commentary

The ins and outs of ABCA[1]

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Population studies have shown a strong inverse relationship between plasma HDL levels and risk for cardiovascular disease, implying that factors associated with HDL metabolism are cardioprotective. One of these factors is ATP-binding cassette transporter A1 (ABCA1), an integral membrane protein that transports cholesterol and phospholipids to lipid-depleted HDL apolipoproteins, such as apolipoprotein A (apoA)-I (1). Studies of human HDL deficiencies and atherosclerosis-susceptible mouse models have shown that ABCA1 protects against atherogenesis and may prevent disease progression (2–4). These observations have made ABCA1 a promising new therapeutic target for treating cardiovascular disease. Thus, it is critical to understand how ABCA1 functions and is regulated.

Despite this strong interest and intensive study over the last 8 years, we still do not have a clear picture of how and where ABCA1 mediates the transfer of cholesterol and phospholipids to apoA-I. Based on structural similarities with other better-characterized ABC transporters, it is assumed by many that ABCA1 pumps lipids to the cell surface where they become accessible for removal by apoA-I. There is evidence, however, that ABCA1-bound apoA-I is endocytosed by macrophages and targeted to late endosomal or lysosomal compartments, where it picks up lipids and exports them from cells by an exocytosis process.

The concept of this retroendocytosis pathway was introduced by Takahashi and Smith (5) before the discovery of ABCA1. They showed that apoA-I is internalized and resecreted by macrophages in association with cholesterol efflux from cells. Since then, several groups have shown that apoA-I and ABCA1 colocalize in endosomal compartments and that ABCA1 rapidly recycles between intracellular compartments and the plasma membrane (6–8). Cycling of proteins through cells, however, does not imply functional significance. It is well established that cultured cells, particularly macrophages, internalize and resecrete exogenous molecules without any apparent biological effect. In addition, cells rapidly recycle membrane proteins, including those bound to the surface.

The possibility that an apoA-I retroendocytosis pathway actually functions to export lipids is supported largely by circumstantial evidence. Cells from subjects with loss-of-function mutations in ABCA1 were shown to have defective late endocytotic trafficking and accumulation of cholesterol and phospholipids in late endocytotic vesicles (7), consistent with a role of ABCA1 in removing lipids from these compartments. Trapping ABCA1 in the plasma membrane by cyclosporine treatment was reported to reduce apoA-I-mediated cholesterol efflux (9). Similarly, deletion of a PEST sequence in ABCA1 inhibited its trafficking to late endosomes/lysosomes, and decreased apoA-I-mediated efflux of cholesterol derived from endocytosed and degraded lipoproteins (8). Taken together, these studies support the idea that ABCA1-bound apoA-I traffics to late endosomal vesicles and/or lysosomes, where it forms nascent lipoprotein particles that are subsequently secreted from the cell.

There is strong evidence, however, that the ABCA1-dependent transfer of cellular lipids to apoA-I occurs at the plasma membrane. Electron microscopy studies have shown that apoA-I selectively interacts with exovesiculated membrane protrusions on the surface of ABCA1-expressing cells (10, 11). Inducing ABCA1 in the absence of apolipoproteins increases the amount of cholesterol that appears in plasma membrane domains accessible to the added enzyme cholesterol oxidase, and subsequent addition of apoA-I removes cholesterol exclusively from these domains (12). These findings indicate that ABCA1 can form lipid domains on the cell surface that are accessible for removal by apolipoproteins even before it has contact with apolipoproteins. It has also been shown that partitioning of ABCA1 to the plasma membrane can increase cholesterol efflux under some conditions (8, 13).

As reported in the current issue of the *Journal of Lipid Research*, Faulkner et al. (14) used a three-pronged approach to test the functional relevance of an apoA-I/ABCA1 retroendocytosis pathway in cultured RAW macrophages. They reasoned that if retroendocytosis plays an important role in apoA-I lipidation, apoA-I should be specifically internalized by cells in response to ABCA1 expression, the mass of apoA-I cycled through the cell should account for most of the lipid efflux, and the degree of apoA-I internalization should correlate with degree of cholesterol efflux. They generated fully functional fluores-

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cent and radiolabeled apoA-I to track its cellular trafficking, secretion, and degradation.

The authors confirmed that ABCA1-expressing macrophages internalized apoA-I, that some of it accumulated in late endosomes or lysosomes, and that a fraction of it was resecreted. They found, however, that the majority of the resecreted apoA-I was degraded. This suggested that the endosomes/lysosome trafficking may be part of the ABCA1 degradation pathway and that ABCA1-bound apoA-I is being dragged along.

The authors devised a formula to estimate the contribution of a retroendocytosis pathway to generation of lipidated apoA-I in the medium. They quantified how much apoA-I appeared as a lipoprotein during 3-hour incubations and estimated how much apoA-I passed through cells during this time from their pulse-chase assays. Although a rough approximation, the authors concluded that only a minor fraction of the HDL particles formed during the 3-hour incubations could be attributed to internalization and resecretion of apoA-I.

Lastly, this study used endocytosis inhibitors to determine if internalization of apoA-I was required for cholesterol efflux. They found that amiloride and monensin blocked apoA-I internalization while having only slight inhibitory effects on cholesterol efflux. This was particularly evident at high concentrations of monensin, where apoA-I was almost completely excluded from the cell while cholesterol efflux was nearly normal. Thus, under the conditions of these experiments, apoA-I uptake and cholesterol efflux could be dissociated pharmacologically. Further evidence for this dissociation was provided by data showing that overexpressing ABCA1 in transfected CHO cells markedly increased apoA-I-mediated cholesterol efflux while it actually decreased internalization of apoA-I.

This paper adds significantly to the view that the plasma membrane is a key site for ABCA1-dependent cholesterol efflux to apoA-I and that internalization of apoA-I is not required for this process. Whether internalization of an apoA-I/ABCA1 complex also plays a role in cholesterol export remains to be resolved. This is largely because there is no evidence that internalized apoA-I becomes lipidated within cells. It is possible that cholesterol that enters the endosomal/lysosomal compartments is rapidly translocated to plasma membrane domains that are accessible to ABCA1 and apoA-I. We still know virtually nothing about the molecular properties and cellular locations of lipid domains targeted for removal by the ABCA1 pathway.

A limitation of the study by Faulkner et al. (14) is that the macrophages were not loaded or labeled with cholesterol derived from scavenger receptor-mediated uptake and lysosomal degradation of lipoproteins, which is the major process in vivo. Labeling cells with cholesterol tracer will selectively incorporate the label into the plasma membrane without passing through intracellular compartments. Thus, it is possible that an apoA-I/ABCA1 retroendocytosis mechanism could emerge when cells accumulate excess lipoprotein-derived cholesterol, particularly in endosomal vesicles and lysosomes. Confirmation of an apoA-I retroendocytosis pathway that functions to export lipids directly from these compartments will require more sophisticated techniques to identify the initial sites of apoA-I lipidation.

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