

Class B Scavenger Receptors, Caveolae and Cholesterol Homeostasis

Gregory A. Graf, Sergey V. Matveev, and Eric J. Smart*

Class B scavenger receptors are predominately localized to cholesterol and sphingomyelin-enriched domains within the plasma membrane, called caveolae. Caveolae and their associated protein, caveolin, have been implicated in cholesterol trafficking and in the regulation of cellular cholesterol homeostasis. Recent studies indicate that scavenger receptor, class B, type I (SR-BI) mediates cholesterol flux between cells and lipoproteins. Caveolae appear to be the sites within the plasma membrane where such exchange occurs, suggesting that the regulation of caveolae and caveolins may be pivotal to the net flux of cholesterol between cells and lipoproteins when they are bound to SR-BI. (Trends Cardiovasc Med 1999;9:221–225). © 2000, Elsevier Science Inc.

Plasma levels of high-density lipoprotein (HDL) cholesterol are negatively correlated with the risk of developing atherosclerosis, the leading cause of death in Western, industrialized countries (Grundy 1986, Tall 1990). The role of HDL in cholesterol metabolism includes the delivery of cholesterol esters to steroidogenic tissues (Andersen and Dietschy 1978, Murphy et al. 1985) and the transfer of cholesterol from peripheral tissues to the liver in a process termed “reverse cholesterol transport” (Fielding and Fielding 1995a, Johnson et al. 1991). Reverse cholesterol transport (RCT) requires the extraction of cholesterol from extrahepatic cells by HDL and the subsequent deliv-

ery of cholesterol in the form of esterified cholesterol to hepatocytes or steroidogenic tissues. Unlike the delivery of cholesterol from low-density lipoprotein (LDL), the cellular uptake of cholesterol esters from HDL is termed “selective.” This terminology is based on the observations of Pittman and his colleagues which illustrated that uptake of HDL cholesterol esters is independent of HDL particle internalization and lipoprotein degradation (Glass et al. 1983, 1985).

Although the selective delivery of HDL cholesterol esters was described almost 20 years ago, the proteins responsible for this activity have only recently been characterized. The class B, type I scavenger receptor (SR-BI) was originally identified as a receptor for modified lipoproteins, but was subsequently shown to bind HDL and to mediate the selective uptake of HDL cholesterol esters (Acton et al. 1994, 1996), and more recently the uptake of cholesterol esters from ApoB containing lipoproteins (Stangl et al. 1998, Swarnakar et al. 1999). It also shares considerable homology

with CD36. CD36 is a pleiotropic protein implicated in a wide variety of physiological and pathophysiological processes including fatty acid transport, cell adhesion, hypertension, and diabetes. Most recently, a splice variant of the SR-BI gene product was identified and designated SR-BII. Each of these receptors binds modified lipoproteins, anionic phospholipid containing vesicles and maleylated BSA in addition to HDL and LDL. However, they vary considerably in their ability to mediate the flux of cholesterol and cholesterol esters between the surface of cells and HDL. Other Class B scavenger receptors include the lysosomal integral membrane protein II (LIMPII) and α -macrosialin; however, the relationship between these receptors, caveolae and cholesterol flux has not been thoroughly examined. For the purposes of this review, “SR-Bs” denotes SR-BI, SR-BII and CD36.

Each of these receptors has been shown by independent laboratories to be enriched in plasma membrane caveolae (Babitt et al. 1997, Lisanti et al. 1994, Webb et al. 1998). Caveolae are cholesterol and sphingomyelin-enriched microdomains within the plasma membrane that appear to be spatial integrators of a variety of signal transduction processes. They can be identified on the surface of cells as 50–100-nm, flask-shaped invaginations, but can also assume a flat morphology and may detach from the surface as vesicles. The cell biology of caveolae and caveolins has recently been reviewed (Anderson 1998, Smart et al. 1999). This review seeks to examine the recent developments in the relationship between SR-Bs, caveolae and cholesterol flux as well as the implications of SR-B localization within caveolae on cellular cholesterol homeostasis.

• **SR-Bs are Localized to Caveolae**

The first suggestion that SR-Bs were localized to plasma membrane caveolae came from the characterization of cave-

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olae-associated proteins from endothelium (Lisanti et al. 1994). Subsequently, SR-BI was localized to plasma membrane caveolae both biochemically and immunohistochemically in cultured cells expressing SR-BI (Babitt et al. 1997). Both SR-BI and CD36 co-immunoprecipitate with caveolin in the presence of anti-caveolin IgG (Smart et al. unpublished observation). SR-BI has been localized to caveola-related domains in a human monocyte cell line (THP-1). Further, conversion of THP-1 monocytes to THP-1 macrophage with PMA up-regulated the expression of caveolin. Caveolae from THP-1 macrophage were highly enriched in SR-BI (Matveev et al. 1999). Finally, the alternatively spliced products of the SR-BI gene, SR-BII, has been localized to plasma membrane caveolae in cultured cells.

The presence of caveolae is not an *a priori* requirement for the expression of SR-Bs. Therefore, a number of published reports have indicated that SR-Bs are not in caveolae based on the absence of caveolins in these cells. CD36 has been localized to a caveola-related domain from platelets; however, caveolins were not observed by immunoblot analysis in these cells (Dorahy et al. 1996). SR-BI is present in cultured rat ovarian granulosa cells as is caveolin; however, upon luteinization of these cells selective uptake of HDL cholesterol esters and SR-BI expression are up-regulated while caveolin expression declines, suggesting that SR-BI is not localized to caveolae in these cells (Azhar et al. 1998). SR-BI was predominately localized to microvillar channels by immuno-electron microscopy. However, morphologically invaginated caveolae were not observed in these cells, despite ample expression of caveolin. Further, in cultured mouse granulosa cells luteinization increased SR-BI expression 20-fold, but increased selective uptake by 40% and had no effect on caveolin expression (Reaven et al. 1999). While luteinization of ovarian granulosa cells clearly upregulates SR-BI, the relationship between SR-BI expression, selective uptake and caveolin expression in steroidogenic cells remains unclear. In addition, the relationship between caveolae and microvillar channels, if any, remains to be determined. While SR-Bs cannot be localized to caveolae in cells devoid of caveolins, SR-Bs are present in low buoyant density micro-

domains within the cell surface in each case in which it was examined. The relationship between caveolae and other low buoyant density, detergent-resistant membranes has recently been reviewed (Smart et al. 1999).

The targeting mechanism for SR-B localization to caveolae has not been examined. Acylation is an attractive candidate, as other membrane-associated proteins including src-like kinases and endothelial nitric oxide synthase are targeted to caveolae by this mechanism (Shaul et al. 1996, Shenoy-Scaria et al. 1994). However, these proteins are only transiently localized to the plasma membrane, and the caveola-targeting signal for transmembrane proteins such as the epidermal growth factor receptor and platelet derived growth factor receptor is not known. The targeting signal does not appear to reside in the C-terminal cytosolic tail, as both SR-BI and SR-BII are present in caveolae, but share no homology within this region (Webb et al. 1997, 1998). Within the N-terminal cytosolic domain, a putative caveolar-localization signal, MGC, is present in human SR-BI and CD36. However, hamster and mouse SR-BI contain MGG and rat SR-BI contains MGK. Mutation of the C-terminal cysteine residues resulted in de-acylated mouse SR-BI indicating no myristylation within the N-terminus of mouse SR-BI. However, caveolar-localization has not been examined in de-acylated SR-Bs.

• Caveolae, Caveolin and Cholesterol

The cytosolic surface of caveolae is decorated with a striated coat. The caveolar marker protein, caveolin, is associated with the caveolar coat and caveolin homooligomers may be a structural component of caveolae (Smart et al. 1999). The association of caveolin with cell surface caveolae is dependent upon cholesterol. Oxidation of caveolar cholesterol by cholesterol oxidase caused plasma membrane-associated caveolin to translocate to the Golgi, while morphologically invaginated caveolae remained at the cell surface (Smart et al. 1994). Likewise, the treatment of cultured endothelial cells with oxidized LDL or cyclodextrins depletes caveolae of cholesterol and results in a redistribution of caveolin to an intracellular membrane compartment (Blair et al. 1999). Caveola morphology is also dependent on cholesterol.

Depletion of membrane cholesterol causes invaginated caveolae to flatten within the plane of the membrane (Rothberg et al. 1990), and treatment of cells with lovastatin reduces the number of invaginated caveolae at the cell surface (Rothberg et al. 1992).

Interestingly, caveolin is a cholesterol-binding protein that forms a chaperone complex with HSP-56, cyclophilin A and cyclophilin 40 to bind a cytosolic pool of cholesterol, presumably with the function of trafficking cholesterol between membrane compartments (Murata et al. 1995, Uittenbogaard et al. 1998). Additionally, newly synthesized cholesterol translocates from the ER to caveolae before diffusing into the bulk plasma membrane in a caveolin-dependent manner, suggesting that caveolin may translocate cholesterol from the ER to the cell surface independent of vesicles (Smart et al. 1996). The expression of caveolin also depends on cellular cholesterol levels. Cholesterol loading of fibroblasts with LDL increased caveolin mRNA (Fielding et al. 1997), while treatment of MDCK cells with an HMG CoA reductase inhibitor decreased caveolin mRNA and protein (Hailstones et al. 1998). One report indicated that modulation of caveolin expression by cholesterol is mediated by sterol-regulatory-like sequences present in the caveolin promoter (Bist et al. 1997). However, there are conflicting reports on the regulation of caveolin expression by 25-hydroxycholesterol, a potent activator of the sterol regulatory element (Bist et al. 1997, Hailstones et al. 1998).

• Cholesterol Exchange between the Cell Surface and Lipoproteins

The mechanisms of cholesterol and cholesterol ester exchange between the cell surface and HDL are not well understood. Diffusion (receptor independent) and receptor-dependent hypotheses have emerged for the transfer of cholesterol and cholesterol ester between the cell surface and HDL (Fielding and Fielding 1995a). In the receptor-independent model, HDL is a passive participant in both uptake of cholesterol esters and efflux of free cholesterol. The selective uptake of HDL cholesterol ester to phospholipid bilayers is similar in both unilamellar vesicles and hepatic plasma membranes, suggesting that HDL-binding proteins are not essential to selective

uptake (Morrison et al. 1994). In contrast, SR-Bs can mediate the selective uptake of cholesterol esters from HDL in cultured cells (Acton et al. 1996, Muraio et al. 1997, Webb et al. 1998). Perhaps the most convincing evidence for an active role of HDL-binding proteins in the selective uptake of HDL cholesterol esters is provided by the comparisons between different class B scavenger receptors. SR-BII, binds HDL with similar affinity to SR-BI but is approximately 70% less efficient at mediating selective uptake (Webb et al. 1998). Similarly, CD36 binds HDL with greater affinity than SR-BI, but promotes virtually no selective uptake (Connelly et al. 1999). SR-BI also has an enhanced ability to mediate the efflux of cholesterol from cells to acceptors relative to other SR-Bs (de la Llera-Moya et al. 1999). The studies demonstrating the unique ability of SR-BI to facilitate the flux of free and esterified cholesterol between the cell surface and extracellular donors and acceptors has recently been reviewed (Williams et al. 1999).

Within the plasma membrane, caveolae appear to be a site of SR-BI dependent cholesterol ester uptake from HDL. In SR-BI transfected cells, HDL-³H-cholesterol ether is initially associated with plasma membrane caveolae which rapidly saturate. Cholesterol ether accumulates within the bulk plasma membrane prior to internalization to a yet to be determined intracellular membrane compartment (Graf et al. 1999). Similar results were obtained with DiI labeled HDL, which indicated an initial punctate pattern of fluorescence within the cell surface that diffused within the bulk plasma membrane (Babitt et al. 1997). Over time, the majority of the label accumulated in the perinuclear region of the cells.

The initial step in selective uptake is the movement of cholesterol ester into the plasma membrane (Knecht and Pittman 1989). Once in the plasma membrane, cholesterol esters can either efflux back to the HDL particle or be internalized (Knecht and Pittman 1989, Rinninger et al. 1993). However, once cholesterol esters are internalized, they cannot be effluxed in the esterified form (Rothblat et al. 1986). Indeed, cholesterol esters associated with caveolae constitute a reversible pool of cholesterol esters within the plasma membrane (Graf et al. 1999). However, once the caveola pool of cholesterol

esters is internalized, cell-associated cholesterol esters can no longer efflux back to HDL in the esterified form.

The efflux of free cholesterol from the cell surface to HDL also appears to occur via caveolae. Free cholesterol, derived *de novo* or from low-density lipoproteins, is effluxed from cell surface caveolae to HDL (Fielding and Fielding 1995b). In cultured fibroblasts, the rate of free cholesterol efflux is correlated with both caveolin mRNA and caveola-free cholesterol content (Fielding et al. 1997). Similarly, SR-BI mediates the efflux of free cholesterol from cells to HDL (de la Llera-Moya et al. 1999, Ji et al. 1997, Jian et al. 1998), presumably from caveolae.

• **Caveolae are Potential Modulators of Cholesterol Flux**

SR-BI has been shown to facilitate the flux of free and esterified cholesterol between the cell surface and lipoproteins. However, this activity is thought to be dependent on established concentration gradients (Williams et al. 1999). The lipid composition of caveolae has a number of implications on the flux of cholesterol between the cell surface and free cholesterol acceptors and cholesterol ester donors. Caveolae are enriched in both cholesterol and sphingomyelin, both of which at high concentrations alter the distribution of cholesterol between the fast and slow kinetic pools within biological

and model membranes (Schroeder et al. 1991). The precise lipid composition of caveolae remains unknown; however, one report suggests that caveolar cholesterol and sphingomyelin concentrations are approximately 30 and 14 mol%, respectively (Brown and Rose 1992). The high content of cholesterol within caveolae may facilitate the aqueous desorption of cholesterol from the plasma membrane, thereby facilitating the efflux of cholesterol from cells to HDL and other acceptors.

In contrast, the solubility of cholesteryl-oleate is sensitive to high concentrations of cholesterol in phosphatidylcholine vesicles, suggesting that the cholesterol content of caveolae may inhibit the uptake of cholesterol esters into the plasma membrane. Recent studies have indicated that caveolin may be a negative regulator of SR-BI-dependent selective cholesterol ester uptake (Smart et al. unpublished observation). Taken together, these data appear paradoxical, since the site of SR-BI-dependent cholesterol ester uptake is highly enriched in both cholesterol and caveolin, two potential inhibitors of SR-BI selective uptake activity. How then can caveolae be the site of cholesterol ester uptake if the fundamental properties of caveolae are inhibitory to SR-BI selective uptake activity?

Based on observations from our laboratory and many others, we have developed a theoretical model for the regula-

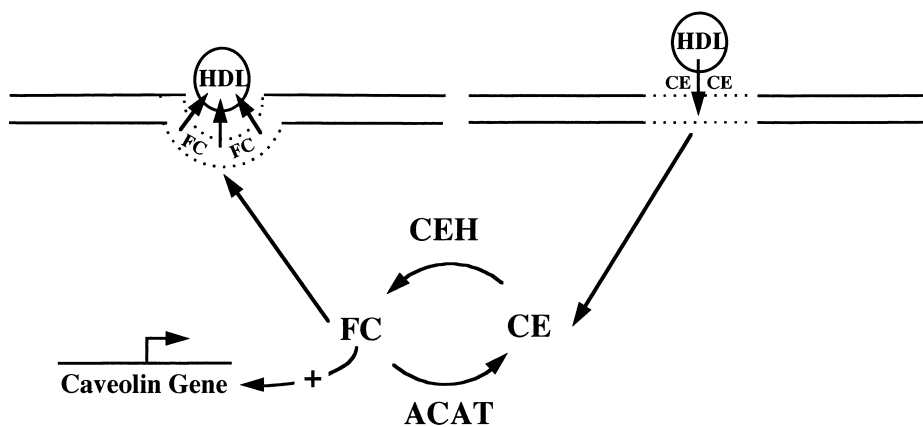


Figure 1. Theoretical model for caveolar regulation of net cholesterol flux mediated by SR-BI. Cells depleted of cholesterol contain less caveolin, caveolae at the cell surface are depleted of cholesterol, and caveolae appear flat within the plane of the plasma membrane. These cells accumulate cholesterol when HDL is bound to SR-BI in the form of cholesterol esters. In contrast, cells enriched in cholesterol upregulate caveolin expression, caveolae at the cell surface are enriched in cholesterol, and caveolae appear invaginated within the plasma membrane. These cells exhibit a net loss of cholesterol when HDL is bound to the cell surface. CEH, cholesterol ester hydrolase; ACAT, acetyl CoA cholesteryl acyl transferase; CE, cholesterol ester; FC, free cholesterol.

tion of SR-BI-dependent cholesterol flux between the cell surface and HDL (Figure 1). Cells that are depleted of cholesterol have decreased expression of caveolin. Caveolae within the plasma membrane are relatively depleted of caveolin and cholesterol and therefore appear flat within the plane of the membrane. In this context, SR-BI bound to HDL favors the net uptake of cholesterol esters. As the cell accumulates cholesterol, caveolin expression increases and traffics cholesterol to the cell surface. Caveolae within the cell surface are relatively enriched in both cholesterol and caveolin. The increased caveola cholesterol content facilitates the efflux of cholesterol to HDL by enhancing the gradient necessary for aqueous diffusion of free cholesterol out of the plasma membrane. In addition, the increased cholesterol content of caveolae decreases the solubility of cholesterol esters within the cell surface and caveolin may directly decrease SR-BI selective uptake activity. The net effect is efflux of cell surface free cholesterol to HDL or other acceptors. Within the context of this model, SR-BI facilitates the flux of cholesterol between the cell surface and extracellular acceptors and donors, while the net movement of cholesterol is determined by the composition of the microdomain wherein SR-BI resides. The composition of caveolae is reflective of the cholesterol content of cells, thereby providing a means to control the net movement of cholesterol between cells and their environment.

• Conclusions

The precise relationship between caveolae and cellular cholesterol requires additional study. At present, caveolae appear to be integrators of cellular cholesterol homeostasis. Caveolin is a cholesterol-trafficking protein that specifically traffics cholesterol to cell surface caveolae. Once in caveolae, cholesterol can diffuse within the plane of the membrane, or is available for efflux to HDL or other acceptors. Alternatively, caveolae can mediate the uptake of cholesterol esters from bound lipoproteins. The net movement of cholesterol between the cell and lipoproteins may therefore be dependent on caveolar function. Regulating the expression of caveolin would provide a means of controlling the net flux of cholesterol mediated by SR-BI.

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Searching for Alternatives to Heparin Sulfated Fucans from Marine Invertebrates

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We describe a variety of sulfated polysaccharides with regular and well-defined structures which are useful tools for elucidating structure/biological function relationship. Several of these compounds have anticoagulant and antithrombotic activities. The most studied and promising polysaccharide is a fucosylated chondroitin sulfate, composed of a chondroitin sulfate-like backbone, substituted at position 3 of the β -D-glucuronic acid with heavily sulfated fucose side chains. The anticoagulant activity of this polysaccharide is mediated by both antithrombin and heparin cofactor II; it has antithrombotic activity when targeted at the intrinsic coagulation pathway. (Trends Cardiovasc Med 1999;9: 225–232). © 2000, Elsevier Science Inc.

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