minireview

# Apolipoproteins, membrane cholesterol domains, and the regulation of cholesterol efflux

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**Abstract** Published data related to both cell membrane biology and apolipoprotein structure are reviewed and used to formulate a new model describing the mechanisms of cholesterol efflux from cell plasma membrane to high density lipoprotein (HDL) particles. The central premise of this model is the existence of heterogenous domains of cholesterol within plasma membranes. We propose that cholesterol efflux from cell membranes is influenced by three factors: *1)* the distribution of cholesterol between cholesterol-rich and cholesterol-poor membrane domains, *2)* the diffusion of cholesterol molecules through the extracellular unstirred water layer, and *3)* the transient interaction of segments of the amphipathic helix of the HDL apolipoprotein with cholesterol-poor membrane domains resulting in enhanced cholesterol efflux.—**Rothblat, G. H., F. H. Mahlberg, W. J. Johnson, and M. C. Phillips.** Apolipoproteins, membrane cholesterol domains, and the regulation of cholesterol efflux. *J. Lipid Res.* 1992. **33:** 1091–1097.

**Supplementary key words** HDL • amphipathic helix • plasma membrane • cholesterol • lipid domains • efflux

The efflux of unesterified (free) cholesterol (FC) from cells to acceptor lipoproteins represents an important process by which cells maintain cholesterol homeostasis. This reaction is the first step in the transport of cholesterol from extrahepatic cells to the liver for excretion, a process termed "reverse cholesterol transport" (1). Within recent years, a large number of investigations have been conducted in an effort to establish the mechanism and quantitative aspects related to the movement of cholesterol from cells to extracellular acceptors. The results from these studies have led to a variety of sometimes conflicting and controversial models. Three general models for cholesterol efflux have been proposed: *1)* HDL receptor-mediated cholesterol translocation and efflux (2, 3), *2)* acceptor retroendocytosis (4), and *3)* passive diffusion of FC through the aqueous phase between the plasma membrane and the acceptor particles. This model has been termed "aqueous diffusion" (5, 6). The HDL receptor model has been enthusiastically embraced by many investigators although neither the existence of a specific HDL receptor on peripheral cells nor its role in cholesterol efflux has yet been conclusively documented. Indeed, it is now generally agreed that the efflux of plasma membrane cholesterol does not require binding of HDL to specific receptors (7, 8) and the results from studies on the intracellular movement and efflux of the lysosomal pool of cholesterol are not consistent with a specific binding model (9). Retroendocytosis of HDL particles appears to occur in some cells, as demonstrated primarily by microscopic techniques (4, 10). The quantitative importance of this process in cellular cholesterol efflux remains to be established. The third general model for cholesterol efflux invokes the passive diffusion of cholesterol molecules through the aqueous phase surrounding cells. This model is supported by a large body of experimental evidence (for reviews see refs. 11, 12). As predicted by this model, the efficiency of an acceptor in stimulating cell cholesterol efflux depends, in large part, on the physical parameters describing the particles (i.e., size and surface area) (6). However, a number of studies, including those from this laboratory, have reported differences in acceptor efficiency that cannot be attributed solely to the physical properties of the acceptors. For instance, under similar experimental conditions the rate of cholesterol efflux varies widely depending on cell type (13). In addition, the apolipoprotein composition of the acceptor particle can influence cholesterol efflux from J774 mouse macrophages (14–16). With these cells, particles reconstituted with apoA-I and phosphatidylcholine stimulate more rapid efflux than similar particles containing either apoA-II or apoC (14, 15). However, this difference is not observed with all cell types, since with cells such as Fu5AH rat hepatoma and rabbit smooth muscle cells, cholesterol removal is independent of

Abbreviations: HDL, high density lipoproteins; FC, free cholesterol; apo, apolipoprotein; PC, phosphatidylcholine; CHO, Chinese hamster ovary; LCAT, lecithin: cholesterol acyltransferase.

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the type of apolipoprotein present in the acceptor (i.e., either apoA-I, apoA-II or apoC) (14, 16). Thus, a simple aqueous diffusion process cannot explain all of the experimental observations. Available data suggest that additional factors, such as apolipoprotein and plasma membrane structure, are involved in the modulation of cellular cholesterol efflux.

The principal objective of this article is to briefly review diverse published data related to both cell membrane biology and apolipoprotein structure in an attempt to link the results of these studies into a new model describing the interaction between cell membranes and acceptor lipoproteins that could modulate the efflux of cholesterol from cells. This model accommodates much of the various data that has been published and suggests new experimental approaches for the investigation of cellular cholesterol efflux.

## PLASMA MEMBRANE CHOLESTEROL DOMAINS

The central premise of this model is the existence of specific domains of cholesterol within the plasma membrane of some types of cells. The considerable evidence documenting the presence of cholesterol domains within biological membranes has been reviewed recently by Schroeder, et al. (17) and by Hui (18). The packing of cholesterol within these domains seems to be governed by a variety of factors including cholesterol to phospholipid ratios, phospholipid composition, and the presence of membrane proteins (17–20). In addition, lipid lateral packing density within the domains would differ and packing defects would be present at domain boundaries (21, 22). Since the rate of desorption of cholesterol from a membrane into the aqueous phase is, in large part, a function of the strength of the interaction of the cholesterol molecule with adjacent phospholipid molecules (11), the individual domains might be expected to exhibit differences in the rate constant for cholesterol desorption. The rate of efflux from sterol-poor domains is predicted to be faster than that from sterol-rich domains since the cholesterol molecules in the sterol-poor domains would probably be less tightly packed and thus more likely to undergo desorption into the aqueous phase (17). Thus, the presence of domains would result in the appearance of different kinetic pools of cholesterol within the membrane. Although these kinetic pools may sometimes be a reflection of the transbilayer distribution of cholesterol between the two leaflets of the membrane (19), the lateral domains of cholesterol within a leaflet are postulated to be of primary importance in the present model.

We propose that, as a consequence of the distribution of cholesterol between distinct domains within the plasma membrane, the efflux of cholesterol from cells can be assigned to three kinetic pools. The existence of these pools was suggested by the studies of Schroeder et al. (17) who, by following the efflux of a fluorescent analog of cholesterol, dehydroergosterol, demonstrated a fast pool with a halftime of 20 min in L-cell mouse fibroblasts incubated with an acceptor at 37°C. These investigators also observed a pool with a $t_{1/2}$ of approximately 2 h (intermediate pool) and a third kinetic pool of slowly exchangeable cholesterol. The rate of desorption from this latter pool could not be determined by the technique used in these studies. In our laboratory, kinetic studies on the efflux of isotopically labeled cholesterol from J774 mouse macrophages and Fu5AH rat hepatoma cells to apolipoprotein/PC discoidal acceptors particles have revealed the presence of two kinetic pools comparable to those described above, one with a $t_{1/2}$ of approximately 2 h (intermediate pool) and another with a $t_{1/2}$ ranging between 15 and 25 h (slowly exchangeable) (16). As opposed to the fluorescence spectrophotometric assay, the use of radiolabeled tracer allows experimental measurements of cholesterol efflux over longer periods of time, thereby permitting discrimination of domains with $t_{1/2}$ in the range of 2 to 30 h. However, very fast transfer with $t_{1/2}$ less than 1.5 h is difficult to accurately resolve using the isotopic approach, particularly if this pool constitutes a small fraction of membrane cholesterol. Therefore, the existence of a fast pool in J774 cells could not be established. Computer modeling of the kinetic data obtained from the cell efflux or exchange experiments demonstrated that the distribution of cholesterol between these pools varied considerably, depending on cell type (**Table 1**). In the L-cells, where the fast pool (P1) was demonstrated, this pool amounted to 14% of the cholesterol, whereas the intermediate pool (P2) contributed about 76% and the slow pool (P3) the remaining 10% (17). The data derived from isotopic efflux experiments using different cell types indicated that the distribution of cholesterol ranged from 50% intermediate/50% slow in hepatoma cells to essentially all slow in the smooth muscle cells (16). We propose that the differences in the distribution of cholesterol between these kinetic pools contributes to the considerable differences previously reported in the $t_{1/2}$ for efflux among different cells (13).

TABLE 1. Predicted distribution of exchangeable cholesterol among plasma membrane kinetic pools of different cells in culture

| | | Pool Size (%) | | | |
|---|---|---|---|---|---|
| Cell | Method[a] | P1 | P2 | P3 | Ref. |
| L-cell | analog | 14 | 76 | 10 | 17 |
| Fu5AH hepatoma | isotope | | 50 | 50 | 16 |
| J774 macrophage | isotope | | 15 | 85 | 16 |
| Rabbit smooth muscle | isotope | | 0 | 100 | 16 |

[a]Kinetics determined using either dehydroergosterol (fluorescent cholesterol analog) or radiolabeled cholesterol.

With both J774 macrophages and Fu5AH hepatoma cells there was approximately a 10-fold difference in the rate constants for efflux from the two kinetic pools that were detected (16). From the theory of transition state kinetics, the difference in free energy of activation of two processes characterized by rate constants $k_1$ and $k_2$ is approximately equal to RT $\ln(k_1/k_2)$. For the situation where $k_1 = 10\ k_2$, the difference in free energy of activation is 1.4 kcal/mol. Any interaction of cholesterol molecules with membrane components that increases the activation energy by this amount will increase the $t_{1/2}$ for transfer by an order of magnitude. The stronger interaction of cholesterol with sphingomyelin as compared to the equivalent phosphatidylcholine can lead to such an effect (23). Thus the differences in efflux from the two kinetic pools could, in part, be linked to the distribution of cholesterol among different phospholipid domains. In addition, it is possible that binding of cholesterol to membrane proteins may also increase the $t_{1/2}$ for exchange.

The kinetic parameters listed in Table 1 are derived from nonlinear regression analysis fitting the time courses for cholesterol efflux (16). This analysis assumes parallel efflux of cholesterol from two independent domains (**Fig. 1A**). This assumption, however, is somewhat arbitrary since there is no experimental evidence eliminating the possibility of exchange between the membrane domains. Such alternate models include parallel release of cholesterol from two pools that interchange cholesterol at a slow rate relative to the release of cholesterol from the membrane (Fig. 1B), or transfer of cholesterol between two membrane pools, only one of which releases cholesterol to the acceptor (Fig. 1C). The derived values describing the size of the pools and the $t_{1/2}$ for cholesterol efflux could be markedly different between the three models shown in Fig. 1. To address this issue we have conducted theoretical modeling of the systems described in Fig. 1B and 1C to determine what the impact of such transfer of cholesterol between domains would have on the estimation of domain parameters. This analysis (data not shown) indicates that: *1)* the kinetics of efflux are biexponential as long as the rate of transfer between slow and fast pools is slower than that for efflux from the fast pool, and *2)* if transfer between membrane cholesterol domains occurs (Fig. 1B and 1C) the actual rate constants for efflux would be less than those obtained by modeling efflux data in terms of model 1A, which assumes no exchange between domains (Fig. 1A). This mathematical modeling leads us to conclude that for those cell systems exhibiting biexponential efflux kinetics the $t_{1/2}$ for exchange between the membrane cholesterol pools must exceed 1 h and the rates of release derived using model 1A may be overestimates of the actual values.

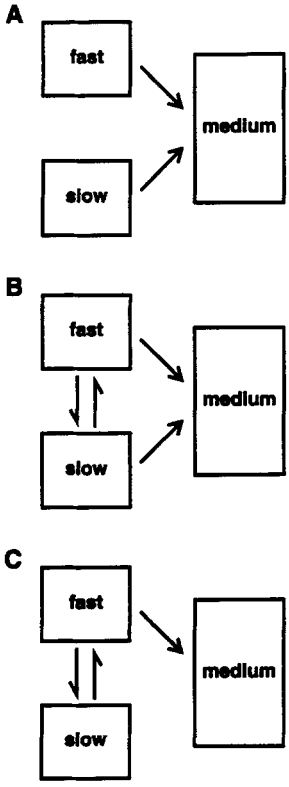


**Fig. 1.** Possible models for the movement of cholesterol from kinetic pools within the plasma membrane to extracellular acceptors. A. Parallel efflux from fast and slow domains without exchange between domains. B. Parallel efflux with exchange between domains. C. Efflux from only the fast domain with exchange between the domains.

## HDL APOLIPOPROTEINS AND THE AMPHIPATHIC HELIX

HDL or subclasses of HDL are believed to be the physiological acceptors of cell cholesterol, functioning to remove excess cholesterol from extrahepatic cells. Major components of HDL particles are the exchangeable apolipoproteins A-I, A-II, A-IV, and C. All of these apolipoproteins are characterized by the presence of amphipathic helixes (24). Based on their structure, amphipathic helixes have been grouped into a number of distinct classes, with those of exchangeable serum apolipoproteins having what has been termed class A structure (25). In class A helixes there is a clustering of positively charged amino acids at the polar-nonpolar interface and negative amino acid residues at the center of the polar face (for a review, see ref. 25). This helical structure, with opposing polar and nonpolar faces, has been shown to be responsible for the association of these apolipoproteins with lipid. Variations in the structure of the amphipathic helixes and in the number of such regions within a specific apolipoprotein underlie the differences in lipid affinity exhibited by the apolipoproteins (25).

The organization of an exchangeable apolipoprotein on the surface of a lipoprotein is a function of both the helical nature of the protein and the size and lipid composition of the particle. It has been demonstrated that HDL particles are dynamic structures on which the apolipoproteins can undergo conformational changes. This ability to manifest a variety of different conformations on the surface of lipoproteins has been documented through the use of both physicochemical techniques (26) and by the expression of different epitopes, as assessed by monoclonal antibodies (27). Thus, changes in the size and/or the lipid composition of the HDL particle will determine the regions of the apolipoprotein that are exposed to either the lipid or aqueous phases at the surface of the lipoprotein. One of the most dramatic examples of this apolipoprotein plasticity is the postulated "hinged domain" of apoA-I. It has been proposed that this domain is comprised of at least one pair of helical segments located in the region of residue 100 of apoA-I (25). Depending on the packing of the apolipoprotein on the surface of a particle, this segment would be either associated with the lipid in the HDL or released from the surface and extending into the aqueous phase surrounding the particle (25).

## INTERACTION OF APOLIPOPROTEINS WITH MEMBRANE LIPID DOMAINS

With the recent advances in our understanding of the helical structure of apolipoproteins, together with the growing body of data supporting the concept of lipid domains within membranes, it is now possible to formulate a working model in which the association of amphipathic helixes of the apolipoproteins with specific lipid domains in membranes would result in the modulation of cholesterol efflux from these membranes (**Fig.2**).

One of the most efficient acceptors of cellular cholesterol has been shown to be small HDL particles that are enriched in both phospholipid and protein and relatively depleted in cholesterol (28). The physicochemical characteristics of these particles are consistent with the nascent HDL discs that arise from the synthesis of HDL by the liver and intestine and from lipolysis of triglyceride-rich lipoproteins (29). The major, and in many cases the only, apolipoprotein associated with such particles is apoA-I. The results from the studies of Segrest et al. (25) would predict that on such a small disc the hinged region of the apoA-I would be in the "open" configuration, i.e., removed from the surface of the disc and extended into the aqueous phase. It could be this extended hinge region that would interact with specific lipid domains in the plasma membrane. Because of the reduced packing pressure that would be present in the cholesterol-poor domains, it can be suggested that it would be these domains that would exhibit the greatest interaction with the apolipoprotein. Thus, the acceptor lipoprotein particle would be loosely and transiently anchored to particular areas of the plasma membrane through the association of amphipathic helixes with membrane lipids. This type of lipoprotein-membrane interaction would be considerably less stringent than ligand-receptor interactions and would be more consistent with the mechanism of association of HDL with cells that was suggested by Tabas and Tall (30) and more recently by Leblond and Marcel (31).

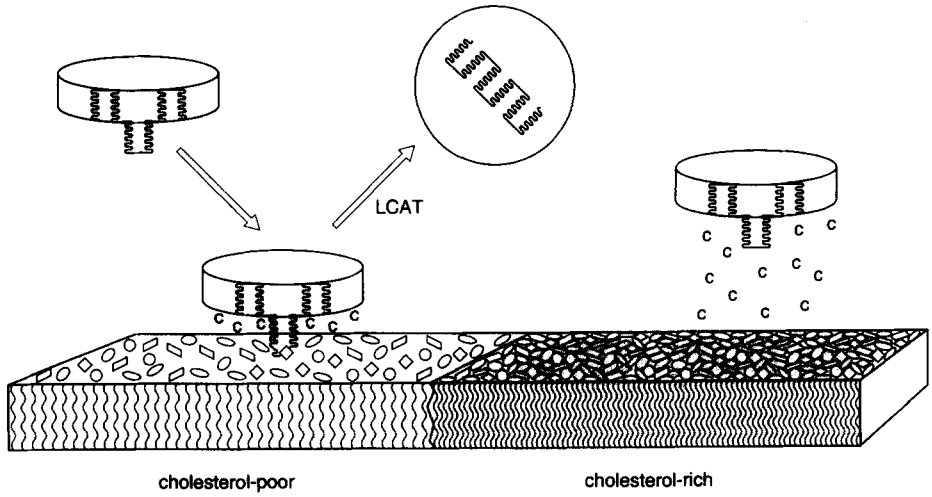


**Fig. 2.** Model for the efflux of cholesterol from cholesterol-poor and cholesterol-rich plasma membrane domains and the interaction with acceptors containing apoA-I. Hinged region of apoA-I on HDL disc interacts with cholesterol-poor domain of the plasma membrane. "C" (cholesterol molecules) move from membrane to the acceptor. Through the action of LCAT the disc is converted to a spherical HDL. The apoA-I does not interact with the cholesterol-rich domain because of the increased lateral packing density and thus flux from this domain occurs through a large unstirred water layer.

If the events described above occurred, one would anticipate that the association of the acceptor lipoprotein with the membrane would serve to enhance the removal of cellular cholesterol by either changing the domain size and/or rate of removal of cholesterol from these domains. According to the aqueous diffusion model, when excess acceptor is present, the rate-limiting step in the efflux of cell cholesterol is the desorption of cholesterol from the membrane into the aqueous phase. However, when acceptor concentration is not in excess, the movement of the desorbed cholesterol molecule through the unstirred water layer becomes an additional rate-limiting step (cf. 11). Under these conditions, the increased local concentration resulting from the anchoring of the acceptor particles to the cholesterol-poor membrane domains would increase efflux of cholesterol from these domains. Depending on the depth to which the hinged region of apoA-I was spread or embedded into the membrane, the distance between the lipoprotein acceptor and the membrane donor would be in the range of 5 to 30 Å. Since the length of a cholesterol molecule is about 17 Å (32), the need to traverse an unstirred water layer would be largely eliminated. If the cholesterol molecule has to traverse only a layer of interfacial water and does not experience a region of bulk water, the rate of transfer should be enhanced. The interfacial water layer, which is only a few molecules thick, has a reduced polarity due to the polarization of water molecules by the membrane surface. This effect can reduce the permittivity from the value of about 80 for bulk water to $<10$ for water interacting with a charged surface (33). Although we have focused this discussion on the hinged region of apoA-I, amphipathic helixes of other apolipoproteins may behave in a similar manner. The diversity of these helixes and their organization on the surface of a lipoprotein particle could explain the differences in acceptor efficiency that are linked to the apolipoprotein composition, and that are independent of the physical parameters of the acceptor lipoprotein particle (14–16, 34).

Because of the greater lipid lateral packing density within the cholesterol-rich domains, we would predict that the affinity of the amphipathic helixes would be reduced or eliminated in these regions. As it appears that plasma membrane lipids contain an average of over 40 mol% cholesterol (35), and at cholesterol concentrations of about 20 mol%, apoA-I will not bind to model membranes (36), then substantial areas of the plasma membrane should not interact with the apolipoproteins. Because of the reduced thermal motions of the cholesterol molecules in these domains, the desorption from the membrane would be slower and the rate constant would not be readily influenced by the apolipoprotein composition of the acceptor. The primary property of the acceptors regulating flux from these membrane domains would then be the physicochemical characteristics of the acceptor particle, such as phospholipid composition and surface area (6, 14).

Cholesterol transport from the intracellular pools to the plasma membrane appears to be fast relative to the transfer from the membrane to extracellular acceptors. For instance, in Fu5AH rat hepatoma cells (9) and CHO cells (37) the $t_{1/2}$ of cholesterol transfer from the lysosomes to the plasma membrane ranged between 20 and 30 min. In the present model, the $t_{1/2}$ of plasma membrane cholesterol efflux (1–2 h) from the cholesterol-poor domain may be sufficiently slow so that intracellular transport would not be rate limiting. However, if more rapid kinetic pools with half-times in the range of 20–30 min were prevalent in the plasma membrane, intracellular cholesterol transport steps would become rate limiting and regulatory, and the apolipoprotein-mediated stimulation of cholesterol efflux from these fast pools could have dramatic metabolic consequences.

In a further extension of the apolipoprotein anchor model described above, it is possible that the acquisition of cell cholesterol by the acceptor particle would serve to regulate the interaction of the lipoprotein with the cell surface. For example, as a consequence of enrichment of a discoidal acceptor particle with cholesterol, and the subsequent esterification of the cholesterol by LCAT, a hydrophobic lipid core would be generated that would convert the disc to a sphere (29). Both the presence of cholesterol in the lipoprotein and the formation of a lipid core would result in the reorganization of the apolipoprotein amphipathic helixes (D. Sparks, and S. Lund-Katz, personal communication). In this new configuration, the helixes would be expected to exhibit greater association with the lipoprotein surface, and thus have reduced interactions with the plasma membrane. This prediction is consistent with the observation that HDL discs are very efficient in stimulating cholesterol efflux (11, 34). Also, in this way the interaction of discoidal lipoprotein particles with the plasma membrane would be self-limiting.

In the model proposed above, the amphipathic helixes of the apolipoproteins play a relatively passive role anchoring the acceptor particle to the cell membrane. However, it can be anticipated that the insertion of regions of the apolipoprotein into membrane lipid domains would produce changes in the organization of the domain which, in turn, could result in the modulation of a variety of metabolic reactions. For example, the kinetics of cellular cholesterol efflux might be modified if the sizes and packing properties of the domains were changed by interactions with helical regions of different apolipoproteins. Increases in the rate of desorption of cholesterol molecules have been observed when apoA-I is added to phospholipid/cholesterol vesicles; this effect has been attributed to perturbations in the interactions of cholesterol

with phospholipid caused by the insertion of the apolipoprotein (38). In agreement with this phenomenon, the efflux dose-response curves obtained upon exposure of fibroblasts to acceptor discs containing either apoA-I or apoC never reached a common minimum $t_{1/2}$, even at very high acceptor concentrations (14). This suggests that the differences in efflux were a reflection of changes in the cells. An even more dramatic example of possible selective effects of apolipoproteins in modulating efflux is the data obtained from studies on the OB17 mouse adipose cell line in which particles containing apoA-I or apoA-IV have been reported to reduce cell cholesterol content, whereas acceptors containing apoA-II or apoE do not (39).

The interaction of the amphipathic helixes of apolipoproteins on the plasma membrane could produce metabolic events not directly related to the efflux of cholesterol. The lipid composition of membranes has been linked to the regulation of membrane-bound enzymes, transporters, and ion channels (for a review see ref. 40). Thus, changes in membrane lipid domains, as induced by the interaction of the amphipathic helixes of apolipoproteins with the plasma membrane may affect $Ca^{2+}$ transport, the activity of membrane phospholipases, or second messenger signaling pathways. These events could be responsible for the variety of cellular responses that have been reported to be triggered by HDL (41–47).

The role of apolipoprotein helixes in modulating membrane cholesterol domains by transient interactions with the plasma membrane remains to be experimentally demonstrated. The recent development of synthetic polypeptides having characteristics similar to apolipoproteins (24, 44, 46) and monoclonal antibodies against specific regions of the apolipoproteins (31, 48) should be very useful in testing this model. Also important for the validation of this model is the need to demonstrate that changes in the cholesterol content of the acceptor particle will change the expression of apolipoprotein epitopes that are critical in the modulation of membrane cholesterol domains and the kinetic parameters describing cholesterol efflux. Finally, the molecular organization of the membrane domains remains to be established. Progress in these areas will provide a greater understanding of the mechanisms of cholesterol efflux and the initial steps in the reverse cholesterol transport pathway.

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