Minireview

Structure and Function of Sphingolipid- and Cholesterol-rich Membrane Rafts*

Deborah A. Brown‡ and Erwin London‡‡

From the Departments of Biochemistry and Cell Biology and Chemistry, State University of New York, Stony Brook, New York 11794-5215

It is well known that separate domains with different lipid compositions can exist in liposomes containing mixtures of different phospholipids. The question of whether cellular membranes contain similar lipid domains has intrigued workers for many years. One type of domain, sphingolipid-rich and cholesterol-based structures called membrane rafts, has received much attention in the last few years. We will review the evidence that rafts exist in cells and focus on their structure, or the organization of raft lipids and proteins. Our discussion of function will focus on the role of rafts in signaling in hematopoietic cells, a particularly well developed area that has provided insights into raft organization in the membrane. Several reviews of rafts (1–4) and of related structures called caveolae (5–7) have appeared recently.

Lipid Phase Behavior and Raft Formation

Sphingolipids differ from most biological phospholipids in containing long, largely saturated acyl chains. This allows them to readily pack tightly together, a property that gives sphingolipids much higher melting temperatures ($T_m$) than membrane (glycero)phospholipids, which are rich in kinked unsaturated acyl chains. It is now clear that tight acyl chain packing is a key feature of raft lipid organization (3, 8, 9). In fact, the differential packing ability of sphingolipids and phospholipids probably leads to phase separation in the membrane. Thus, sphingolipid-rich rafts co-exist with phospholipid-rich domains that are in the familiar, loosely packed disordered state (variously abbreviated as $L_a$, $L_d$, or $L_1$). Phase separation between lipids in different physical states, most often the $L_1$ and the solid-like gel phases, has been well characterized in model membranes. Indeed, the gel phase is the most familiar state in which acyl chains are highly ordered.

However, because of the high concentration of cholesterol in the plasma membrane and other membranes in which rafts form, raft lipids do not exist in the gel phase. Cholesterol has important effects on phase behavior. It is well known that addition of cholesterol to a pure phospholipid bilayer abolishes the normal sharp thermal transition between gel and $L_1$ phases, giving the membrane properties intermediate between the two phases. This effect initially suggested that domains in ordered and disordered states cannot co-exist at high cholesterol levels. However, further work showed that a different kind of phase separation can occur in binary mixtures of individual phospholipids with cholesterol. In these mixtures, domains in an $L_1$-like phase co-exist with domains in a new state, the liquid-ordered ($L_o$) phase. Acyl chains of lipids in the $L_1$ phase are extended and tightly packed, as in the gel phase, but have a high degree of lateral mobility (3).

Rafts probably exist in the $L_1$ phase or a state with similar properties. In support of this model, detergent-insoluble membranes that can be isolated from cell lysates and are likely to be derived from rafts (discussed below) are in the $L_1$ phase (10, 11). Model membrane studies that do not involve detergents also support the idea that $L_1$ and $L_o$ phase domains could co-exist in biological membranes. These studies showed that phase separation can occur in ternary mixtures of cholesterol with two phospholipids (or a phospholipid and a sphingolipid) that have different $T_m$ and thus different tendencies to form an ordered phase (8, 12). In these mixtures, $L_1$ phase domains enriched in the high $T_m$ lipid separate from $L_o$ phase domains enriched in the low $T_m$ lipid. Because of the significant difference in $T_m$ between sphingolipids and biological phospholipids, these lipid mixtures are a reasonable (though crude) model of cholesterol-containing cell membranes like the plasma membrane.

Cholesterol has another important effect on phase behavior. As discussed above, there are parallels between $L_1/L_d$ phase separation and gel/$L_1$ phase separation. In both cases, a phase in which acyl chains are highly ordered (gel or $L_1$) separates from a phase in which they are disordered ($L_d$). Thus, lipid mixtures can undergo either gel/$L_1$ phase separation in the absence of cholesterol or $L_1/L_d$ phase separation in its presence. Comparing the phase behavior of mixtures with and without cholesterol shows that the sterol can sometimes promote phase separation (8, 12), apparently because of favorable packing interactions between saturated lipids and sterol (13). Thus, in phospholipid/sphingolipid mixtures, less sphingolipid is required to form the $L_1$ phase (in the presence of cholesterol) than to form the gel phase in its absence (8, 9). This cholesterol effect probably explains why rafts can form in cell membranes that contain relatively low levels of sphingolipids. It also explains why cholesterol depletion can disrupt rafts and affect raft function. Finally, it probably explains why sphingomyelin, with a $T_m$ of 37–41 °C, can be essentially as effective as glycosphingolipids (which can have much higher $T_m$) in promoting raft formation (11). Any difference in raft stability that might result from the difference in $T_m$ between the two lipids is minor compared with the strong raft-stabilizing effect of cholesterol.

Because headgroup structure is an important modulator of lipid packing, headgroup as well as acyl chain structure may be important in raft formation. For instance, phosphatidylethanolamines (PE), with their small headgroup, have much higher $T_m$ than the corresponding phosphatidylcholines (PC). This effect may be especially important in the sphingolipid-poor (but PE-rich) inner bilayer leaflet, where raft structure is very poorly understood.

Rafts and Detergent-insoluble Membranes

Membrane fragments that are insoluble in non-ionic detergents (DRMs, also termed DIGs (detergent-insoluble glycolipid-enriched membranes), GEMs (glycolipid-enriched membranes), and TIFF (Triton-insoluble floating fraction)) can be isolated from most mammalian cells (14). DRMs appear to be derived from rafts; they are rich in cholesterol and sphingolipids and are in the $L_1$ phase when isolated from cells (10). Furthermore, $L_1$ phase liposomes are also detergent-insoluble under the conditions used to extract cells (9). Thus, there is a close relation between rafts and DRMs, and isolation of DRMs is one of the most widely used methods for studying rafts.

The tight acyl chain packing of both gel and $L_1$ phase lipids is probably responsible for their detergent insolubility. This provides a rational explanation for the detergent insolubility of DRMs, which was initially puzzling; in a tightly packed state, lipid-lipid interactions can be more stable than lipid-detergent interactions. DRM Proteins—A number of proteins are enriched in DRMs. Some of these are targeted to rafts by modification with saturated chain lipid groups, which pack well into an ordered lipid environ-

* This minireview will be reprinted in the 2000 Minireview Compendium, which will be available in December, 2000. This work was supported by National Institutes of Health Grants GM 47897 (to D. A. B.) and GM 48596 (to E. L.).

‡ To whom correspondence should be addressed. Tel.: 631-632-8563; E-mail: deborah.brown@sunysb.edu.

‡‡ The abbreviations used are: $T_m$, melting temperature; $L_1$, liquid crystalline; $L_d$, liquid disordered; $L_o$, liquid ordered; PE, phosphatidylethanolamine; PC, phosphatidylcholine; DRM, detergent-resistant membrane; GPL, glycosylphosphatidylinositol; POPC, palmitoyl oleoyl PC; TCR, T cell receptor.
ment. These modifications include glycosylphosphatidylinositol (GPI) anchors and closely spaced myristate and palmitate or dual palmitate chains (2, 15–18). In contrast, both membrane-spanning proteins and prenyl groups (which are bulky and branched) should be difficult to accommodate in a highly ordered environment. Indeed, DRMs are relatively poor in transmembrane proteins and contain very low levels of prenylated proteins (17).

Nevertheless, several specific transmembrane proteins are enriched in DRMs. Very little is known about how this occurs. Palmitoylation can contribute to DRM targeting (16, 17), although not all palmitoylated transmembrane proteins are in DRMs and not all transmembrane DRM proteins are palmitoylated. As might be expected, the sequence of the membrane-spanning domain (which could affect the way the protein interacts with lipids) can affect DRM localization (19–21). However, mutations in cytoplasmic domains, which seem unlikely to interact directly with lipids, can also affect DRM association (22–25). Although the mechanism of this effect is not known, such mutants might fail to interact with binding partners that themselves associate directly with raft lipids or might be mistargeted to membranes whose lipid composition cannot support raft formation.

Clustering and DRM Affinity—The affinity of gangliosides (26) and lipid-linked proteins (15, 27) for DRMs (and presumably also for rafts) can be increased by clustering or oligomerization because of the increase in the number of saturated acyl chains per molecule or cluster. Enhancement of raft affinity by clustering of molecules that individually have more modest raft affinity is supported by theoretical considerations and may have important physiological consequences (2, 27). This effect probably explains why several receptors on the surface of hematopoietic cells are recruited to DRMs when they are clustered following antigen binding (discussed below), although the structural features of these proteins that confer an affinity for rafts have not been identified.

Limitations of the DRM Method—Although DRM association is a useful way of showing that a protein or lipid has an affinity for rafts, it cannot be used to quantitate the fraction of the molecule that is present in rafts in the intact cell. This is partly because cells must generally be chilled before detergent extraction in order to isolate DRMs. Chilling is necessary to stabilize the l phase and enhance its detergent resistance. However, because phase separation is also strongly temperature-dependent, more of the membrane is probably in the l phase at 0 than at 37 °C. This may explain why a surprisingly high fraction of plasma membrane lipids can be detergent-insoluble (reviewed in Refs. 2 and 3) and why the phospholipid composition of DRMs can be similar to that of the plasma membrane (28). On the other hand, in some cases detergent may partially solubilize raft lipids and proteins even after chilling, leading to an underestimation of the fraction of these molecules in rafts (11, 15, 29).

These temperature effects raise the question of whether rafts exist at all in cell membranes at physiological temperatures and highlight the importance of detergent-independent methods (described below) in providing evidence for the existence of rafts.

Other Features of Raft Structure:
Outstanding Questions

Visualization of Rafts—If rafts are present in cells, it should be possible to detect them by microscopy. Nevertheless, molecules such as GPI-anchored proteins and gangliosides, taken as putative raft markers because of their enrichment in DRMs, often appear uniformly distributed on the cell surface when detected microscopically (reviewed in Refs. 3 and 4). However, the distribution of these markers can change dramatically upon clustering with antibodies or other agents. In some cases, clustering of one marker can cause redistribution of another, although the two are unlikely to interact directly (2, 27, 30–32) (discussed below in the section on hematopoietic cell signaling). The implication that the two markers colocalize because both are associated with rafts provides some of the strongest evidence to date that rafts are present in cell membranes.

Why are rafts difficult to see without clustering? At least three explanations are possible. First, rafts may be too small to see by microscopy. If so, clustering might cause coalescence of rafts into larger, visible units. Second, if individual markers have only a moderate affinity for rafts, they may not be highly concentrated in the domains. For instance, rafts would be difficult to detect visually if the concentration of a marker there were only 3- or 4-fold higher than in the rest of the membrane. These values are plausible because phase diagrams indicate that saturated acyl chains can sometimes give lipids only a moderate preference for ordered membrane domains (33). As discussed earlier, clustering could increase the affinity of these markers for rafts, increasing their concentration in the domains and facilitating detection. Finally, it is even possible that rafts do not exist constitutively but that clustering of components that have an affinity for an ordered state induces raft formation. We have discussed these alternate models of raft structure and dynamics previously (3), and they are illustrated in Fig. 1.

Raft Size—As is clear from the previous discussion, raft size is very poorly understood. Microscopically detectable lipid domains can sometimes be observed in model membranes without cholesterol (34, 35), but it is not yet clear how cholesterol affects domain size. Single-particle tracking experiments in cells showed that a ganglioside and a GPI-anchored protein were transiently confined to domains of about 200–300 nm that were sensitive to a glycosphingolipid synthesis inhibitor (4). Two other groups have used resonance energy transfer to search for clustering of GPI-anchored proteins in the membrane (36, 37). One group (36) found cholesterol-dependent clustering into domains of <70 nm, too small to see by microscopy, whereas the other found no evidence for clustering (37). This discrepancy would be reconciled if in fact only a small fraction of the molecules were closely clustered, a possibility that is consistent with both studies (37). It should be noted, however, that preferential partitioning of proteins into rafts does not necessarily result in their close clustering. For instance, GPI-anchored proteins could be present at a relatively low concentration in rafts if they had only a moderate affinity for the domains. Furthermore, as there is no reason to suspect that GPI-anchored proteins in rafts interact with each other more strongly than with raft lipids, they may be uniformly distributed within rafts. Thus, GPI-anchored proteins could be present at low local density if a large fraction of the membrane formed rafts.

Membrane Distribution—Rafts are likely to be most abundant in membranes that are rich in cholesterol and sphingolipids, including the plasma membrane, late secretory pathway, and endocytic compartments. In the plasma membrane, rafts appear to have a
preferential association with 50–100-nm pits called caveolae, which are present in many mammalian cell types (5–7). It should be noted, though, that rafts (as detected by DRM formation and by the co-clustering of several putative raft components) are not restricted to caveolae and are abundant in cells that lack caveolae. It is not yet known whether localization in caveolae affects the structure of rafts.

The behavior of rafts in the cytoplasmic leaflet of the bilayer is an important outstanding question. Although sphingolipids are largely restricted to the outer leaflet, several observations suggest that rafts are present in the inner leaflet and that rafts in the two leaflets are coupled. First, DRMs have a bilayer appearance when isolated and are enriched in dually acylated cytoskeletal membrane proteins, such as Src family kinases and G protein α subunits. Second, Src family kinases can co-redistribute when cell-surface GPI-anchored proteins or gangliosides are clustered using antibodies or toxins (27, 31).

Recent advances in tandem electrospray mass spectrometry have shown that plasma membrane phospholipids are more highly saturated than those in intracellular membranes (28, 38) and thus may form rafts readily in the presence of cholesterol. The monounsaturated than those in intracellular membranes (28, 38) and thus may form rafts readily in the presence of cholesterol. The monounsaturated phospholipid palmitoyl oleoyl phosphatidylcholine (POPC) may form the I phase when mixed with cholesterol (39). In addition, mixtures of brain PC (which is rich in POPC) and cholesterol are partially Triton-insoluble (in the cold) in the absence of sphingolipids (9). Finally, as mentioned earlier, the high concentration of relatively high T_{PE} in the inner leaflet may promote raft formation. Together, these observations suggest that formation of I_{1} phase rafts in the sphingolipid-poor cytoplasmic membrane leaflet is plausible.

How these rafts might be coupled with outer leaflet rafts is very poorly understood. There is some evidence for monolayer coupling of sphingolipid-rich domains in model membranes (40). More recently, confocal imaging and fluorescence correlation spectroscopy were used to show a correlation between like phases in the two leaflet model membranes containing two phospholipid species (34). Further characterization of the basis of this effect will probably shed light on the behavior of rafts in cells.

**Introduction to Raft Function**

In principle, targeting of proteins to rafts might affect function in either of two ways. First, concentration of proteins in rafts could facilitate interactions between them. (Similarly, segregation of raft and non-raft proteins could separate them during sorting.) Second, the ordered lipid environment might directly affect function, possibly by altering protein conformation. There are no clear examples of this second possibility, although cholesterol concentration and bilayer width can affect transmembrane helix orientation and helix-helix interaction (41, 42). In addition, cholesterol depletion (which can disrupt raft function) alters the function of a raft-associated potassium channel (43).

Several approaches have been taken to investigate raft function. One is to show that a protein is enriched in DRMs. This is consistent with a role for rafts in the function of that protein but does not prove it. More suggestive is showing that several proteins that must interact to function all redistribute and colocalize with each other when one raft component is experimentally clustered. Another approach is to show that disrupting the association of a protein with rafts disrupts function. For example, mutation of palmitoylation sites on two proteins (Lck and LAT, described below) simultaneously abolished DRM association and affected function. Finally, raft disruption by depletion of cholesterol (or occasionally sphingolipids) can affect function. Although this method is useful, depletion may have pleiotropic effects on membrane structure and lipid-protein interactions in addition to disrupting rafts. The strongest evidence for the involvement of rafts in function is provided when several approaches point to the same conclusion. This is well illustrated in the case of signaling in hematopoietic cells, particularly T cells and basophils.

Before examining this topic in detail, we will briefly mention other processes in which rafts have been implicated. Rafts were first proposed to mediate sorting in the trans-Golgi network, especially in polarized epithelial cells and neurons (1, 2, 44–46). Recent results suggest that rafts may also be important in sorting in the endocytic pathway (47). Rafts can serve as docking sites for certain pathogens and toxins (48). In addition, they may be important in the aberrant amyloid precursor protein processing that contributes to Alzheimer’s disease (6, 7). Integrin receptors may also function in rafts. Several integrins have been found in DRMs (2, 7, 49, 50). In one study, integrins, the integrin-associated protein IAP (which can regulate integrin function), and heterotrimeric G proteins formed a stable cholesterol-dependent complex that was enriched in DRMs (49). Finally, rafts polarize to the front of adenocarcinoma cells migrating in a chemotactic gradient (51). In these cells, development of front-rear polarity, which is required for directed migration, is abolished by cholesterol depletion.

**Signaling in Hematopoietic Cells**

Although different hematopoietic cells play distinct roles in the immune response, their antigen-responsive signaling pathways have several features in common with each other. Multisubunit receptors on T and B lymphocytes bind antigen directly, whereas those on other hematopoietic cells constitutively bind the Fc domains of different classes of antibodies and thus bind antigen indirectly (52). As examples of the latter case, FcγR on neutrophils binds IgG, FcεRI on basophils and mast cells binds IgE, and FcγR on several myeloid cells binds IgA. In each case, antigen binding triggers receptor cross-linking, leading to activation of specific Src family tyrosine kinases. The activated kinases phosphorylate tyrosine residues in the cytoplasmic domains of one or more receptor subunits. These events initiate signaling cascades, via recruitment of downstream signaling proteins, that culminate in cell-type-specific responses. As a specific example, an early signaling event in T cells is the heavy tyrosine phosphorylation of the linker for activation of T cells (LAT) protein. Phosphorylated tyrosine residues on LAT serve as the docking site for a number of downstream signaling proteins (16).

In several cases, receptors appear uniformly distributed on resting cells but can be experimentally clustered into large patches using a specific process that mimics physiological clustering of receptors by antigen. As discussed later, this receptor clustering can induce clustering of rafts.

**DRM/Raft Localization**—Several of the signaling proteins described above are enriched in DRMs. Src family kinases and LAT (16), both of which require acylation for DRM targeting, are present in DRMs constitutively. Antibody-mediated clustering can recruit receptors on several cell types to DRMs. These include the T cell receptor (TCR) (53), the B cell receptor (54), FcεRI (29), FcγR (55), and CD20 (23) (a protein whose cross-linking activates B cells). There is some information on structural features of these transmembrane receptors that is required for their targeting to DRMs (21, 23, 55), although no general patterns have emerged.

In several cases, receptor clustering induces redistribution of other putative raft markers. As this co-clustering involves two sets of molecules that are not believed to interact directly, it suggests that both molecules associate with the same rafts and that these coalesce into larger domains upon clustering of one component. As an example, the ganglioside GM1 and other order-prefering lipids, taken as raft markers, colocalize with clustered FceRI on basophils (2). (It should be noted that GM1 is generally detected using cholera toxin. As this toxin is pentavalent, it should induce GM1 clustering, enhancing raft association.) In another example, the Src family kinase Lyn, a signaling partner of FcεRI in basophils, colocalizes with clustered FceRI in a cholesterol-dependent manner (56). Similarly, contact of T cells with beads coated with antibodies to the CD3 component of the TCR and to the co-stimulatory protein CD28 induces clustering of GM1 at the contact site (30). Clustering of other lipids on T cells is also observed upon co-clustering of the TCR, LAT, and the Src family kinase Lck (31, 57).

**Functional Importance of Raft Localization**—Several observations support a functional role for rafts in hematopoietic cell signaling (reviewed in Refs. 58 and 59). FcεRI becomes tyrosine-phosphorylated by Lyn with the same kinetics with which it becomes recruited to DRMs, and the fraction of the receptor that partitions into DRMs is selectively phosphorylated (29). Cholesterol depletion inhibits receptor tyrosine phosphorylation in T cells (60) and basophils (56) and signaling in T cells (60). Recruitment of CD48 (a raft-associated GPI-anchored protein) to the site of contact
between the TCR and antigen-presenting cell (APC) can enhance signaling in a cholesterol-dependent manner (61). Another study showed that patching of gangliosides in T cells stimulates signaling (31).

Mutagenesis has been used to suggest that two proteins, Lck and LAT, must be in rafts to function. Stimulation of signaling in T cells by ganglioside patching (31) was abolished in cells expressing only a mutant, non-palmitoylated form of Lck that cannot associate with rafts. Strikingly, signaling was rescued when separate clusters of the mutant Lck and of gangliosides were brought together using bridging antibodies, demonstrating that the Lck was not inactivated by mutation. These results suggest that Lck must be physically close to its signaling partners for productive signaling and that this association normally requires clustering of the proteins in rafts.

A similar approach suggests that LAT must be in rafts to function (62). A mutant non-palmitoylated LAT is localized correctly to a region close to its signaling partners for productive signaling and bridging antibodies, demonstrating that the Lck was not inactivated by mutation. These results suggest that LAT must be physically close to its signaling partners for productive signaling and that this association normally requires clustering of the proteins in rafts.

**Signaling in Other Cells—Rafts may play a role in signaling outside hematopoietic cells**, although this area has not yet been well developed. One example involves ephrin B proteins, which are important in the developing nervous system. Ephrin B proteins, which are in DRMs, were recently shown to recruit multiprotein signaling complexes to DRMs when expressed exogenously (24). Other studies using fibroblasts showed that cholesterol depletion inhibited the hormone-stimulated phosphatidylinositol turnover, probably by deforming phospholipid membranes and reducing the ability of raft-membrane proteins to interact properly. This results in hyperactivation of extracellular signal-regulated kinase in response to epidermal growth factor (68). Finally, a number of studies suggest that caveolae are important signaling centers (5, 7).