X100-resistant membrane domains (Drobnik et al. 2002; Mendez et al. 2001). However, a significant fraction of ABCA1 was found in the "larger", Lubrol WX-resistant membrane domains from human macrophages, but not from fibroblasts (Drobnik et al. 2002; Bared et al. 2004). In addition, cholesterol efflux from the cells to apoA-I derived from rafts whereas cholesterol efflux to HDL did not (Drobnik et al. 2002; Mendez et al. 2001). Furthermore, relationships with rafts were not straightforward since the lipid acceptor apoA-I was found to interact with lipid rafts in macrophages (Gaus et al. 2004) but not in ABCA1-overexpressing BHK cells (Landry et al. 2006). The composition of the lipids secreted by ABCA1 could also give indications on their origin in the cell membrane: the two types of lipid-poor, nascent HDL particles secreted by macrophages expressing upregulated ABCA1 came respectively from liquid-ordered and disordered membrane domains, and the microparticles released by the cells likely came from raft-like domains (Duong et al. 2006; Liu et al. 2003). This cellular release of lipid microparticles (devoid of apoA-I) is suggestive of a marked membrane destabilization induced by ABCA1 presence and/or activity; indeed, ABCA1-transfected BHK cells were recently shown to exhibit disrupted cholesterol- and sphingomyelin-rich rafts with redistribution of caveolin, while the cells transfected by an inactive ABCA1 mutant were unperturbed (Landry et al. 2006). In line with these data was the observation of an increased sensitivity to high MβCD concentrations of cells treated by 8Br-cAMP inducing ABCA1 expression, as compared to the non-treated control cells (Smith et al. 2004). Another manifestation of such a membrane deorganization is the report of an increased sensitivity to cholesterol oxidase of the membrane cholesterol in ABCA1-overexpresing BHK cells; interestingly, this cholesterol pool can either be transferred to extracellular apoA-I (but not HDL) or to intracellular esterifying enzyme ACAT (Vaughan and Oram 2003). ABCA1-mediated lipids redistribution in the cell membrane has been also evidenced by measuring the exofacial exposure of fluorescent phospholipid analogues, showing a preferential outward movement of phosphatidylserine and phosphatidylethanolamine (Alder-Baerens et al. 2005). This translocase activity is likely to be related to the modulation of ABCA1 ATPase activity recently determined on proteoliposomes reconstituted with purified ABCA1, and showing a preferential stimulation by

choline-containing lipids (Takahashi et al. 2006). Also, some cellular morphological alterations, typically echinocyte-like protrusions, observed on ABCA1-transfected HEK293 cells, were highly suggestive of an asymmetry of the cell membrane likely induced by a flip-floppase activity (Wang et al. 2000b). Actually, the exact role of these kinds of membrane disturbances and their relationships with cholesterol-rich rafts and cholesterol traffic remain to be further elucidated (Jessup et al. 2006).

Notably, ABCA1 also displays various cellular effects concerning general membrane trafficking; indeed, Tangier fibroblasts (deficient in ABCA1) display enhanced endocytosis (Zha et al. 2001) and phagocytosis (Bared et al. 2004), while ABCA1 overexpression decreased both receptor-mediated and fluid-phase endocytosis (Alder-Baerens et al. 2005). However, Tangier fibroblasts exhibit altered membrane trafficking, including caveolae, between Golgi and plasma membrane (Orso et al. 2000). More precisely, Tangier fibroblasts accumulated both cholesterol and sphingomyelin in the late endocytosis compartment, and ABCA1 transfection induced a correction of this trafficking defect along with the location of tagged ABCA1 in endosome vesicles (Neufeld et al. 2004). Interestingly, vesicular transport from Golgi to plasma membrane increased during ABCA1-mediated cholesterol efflux from both macrophages and fibroblasts (Zha et al. 2003). This is consistent with the observation of preferential cholesterol efflux from "internal pools" (i.e. late endosomes and lysosomes) fed by LDL endocytosis than from "superficial pools" (i.e. recycling endosomes and trans-Golgi) fed by soluble donors such as serum or cyclodextrin (Chen et al. 2001). Although ABCA1 has been repeatedly reported to be expressed mainly at the cell surface (Landry et al. 2006; Wang et al. 2000b), these data highlight that the (small) intracellular fraction of ABCA1 is likely to have a clear functional importance (Chen et al. 2005; Neufeld et al. 2001; Neufeld et al. 2004), in particular revealing an unexpected possible functional synergy with NPC1 (Chen et al. 2001; Neufeld et al. 2004). This would be in line with the regulation of the internal localization of ABCA1 by various protein partners, such as syntrophins (Munehira et al. 2004; Okuhira et al. 2005) and syntaxins (Bared et al. 2004). Finally, cholesterol-induced regulation of ABCA1 intracellular trafficking and its relationships with retroendocytosis still remain to be clarified.

ABCG1: an homodimeric ABC transporter transferring cholesterol to acceptors

Within the frame of cholesterol homeostasis in the body, the HDL-mediated reverse cholesterol transport from peripheral cells to hepatocytes has received much attention,

in particular considering the efflux of cholesterol from macrophages, since they are involved in the first steps of atheromatosis when they incorporate uncontrolled amounts of cholesterol leading to foam cells. Indeed, on the basis of their expression regulation providing adapted responses to loading/depletion of cellular cholesterol, three ABC transporters appear to be involved in such an export of cholesterol to ApoAI-containing lipoproteins: A1 (see above), G1 and G4 (Klucken et al. 2000). Cell transfection experiments confirmed that ABCG1 and G4 (a very close homologue to G1) induce cholesterol transfer to HDL (Wang et al. 2004). The use of gene-disrupted and transgenic mice for ABCG1 brought further evidence of its involvement in preventing cholesterol accumulation in tissues (Kennedy et al. 2005). Furthermore, it appears now that ABCG1 displays a complementary role to that of ABCA1, since ABCA1 can transfer cholesterol to lipid-free/poor apoA1 while ABCG1 can transfer cholesterol to "nascent" HDL particles (i.e. discoidal preβ-HDL, formed in medium conditioned by ABCA1-expressing cells), to finally form mature HDL (Baldan et al. 2006; Gelissen et al. 2006).

Under the perspective of possible functionally relevant interactions of ABCG1 with membrane cholesterol, very few is presently known. First, it has been observed that human ABCG1 overexpressed in BHK cells induced an increased amount of cholesterol in the plasma membrane accessible to the soluble enzyme cholesterol oxidase; this cholesterol pool was either removed by HDL or devoted to esterification in internal compartments in the absence of external cholesterol acceptors (Vaughan and Oram 2005). Such an influence of ABCG1 on cellular distribution and trafficking of cholesterol could be related to an alteration of cholesterol-rich membrane microdomains. In addition, in transfected HEK293 cells, ABCG1 has been shown to efflux cholesterol to external acceptors, along with sphingomyelin preferentially to phosphatidylcholine (Kobayashi et al. 2006). Second, concerning its intracellular localization and trafficking, some controversial data have been reported: ABCG1 appears either mainly perinuclear (Gelissen et al. 2006; Lorkowski et al. 2001), or constitutively at the plasma membrane (Kobayashi et al. 2006), or redistributed to the cell surface by LXR activation (Wang et al. 2006b). This leads to two alternative possibilities allowing ABCG1 to redistribute cholesterol in the cell: either it handles cholesterol at the plasma membrane level, or it processes cholesterol in some internal membranes before it becomes exposed at the cell surface thanks to membrane traffic (Baldan et al. 2006). Anyway, it seems likely that this could lead to the formation of cholesterol-enriched microdomains in the cell membrane, which should then favor cholesterol transfer to ApoAI-containing lipoproteins or even other cholesterol acceptors.

ABCG5/G8: an heterodimeric ABC transporter expelling sterols

The recent cloning of two genes responsible for the dyslipidemic disease sitosterolemia has highlighted the ABC family as providing new partners in the control of cholesterol homeostasis in the body (Berge et al. 2000). Indeed, studies on mice bearing a deletion for either of the gene coding for ABCG5 or ABCG8 (Yu et al. 2002a), or overexpressing both of these genes (Wu et al. 2004; Yu et al. 2002b), have shown that these two "half-transporters" are involved in biliary secretion of cholesterol and other sterols, in particular phytosterols like sitosterol. Furthermore, these half-transporters (i.e. each harboring only one nucleotide binding domain) were demonstrated to be necessarily coexpressed as an heterodimer in order to exhibit their full functionality (Graf et al. 2003; Wang et al. 2006c). They are present in hepatocytes at the apical canalicular membrane, where they participate to bile formation (along with BSEP = ABCB11 and MDR3 = ABCB4, respectively secreting bile salts and phosphatidylcholine), and also in the intestine where they are believed to limit sterol absorption (mainly in jejunum and ileon) (Duan et al. 2004; Hazard and Patel 2007).

The molecular mechanisms of ABCG5/G8 are still very elusive, in particular regarding its relationships with membrane environment and its modulation by the cellular parameters. Some points can nevertheless be considered as potentially relevant as regards functional links with cholesterol regulating its activity. First, intracellular trafficking of ABCG5/G8 is carefully controlled so that each monomer is stabilized by the other one, and only heterodimerization will allow its expression at the cell surface (at the apical pole when the cell is polarized) (Graf et al. 2002), which requires an efficient protein sorting (Okiyoneda et al. 2006). Second, from recent in-vitro data on inside-out vesicles receiving cholesterol from donor liposomes, ABCG5/G8 seems to operate an efficient cholesterol transfer to the luminal leaflet of the vesicle membrane thanks to a floppase mechanism since the vesicle lumen were devoid of any cholesterol acceptor, suggesting that spontaneous cholesterol flip-flop rate would be limiting in the membranes containing ABCG5/G8 (Wang et al. 2006a). If this could be confirmed under more physiological conditions, it would imply that ABCG5/G8 could play an important role in the intramembrane distribution of cholesterol. Third, from in vivo experiments on intraveinously infused ABCG5/G8-KO mice with high concentrations of hydrophobic bile salts, the observation of a depletion of cholesterol present in the canalicular membrane, leading to bile duct lesion and cholestasis, led to infer that ABCG5/G8 can have a protective effect against the detergent action of the bile acids (Kosters et al. 2006). Furthermore, the absence of any

stimulation of biliary cholesterol secretion under these conditions led the authors to consider that the limiting step should be the intramembrane translocation of cholesterol, and thus to suggest that this transporter acts as a floppase (Kosters et al. 2006). In that case, the observation of the extended selectivity of this transporter to handle various natural sterols, in order to exclude them from the body in spite of their abundance in the diet (Yu et al. 2004), would raise the question of their intramembrane distribution (between the two leaflets as well as regarding the cholesterol-rich microdomains likely to be present). As a mater of fact, it has been observed on model membranes that the phytosterols, sitosterol and stigmasterol, either behave approximately as cholesterol in promoting ordered, detergent-resistant microdomains (Xu et al. 2001), or are less able to favorably interact with phospholipid bilayers and stabilize microdomains (Halling and Slotte 2004). However, no direct investigation of the relationships between ABCG5/G8 and membrane microdomains have been presently reported, and this would thus be desirable.

P-gp: a (possible) model ABC transporter for relationships with cholesterol

P-glycoprotein (P-gp = ABCB1) is well-described for almost three decades for being responsible for the multidrug resistance (MDR) phenotype of some tumor cells (Gerlach et al. 1986). Its gene cloning about twenty years ago has shown that it belongs to the superfamily of ABC active membrane transporters (Bosch and Croop 1996). P-gp works as expelling by an ATP-dependent process various cytotoxic drugs out of the tumor cells which express it in the plasma membrane (Gottesman and Pastan 1993), leading to anticancer chemotherapy failures (Nooter and Herweijer 1991). In the nineties, it became progressively clear that P-gp, which is also expressed in some healthy tissues such as intestine mucosa, blood-brain barrier, biliary canalicule and kidney tubule, can handle a very large number of amphiphilic molecules of pharmaceutical interest, and is consequently involved in their pharmacokinetic characteristics (such as digestive absorption, CNS disposition, biliary and urinary excretion) (Schinkel et al. 1997). P-gp is thus characterized by the rather unique property of multispecific transported substrate recognition, concerning a very much broad spectrum of molecular structures, provided they are amphiphilic, neutral or cationic, and presenting a size ranging between about 250 and 1,250 Da (Ambudkar et al. 1999). The underlying molecular mechanisms only begin to be unraveled (Garrigues et al. 2002b; Shilling et al. 2006); in particular, a body of evidences have shown that the various, more or less hydrophobic substrates recognized P-gp at the level of its transmembrane domains after partitioning in the surrounding lipid phase (Ferte 2000; Higgins and Gottesman 1992; Sharom 1997).

Various steroids belong to the number of substrates transported by P-gp, as they notably exhibit reduced cellular accumulation in MDR cells and inhibition of P-gp-mediated drug transport, and among them cholesterol behaves similarly (Barnes et al. 1996; Wang et al. 2000a). In addition, cholesterol modulates ATPase activity of P-gp contained in native inside-out membrane vesicles prepared from MDR (selected or transfected) cells (Barakat et al. 2005; Garrigues et al. 2002a; Gayet et al. 2005; Rothnie et al. 2001; Wang et al. 2000a). It has then been demonstrated in-vitro on such P-gp-containing inside-out vesicles that exogenous radiolabeled cholesterol associates in an ATP-dependent manner, and in addition that endogenous cholesterol becomes less accessible to the exogenously added soluble enzyme cholesterol oxidase also in an ATP-dependent manner (Garrigues et al. 2002a). Taken as a whole, these data provide the evidence that P-gp actively translocates membrane cholesterol from the cytosolic leaflet to the exoplasmic one. Such an intramembrane redistribution of cholesterol, leading to an increased exposure at the cell surface and/or a stabilization of membrane microdomains, is consistent with the observation of alterations of cholesterol interaction with the external medium, including resistance to permeabilization by digitonin (Ramu et al. 1991), and variable efficiency of MβCD extraction (Le Goff et al. 2006; Reungpatthanaphong et al. 2004). However, cellular efflux of cholesterol to MβCD appears rather cell type-dependent since it was increased in stably transfected LLC-MDR1 but not in conditionally transfected HeLa-MDR/Tet cells (Le Goff et al. 2006). Also, cellular cholesterol depletion by MβCD was less efficient in MDR K562 versus parental sensitive cells (Reungpatthanaphong et al. 2004), and in that case an increased recycling of cholesterol from internal stores can be invoked as well as an altered cholesterol distribution/organization within the plasma membrane. Indeed, the whole cellular trafficking of cholesterol has been observed to be stimulated in some MDR cells, with increased biosynthesis (Metherall et al. 1996) and esterification (Debry et al. 1997; Field et al. 1995), although this has been subsequently contested (Issandou and Grand-Perret 2000), and appears once more as depending on the cell type considered (Luker et al. 1999). Anyway, such an increased membrane trafficking can provide a fair explanation to the apparently unexpected observation of an increased influx of cholesterol from mixed micelles to intestinal cells overexpressing P-gp (Tessner and Stenson 2000). Finally, in the double KO, *mdr1a-/mdr1b*-disrupted mice, the observation of a decreased cholesterol accumulation with an increased cholesterol esterification in the hepatocytes, but not in the other tissues, when cholesterol was administered orally, but not intraveinously (Luker et al.

2001), illustrates that all the consequences, both direct and indirect, of P-gp expression on the cellular traffic and metabolism of cholesterol are not yet deciphered.

The involvement of P-gp in cell cholesterol homeostasis is nevertheless indicated by the regulation of its gene expression by the cholesterol loading level of the cell: human macrophages treated by HDL for cholesterol depletion, or by LDL for cholesterol repletion, respectively up-regulated and down-regulated P-gp expression (Klucken et al. 2000). As a mater of fact, MDR cells submitted to cholesterol starvation during culture felt a much smaller cholesterol level decrease than the sensitive counterparts (Mazzoni and Trave 1993). Furthermore, it has been observed that in the MDR cells, in contrast to sensitive tumor cells, cholesterol level is mainly regulated via its efflux to HDL (Liscovitch and Lavie 2000). This could be viewed as consistent with the observation of an up-regulation of Cav1 expression in some MDR cells (Lavie et al. 1998; Pang et al. 2004; Yang et al. 1998), although this could not be considered as a general rule (Lavie et al. 1998).

Reciprocally, P-gp function has been amply shown to be influenced by the nature, the composition and the structural organization of its surrounding membrane. Actually, P-gp often localized (at least partially) in cholesterol-rich, detergent-resistant membrane microdomains (Barakat et al. 2005; Luker et al. 2000; Troost et al. 2004), possibly depending on the detergent used (Hinrichs et al. 2004; Radeva et al. 2005), at variance with a report using a detergent-free method for P-gp isolation (Reungpatthanaphong et al. 2004). Furthermore, P-gp colocalized with some membrane proteins included in rafts, and these protein-protein interactions were shown to functionally regulate P-gp (Demeule et al. 2000; Ghetie et al. 2004; Luciani et al. 2002). However, P-gp function was also modulated by treatments altering rafts (Barakat et al. 2005; Luker et al. 2000; Troost et al. 2004), although these experimental conditions rather targeted the cholesterol presence in the cell membrane. The interpretation of such experimental results are thus not always unambiguous concerning the respective roles of free cholesterol versus cholesterol composing the rafts in the membrane. In addition, P-gp has also been described in internal membranes (Molinari et al. 1994), where its functional implication has been discussed in relation to intracellular membrane trafficking (Kim et al. 1997; Lee et al. 2001; Rajagopal and Simon 2003; Shapiro et al. 1998); furthermore, intracellular trafficking leading to apical polarized expression of P-gp appeared to be dependent on cholesterol (Slimane et al. 2003). As a whole, it appears that P-gp establishes privileged relationships with membrane cholesterol, even if its inclusion in the rafts is likely partial and dynamic in essence, and clearly depends on the cell type considered as well as the exact nature of the

microdomain involved (e.g. presence of Cav1, detergent-type resistance and MβCD sensitivity) (Orlowski et al. 2006). Thus, even if P-gp could be considered as a (both theoretical and experimental) guideline for other ABC transporters involved in lipid traffic, many points still remain unclear and should be addressed in order to gain the undisputed status of "model ABC transporter". However, it could be envisioned that the different ABC transporters, considered here to be involved in cellular cholesterol transport, all present an active floppase function of rather broad selectivity for various lipids, which may be converted to an (active) "extrudase" function if the transporter specifically interacts with a lipid acceptor (such as a lipoprotein).

Synthesis and perspectives

Cholesterol homeostasis is of primary importance for body health and cell physiology. It involves various membrane proteins which mediate cholesterol transfer from a donor in an aqueous medium to an acceptor in another aqueous compartment: cell membrane is thus a limiting step in such cholesterol "transport". In this frame, the molecular mechanisms of these cholesterol "transporters", whenever they behave as "translocases" or "transferases", is thus desirable to be analyzed and elucidated, since this determines the resultant biological processes which ultimately are considered as relevant for public health (e.g. hypercholesterolemia leading to atherosclerotic diseases). Indeed, understanding cellular physiology of cholesterol metabolism and traffic needs the description of the lower integration levels, i.e. the membrane and within it the proteins, which are the actual pharmacological targets. As a matter of fact, lipid composition and structural organization of the cell membrane are well-known to be essential for the biological activity of the membrane proteins, especially membrane transporters. The cholesterol-rich microdomains, lipid rafts, are then prominent candidates for regulating cholesterol transporters. They indeed appear to play noticeable roles in the various proteins we discussed above. However, it is not always clear whether this is realized directly by providing either a specific close environment or an alteration of the local biophysical properties of the membrane (curvature, asymmetry, thickness, etc.), or indirectly by modulating the transporter activity within the membrane, for example by altering membrane trafficking or also by recruiting regulatory protein partners: this will need further work in every case.

As a whole, it is noteworthy that the main cholesterol transporters considered here show variable intracellular distribution or presence in the membrane microdomains, often depending on the cell type and on the experimental procedure. Obviously, some methodological pitfalls are always

possibly underlying the works cited here, such as: (1) cell and membrane fractionation, generally based on the use of detergents under specific conditions, (2) cell cholesterol manipulation using cyclodextrin, not always straightforward for preserving the integrity of the cell physiology, and (3) protein colocalizations using fluorescence microscopy, always subjected to the limitations due to the optical microscopy resolution. However, the whole data presented above fairly suggest that the cholesterol "transporters" display a highly dynamic behavior with respect to their membrane environment, taking full benefit of the intracellular traffic. This should be considered as an evidence of all the regulation processes these membrane proteins can undergo, and as an illustration of their likely functional versatility when considering the cellular consequences of their molecular working.

The cholesterol "transporters" can be classified in two categories, according to their passive (i.e. acting as facilitators) or active (i.e. energy-dependent) activity. The second case, mostly concerning the members of the ABC family, raises the interesting question of the relationships between the cholesterol flip-flop in the membrane and the "raft determinism". Since the lipid rafts in plasma membrane are currently described as presenting an asymmetry between the two membrane leaflets, as regards their lipid composition, the actual question is whether these microdomains are at thermodynamic equilibrium. Considering their highly dynamic behavior and their involvement in the whole membrane trafficking, these membrane microdomains are likely out of equilibrium (as is the biological membrane as a whole!). The following question is about the nature of the molecule(s) taken as the primary substrate of an active intramembrane translocation and whose asymmetric distribution provides the driving force for the whole raft formation and then raft stability at the steady state. Although it is difficult to claim that it is the only lipid component to be concerned, cholesterol is indeed outwardly translocated ("flopped") by some ABC transporters, and this may then be at least part of the global mechanism of raft determinism (also involving, among others, sphingomyelin). In addition, this translocation is not necessarily limited within the rafts themselves, since cholesterol may also be actively flopped at any place of the membrane, allowing to passively diffuse laterally in the exoplasmic leaflet up to incorporate a raft domain. As a matter of fact, the representative and long-known ABC transporter P-gp is able to establish an altered cholesterol distribution between the two membrane leaflets when assayed in-vitro (Garrigues et al. 2002a). This straight experimental observation clearly demonstrates that the active intramembrane flux of cholesterol is faster than its passive back flux in that case, but this could also be achieved by other ABC transporters under different conditions.

Furthermore, for a more general point of view, and given the rather rapid rate at which cholesterol can spontaneously transfer between membrane pools, we can wonder whether the function of a cholesterol "transporter" is to regulate exchange with the extracellular medium or to maintain the disequilibrium of sterol between cholesterol-rich and cholesterol-poor domains. It seems fair to assume that an heterogenous cholesterol abundance between the various membrane pools is necessary to determine cholesterol exchange rates with the extracellular medium as part of the general process of lipid (and membrane proteins) sorting among the intracellular compartments. Thus, the various cholesterol transporters, at least the active ones, would be as well involved in regulating the different intracellular cholesterol pools as in ensuring the actual fluxes of cholesterol with extracellular donors and acceptors. In any case, these active transporters could be considered as participating in a global process of feeding a continuous cholesterol turn-over within the cells or in the organism, which is inherent to the homeostasis of this important biological component.

Anyway, all these possible aspects of functioning of these cholesterol transporters make them likely to be sensitive to the presence of cholesterol in their membrane environment. In the case of SR-BI, the cellular consequences of a local facilitating effect on the passive exchange of cholesterol between the membrane phase and a soluble complexing agent is obviously dependent on various conditions imposed by the metabolic status of the cell, especially regarding its lipid loading level: this could determine the direction of the mediated cholesterol flux. Also, when cholesterol has to "choose" between different pathways for cellular export, rafts may play a role (Fielding and Fielding 2003; Jessup et al. 2006), in particular in relation with the cell polarization. In the case of NPC1L1, the strong homology with NPC1 indicates that it could be involved in vesicular trafficking, which is known to depend on cellular rafts (Rajendran and Simons 2005). In the case of members of the ABC family, which most likely mediate an active process in the membrane, the question remains about the relationship between a local interleaflet translocation of cholesterol and the resulting effect in the cell, taking into account the lipid trafficking under the influence of the rafts (Orlowski et al. 2006). In all cases, the effect of the rafts should also depend on the substrate selectivity of the various cholesterol transporters, since they all are known or suspected to handle a more or less broad spectrum of lipids. Finally, the rafts can also well be a factor favoring the "functional harmonization" of the different lipid transporters involved in cholesterol cellular traffic and presenting possible functional redundancy and/or complementation, allowing for subtle coregulations and optimization: this will undoubtly warrant further investigations in the field.

Anyway, cholesterol-rich membrane microdomains appear to provide a novel way, besides the more described genic regulations involving various nuclear factors, for cholesterol transport regulation based on chemico-physical and biophysical properties of the membrane.

References

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

Alder-Baerens N, Muller P, Pohl A, Korte T, Hamon Y, Chimini G, Pomorski T, Herrmann A (2005) Headgroup-specific exposure of phospholipids in ABCA1-expressing cells. J Biol Chem 280:26321–26329

Altmann SW, Davis HR Jr, Yao X, Laverty M, Compton DS, Zhu LJ, Crona JH, Caplen MA, Hoos LM, Tetzloff G, Priestley T, Burnett DA, Strader CD, Graziano MP (2002) The identification of intestinal scavenger receptor class B, type I (SR-BI) by expression cloning and its role in cholesterol absorption. Biochim Biophys Acta 1580:77–93

Altmann SW, Davis HR Jr, Zhu LJ, Yao X, Hoos LM, Tetzloff G, Iyer SP, Maguire M, Golovko A, Zeng M, Wang L, Murgolo N, Graziano MP (2004) Niemann-Pick C1 Like 1 protein is critical for intestinal cholesterol absorption. Science 303:1201–1204

Ambudkar SV, Dey S, Hrycyna CA, Ramachandra M, Pastan I, Gottesman MM (1999) Biochemical, cellular, and pharmacological aspects of the multidrug transporter. Annu Rev Pharmacol Toxicol 39:361–398

Assanasen C, Mineo C, Seetharam D, Yuhanna IS, Marcel YL, Connelly MA, Williams DL, de la Llera-Moya M, Shaul PW, Silver DL (2005) Cholesterol binding, efflux, and a PDZ-interacting domain of scavenger receptor-BI mediate HDL-initiated signaling. J Clin Invest 115:969–977

Babitt J, Trigatti B, Rigotti A, Smart EJ, Anderson RG, Xu S, Krieger M (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

Baldan A, Tarr P, Lee R, Edwards PA (2006) ATP-binding cassette transporter G1 and lipid homeostasis. Curr Opin Lipidol 17:227–232

Barakat S, Gayet L, Dayan G, Labialle S, Lazar A, Oleinikov V, Coleman AW, Baggetto LG (2005) Multidrug-resistant cancer cells contain two populations of P-glycoprotein with differently stimulated P-gp ATPase activities: evidence from atomic force microscopy and biochemical analysis. Biochem J 388:563–571

Bared SM, Buechler C, Boettcher A, Dayoub R, Sigruener A, Grandl M, Rudolph C, Dada A, Schmitz G (2004) Association of ABCA1 with syntaxin 13 and flotillin-1 and enhanced phagocytosis in tangier cells. Mol Biol Cell 15:5399–5407

Barnes KM, Dickstein B, Cutler GB Jr, Fojo T, Bates SE (1996) Steroid treatment, accumulation, and antagonism of P-glycoprotein in multidrug-resistant cells. Biochemistry 35:4820–4827

Berge KE, Tian H, Graf GA, Yu L, Grishin NV, Schultz J, Kwiterovich P, Shan B, Barnes R, Hobbs HH (2000) Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters. Science 290:1771–1775

Bietrix F, Yan D, Nauze M, Rolland C, Bertrand-Michel J, Comera C, Schaak S, Barbaras R, Groen AK, Perret B, Terce F, Collet X (2006) Accelerated lipid absorption in mice overexpressing intestinal SR-BI. J Biol Chem 281:7214–7219

Bosch I, Croop J (1996) P-glycoprotein multidrug resistance and cancer. Biochim Biophys Acta 1288:F37–F54

Briand O, Lestavel S, Pilon A, Torpier G, Fruchart JC, 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 1631:42–50

Burgos PV, Klattenhoff C, de la Fuente E, Rigotti A, Gonzalez A (2004) Cholesterol depletion induces PKA-mediated basolateral-to-apical transcytosis of the scavenger receptor class B type I in MDCK cells. Proc Natl Acad Sci USA 101:3845–3850

Cai L, Eckhardt ER, Shi W, Zhao Z, Nasser M, de Villiers WJ, van der Westhuyzen DR (2004) Scavenger receptor class B type I reduces cholesterol absorption in cultured enterocyte CaCo-2 cells. J Lipid Res 45:253–262

Cavelier C, Lorenzi I, Rohrer L, von Eckardstein A (2006) Lipid efflux by the ATP-binding cassette transporters ABCA1 and ABCG1. Biochim Biophys Acta 1761:655–666

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

Chen W, Sun Y, Welch C, Gorelik A, Leventhal AR, Tabas I, Tall AR (2001) Preferential ATP-binding cassette transporter A1-mediated cholesterol efflux from late endosomes/lysosomes. J Biol Chem 276:43564–43569

Chen W, Wang N, Tall AR (2005) A PEST deletion mutant of ABCA1 shows impaired internalization and defective cholesterol efflux from late endosomes. J Biol Chem 280:29277–29281

Connelly MA, de la Llera-Moya M, Peng Y, Drazul-Schrader D, Rothblat GH, Williams DL (2003) Separation of lipid transport functions by mutations in the extracellular domain of scavenger receptor class B, type I. J Biol Chem 278:25773–25782

Cruz JC, Sugii S, Yu C, Chang TY (2000) Role of Niemann-Pick type C1 protein in intracellular trafficking of low density lipoprotein-derived cholesterol. J Biol Chem 275:4013–4021

Davies JP, Chen FW, Ioannou YA (2000) Transmembrane molecular pump activity of Niemann-Pick C1 protein. Science 290:2295–2298

Davies JP, Ioannou YA (2000) Topological analysis of Niemann-Pick C1 protein reveals that the membrane orientation of the putative sterol-sensing domain is identical to those of 3-hydroxy-3-methylglutaryl-CoA reductase and sterol regulatory element binding protein cleavage-activating protein. J Biol Chem 275:24367–24374

Davies JP, Ioannou YA (2006) The role of the Niemann-Pick C1-like 1 protein in the subcellular transport of multiple lipids and their homeostasis. Curr Opin Lipidol 17:221–226

Davies JP, Scott C, Oishi K, Liapis A, Ioannou YA (2005) Inactivation of NPC1L1 causes multiple lipid transport defects and protects against diet-induced hypercholesterolemia. J Biol Chem 280:12710–12720

Davis HR Jr, Zhu LJ, Hoos LM, Tetzloff G, Maguire M, Liu J, Yao X, Iyer SP, Lam MH, Lund EG, Detmers PA, Graziano MP, Altmann SW (2004) Niemann-Pick C1 Like 1 (NPC1L1) is the intestinal phytosterol and cholesterol transporter and a key modulator of whole-body cholesterol homeostasis. J Biol Chem 279:33586–33592

de la Llera-Moya M, Connelly MA, Drazul D, Klein SM, Favari E, Yancey PG, Williams DL, Rothblat GH (2001) Scavenger receptor class B type I affects cholesterol homeostasis by magnifying cholesterol flux between cells and HDL. J Lipid Res 42:1969–1978

de la Llera-Moya M, Rothblat GH, Connelly MA, Kellner-Weibel G, Sakr SW, Phillips MC, Williams DL (1999) Scavenger receptor

BI (SR-BI) mediates free cholesterol flux independently of HDL tethering to the cell surface. J Lipid Res 40:575–580

Debry P, Nash EA, Neklason DW, Metherall JE (1997) Role of multidrug resistance P-glycoproteins in cholesterol esterification. J Biol Chem 272:1026–1031

Demeule M, Jodoin J, Gingras D, Beliveau R (2000) P-glycoprotein is localized in caveolae in resistant cells and in brain capillaries. FEBS Lett 466:219–224

Drobnik W, Borsukova H, Bottcher A, Pfeiffer A, Liebisch G, Schutz GJ, Schindler H, Schmitz G (2002) Apo AI/ABCA1-dependent and HDL3-mediated lipid efflux from compositionally distinct cholesterol-based microdomains. Traffic 3:268–278

Duan LP, Wang HH, Wang DQ (2004) Cholesterol absorption is mainly regulated by the jejunal and ileal ATP-binding cassette sterol efflux transporters Abcg5 and Abcg8 in mice. J Lipid Res 45:1312–1323

Duong PT, Collins HL, Nickel M, Lund-Katz S, Rothblat GH, Phillips MC (2006) Characterization of nascent HDL particles and microparticles formed by ABCA1-mediated efflux of cellular lipids to apoA-I. J Lipid Res 47:832–843

Engelman DM (2005) Membranes are more mosaic than fluid. Nature 438:578–580

Ferte J (2000) Analysis of the tangled relationships between P-glycoprotein-mediated multidrug resistance and the lipid phase of the cell membrane. Eur J Biochem 267:277–294

Field FJ, Born E, Chen H, Murthy S, Mathur SN (1995) Esterification of plasma membrane cholesterol and triacylglycerol-rich lipoprotein secretion in CaCo-2 cells: possible role of p-glycoprotein. J Lipid Res 36:1533–1543

Fielding CJ, Fielding PE (2000) Cholesterol and caveolae: structural and functional relationships. Biochim Biophys Acta 1529:210–222

Fielding CJ, Fielding PE (2003) Relationship between cholesterol trafficking and signaling in rafts and caveolae. Biochim Biophys Acta 1610:219–228

Fielding PE, Nagao K, Hakamata H, Chimini G, Fielding CJ (2000) A two-step mechanism for free cholesterol and phospholipid efflux from human vascular cells to apolipoprotein A-1. Biochemistry 39:14113–14120

Frank PG, Marcel YL, Connelly MA, Lublin DM, Franklin V, Williams DL, Lisanti MP (2002) Stabilization of caveolin-1 by cellular cholesterol and scavenger receptor class B type I. Biochemistry 41:11931–11940

Garcia-Calvo M, Lisnock J, Bull HG, Hawes BE, Burnett DA, Braun MP, Crona JH, Davis HR Jr, Dean DC, Detmers PA, Graziano MP, Hughes M, Macintyre DE, Ogawa A, O'Neill K A, Iyer SP, Shevell DE, Smith MM, Tang YS, Makarewicz AM, Ujjainwalla F, Altmann SW, Chapman KT, Thornberry NA (2005) The target of ezetimibe is Niemann-Pick C1-Like 1 (NPC1L1). Proc Natl Acad Sci USA 102:8132–8137

Garrigues A, Escargueil AE, Orlowski S (2002a) The multidrug transporter, P-glycoprotein, actively mediates cholesterol redistribution in the cell membrane. Proc Natl Acad Sci USA 99:10347–10352

Garrigues A, Loiseau N, Delaforge M, Ferte J, Garrigos M, Andre F, Orlowski S (2002b) Characterization of two pharmacophores on the multidrug transporter P-glycoprotein. Mol Pharmacol 62:1288–1298

Garver WS, Erickson RP, Wilson JM, Colton TL, Hossain GS, Kozloski MA, Heidenreich RA (1997) Altered expression of caveolin-1 and increased cholesterol in detergent insoluble membrane fractions from liver in mice with Niemann-Pick disease type C. Biochim Biophys Acta 1361:272–280

Garver WS, Heidenreich RA, Erickson RP, Thomas MA, Wilson JM (2000) Localization of the murine Niemann-Pick C1 protein to two distinct intracellular compartments. J Lipid Res 41:673–687

Garver WS, Hossain GS, Winscott MM, Heidenreich RA (1999) The Npc1 mutation causes an altered expression of caveolin-1, annexin II and protein kinases and phosphorylation of caveolin-1 and annexin II in murine livers. Biochim Biophys Acta 1453:193–206

Gaus K, Kritharides L, Schmitz G, Boettcher A, Drobnik W, Langmann T, Quinn CM, Death A, Dean RT, Jessup W (2004) Apolipoprotein A-1 interaction with plasma membrane lipid rafts controls cholesterol export from macrophages. FASEB J 18:574–576

Gayet L, Dayan G, Barakat S, Labialle S, Michaud M, Cogne S, Mazane A, Coleman AW, Rigal D, Baggetto LG (2005) Control of P-glycoprotein activity by membrane cholesterol amounts and their relation to multidrug resistance in human CEM leukemia cells. Biochemistry 44:4499–4509

Gelissen IC, Harris M, Rye KA, Quinn C, Brown AJ, Kockx M, Cartland S, Packianathan M, Kritharides L, Jessup W (2006) ABCA1 and ABCG1 synergize to mediate cholesterol export to apoA-I. Arterioscler Thromb Vasc Biol 26:534–540

Gerlach JH, Kartner N, Bell DR, Ling V (1986) Multidrug resistance. Cancer Surv 5:25–46

Ghetie MA, Marches R, Kufert S, Vitetta ES (2004) An anti-CD19 antibody inhibits the interaction between P-glycoprotein (P-gp) and CD19, causes P-gp to translocate out of lipid rafts, and chemosensitizes a multidrug-resistant (MDR) lymphoma cell line. Blood 104:178–183

Gong Y, Duvvuri M, Duncan MB, Liu J, Krise JP (2006) Niemann-Pick C1 protein facilitates the efflux of the anticancer drug daunorubicin from cells according to a novel vesicle-mediated pathway. J Pharmacol Exp Ther 316:242–247

Gottesman MM, Ambudkar SV (2001) Overview: ABC transporters and human disease. J Bioenerg Biomembr 33:453–458

Gottesman MM, Pastan I (1993) Biochemistry of multidrug resistance mediated by the multidrug transporter. Annu Rev Biochem 62:385–427

Graf GA, Connell PM, van der Westhuyzen DR, Smart EJ (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

Graf GA, Li WP, Gerard RD, Gelissen I, White A, Cohen JC, Hobbs HH (2002) Coexpression of ATP-binding cassette proteins ABCG5 and ABCG8 permits their transport to the apical surface. J Clin Invest 110:659–669

Graf GA, Yu L, Li WP, Gerard R, Tuma PL, Cohen JC, Hobbs HH (2003) ABCG5 and ABCG8 are obligate heterodimers for protein trafficking and biliary cholesterol excretion. J Biol Chem 278:48275–48282

Gu X, Kozarsky K, 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

Halling KK, Slotte JP (2004) Membrane properties of plant sterols in phospholipid bilayers as determined by differential scanning calorimetry, resonance energy transfer and detergent-induced solubilization. Biochim Biophys Acta 1664:161–171

Hamon Y, Broccardo C, Chambenoit O, Luciani MF, Toti F, Chaslin S, Freyssinet JM, Devaux PF, McNeish J, Marguet D, Chimini G (2000) ABC1 promotes engulfment of apoptotic cells and transbilayer redistribution of phosphatidylserine. Nat Cell Biol 2:399–406

Hansen GH, Niels-Christiansen LL, Immerdal L, Danielsen EM (2003) Scavenger receptor class B type I (SR-BI) in pig enterocytes: trafficking from the brush border to lipid droplets during fat absorption. Gut 52:1424–1431

Hauser H, Dyer JH, Nandy A, Vega MA, Werder M, Bieliauskaite E, Weber FE, Compassi S, Gemperli A, Boffelli D, Wehrli E, Schulthess G, Phillips MC (1998) Identification of a receptor

mediating absorption of dietary cholesterol in the intestine. Biochemistry 37:17843–17850

Hazard SE, Patel SB (2007) Sterolins ABCG5 and ABCG8: regulators of whole body dietary sterols. Pflugers Arch 453:745–752

Higgins CF (1992) ABC transporters: from microorganisms to man. Annu Rev Cell Biol 8:67–113

Higgins CF, Gottesman MM (1992) Is the multidrug transporter a flippase? Trends Biochem Sci 17:18–21

Hinrichs JW, Klappe K, Hummel I, Kok JW (2004) ATP-binding cassette transporters are enriched in non-caveolar detergent-insoluble glycosphingolipid-enriched membrane domains (DIGs) in human multidrug-resistant cancer cells. J Biol Chem 279:5734–5738

Hui DY, Howles PN (2005) Molecular mechanisms of cholesterol absorption and transport in the intestine. Semin Cell Dev Biol 16:183–192

Ikemoto M, Arai H, Feng D, Tanaka K, Aoki J, Dohmae N, Takio K, Adachi H, Tsujimoto M, Inoue K (2000) Identification of a PDZ-domain-containing protein that interacts with the scavenger receptor class B type I. Proc Natl Acad Sci USA 97:6538–6543

Ikonen E (2006) Mechanisms for cellular cholesterol transport: defects and human disease. Physiol Rev 86:1237–1261

Ioannou YA (2005) Guilty until proven innocent: the case of NPC1 and cholesterol. Trends Biochem Sci 30:498–505

Issandou M, Grand-Perret T (2000) Multidrug resistance P-glycoprotein is not involved in cholesterol esterification. Biochem Biophys Res Commun 279:369–377

Iyer SP, Yao X, Crona JH, Hoos LM, Tetzloff G, Davis HR Jr, Graziano MP, Altmann SW (2005) Characterization of the putative native and recombinant rat sterol transporter Niemann-Pick C1 Like 1 (NPC1L1) protein. Biochim Biophys Acta 1722:282–292

Jessup W, Gelissen IC, Gaus K, Kritharides L (2006) Roles of ATP binding cassette transporters A1 and G1, scavenger receptor BI and membrane lipid domains in cholesterol export from macrophages. Curr Opin Lipidol 17:247–257

Kellner-Weibel G, de la Llera-Moya M, Connelly MA, Stoudt G, Christian AE, Haynes MP, Williams DL, Rothblat GH (2000) Expression of scavenger receptor BI in COS-7 cells alters cholesterol content and distribution. Biochemistry 39:221–229

Kennedy MA, Barrera GC, Nakamura K, Baldan A, Tarr P, Fishbein MC, Frank J, Francone OL, Edwards PA (2005) ABCG1 has a critical role in mediating cholesterol efflux to HDL and preventing cellular lipid accumulation. Cell Metab 1:121–131

Kim H, Barroso M, Samanta R, Greenberger L, Sztul E (1997) Experimentally induced changes in the endocytic traffic of P-glycoprotein alter drug resistance of cancer cells. Am J Physiol 273:C687–C702

Klucken J, Buchler C, Orso E, Kaminski WE, Porsch-Ozcurumez M, Liebisch G, Kapinsky M, Diederich W, Drobnik W, Dean M, Allikmets R, Schmitz G (2000) ABCG1 (ABC8), the human homolog of the Drosophila white gene, is a regulator of macrophage cholesterol and phospholipid transport. Proc Natl Acad Sci USA 97:817–822

Ko DC, Gordon MD, Jin JY, Scott MP (2001) Dynamic movements of organelles containing Niemann-Pick C1 protein: NPC1 involvement in late endocytic events. Mol Biol Cell 12:601–614

Kobayashi A, Takanezawa Y, Hirata T, Shimizu Y, Misasa K, Kioka N, Arai H, Ueda K, Matsuo M (2006) Efflux of sphingomyelin, cholesterol, and phosphatidylcholine by ABCG1. J Lipid Res 47:1791–1802

Kosters A, Kunne C, Looije N, Patel SB, Oude Elferink RP, Groen AK (2006) The mechanism of ABCG5/ABCG8 in biliary cholesterol secretion in mice. J Lipid Res 47:1959–1966

Krieger M (2001) Scavenger receptor class B type I is a multiligand HDL receptor that influences diverse physiologic systems. J Clin Invest 108:793–797

Lai EC (2003) Lipid rafts make for slippery platforms. J Cell Biol 162:365–370

Landry YD, Denis M, Nandi S, Bell S, Vaughan AM, Zha X (2006) ATP-binding cassette transporter A1 expression disrupts raft membrane microdomains through its ATPase-related functions. J Biol Chem 281:36091–36101

Lavie Y, Fiucci G, Liscovitch M (1998) Up-regulation of caveolae and caveolar constituents in multidrug-resistant cancer cells. J Biol Chem 273:32380–32383

Le Goff W, Settle M, Greene DJ, Morton RE, Smith JD (2006) Reevaluation of the role of the multidrug-resistant P-glycoprotein in cellular cholesterol homeostasis. J Lipid Res 47:51–58

Lee G, Schlichter L, Bendayan M, Bendayan R (2001) Functional expression of P-glycoprotein in rat brain microglia. J Pharmacol Exp Ther 299:204–212

Levy E, Menard D, Suc I, Delvin E, Marcil V, Brissette L, Thibault L, Bendayan M (2004) Ontogeny, immunolocalisation, distribution and function of SR-BI in the human intestine. J Cell Sci 117:327–337

Liscovitch M, Lavie Y (2000) Multidrug resistance: a role for cholesterol efflux pathways? Trends Biochem Sci 25:530–534

Liu B, Krieger M (2002) Highly purified scavenger receptor class B, type I reconstituted into phosphatidylcholine/cholesterol liposomes mediates high affinity high density lipoprotein binding and selective lipid uptake. J Biol Chem 277:34125–34135

Liu L, Bortnick AE, Nickel M, Dhanasekaran P, Subbaiah PV, Lund-Katz S, Rothblat GH, Phillips MC (2003) Effects of apolipoprotein A-I on ATP-binding cassette transporter A1-mediated efflux of macrophage phospholipid and cholesterol: formation of nascent high density lipoprotein particles. J Biol Chem 278:42976–42984

Liu T, Krieger M, Kan HY, Zannis VI (2002) The effects of mutations in helices 4 and 6 of ApoA-I on scavenger receptor class B type I (SR-BI)-mediated cholesterol efflux suggest that formation of a productive complex between reconstituted high density lipoprotein and SR-BI is required for efficient lipid transport. J Biol Chem 277:21576–21584

Lorkowski S, Kratz M, Wenner C, Schmidt R, Weitkamp B, Fobker M, Reinhardt J, Rauterberg J, Galinski EA, Cullen P (2001) Expression of the ATP-binding cassette transporter gene ABCG1 (ABC8) in Tangier disease. Biochem Biophys Res Commun 283:821–830

Lucero HA, Robbins PW (2004) Lipid rafts-protein association and the regulation of protein activity. Arch Biochem Biophys 426:208–224

Luciani F, Molinari A, Lozupone F, Calcabrini A, Lugini L, Stringaro A, Puddu P, Arancia G, Cianfriglia M, Fais S (2002) P-glycoprotein-actin association through ERM family proteins: a role in P-glycoprotein function in human cells of lymphoid origin. Blood 99:641–648

Luker GD, Dahlheimer JL, Ostlund RE Jr, Piwnica-Worms D (2001) Decreased hepatic accumulation and enhanced esterification of cholesterol in mice deficient in mdr1a and mdr1b P-glycoproteins. J Lipid Res 42:1389–1394

Luker GD, Nilsson KR, Covey DF, Piwnica-Worms D (1999) Multidrug resistance (MDR1) P-glycoprotein enhances esterification of plasma membrane cholesterol. J Biol Chem 274:6979–6991

Luker GD, Pica CM, Kumar AS, Covey DF, Piwnica-Worms D (2000) Effects of cholesterol and enantiomeric cholesterol on P-glycoprotein localization and function in low-density membrane domains. Biochemistry 39:7651–7661

Lusa S, Blom TS, Eskelinen EL, Kuismanen E, Mansson JE, Simons K, Ikonen E (2001) Depletion of rafts in late endocytic membranes is controlled by NPC1-dependent recycling of cholesterol to the plasma membrane. J Cell Sci 114:1893–1900

Mardones P, Quinones V, Amigo L, Moreno M, Miquel JF, Schwarz M, Miettinen HE, Trigatti B, Krieger M, VanPatten S, Cohen DE, Rigotti A (2001) Hepatic cholesterol and bile acid metabolism and intestinal cholesterol absorption in scavenger receptor class B type I-deficient mice. J Lipid Res 42:170–180

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

Matveev S, van der Westhuyzen DR, Smart EJ (1999) Co-expression of scavenger receptor-BI and caveolin-1 is associated with enhanced selective cholesteryl ester uptake in THP-1 macrophages. J Lipid Res 40:1647–1654

Maxfield FR (2002) Plasma membrane microdomains. Curr Opin Cell Biol 14:483–487

Mazzoni A, Trave F (1993) Cytoplasmic membrane cholesterol and doxorubicin cytotoxicity in drug-sensitive and multidrug-resistant human ovarian cancer cells. Oncol Res 5:75–82

Mendez AJ, Lin G, Wade DP, Lawn RM, Oram JF (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

Metherall JE, Li H, Waugh K (1996) Role of multidrug resistance P-glycoproteins in cholesterol biosynthesis. J Biol Chem 271:2634–2640

Millard EE, Gale SE, Dudley N, Zhang J, Schaffer JE, Ory DS (2005) The sterol-sensing domain of the Niemann-Pick C1 (NPC1) protein regulates trafficking of low density lipoprotein cholesterol. J Biol Chem 280:28581–28590

Millard EE, Srivastava K, Traub LM, Schaffer JE, Ory DS (2000) Niemann-pick type C1 (NPC1) overexpression alters cellular cholesterol homeostasis. J Biol Chem 275:38445–38451

Molinari A, Cianfriglia M, Meschini S, Calcabrini A, Arancia G (1994) P-glycoprotein expression in the Golgi apparatus of multidrug-resistant cells. Int J Cancer 59:789–795

Munehira Y, Ohnishi T, Kawamoto S, Furuya A, Shitara K, Imamura M, Yokota T, Takeda S, Amachi T, Matsuo M, Kioka N, Ueda K (2004) Alpha1-syntrophin modulates turnover of ABCA1. J Biol Chem 279:15091–15095

Munro S (2003) Lipid rafts: elusive or illusive? Cell 115:377–388

Neufeld EB, Remaley AT, Demosky SJ, Stonik JA, Cooney AM, Comly M, Dwyer NK, Zhang M, Blanchette-Mackie J, Santamarina-Fojo S, Brewer HB Jr (2001) Cellular localization and trafficking of the human ABCA1 transporter. J Biol Chem 276:27584–27590

Neufeld EB, Stonik JA, Demosky SJ Jr, Knapper CL, Combs CA, Cooney A, Comly M, Dwyer N, Blanchette-Mackie J, Remaley AT, Santamarina-Fojo S, Brewer HB Jr (2004) The ABCA1 transporter modulates late endocytic trafficking: insights from the correction of the genetic defect in Tangier disease. J Biol Chem 279:15571–15578

Neufeld EB, Wastney M, Patel S, Suresh S, Cooney AM, Dwyer NK, Roff CF, Ohno K, Morris JA, Carstea ED, Incardona JP, Strauss JF 3rd, Vanier MT, Patterson MC, Brady RO, Pentchev PG, Blanchette-Mackie EJ (1999) The Niemann-Pick C1 protein resides in a vesicular compartment linked to retrograde transport of multiple lysosomal cargo. J Biol Chem 274:9627–9635

Nooter K, Herweijer H (1991) Multidrug resistance (mdr) genes in human cancer. Br J Cancer 63:663–669

Ohgami N, Ko DC, Thomas M, Scott MP, Chang CC, Chang TY (2004) Binding between the Niemann-Pick C1 protein and a photoactivatable cholesterol analog requires a functional sterol-sensing domain. Proc Natl Acad Sci USA 101:12473–12478

Ohsaki Y, Sugimoto Y, Suzuki M, Hosokawa H, Yoshimori T, Davies JP, Ioannou YA, Vanier MT, Ohno K, Ninomiya H (2006) Cholesterol depletion facilitates ubiquitylation of NPC1 and its association with SKD1/Vps4. J Cell Sci 119:2643–2653

Okiyoneda T, Kono T, Niibori A, Harada K, Kusuhara H, Takada T, Shuto T, Suico MA, Sugiyama Y, Kai H (2006) Calreticulin facilitates the cell surface expression of ABCG5/G8. Biochem Biophys Res Commun 347:67–75

Okuhira K, Fitzgerald ML, Sarracino DA, Manning JJ, Bell SA, Goss JL, Freeman MW (2005) Purification of ATP-binding cassette transporter A1 and associated binding proteins reveals the importance of beta1-syntrophin in cholesterol efflux. J Biol Chem 280:39653–39664

Oliver WR Jr, Shenk JL, Snaith MR, Russell CS, Plunket KD, Bodkin NL, Lewis MC, Winegar DA, Sznaidman ML, Lambert MH, Xu HE, Sternbach DD, Kliewer SA, Hansen BC, Willson TM (2001) A selective peroxisome proliferator-activated receptor delta agonist promotes reverse cholesterol transport. Proc Natl Acad Sci USA 98:5306–5311

Orlowski S, Martin S, Escargueil A (2006) P-glycoprotein and 'lipid rafts': some ambiguous mutual relationships (floating on them, building them or meeting them by chance?). Cell Mol Life Sci 63(9):1038–1059

Orso E, Broccardo C, Kaminski WE, Bottcher A, Liebisch G, Drobnik W, Gotz A, Chambenoit O, Diederich W, Langmann T, Spruss T, Luciani MF, Rothe G, Lackner KJ, Chimini G, Schmitz G (2000) Transport of lipids from golgi to plasma membrane is defective in tangier disease patients and Abc1-deficient mice. Nat Genet 24:192–196

Ortegren U, Yin L, Ost A, Karlsson H, Nystrom FH, Stralfors P (2006) Separation and characterization of caveolae subclasses in the plasma membrane of primary adipocytes; segregation of specific proteins and functions. FEBS J 273:3381–3392

Pagler TA, Rhode S, Neuhofer A, Laggner H, Strobl W, Hinterndorfer C, Volf I, Pavelka M, Eckhardt ER, van der Westhuyzen DR, Schutz GJ, Stangl H (2006) SR-BI-mediated high density lipoprotein (HDL) endocytosis leads to HDL resecretion facilitating cholesterol efflux. J Biol Chem 281:11193–11204

Pang A, Au WY, Kwong YL (2004) Caveolin-1 gene is coordinately regulated with the multidrug resistance 1 gene in normal and leukemic bone marrow. Leuk Res 28:973–977

Parathath S, Connelly MA, Rieger RA, Klein SM, Abumrad NA, de la Llera-Moya M, Iden CR, Rothblat GH, Williams DL (2004) Changes in plasma membrane properties and phosphatidylcholine subspecies of insect Sf9 cells due to expression of scavenger receptor class B, type I, and CD36. J Biol Chem 279:41310–41318

Peng Y, Akmentin W, Connelly MA, Lund-Katz S, Phillips MC, Williams DL (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

Pike LJ (2004) Lipid rafts: heterogeneity on the high seas. Biochem J 378:281–292

Pohl J, Ring A, Ehehalt R, Schulze-Bergkamen H, Schad A, Verkade P, Stremmel W (2004) Long-chain fatty acid uptake into adipocytes depends on lipid raft function. Biochemistry 43:4179–4187

Pownall HJ (2006) Detergent-mediated phospholipidation of plasma lipoproteins increases HDL cholesterophilicity and cholesterol efflux via SR-BI. Biochemistry 45:11514–11522

Puri V, Watanabe R, Dominguez M, Sun X, Wheatley CL, Marks DL, Pagano RE (1999) Cholesterol modulates membrane traffic along the endocytic pathway in sphingolipid-storage diseases. Nat Cell Biol 1:386–388

Radeva G, Perabo J, Sharom FJ (2005) P-glycoprotein is localized in intermediate-density membrane microdomains distinct from classical lipid rafts and caveolar domains. FEBS J 272:4924–4937

Rajagopal A, Simon SM (2003) Subcellular localization and activity of multidrug resistance proteins. Mol Biol Cell 14:3389–3399

Rajendran L, Simons K (2005) Lipid rafts and membrane dynamics. J Cell Sci 118:1099–1102

Ramu A, Ramu N, Rosario LM (1991) Circumvention of multidrug-resistance in P388 cells is associated with a rise in the cellular content of phosphatidylcholine. Biochem Pharmacol 41:1455–1461

Reaven E, Cortez Y, Leers-Sucheta S, Nomoto A, Azhar S (2004) Dimerization of the scavenger receptor class B type I: formation, function, and localization in diverse cells and tissues. J Lipid Res 45:513–528

Reaven E, Leers-Sucheta S, Nomoto A, 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

Reaven E, Zhan L, Nomoto A, Leers-Sucheta S, Azhar S (2000) Expression and microvillar localization of scavenger receptor class B, type I (SR-BI) and selective cholesteryl ester uptake in Leydig cells from rat testis. J Lipid Res 41:343–356

Reboul E, Klein A, Bietrix F, Gleize B, Malezet-Desmoulins C, Schneider M, Margotat A, Lagrost L, Collet X, Borel P (2006) Scavenger receptor class B type I (SR-BI) is involved in vitamin E transport across the enterocyte. J Biol Chem 281:4739–4745

Remaley AT, Rust S, Rosier M, Knapper C, Naudin L, Broccardo C, Peterson KM, Koch C, Arnould I, Prades C, Duverger N, Funke H, Assman G, Dinger M, Dean M, Chimini G, Santamarina-Fojo S, Fredrickson DS, Denefle P, Brewer HB Jr (1999) Human ATP-binding cassette transporter 1 (ABC1): genomic organization and identification of the genetic defect in the original Tangier disease kindred. Proc Natl Acad Sci USA 96:12685–12690

Reungpatthanaphong P, Marbeuf-Gueye C, Le Moyec L, Salerno M, Garnier-Suillerot A (2004) Decrease of P-glycoprotein activity in K562/ADR cells by MbetaCD and filipin and lack of effect induced by cholesterol oxidase indicate that this transporter is not located in rafts. J Bioenerg Biomembr 36:533–543

Rhainds D, Bourgeois P, Bourret G, Huard K, Falstrault L, Brissette L (2004) Localization and regulation of SR-BI in membrane rafts of HepG2 cells. J Cell Sci 117:3095–3105

Rothnie A, Theron D, Soceneantu L, Martin C, Traikia M, Berridge G, Higgins CF, Devaux PF, Callaghan R (2001) The importance of cholesterol in maintenance of P-glycoprotein activity and its membrane perturbing influence. Eur Biophys J 30:430–442

Sagiv Y, Hudspeth K, Mattner J, Schrantz N, Stern RK, Zhou D, Savage PB, Teyton L, Bendelac A (2006) Cutting edge: impaired glycosphingolipid trafficking and NKT cell development in mice lacking Niemann-Pick type C1 protein. J Immunol 177:26–30

Sahoo D, Darlington YF, Pop D, Williams DL, Connelly MA (2007) Scavenger receptor class B Type I (SR-BI) assembles into detergent-sensitive dimers and tetramers. Biochim Biophys Acta (in press)

Sane AT, Sinnett D, Delvin E, Bendayan M, Marcil V, Menard D, Beaulieu JF, Levy E (2006) Localization and role of NPC1L1 in cholesterol absorption in human intestine. J Lipid Res 47:2112–2120

Schinkel AH, Mayer U, Wagenaar E, Mol CA, van Deemter L, Smit JJ, van der Valk MA, Voordouw AC, Spits H, van Tellingen O, Zijlmans JM, Fibbe WE, Borst P (1997) Normal viability and altered pharmacokinetics in mice lacking mdr1-type (drug-transporting) P-glycoproteins. Proc Natl Acad Sci USA 94:4028–4033

Schuck S, Honsho M, Ekroos K, Shevchenko A, Simons K (2003) Resistance of cell membranes to different detergents. Proc Natl Acad Sci USA 100:5795–5800

Shapiro AB, Fox K, Lee P, Yang YD, Ling V (1998) Functional intracellular P-glycoprotein. Int J Cancer 76:857–864

Sharom FJ (1997) The P-glycoprotein efflux pump: how does it transport drugs? J Membr Biol 160:161–175

Shetty S, Eckhardt ER, Post SR, van der Westhuyzen DR (2006) Phosphatidylinositol-3-kinase regulates scavenger receptor class B type I subcellular localization and selective lipid uptake in hepatocytes. Arterioscler Thromb Vasc Biol 26:2125–2131

Shilling RA, Venter H, Velamakanni S, Bapna A, Woebking B, Shahi S, van Veen HW (2006) New light on multidrug binding by an ATP-binding-cassette transporter. Trends Pharmacol Sci 27:195–203

Simons K, Ikonen E (2000) How cells handle cholesterol. Science 290:1721–1726

Simons K, Toomre D (2000) Lipid rafts and signal transduction. Nat Rev Mol Cell Biol 1:31–39

Slimane TA, Trugnan G, Van ISC, Hoekstra D (2003) Raft-mediated trafficking of apical resident proteins occurs in both direct and transcytotic pathways in polarized hepatic cells: role of distinct lipid microdomains. Mol Biol Cell 14:611–624

Smart EJ, De Rose RA, Farber SA (2004) Annexin 2-caveolin 1 complex is a target of ezetimibe and regulates intestinal cholesterol transport. Proc Natl Acad Sci USA 101:3450–3455

Smith JD, Le Goff W, Settle M, Brubaker G, Waelde C, Horwitz A, Oda MN (2004) ABCA1 mediates concurrent cholesterol and phospholipid efflux to apolipoprotein A-I. J Lipid Res 45:635–644

Soccio RE, Breslow JL (2004) Intracellular cholesterol transport. Arterioscler Thromb Vasc Biol 24:1150–1160

Stangl H, Hyatt M, Hobbs HH (1999) Transport of lipids from high and low density lipoproteins via scavenger receptor-BI. J Biol Chem 274:32692–32698

Takahashi K, Kimura Y, Kioka N, Matsuo M, Ueda K (2006) Purification and ATPase activity of human ABCA1. J Biol Chem 281:10760–10768

te Vruchte D, Lloyd-Evans E, Veldman RJ, Neville DC, Dwek RA, Platt FM, van Blitterswijk WJ, Sillence DJ (2004) Accumulation of glycosphingolipids in Niemann-Pick C disease disrupts endosomal transport. J Biol Chem 279:26167–26175

Tessner TG, Stenson WF (2000) Overexpression of MDR1 in an intestinal cell line results in increased cholesterol uptake from micelles. Biochem Biophys Res Commun 267:565–571

Thuahnai ST, Lund-Katz S, Anantharamaiah GM, Williams DL, Phillips MC (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

Thuahnai ST, Lund-Katz S, Dhanasekaran P, de la Llera-Moya M, Connelly MA, Williams DL, Rothblat GH, Phillips MC (2004) Scavenger receptor class B type I-mediated cholesteryl ester-selective uptake and efflux of unesterified cholesterol. Influence of high density lipoprotein size and structure. J Biol Chem 279:12448–12455

Troost J, Lindenmaier H, Haefeli WE, Weiss J (2004) Modulation of cellular cholesterol alters P-glycoprotein activity in multidrug-resistant cells. Mol Pharmacol 66:1332–1339

Truong TQ, Aubin D, Bourgeois P, Falstrault L, Brissette L (2006) Opposite effect of caveolin-1 in the metabolism of high-density and low-density lipoproteins. Biochim Biophys Acta 1761:24–36

Uittenbogaard A, Everson WV, Matveev SV, Smart EJ (2002) Cholesteryl ester is transported from caveolae to internal membranes as part of a caveolin-annexin II lipid-protein complex. J Biol Chem 277:4925–4931

Uittenbogaard A, Ying Y, Smart EJ (1998) Characterization of a cytosolic heat-shock protein-caveolin chaperone complex. Involvement in cholesterol trafficking. J Biol Chem 273:6525–6532

Vainio S, Bykov I, Hermansson M, Jokitalo E, Somerharju P, Ikonen E (2005) Defective insulin receptor activation and altered lipid rafts in Niemann-Pick type C disease hepatocytes. Biochem J 391:465–472

Valasek MA, Weng J, Shaul PW, Anderson RG, Repa JJ (2005) Caveolin-1 is not required for murine intestinal cholesterol transport. J Biol Chem 280:28103–28109

885

Vaughan AM, Oram JF (2003) ABCA1 redistributes membrane cholesterol independent of apolipoprotein interactions. J Lipid Res 44:1373–1380

Vaughan AM, Oram JF (2005) ABCG1 redistributes cell cholesterol to domains removable by high density lipoprotein but not by lipid-depleted apolipoproteins. J Biol Chem 280:30150–30157

Wang E, Casciano CN, Clement RP, Johnson WW (2000a) Cholesterol interaction with the daunorubicin binding site of P-glycoprotein. Biochem Biophys Res Commun 276:909–916

Wang J, Sun F, Zhang DW, Ma Y, Xu F, Belani JD, Cohen JC, Hobbs HH, Xie XS (2006a) Sterol transfer by ABCG5 and ABCG8: in vitro assay and reconstitution. J Biol Chem 281:27894–27904

Wang L, Connelly MA, Ostermeyer AG, Chen HH, Williams DL, Brown DA (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

Wang N, Lan D, Chen W, Matsuura F, Tall AR (2004) ATP-binding cassette transporters G1 and G4 mediate cellular cholesterol efflux to high-density lipoproteins. Proc Natl Acad Sci USA 101:9774–9779

Wang N, Ranalletta M, Matsuura F, Peng F, Tall AR (2006b) LXR-induced redistribution of ABCG1 to plasma membrane in macrophages enhances cholesterol mass efflux to HDL. Arterioscler Thromb Vasc Biol 26:1310–1316

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

Wang N, Silver DL, Thiele C, Tall AR (2001) ATP-binding cassette transporter A1 (ABCA1) functions as a cholesterol efflux regulatory protein. J Biol Chem 276:23742–23747

Wang Z, Stalcup LD, Harvey BJ, Weber J, Chloupkova M, Dumont ME, Dean M, Urbatsch IL (2006c) Purification and ATP hydrolysis of the putative cholesterol transporters ABCG5 and ABCG8. Biochemistry 45:9929–9939

Webb NR, Connell PM, Graf GA, Smart EJ, de Villiers WJ, de Beer FC, van der Westhuyzen DR (1998) SR-BII, an isoform of the scavenger receptor BI containing an alternate cytoplasmic tail, mediates lipid transfer between high density lipoprotein and cells. J Biol Chem 273:15241–15248

Williams DL, Wong JS, Hamilton RL (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

Wu JE, Basso F, Shamburek RD, Amar MJ, Vaisman B, Szakacs G, Joyce C, Tansey T, Freeman L, Paigen BJ, Thomas F, Brewer HB Jr, Santamarina-Fojo S (2004) Hepatic ABCG5 and ABCG8 overexpression increases hepatobiliary sterol transport but does not alter aortic atherosclerosis in transgenic mice. J Biol Chem 279:22913–22925

Xu X, Bittman R, Duportail G, Heissler D, Vilcheze C, London E (2001) Effect of the structure of natural sterols and sphingolipids on the formation of ordered sphingolipid/sterol domains (rafts). Comparison of cholesterol to plant, fungal, and disease-associated sterols and comparison of sphingomyelin, cerebrosides, and ceramide. J Biol Chem 276:33540–33546

Yancey PG, de la Llera-Moya M, Swarnakar S, Monzo P, Klein SM, Connelly MA, Johnson WJ, Williams DL, Rothblat GH (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

Yang CP, Galbiati F, Volonte D, Horwitz SB, Lisanti MP (1998) Upregulation of caveolin-1 and caveolae organelles in Taxol-resistant A549 cells. FEBS Lett 439:368–372

Young SG, Fielding CJ (1999) The ABCs of cholesterol efflux. Nat Genet 22:316–318

Yu L, Bharadwaj S, Brown JM, Ma Y, Du W, Davis MA, Michaely P, Liu P, Willingham MC, Rudel LL (2006) Cholesterol-regulated translocation of NPC1L1 to the cell surface facilitates free cholesterol uptake. J Biol Chem 281:6616–6624

Yu L, Hammer RE, Li-Hawkins J, Von Bergmann K, Lutjohann D, Cohen JC, Hobbs HH (2002a) Disruption of Abcg5 and Abcg8 in mice reveals their crucial role in biliary cholesterol secretion. Proc Natl Acad Sci USA 99:16237–16242

Yu L, Li-Hawkins J, Hammer RE, Berge KE, Horton JD, Cohen JC, Hobbs HH (2002b) Overexpression of ABCG5 and ABCG8 promotes biliary cholesterol secretion and reduces fractional absorption of dietary cholesterol. J Clin Invest 110:671–680

Yu L, von Bergmann K, Lutjohann D, Hobbs HH, Cohen JC (2004) Selective sterol accumulation in ABCG5/ABCG8-deficient mice. J Lipid Res 45:301–307

Zha X, Gauthier A, Genest J, McPherson R (2003) Secretory vesicular transport from the Golgi is altered during ATP-binding cassette protein A1 (ABCA1)-mediated cholesterol efflux. J Biol Chem 278:10002–10005

Zha X, Genest J Jr, McPherson R (2001) Endocytosis is enhanced in Tangier fibroblasts: possible role of ATP-binding cassette protein A1 in endosomal vesicular transport. J Biol Chem 276:39476–39483

Zhang M, Dwyer NK, Love DC, Cooney A, Comly M, Neufeld E, Pentchev PG, Blanchette-Mackie EJ, Hanover JA (2001a) Cessation of rapid late endosomal tubulovesicular trafficking in Niemann-Pick type C1 disease. Proc Natl Acad Sci USA 98:4466–4471

Zhang M, Dwyer NK, Neufeld EB, Love DC, Cooney A, Comly M, Patel S, Watari H, Strauss JF 3rd, Pentchev PG, Hanover JA, Blanchette-Mackie EJ (2001b) Sterol-modulated glycolipid sorting occurs in niemann-pick C1 late endosomes. J Biol Chem 276:3417–3425