



Thematic Review Series: Biology of Lipid Rafts

The ins and outs of lipid rafts: functions in intracellular cholesterol homeostasis, microparticles, and cell membranes

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Abstract Cellular membranes are not homogenous mixtures of proteins; rather, they are segregated into microdomains on the basis of preferential association between specific lipids and proteins. These microdomains, called lipid rafts, are well known for their role in receptor signaling on the plasma membrane (PM) and are essential to such cellular functions as signal transduction and spatial organization of the PM. A number of disease states, including atherosclerosis and other cardiovascular disorders, may be caused by dysfunctional maintenance of lipid rafts. Lipid rafts do not occur only in the PM but also have been found in intracellular membranes and extracellular vesicles (EVs). Here, we focus on discussing newly discovered functions of lipid rafts and microdomains in intracellular membranes, including lipid and protein trafficking from the ER, Golgi bodies, and endosomes to the PM, and we examine lipid raft involvement in the production and composition of EVs. Because lipid rafts are small and transient, visualization remains challenging. Future work with advanced techniques will continue to expand our knowledge about the roles of lipid rafts in cellular functioning.—Ouweneel, A. B., M. J. Thomas, M. G. Sorci-Thomas. **The ins and outs of lipid rafts: functions in intracellular cholesterol homeostasis, microparticles, and cell membranes.** *J. Lipid Res.* 2020. 61: 676–686.

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MICRODOMAINS AND CELLULAR CHOLESTEROL HOMEOSTASIS

Cellular membranes are not homogenous mixtures of lipids and proteins, rather, they are composed of lipids and proteins, some of which segregate into lipid microdomains called lipid rafts, which are enriched in free cholesterol (FC) and glycosphingolipids, like SM, and are resistant to detergent extraction. In this review, domains enriched in FC and SM will be designated as “lipid rafts,” while other lipid domains that are not as well characterized, particularly those in mitochondria, will be referred to as lipid microdomains. The segregation of these structures regulates cellular polarity and vesicular traffic as well as cell signaling pathways affecting a plethora of biological processes (1). However, over two decades since the functional lipid raft model of the plasma membrane (PM) was first published by Simons and Ikonen (2), many of the fundamental questions about the biogenesis and structure of lipid rafts still remain unanswered. Although less so today than in the past, experimental obstacles in visualizing lipid rafts have hampered progress in understanding raft size, when and where they are formed, and how they are turned over or removed from the cell. However, new techniques have been developed that provide a clearer vision of how lipid rafts form and how they function. Since our previous

Abbreviations: AMBRA1, autophagy and beclin 1 regulator 1; Aster, ER-resident cholesterol transport protein; CAV1, caveolin-1; CL, cardiolipin; CE, cholesteryl ester; DLPI, dynamin-like protein 1; DRM, detergent-resistant membrane; EV, extracellular vesicle; FC, free cholesterol; GD3, disialoganglioside with three glycosyl groups; LDLr, LDL receptor; MAM, mitochondria-associated membrane; Mfn, mitofusin; nHDL, nascent HDL; NPC, Niemann-Pick type C; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PM, plasma membrane; PS, phosphatidylserine; SR-BI, scavenger receptor BI; TG, triglyceride; TGN, *trans*-Golgi network.

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review of lipid rafts in inflammatory receptor signaling and atherosclerosis (1), a great deal of information has emerged providing new insights into how lipid rafts affect a number of disease states. In this current review, we will discuss newly discovered functions of lipid rafts as well as the contribution of lipid rafts to the composition of extracellular vesicles (EVs).

Two common types of lipid rafts have been described: planar lipid rafts and invaginated lipid rafts, which curve inward and are termed caveolae. Caveolae are specialized lipid rafts found in very high numbers in adipocytes, which associate with the protein caveolin-1 (CAV1) to form concave regions on the cell membrane (3). Formation of lipid rafts is due to phase separation of lipids within the membrane that is thermodynamically driven and depends on the amounts and types of membrane lipids and the amounts of FC and SM, both essential components of planar lipid rafts and caveolae. Regions of the PM that are rich in FC and SM are more ordered and often referred to as the liquid-ordered phase as compared with the rest of the lipid membrane, which is called the liquid-disordered phase. The size of lipid rafts has been estimated to average around 500 nm², and they have lifetimes on the PM that range from seconds to minutes (4). Because lipid rafts are mobile and “float” like an iceberg in the liquid-disordered lipid phase, they can also associate to form larger raft-domains, when proteins that associate with liquid-ordered domains facilitate oligomerization of protein complexes. Studies of both rabbit erythrocytes and Chinese hamster ovary cells suggest that PMs accommodate lipid rafts having different lipid packing and sizes (5, 6).

Changes in the FC composition, which modulates lipid raft content, can affect cell mobility. For example, increasing the FC concentration in THP1 cells leads to increased chemotactic response to monocyte chemoattractant protein-1 (MCP-1) due to alterations in C-C motif chemokine receptor 2 (CCR2) levels in lipid rafts. Modulating human monocyte FC affected rolling of these cells on E-selectin-coated surfaces by changing E-selectin counterreceptor CD44 distribution on the lipid rafts, suggesting a mechanism through which FC concentration modulates monocyte adhesion by regulation of receptor mobility (7, 8).

All mammalian cells have an outer protective barrier made up of a phospholipid bilayer that associates with a variety of proteins, aiding in cellular integrity and communication. The PM is highly organized and displays asymmetry with respect to the types of phospholipids found in the inner and outer bilayer leaflets and with respect to FC distribution. For example, phosphatidylcholine (PC) predominates in the bilayer with a higher percentage of PC and SM in the outer leaflet, while phosphatidylserine (PS) and phosphatidylethanolamine (PE) are preferentially found in the inner leaflet (9, 10). Bilayers associated with intracellular organelles have somewhat different phospholipid to FC ratios, and FC is reported to be asymmetrically distributed between the inner and outer leaflets (11–13). Recent studies suggest that long chain C24 sphingolipids modulate this membrane bilayer asymmetry (14).

Despite years of research, membrane lipid rafts remain controversial. Because of their dynamic nature the proportions of raft to non-raft membrane lipids are difficult to quantify and image in real time. The most common method to study lipid raft composition is to isolate detergent-resistant membrane (DRM) from cells (15). DRMs from model membranes and cell membranes contain a subset of lipid-anchored and integral PM proteins. Intact membrane-associated regions can be visualized using fluorescently labeled antibodies that recognize proteins or fluorescent ganglioside analogs (16) sequestered in the raft regions. Newer, non-detergent methods for isolating bulk lipid rafts from different cell types have been developed permitting analysis of their lipid content and associated proteins (17, 18). More detailed investigations have recently employed Foster resonance energy transfer (FRET), atomic force microscopy, stimulated emission depletion microscopy (STED), and other super-resolution techniques. An overall consensus is that lipid rafts range in diameter estimated from 5 to 80 nm, with relatively short lifetimes, rapidly responding to cellular needs. With the refinement of the super-resolution techniques, we are now poised to discover new information on factors leading to how these elusive and highly dynamic structures condense and dissolve.

CONTRIBUTION OF CHOLESTEROL SYNTHESIS

The FC found in lipid rafts can be derived endogenously by de novo FC synthesis in the ER or exogenously from lipoproteins or vesicles. Newly synthesized FC rapidly leaves the ER, mostly through nonvesicular mechanisms bypassing ER-Golgi membrane transport (19, 20), maintaining low ER membrane sterol content, as illustrated in **Fig. 1**. The newly produced FC can be transported to the PM, thereby becoming available to extracellular acceptors (21), or it can be transported to cholesterol pools in other intracellular compartments, e.g., endosomes (22). Interestingly, a membrane-FC-gradient exists in cells with the FC levels lowest in the ER, and increasing as it approaches the PM. Because excess FC is cytotoxic and cannot be effectively removed by catabolism, cells devote considerable effort to sensing intracellular FC levels and adjusting synthesis and receptor-mediated lipoprotein uptake as well as FC efflux to balance the gradient and maintain proper cellular cholesterol levels. When a cholesterol-rich diet is consumed, the excess FC perturbs cellular cholesterol balance, thus impacting lipid raft homeostasis (23). Excess FC is either stored as cholesteryl ester (CE) or actively exported through efflux pathways. Cholesterol exported from peripheral tissues is then transported back to the liver by HDLs for recycling, conversion to bile acids, or excretion in feces, accounting for about 1.1 g of FC per day (24). Cellular FC balance is mediated in a complex manner by two main nuclear receptor systems, SREBPs and LXRs. Activated SREBP undergoes assisted migration to the Golgi where it is further cleaved releasing the SREBP-nuclear element, which migrates to the nucleus causing increased

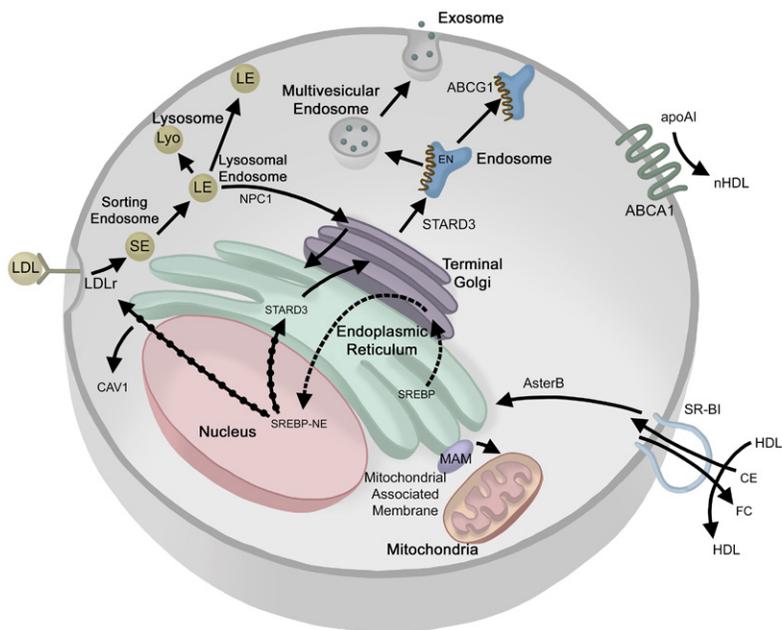


Fig. 1. Ins and outs of cellular cholesterol transport. EN, endosomes; EX, exosome; LE, late endosome; LY, lysosome; MVE, multivesicular endosome; NUC, nucleus; SE, sorting endosome; TG, *trans*-Golgi; SREBP-NE, SREBP-nuclear element. Cholesterol movement (solid arrows); activation (solid arrows with black dots); SREBP movement (dashed arrow).

transcription of gene products leading to an increase in cellular FC from de novo synthesis or uptake of cholesterol from circulating lipoproteins (25). LXR activation by oxysterols promotes FC efflux (26). Key processes in the maintenance of the FC pool take place at several different subcellular localizations; therefore, FC transport between these sites is important for the overall regulation of intracellular FC concentration. FC can be transferred between subcellular membranes by vesicular transport and by non-vesicular mechanisms [reviewed in (27)]. Figure 1 illustrates our current knowledge on the movement of FC within the cell and at the cell surface.

Modulation of cellular FC levels may contribute to certain disease conditions. For example, metastatic ovarian tumor cells reportedly promote FC efflux from tumor-associated macrophages, thereby depleting FC in macrophage lipid rafts, which in turn reduces interferon- γ gene expression and increases interleukin-4-mediated reprogramming. This series of events explains how these macrophages are forced to adopt a tumor-promoting immunosuppressive response rather than an intrinsic tumoricidal activity, which would normally suppress tumor development (28).

CONTRIBUTION OF LIPOPROTEIN CHOLESTEROL

An alternative to FC acquisition via de novo synthesis is through receptor-mediated uptake of FC contained in plasma lipoproteins. apoB- or apoE-containing particles, such as chylomicron remnants, VLDLs, and LDLs, bind to the LDL receptor (LDLr) bound to the PM by a transmembrane domain, but not associated with a lipid raft. After binding the LDLr, the LDL-LDLr complex is endocytosed through clathrin-coated vesicles into a sorting endosome (Fig. 1) where LDL and LDLr dissociate at lower pH. LDLr is then recycled to the cell surface, while the LDL is transported to acidic lysosomal compartments where CEs are hydrolyzed to FC. Interestingly, although multivesicular

bodies harbor most of the FC in the endocytic pathway (29), lysosomal membranes are normally FC poor (30). Multivesicular late endosomes contain two lysosomal proteins, Niemann-Pick type C (NPC)1 and NPC2, which are essential for moving FC out of the endosomal system. Genetic and phenotypic evidence in mutant mice suggest that the NPC proteins participate in different steps of the same pathway and that neither can compensate for the other (31). Deficiency of either protein leads to the accumulation of LDL-derived FC in late endocytic organelles (32). Recent advances have revealed new insights into the architecture of NPC1, including luminal interaction with NPC2 (33, 34), a membrane-embedded sterol-sensing domain (35) and a C-terminal luminal domain (36). Current hypotheses suggest that NPC2 binds FC in the lysosome and then hands it off to NPC1, which has membrane-spanning domains in the lysosomal/endosomal membrane. Transfer of FC by NPC1 to the membrane or the cytoplasm is still under active investigation. NPC2, but not NPC1, was suggested to be essential for ABCA1-mediated flux of FC from the (endo)lysosomal compartment toward the PM (37).

ENDOSOMAL CHOLESTEROL TRAFFICKING AND EXOSOMES

The endosomal compartment is at the crossroads of vesicular flow between the Golgi, lysosomes, ER, PM, and other cellular compartments. Sorting (early) endosomes give rise to recycling endosomes, which are in constant exchange with the PM. They are also the source of multivesicular endosomes, which mature into late endosomes that are in exchange with either the *trans*-Golgi network (TGN) or lysosomes, or fuse with the PM to release exosomes. After release from the endolysosomal system, FC is delivered to other membranes, such as the PM, ER, recycling endosomes, and, in steroid hormone-producing cells, the mitochondria. When the PM concentration of FC

exceeds ~30 mol% of total PM lipid, an accessible pool of mobile FC forms, some of which moves to the ER to signal a FC excess and to moderate activation of SREBP (38, 39). Exactly which membrane(s) serve as the initial acceptor of endosomal FC has not yet been determined; however, recent findings argue that the bulk of LDL-cholesterol is transported to the PM (38, 39). Because SREBP-mediated FC sensing takes place in the ER, lipoprotein-derived cholesterol also needs to be delivered to the ER. However, the mechanisms of post-NPC1 endosome to ER trafficking are still largely unknown (Fig. 1), despite the presence of FC-binding and ER-interacting domains in late endosomal membrane proteins (40). Oxysterol-binding protein-related protein 1L (ORP1L) and StAR-related lipid transfer protein 3 (STARD3) can bind vesicle-associated membrane protein (VAP) in the ER, bridging between late endosomes and the ER. Interestingly, it was recently reported that both ORP1L and STARD3 also transfer FC from the ER to endosomes, employing VAP as the ER partner (41, 42).

ROLE OF NON-ENDOSOMAL CHOLESTEROL MOVEMENT

Scavenger receptor BI (SR-BI) is a PM receptor that mediates the selective uptake of CE from lipoproteins, and is known as the HDL receptor. The selective HDL-cholesterol uptake pathway is distinct from the LDLr pathway, as it does not require clathrin-dependent receptor-mediated whole particle uptake and lysosomal degradation (Fig. 1). SR-BI removes and transports CE from HDL into the cells, which are then hydrolyzed by a neutral CE hydrolase releasing FC (43). The identity of the hydrolase depends on the cell type and carboxyesterase 1 has been suggested as the neutral hydrolase in liver tissue (44). Recently three ER-resident cholesterol transport proteins (Aster-A, -B, and -C) were described that bind FC and facilitate its removal from the PM (45). Moreover, Aster-B was shown to move FC from the PM to the ER downstream of SR-BI and, in that way, enable the ER to sense fluctuations in the pool of PM FC and to link this with regulation of the sterol-sensing SREBP-2 pathway. When PM FC levels rise above homeostatic levels, Asters form bridges between the PM and the ER, and the sterol-binding ASTER domain extracts FC from the PM and moves it down the concentration gradient to the ER (45) (Fig. 1).

ROLE OF CHOLESTEROL EFFLUX

Efflux or removal of excess cholesterol from cells is an ATP-dependent process carried out by ABCA1, which mediates FC movement across the PM to an acceptor such as apoA-I for generating new HDL by the liver or for peripheral tissues to send FC back to the liver in a process called reverse cholesterol transport (46). apoA-I binds with ABCA1, which then packages FC, SM, and phospholipids into newly formed or nascent HDL (nHDL) particles (18). These FC-rich particles are then remodeled by LCAT, also called PC-sterol acyltransferase, into particles having a core

of CE. Alternatively, ABCA1 will also efflux FC to other proteins, e.g., apoA-II, apoA-IV, apoC-I, apoC-II, apoC-III, and apoE (47). ABCA1 is present on the PM, not in lipid rafts, but generates nHDL that has a lipid composition typical of the lipid raft. ABCG1 participates in intracellular sterol movement (48) but has not been shown to directly transfer cholesterol to apolipoproteins or lipoproteins at the surface of the cells (48, 49) as originally proposed (50, 51). Given the ubiquitous cellular presence of ABCA1, FC efflux to apoA-I appears to be a continuous housekeeping function of all cells. However, most cells, with the exception of hepatocytes and enterocytes, do not produce apoA-I (52). They must utilize apoE, which is synthesized by many peripheral tissues (53), or they must generate lipid-poor apoA-I from mature HDL following CE removal by SR-BI, although there is evidence that partially lipidated apoA-I is a poor substrate for ABCA1 (54). SR-BI has also been implicated as a cholesterol efflux transporter, as it induces FC flux down an FC-concentration gradient (55, 56). Efficient SR-BI-mediated CE uptake and possibly FC efflux require the presence of an extracellular matrix protein, procollagen C-endopeptidase enhancer protein 2 (PCPE2) (57, 58). However, the mechanism by which PCPE2 influences SR-BI function remains to be fully elucidated.

Lipid rafts provide a platform for organizing the signaling of many receptors and proteins. Because many of these processes involve inflammation-related pathways, lipid raft composition is carefully regulated. Available FC is either utilized for cellular membrane maintenance, stored, or moved to a substrate pool for export via ABCA1 (59). ABCA1, whose expression is modulated by LXR, is a uniquely sensitive master controller of membrane FC that is essential for maintaining lipid raft composition and function (60–66) (Fig. 1). In accordance with this notion, the lipid composition of nHDL was found to be identical to that typically found in lipid rafts isolated from cells (18, 67). Synthesis of nHDL containing raft FC from three molecules of lipid-poor apoA-I yields an ~9–11 nm diameter particle that contains ~240 molecules of lipid, 100 of which are FC (18). Therefore, ABCA1 exports FC from peripheral tissues, which helps modulate the FC available to form lipid rafts.

ROLE OF INTRACELLULAR BILAYER MEMBRANES

The formation of lipid rafts is not merely confined to the PM. Prior to proposing the raft as an essential component of the PM, lipid rafts were operationally defined as a lipid-regulated sorting mechanism for transport of cholesterol from the TGN to the PM. In 1993, Fiedler et al. (68) found that both the Golgi and the PM formed protein-sphingolipid microdomains. Indeed, both ER and mitochondria reportedly have lipid raft-like microdomains (69, 70). Several studies have shown that these organelles differ both quantitatively and qualitatively in their lipid content [reviewed in (9)].

PMs are enriched in sphingolipids and FC (typically 20–25%), which are packed at a higher density than glycerophospholipids and are more resistant to mechanical stress.

FC is also abundant in the endocytic recycling compartment and part of the Golgi complex, with an enrichment toward the TGN. In contrast, ER membranes are, despite the organelle being the main site of FC synthesis, extremely low in sterol and complex sphingolipid content. Mitochondrial membranes are also relatively low in sterols, except for mitochondria in sterol-producing cells, which import and utilize FC for sterol production. A substantial amount of cellular lipid metabolism and synthesis occurs in the mitochondria. Lipids present in the mitochondrial membrane include lysophosphatidic acid, phosphatidic acid, phosphatidylglycerol, and cardiolipin (CL), a lipid that is unique to the mitochondrial membrane. The presence of phosphatidylglycerol and high CL content in the inner membrane in addition to a high PE/PC ratio are reminiscent of the bacterial origin of this membrane and are probably required to support oxidative phosphorylation.

ROLE OF GOLGI AND TGN

The classical secretory route for many proteins is from the ER to the Golgi and from the TGN to the PM, following the FC and SM gradient, which increases from ER to Golgi to PM (71). FC content is critical for Golgi-related vesicle transport, as Golgi morphology and intra-Golgi transport are disrupted by reducing FC content by 10% (72). Both raft and non-raft domains in the Golgi/TGN have been shown to be important for vesicle transport within or from the Golgi (73, 74). Non-raft coated vesicles are instrumental for trafficking proteins to the basolateral PM or within the Golgi-ER compartment. Specific raft-associated proteins are important for sorting and transport from the TGN to the PM (75, 76). Whether lipids or proteins initiate and mediate the raft formation or sorting process is still under debate.

However, there are nonvesicular transport mechanisms that include proteins in the glycolipid transport protein superfamily (77–79), oxysterol binding proteins (80), and ceramide transport protein (81, 82). Moreover, specific lipid signals at the TGN, the phosphatidylinositol phosphates PI₄P and PI_{4,5}P₂, can be recognized by lipid transport proteins (83). Phosphatidylinositol phosphates can be localized in rafts and in that way contribute to transport and metabolism of other raft lipids in a process that has yet to be elucidated (83). Therefore, it is likely that Golgi-related vesicle trafficking and protein sorting require the proper lipid environment, which includes lipid rafts.

MITOCHONDRIAL INTERACTIONS WITH THE ER

The interaction of the ER with mitochondria occurs via certain subdomains of the ER, named mitochondria-associated membranes (MAMs), which allow membrane scrambling between these organelles and contribute to the complex series of ER functions (84) (Fig. 1). In mammalian cells, MAMs play a critical role in the early steps in the formation of autophagosomes (85, 86). MAMs are enriched in CAV1 (87), lipid synthesis enzymes (88, 89),

and FC (90), with more cholesterol present in MAMs than in either the ER or the mitochondria (91). The latter suggests that they act as sites of nonvesicular lipid transfer between the ER and mitochondria. Moreover, the ganglioside GM1, which contains one sialic acid residue, can accumulate at the ER membranes and can promote the juxtaposition of ER and mitochondria at the MAMs.

Calnexin (CANX), a prototypical calcium-binding ER palmitoylated chaperone protein enriched in the MAMs, was recruited to the detergent TX-100-insoluble fractions corresponding to lipid microdomains during autophagy-stimulating conditions (92). Disialoganglioside having three glycosyl groups (GD3) was detectable in the MAMs of cells under autophagic stimulation (92) and enhanced the association of GD3 with autophagy and beclin 1 regulator 1 (AMBRA1) and increased the association of AMBRA1 with WD repeat domain phosphoinositide-interacting protein 1. siRNA-mediated inhibition of GD3 synthesis in stimulated conditions reduced autophagy and CANX-AMBRA1 association, suggesting that the protein composition of MAMs was modulated by the concentration of GD3, leading to impaired starvation-induced association of core complex molecules at the MAMs (92). Together, these data suggest that lipid microdomains may participate in autophagy and organelle recycling.

MITOCHONDRIAL DYNAMICS AND APOPTOSIS

Functional studies suggest that mitochondrial lipid microdomains participate in the fusion, fission, and apoptosis of the mitochondrial network during remodeling (93). Mitochondrial fusion induced by mitochondrial division inhibitor 1 (Mdivi-1) required GD3 to co-associate with mitofusin (Mfn)2 to participate in the fusion process (94). The mitochondrial network constantly remodels its shape through fusion and fission to regulate their number and function. In human cells, mitochondrial fusion is regulated by the mitofusins Mfn1 and Mfn2, which are embedded in the mitochondrial outer membrane, and by the optic atrophy 1 protein, located in the mitochondrial inner membrane (95, 96). Mitochondrial fission is directed by recruitment of the soluble dynamin-related GTPase called dynamin-like protein 1 (DLP1) from the cytosol to mitochondria (97, 98). This GTPase localization marks sites of future fission. Human mitochondrial fission 1 protein (hFis1), an integral outer mitochondrial membrane protein, has an essential role in completing fission (99).

Mitochondrial fission is required during cell proliferation and apoptosis (100). Glycosphingolipid microdomains were proposed to mediate multiple steps in the apoptosis cascade including recruitment of Fas cell surface death receptor and TNF- α receptors (101–103) and recruitment of the proapoptotic Bcl-2 family proteins, including truncated Bid, t-Bid, and Bax, following Fas cell surface death receptor triggering (69). The activation of Fas cell surface death receptor and TNF- α receptor may also induce an intracellular movement of lipid raft components, such as GD3, toward mitochondria (69, 104, 105). During apoptosis, CL may be

present in lipid microdomains, where it acts as an activation platform for caspase-8 translocation on mitochondria, at contact sites between inner and outer membranes, facilitating self-activation (69, 106). Proapoptotic members of the Bcl-2 family were suggested to associate with mitochondrial fission sites and mitochondrial fission proteins during apoptosis (107). hFis1 is constitutively associated with mitochondrial lipid microdomains, while DLP1 is recruited to lipid microdomains only on apoptotic Fas cell surface death receptor triggering (93). Disruption of lipid rafts leads to an impairment of DLP1 recruitment, a reduction of mitochondrial fission, and a significant reduction of apoptosis (93, 107). Mitochondria depolarization and cytochrome c release may also be dependent on lipid microdomain integrity, because disruption of lipid microdomains prevented depolarization, cytochrome c release, and apoptosis upon apoptotic triggering (69). These studies suggest that mitochondrial lipid microdomains act as activating platforms where key reactions take place that determine cell fate.

When protein content of mitochondrial DRM was investigated using stable isotope labeling by amino acids in cell culture, quantitative mass spectrometric proteomic analysis identified F1/F0 ATPase subunits, voltage-dependent anion selective channel proteins, and several other mitochondrial proteins. However, the amounts of these proteins were not sensitive to FC disruption and they were not enriched in DRMs compared with whole cell membranes (108) suggesting that these proteins at best are partially copurifying contaminants. Proteins that participate in mitochondrial fusion or fission were not detected in the mass spectrometric analysis suggesting that these proteins do not reside in lipid raft-like domains or that their concentration was below the limit of detection.

EVS: EXOMERES, EXOSOMES, MICROPARTICLES, AND LIPOPROTEINS

Communication between cells and tissues is essential for maintaining biochemical integrity. The role of secreted proteins, peptides, and eicosanoid lipids in intercellular communication has been recognized for many years; however, communication between cells by EVs is receiving more attention. Non-lipoprotein EVs or microparticle vesicles are being investigated as a means for transferring information among cells in both animals (109) and plants (110). This is an ancient system that has been reported in several species of bacteria and fungi (111). These particles are reported to carry proteins, lipids, and RNA.

A large number and variety of EVs are released by mammalian tissues and have been recently reviewed (112). These vesicles are reported to transport a variety of proteins, RNAs, and lipids among cells. Another class of particles that are often co-isolated with EVs include the following lipoproteins: HDL, LDL, IDL, VLDL, and chylomicrons. These particles are reported to carry many proteins, microRNAs, and lipids among cells. In particular, lipoproteins are primarily responsible for cholesterol exchange

among cells via lymph and plasma. Lipoproteins consist of an exterior monolayer of phospholipids that interacts with apolipoprotein(s) on the surface of the particle. The interior consists of a mixture of hydrophobic lipids, like FC, CE, and triglycerides (TGs). HDL and LDL have well-defined roles in nutrient transfer, while HDL may have additional anti-inflammatory properties. Proteomic analyses suggest that these particles associate with a variety of proteins (113, 114), but the principal proteins for each particle are apoB₁₀₀ for LDL, IDL, and VLDL, and apoA-I for HDL and chylomicrons. Other characteristic apolipoproteins carried are apoE on IDL, VLDL, chylomicrons, and HDL, and apoA-II and apoM on HDL. Chylomicrons carry a truncated form of apoB, apoB₄₈, which has 48% of the apoB₁₀₀ sequence. VLDL is secreted by the liver with preliminary assembly of apoB₁₀₀ and TG taking place in the ER with final assembly in the Golgi (115). Secretory granules from the Golgi merge with the PM to release VLDL. IDL and LDL are generated in the plasma by remodeling of VLDL, while HDL is generated outside of the cell by lipidation of apoA-I by ABCA1 with the primary site of lipidation at the liver and intestine. Lipids carried on the surface of the lipoproteins are predominantly the amphipathic lipids PC and SM, while the interior or core carries hydrophobic lipids like TG, FC, and CE. VLDL and chylomicrons are particularly rich in TGs, while HDL and LDL carry predominantly CE with lesser amounts of FC and TGs.

A thorough discussion of criteria for defining non-lipoprotein EVs is given in a consensus paper by They et al. (116). EVs are often divided into three classes based on diameter: exomeres, 30–50 nm; exosomes, 50–150 nm; and microparticles, 100–1,000 nm. These size ranges overlap with diameters of lipoproteins like HDL and LDL (5–35 nm) and VLDL and chylomicrons (20–1,200 nm) and may be isolated with EVs (117). In addition to having diameters similar to those of EVs, the densities of lipoproteins overlap with the density ranges that characterize exosomes and microparticles (118). Thus, the relative contributions of EVs and lipoproteins will require increased attention to the details of particle isolation.

Exosomes and microparticles are generated by different cellular processes. Exosomes are secreted through multivesicular bodies and are enriched in tetraspanins (119), while microparticles are formed by budding of the PM (120), which requires modulation of ATP-dependent transporters, e.g., flippase and floppase, and scramblase activity. The primary cellular mechanism for assembling multivesicular bodies uses the endosomal sorting complex required for transport (ESCRT) of intraluminal vesicles into the endosome (121, 122). An ESCRT-independent pathway has been identified that may be ceramide dependent (123) and may have significant effect on the secretion of selected proteins (124). Most cells shed EVs into the tissue environment and these products are taken up by other cells by endocytosis, receptor mediated uptake of EV-bound ligands, and fusion of EV lipid with cell membranes.

EVs have been shown to carry proteins, lipids, and RNAs from an origin cell to a receptive cell (109, 125). The cargo is most often antigens, cell surface proteins, cytoplasmic

contents, and various nuclear components. Under healthy conditions, *in vivo* levels of EVs are generally relatively low, but these levels often increase in disease states or during muscle repair (126), and it has been suggested that their concentration and composition might be used for diagnosis and prognosis (125, 127). EVs from platelets participate in normal procoagulant activity and thrombin production, and their production is crucial for physiologic coagulation (128). The absence of these platelet-derived EVs leads to the development of a bleeding disorder called the Scott syndrome.

In other cases, EVs facilitate movement of proteins from cells expressing these proteins to cells that do not express these proteins, e.g., the transfer of chemokine CCR5 (129), which enables retrograde signaling through EV-mediated transport of synaptotagmin 4 (130), or the transfer of oncogenic receptor epidermal growth factor receptor variant III (EGFRvIII) from EGFRvIII expressing cells to EGFRvIII-negative cells (131). A recent study of adipocyte-specific knockout of CAV1 showed that the essential adipocyte protein CAV1 could be transferred by EVs from endothelial cells normally resident in fat tissue to adipocytes (132). Adipocytes are also reported to release lipid-filled vesicles that are a source of lipid for local macrophages (133). Health-promoting functional roles for EVs have been established for many cell types; however, in some cases exosomes promote disease states. CD63⁺/CD66b⁺ exosomes from neutrophils carrying α 1-antitrypsin-insensitive neutral elastase cause emphysema in mice by binding and degrading extracellular matrix. CD66b⁺/NE⁺ PMN exosomes isolated from COPD patients were found to induce a COPD phenotype in mice (134). EVs have been connected with information exchange within the tumor microenvironment (118, 131) and implicated as participants in diabetes, myocardial infarction, and metabolic syndrome (125, 135, 136).

LIPID RAFTS AND EV LIPIDS

Exosomes and microparticles are characterized by having a lipid bilayer that contains intraparticle proteins and metabolites, which support transmembrane and surface bound proteins. In contrast, exomers generally carry lesser amounts of lipids (137). Some groups have described exosomes as having membranes with lipid compositions that are lipid raft-like (138–140), suggesting that they might be

directly formed from lipid rafts. Table 1 summarizes lipid compositions for exosomes with diameters of around 50–100 nm. For comparison, the composition of both nHDL, the first-formed HDL particles generated by ABCA1, and one defined mature HDL particle, HDL2b, are included. Both exosomes and nHDL show an enrichment in both FC and SM, which is usually associated with lipid rafts (2, 18, 141, 142), an enrichment not found in mature HDL, suggesting that exosomes and nHDL have origins associated with lipid rafts. EVs in the first four columns of Table 1 show high PS levels (138, 143). Higher concentrations of PS on the outer leaflet of the lipid bilayer would promote uptake by other cells, like macrophages (144, 145). Exosomes from adipocytes have little PS. Increased levels of FC and SM strongly suggest that the lipid profile of exosomes is more like lipid rafts, but it is unclear at this time if this lipid composition has functional significance or is simply related to the site of vesicle generation where the predominant composition is that of a lipid raft.

CONCLUDING REMARKS

Lipid rafts are essential for maintaining cellular functions, including spatial PM organization, signal transduction, and receptor activation, as well as newer functions involving intracellular lipid and protein trafficking from the ER, Golgi, and endosomes to the PM. Lipid microdomains have also been shown to be involved in mitochondrial function by facilitating interaction between the ER and mitochondrial membranes for the exchange of lipid and proteins. Most recently, cellular EVs are now recognized as important elements in the transfer of information and/or biomolecules among cells. Exosomes, representing one EV size class, are enriched in FC and SM, similar to that found in lipid rafts. These findings suggest that EVs may be derived from endosomes that originated from lipid rafts, thereby reinforcing the concept that the formation, stability, and turnover of lipid rafts is critically dependent on the maintenance of a cellular FC gradient, where FC content increases markedly proceeding from intracellular organelle membranes to the PM. The PM FC concentration gradient is maintained through tight feedback control of FC synthesis, uptake, and efflux.

Because of their transient nature and small size, lipid rafts appear to lack structural boundaries, making their visualization challenging. Advances in our technical ability

TABLE 1. Cellular exosome lipid composition compared with HDL

Lipid Class	PC-3 Cells (139)	DiFi Cells (137)	Oli-neu Cells (123)	Platelets (146)	Adipocytes (147)	nHDL (18) (Diameter = 12 nm)	HDL(2b) (148, 149) (Diameter = 10.4 nm)
FC	43.5	60.3	43	42.5	64	45.7	8.2
CE	0.1	6.7	—	4.1	—	3	39.9
SM	16.3	6.5	8.2	12.5	8	10.3	10.4
PC	15.3	16.8	26.7	15.9	22	39.5	38.4
PE	5.8	3	10.9	3.1	3	0.6	0.83
PS	11.7	6.5	14.9	10.5	1	0.9	0.02
PI	0.13	—	—	5.2	2	1.6	0.83

Mole percent lipid composition of exosomes from several cellular sources compared with nHDL and mature plasma HDL. For some of these reports the mole percentages were estimated from mass data. PI, phosphatidylinositol.

to study and define these lipid microdomains will be needed in order to expand our knowledge of their roles in cellular functioning. These advances will no doubt show that lipid microdomains participate and regulate even more cellular processes than we can currently know. **6**

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