REVIEW ARTICLE
Lipid rafts: heterogeneity on the high seas

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Lipid rafts are membrane microdomains that are enriched in cholesterol and glycosphingolipids. They have been implicated in processes as diverse as signal transduction, endocytosis and cholesterol trafficking. Recent evidence suggests that this diversity of function is accompanied by a diversity in the composition of lipid rafts. The rafts in cells appear to be heterogeneous both in terms of their protein and their lipid content, and can be localized to different regions of the cell. This review summarizes the data supporting the concept of heterogeneity among lipid rafts and outlines the evidence for cross-talk between raft components. Based on differences in the ways in which proteins interact with rafts, the Induced-Fit Model of Raft Heterogeneity is proposed to explain the establishment and maintenance of heterogeneity within raft populations.

Key words: caveola, cholesterol, detergent, glycosphingolipid, lipid raft.

INTRODUCTION

Lipid rafts are membrane microdomains that are enriched in cholesterol and glycosphingolipids. Hence, names such as CEMs (cholesterol-enriched membranes), GEMs (glycosphingolipid-enriched membranes), DIGs (detergent-insoluble, glycosphingolipid-enriched membranes) and DRMs (detergent-resistant membranes) have been given to these domains. The terms DIG and DRM reflect the observation that these domains are not readily solubilized in non-ionic detergents, a property that is the result of the tight packing of the lipid acyl chains in rafts. The low buoyant density of the detergent-insoluble domains is referred to in the name TIFF (Triton-X-100-insoluble floating fraction).

The original raft-like domain, and still the only one that is identifiable morphologically, is the caveola. Caveolae are small plasma membrane invaginations with a diameter of approx. 25–150 nm [1]. Caveolae are found as single entities or in grape-like clusters at the plasma membrane of many different cells. The invaginated structure of caveolae seems to be stabilized by the protein, caveolin-1 [2,3].

Originally, caveolae were isolated by extracting cells with 1 % Triton X-100 and then floating the lysate on a 5–30 % sucrose gradient [4]. A variety of proteins, including caveolin [5], GPI (glycosylphosphatidylinositol)-linked proteins [4] and numerous proteins involved in cell signalling [6,7] were found associated with these low-density detergent-resistant membrane fractions. The identification of signalling proteins in preparations of lipid rafts has led to the hypothesis that these domains are intimately involved in the process of signal transduction. Numerous studies support this view (for a review see [8–10]). Rafts have also been implicated in endocytic events [11,12] as well as in the trafficking of cholesterol [13].

Using a non-detergent method to prepare caveolae, Schnitzer et al. [14] showed that invaginated caveolae could be separated cleanly from a low-density Triton-X-100-resistant membrane fraction that contained all of the GPI-linked proteins. These findings suggested that detergent-resistant membranes comprise at least two types of domains – those that contain caveolin, which

are referred to as caveolae, and those that lack caveolin, which are now referred to as lipid rafts. In the present review, the term lipid raft will be used generically to apply to both caveolae and flat rafts, since these two membrane domains are typically isolated together during subcellular fractionation and are not often specifically distinguished from each other experimentally.

The work of Schnitzer et al. [14] provided the first indication that there were different types of domains within the total population of low-density detergent-resistant membranes. More recent evidence suggests that additional heterogeneity exists among these domains that includes variation in both the protein and lipid composition of individual rafts. These differences are often reflected by differences in the detergent solubility of individual raft proteins. The present review summarizes the evidence for heterogeneity in both raft lipids and raft proteins, and describes the evidence for cross-talk among raft constituents. This cross-talk permits rafts to adapt to alterations in the availability of raft components through compensatory changes in other raft lipids and proteins. Based on differences in the ways in which proteins interact with rafts, a mechanism is proposed to explain the establishment and maintenance of heterogeneity within the raft population.

WHAT IS A LIPID RAFT? DIFFERENT METHOD, DIFFERENT ANSWER

Like the proverbial skinning of a cat, there are many ways to make a lipid raft. Thus any analysis of raft heterogeneity must begin with a consideration of the different methods used to prepare rafts and what they tell us about the general structure of these domains. The finding that no two raft preparations yield the same answer to the question, what is a lipid raft? supports the notion that lipid rafts are not uniform entities, but rather represent a collection of related domains with similar physical properties.

Detergent-based methods

Most biochemical purifications of lipid rafts are based on an operational definition of these domains – namely that they are

insoluble in Triton X-100 and have low buoyant density. The initial and now-traditional definition of a lipid raft is a membrane domain that is resistant to extraction in cold 1 % Triton X-100 and that floats in the upper half of a 5–30 % sucrose density gradient [4]. A similar, simplified definition of rafts is the 1 % Triton-X-100-insoluble material that floats at the interface of a 5 %/30 % sucrose step gradient [15].

Analyses of this type of raft preparation have revealed a membrane domain of unique lipid composition. Cholesterol is enriched 3- to 5-fold in the detergent-resistant fraction as compared with total membranes, and represents one-third to one-half of the total membrane lipid [4,16,17]. Sphingomyelin is similarly enriched and represents 10–15 % of total membrane lipid in rafts [4,16,17]. An additional 10–20 % of raft lipid comprises glycosphingolipids, such as cerebrosides and gangliosides [4,17]. Glycerophospholipids, including the major membrane phospholipids, phosphatidylcholine and phosphatidylethanolamine, comprise ⩽ 30 % raft lipids as compared with approx. 60 % of lipids in total membranes [4,16]. Inner-leaflet lipids, such as phosphatidylethanolamine and anionic phospholipids, are particularly depleted [10]. Thus Triton-X-100-resistant lipid rafts are distinguished from bulk plasma membrane because they are enriched in cholesterol and sphingolipids, but are relatively depleted in glycerophospholipids.

In the liquid-ordered phase of lipid rafts [18], the acyl chains of lipids are in their extended conformation and are tightly packed. Theoretically, such a liquid-ordered phase should show a preference for saturated fatty acyl chains in any glycerophospholipids that are incorporated into this phase. This prediction has been borne out experimentally in that, within the limited amount of glycerophospholipids present in Triton-X-100-resistant lipid rafts, there is a preference for saturated fatty acids [16,19]. However, the preference for saturated acyl side chains is greater among the phosphatidylethanolamine species and the anionic phospholipids as compared with the phosphatidylcholine species [10,16,19].

The observation that there is a preference for saturated fatty acid side chains in lipids that are found predominantly on the cytofacial leaflet, such as phosphatidylethanolamine, suggests that liquid-ordered domains on the inner leaflet may be more dependent on the saturated acyl chains provided by glycerophospholipids than liquid-ordered domains on the exofacial leaflet. Sphingolipids, including sphingomyelin and gangliosides, partition preferentially into the exofacial leaflet of membranes and hence are unlikely to contribute significantly to the formation of rafts on the inner leaflet of the membrane. An increase in the number of saturated acyl chains on glycerophospholipids on the cytofacial leaflet may therefore be required to compensate for the absence of the saturated acyl chains contributed by sphingolipids to rafts present on the outer leaflet of the membrane.

Subsequent to the identification of low-density detergent-resistant domains in Triton X-100 extracts of cells, a variety of other detergents including Lubrol WX, Lubrol PX, Brij 58, Brij 96, Brij 98, Nonidet P40, CHAPS, and octylglucoside have been employed at different concentrations to prepare detergent-resistant membrane domains [20–24]. Unsurprisingly, use of these different detergents in the preparation of rafts yields membrane domains with lipid compositions different from those of standard, Triton-X-100-resistant membranes [25]. For example, Schuck et al. [25] presented evidence that rafts prepared in Triton X-100 or CHAPS are strongly enriched in sphingolipids and cholesterol as compared with total cell membranes. However, extraction of membranes with Tween 20, Brij 58 or Lubrol WX yielded low-density membranes that showed relatively little enrichment in either of these traditional raft lipids. Rafts prepared by solubilization of cells with Brij 96 or Brij 98 were moderately enriched in cholesterol and sphingomyelin, but were not as enriched in these lipids as rafts prepared using Triton X-100 or CHAPS. Schuck et al. [25] described these differences as being due to the 'DRM selectivities' of the different detergents. Triton X-100 and CHAPS were the most selective, whereas Tween 20 and Brij 58 were the least selective.

The observation that extraction of cells with different detergents leads to the isolation of rafts of different composition suggests that there is some underlying heterogeneity among rafts that gives rise to these differences. However, caution must be exercised when interpreting the results of such studies because there are a number of problems associated with the extraction of membranes with detergents [26]. The notion of 'DRM selectivity' is that certain detergents are able to more completely extract non-raft lipids and proteins from the periphery of rafts. However, it is also possible that some detergents selectively extract subsets of proteins or lipids from within rafts themselves, leaving behind a 'raft' that does not resemble the original membrane domain. Furthermore, a variety of studies have suggested that detergent extraction causes fusion of rafts, as well as lipid exchange between membranes [20,26–28]. Extraction of cells plated on coverslips with Triton X-100 results in the production of large sheets of detergent-resistant membrane, rather than small individual domains, consistent with the possibility that fusion of rafts occurs as a result of detergent treatment [27]. In addition, cross-linking experiments show that GPI-anchored proteins aggregate into large clusters in the presence of detergent, but not in its absence [29]. Thus, although the use of different detergents produces rafts of variable lipid compositions, it is not clear whether this heterogeneity pre-existed in the rafts or was induced by the application of the detergent.

Non-detergent methods

Non-detergent methods have also been developed for isolating lipid rafts [14,30–32]. The preparations of Song et al. [30] and Smart et al. [31] involve the sonication of membranes to release lipid rafts in small membrane pieces, followed by the separation of the light membrane fraction by density gradient centrifugation. The method of Smart et al. [31] yields a more purified raft fraction because it starts out with purified plasma membranes rather than a total cell lysate. An additional non-detergent method for isolating invaginated caveolae involving surface coating of plasma membranes with silica has also been reported [14].

Mass spectrometry was used to analyse the lipids in a raft preparation made by the method of Smart et al. [31]. Like detergent-resistant membranes, these non-detergent lipid rafts were shown to be enriched in both cholesterol and sphingomyelin relative to bulk plasma membrane [16]. However, unlike rafts made by Triton X-100 extraction, these rafts were not depleted of glycerophospholipids. In fact, phosphatidylserine was enriched nearly 4-fold in rafts as compared with plasma membranes, suggesting that rafts may serve as a reservoir for this phospholipid.

Because non-detergent methods do not involve the dissolution of membranes, these methods largely obviate problems such as membrane mixing and the selective extraction of lipids. In addition, these preparations seem to retain a greater fraction of inner-leaflet-membrane lipids [16] than detergent-extracted rafts do and may therefore yield domains in which the coupling between raft leaflets [33–35] is maintained. For these reasons, rafts prepared by non-detergent methods seem more likely to reproduce the *in vivo* composition of these microdomains accurately. However, because they are isolated solely on the basis of their low density, it is possible that non-raft lipids and proteins remain associated with the rafts during purification and are retained in the final preparation.

Because few studies have analysed the components of non-detergent lipid rafts or have tried to separate subpopulations of rafts from these preparations, little is known about heterogeneity among rafts prepared by these methods. Based on their ratio of cholesterol to glycerophospholipids, non-detergent lipid rafts [16] appear to be similar to rafts prepared by solubilization in 'moderately selective' detergents such as Brij 96 or Brij 98 [25]. Whether this lowered 'selectivity' is due to the ability of non-detergent methods to isolate varieties of rafts that are less resistant to detergent or to the propensity of non-raft material to contaminate these preparations remains to be determined. Nonetheless, the finding that non-detergent rafts are similar to some forms of detergent-resistant rafts suggests that there is some overlap in the type of domain that is being isolated by these different methods.
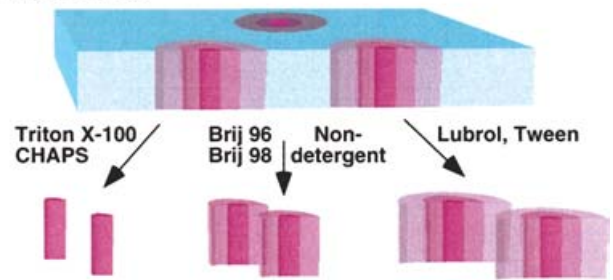
What is a lipid raft?

Ultimately, a lipid raft must be defined by its function, not by the method used to isolate it. Thus there is a need to get away from the early operational definition of a raft. The observation that raft composition depends heavily on the method used to make it suggests that a raft is a moving target. It does not seem to have a readily defined structure with a uniform lipid composition. Under some conditions, it appears to be a well-ordered, cholesterol- and glycosphingolipid-enriched domain. Under other conditions, it seems to be a more diverse collection of lipids, showing variable enrichment in cholesterol and sphingolipids. Clearly, differences between detergent and non-detergent methods for the preparation of lipid rafts could give rise to the observed variability in the lipid composition of the isolated rafts. But when looked at *in toto*, the results are consistent with the notion that there is underlying heterogeneity at some level in the structure of lipid rafts. If lipid rafts were distinct membrane compartments, like clathrin-coated pits or mitochondria, more similarity would be evident among rafts made by different methodologies. The fact that there is significant diversity in the composition of different lipid raft preparations suggests that there is diversity among rafts themselves.
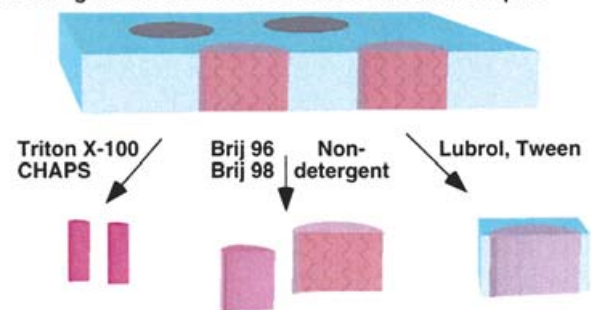
Based on the analyses of both detergent and non-detergent lipid rafts, three models for raft structure and behaviour can be proposed that are consistent with the observed experimental results. These are shown in Figure 1. In model I, lipid rafts contain a well-ordered central core, composed mainly of cholesterol and sphingolipids, that is surrounded by a zone (or zones) of decreasing lipid order. Detergents such as Triton X-100 and CHAPS would solubilize all but the most highly ordered core, giving rise to a membrane fraction that is extremely enriched in cholesterol and sphingolipids. In contrast, detergents such as Lubrol WX or Tween 20 would solubilize only the extremely disordered parts of the membrane, generating a domain that is only slightly different from bulk plasma membrane. Between these two extremes are detergents such as Brij 96 or Brij 98 that would solubilize the outermost zone of lipids, producing a membrane fraction that is somewhat enriched in cholesterol and sphingolipids, but which still contains a significant amount of glycerophospholipids. Non-detergent lipid raft preparations would isolate the inner raft zones, but not the outermost shell, and hence would be similar in properties to rafts solubilized in Brij 96 and Brij 98.

In model II (Figure 1), rafts are homogeneous in composition, but raft constituents have different affinities for lipid rafts and detergents are able to extract these components selectively. Triton X-100 and CHAPS are the most effective in membrane solubilization and hence selectively extract glycerophospholipids (and possibly some proteins), yielding a raft that is highly enriched in cholesterol and sphingolipids. Brij detergents are somewhat less effective in removing glycerophospholipids than are Triton X-100
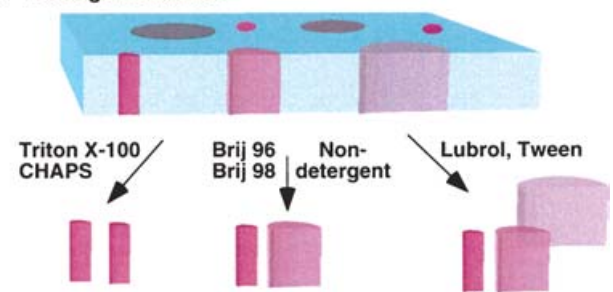

Figure 1   Models of raft structure based on the findings of different raft isolation protocols

I. Layered rafts. Rafts are composed of concentric layers of lipids ranging from a well-ordered cholesterol- and glycosphingolipid-enriched core through less ordered regions that ultimately grade into the disordered structure of the bulk plasma membrane. Different detergents solubilize different layers of the raft leading to the generation of rafts of different protein and lipid content. II. Homogeneous rafts with selective extraction of lipids. Rafts are homogeneous, well-ordered domains that are surrounded by the disordered phase of the bulk plasma membrane. The tendency of different detergents to release low-density domains of different composition is the result of the ability of different detergents to selectively extract lipids and/or proteins from these homogeneous rafts. III. Heterogeneous rafts. Rafts with different lipid and protein composition exist in the membrane and are differentially sensitive to solubilization by individual detergents.

and CHAPS, and hence generate rafts that are moderately depleted in these lipids and moderately enriched in cholesterol and sphingolipids. Lubrol WX and Tween 20 extract very few lipids and produce a raft that is not very different from bulk plasma membrane. Because there is no detergent to extract lipids selectively, non-detergent methods isolate the homogenous, intact raft.

In model III (Figure 1), rafts with distinct lipid compositions co-exist in cells. 'Traditional' rafts contain primarily cholesterol and sphingolipids, and are highly structured. 'Atypical' or 'variant' rafts contain lower levels of cholesterol and glycosphingolipids, and higher levels of glycerophospholipids, particularly those with long, saturated acyl chains. The differences in lipid composition

give rise to differential sensitivity to extraction by detergents. The domains containing mainly cholesterol and sphingolipids are resistant to extraction by Triton X-100, whereas other domains that contain lower concentrations of cholesterol or sphingolipids are solubilized by Triton X-100 and hence are lost from the preparations. Methods using no detergent or less selective detergents disrupt fewer of the 'atypical' or 'variant' rafts and result in the isolation of more subtypes of rafts.

Although all the models can explain the results of analyses of the total cellular raft fraction, they have very different predictions when individual rafts are considered. Models I and II predict that all rafts isolated by the same method would have similar compositions. Heterogeneity is introduced into the system as a result of the isolation procedures. In contrast, model III predicts that rafts isolated by the same method could be heterogeneous in composition because the domains themselves were heterogeneous to begin with. As described below, the results of studies on the protein composition of lipid rafts are much more readily understood within the context of model III than either model I or model II.

EVIDENCE FOR PRE-EXISTING HETEROGENEITY IN THE LIPID RAFT POPULATION

Three types of experiments have been used to provide evidence of heterogeneity in the protein composition of lipid rafts: (i) differential detergent sensitivity of raft proteins; (ii) separation of raft proteins by immunoaffinity chromatography; and (iii) direct visualization of raft proteins and lipids in spatially distinct regions of the cell. Although some of these approaches do not demonstrate unequivocally that heterogeneity exists in the lipid raft population *in vivo*, in combination, the findings strongly support the conclusion that rafts of different protein and lipid composition coexist within cells.

Differential detergent sensitivity

GPI-anchored proteins were the first group of proteins reported to be enriched in lipid rafts [4]. Differential detergent sensitivity has been used to provide evidence that GPI-anchored proteins associate with different types of rafts than do proteins containing membrane spanning domains. Roper et al. [23] showed that GPI-anchored placental alkaline phosphatase was present in detergent-insoluble domains isolated from cells extracted with either 0.5 % Triton X-100 or 0.5 % Lubrol WX. In contrast, prominin, a penta-span membrane protein, was soluble in Triton X-100, but was insoluble in 0.5 % Lubrol. Similarly, Slimane et al. [24] showed that GPI-anchored proteins and single transmembrane domain proteins are trafficked in lipid rafts that are insoluble in both Triton X-100 and Lubrol WX, whereas polytopic membrane proteins are trafficked in rafts that are Lubrol-WX-insoluble, but Triton-X-100-soluble. Furthermore, a GPI-linked form of GFP (green fluorescent protein), but not a GFP-fused form of the multi-drug resistance transporter, MDR1, was found to localize to the low-density detergent-resistant fraction when HepG2 cells were solubilized in 1 % Triton X-100. However, both proteins were recovered in the detergent-resistant fraction when 1 % Lubrol WX was used to lyse the cells. These observations suggest that different types of proteins partition into domains that can be distinguished on the basis of their resistance to extraction by different detergents.

Differential detergent sensitivity has also documented differences in the behaviour of members of a single class of raft proteins. In murine T-lymphoma P1798 cells, Thy-1, a GPI-anchored protein, was found primarily in the low-density detergent-resistant membrane fraction when the cells were solubilized in either 1 %

Triton X-100 or 60 mM octylglucoside. However, another GPI-anchored protein, the heat-stable antigen protein, was found primarily in the low-density raft fraction when solubilization was carried out in Triton X-100, but only 42 % of this protein was in the raft fraction if the cells were extracted with 60 mM octylglucoside [22].

In rat brain membranes, Thy-1 was associated with rafts after extraction with either 0.5 % Triton X-100 or 0.5 % Brij 96, whereas NCAM-120, another GPI-linked protein was completely solubilized by both detergents [20]. In the same system, a third GPI-linked protein, the prion protein, was found almost exclusively in rafts after solubilization in 0.5 % Brij 96, but was divided evenly between the raft and non-raft fractions when 0.5 % Triton X-100 was used. Thus, even in the same cell type, different GPI-anchored proteins appear to associate with lipid rafts that can be distinguished based on their sensitivity to solubilization in non-ionic detergents.

As was true for the analyses of the lipid composition of rafts prepared by different methods, these data could be explained by any of the models shown in Figure 1, if one assumes that there are differences in the way in which different GPI-anchored proteins interact with rafts. The need to introduce such an assumption suggests that there is in fact some pre-existing heterogeneity in the way lipid rafts are put together. Thus, although these data do not distinguish among the various models shown in Figure 1, they are consistent with the idea that rafts are heterogeneous at some level.

Immunological separation of subpopulations of lipid rafts

The best example of the application of this type of methodology to address the question of raft heterogeneity are the studies that demonstrated that invaginated caveolae are distinct from flat non-caveolin-containing lipid rafts. Using anti-caveolin immunoaffinity chromatography, Oh and Schnitzer [36] showed that caveolin-containing caveolar membranes could be separated physically from GPI-anchored proteins present in the same low-density Triton-X-100-resistant membrane fraction. Later studies showed that the heterotrimeric G-proteins, $G_{\alpha i}$ and $G_{\alpha s}$, also partitioned into the fraction containing the GPI-linked proteins, rather than into the caveolin-enriched fraction [37]. Consistent with these findings, Stan et al. [32] used anti-caveolin affinity chromatography to show that vesicles that were highly enriched in caveolin-1 were largely depleted of other proteins typically found in detergent-resistant membrane preparations, including heterotrimeric G-proteins, endothelial NO synthase and non-receptor tyrosine kinases. Use of immunoaffinity techniques directed at raft constituents other than caveolin confirmed that not all rafts contain this protein. For example, anti-GM3 affinity chromatography was applied to detergent-resistant membranes from B16 melanoma cells. This yielded a raft subfraction that contained sphingomyelin, cholesterol, c-Src and Rho A, but not caveolin [38]. These studies demonstrated that it was possible to separate low-buoyant-density detergent-resistant membrane domains into subtypes based on their reactivity with antibodies directed against specific raft constituents.

More recently, immunoaffinity-based methods have been used to demonstrate that different GPI-anchored proteins reside in distinct subsets of lipid rafts. Using antibodies against the GPI-anchored prion protein, Madore et al. [20] showed that immunoaffinity purification of detergent-resistant membranes from rat and mouse brain membranes resulted in the isolation of a subset of rafts that contained essentially all of the prion protein, but less than 10 % of the Thy-1 protein. Similarly, Drevot et al. [21] immunoprecipitated raft membrane vesicles from 3A9 cells with

either a Thy-1-specific or a CD3ε-specific monoclonal antibody. They found that immunodepletion of vesicles with the anti-CD3ε antibody did not significantly affect the amount of Thy-1 that could subsequently be immunoprecipitated with anti-Thy-1 antibodies. These findings suggest that prion protein or CD3ε is present in only a small subset of lipid rafts, whereas the bulk of the Thy-1 is distributed into other detergent-resistant domains.

These immunopurification studies clearly show that not all rafts share the same protein components. These observations cannot be explained by models I and II in Figure 1, since a single method of raft preparation was shown to yield subsets of rafts that contained different proteins. These observations suggest that there is intrinsic heterogeneity in the raft population and are only consistent with model III.

Direct visualization of raft heterogeneity

Immunofluorescence was used to demonstrate that different GPI-anchored proteins exist in discrete domains in intact cells. GPI-anchored folate receptors labelled with fluorescent monoclonal antibodies were shown to be distributed diffusely over the cell surface. Upon cross-linking with a secondary antibody, the folate receptors redistributed into punctate foci. However, Thy-1 remained diffusely distributed [39]. These findings indicate that these two GPI-anchored proteins reside in distinct rafts that have no significant interaction with each other.

The most striking demonstrations of heterogeneity among lipid rafts are those that use immunofluorescence to show the discrete localization of raft proteins and lipids in living cells. Gomez-Mouton et al. [40] demonstrated that ganglioside GM3 and the GPI-anchored protein, urokinase plasminogen activator receptor, were localized exclusively at the leading edge of the polarized T-cells. Conversely, ganglioside GM1 and the raft-localized transmembrane domain protein, CD44, were localized at the trailing edge of the cells. All four of these components were present in the low-density detergent-insoluble fraction of Jurkat cells and all four markers were lost from this fraction when the cells were treated with methyl-β-cyclodextrin to deplete cholesterol from the cells. Thus, although these raft markers were present in the total lipid raft fraction that could be isolated biochemically from the cells, in intact cells the rafts that contained these markers were physically distinct and spatially segregated from each other. Similar findings have been reported in mating yeast [41]. Following stimulation with α-factor, yeast undergo cell-cycle arrest and grow in a polarized fashion towards their mating partner. Under these conditions, a variety of mating-specific proteins, such as Fus1p and Gas1p, are expressed and become localized to the tip of the mating projection. In addition, ergosterol is concentrated at the tip of the mating shmoo. Both mating projection proteins, as well as ergosterol, are found in low-density detergent-resistant fractions of the cells. However, the glucose transporter Hxt2, which is also recovered in the low-density detergent-resistant fraction of the cells, is excluded from the tip of the mating projection. These data suggest that there is selective migration of a subset of lipid rafts into the tip of the mating projection where they are segregated from other lipid rafts that contain proteins that are not involved in the mating process. Besides demonstrating heterogeneity in rafts, these findings have a broader implication – namely that compositionally different rafts subserve different functions within the cell.

These immunofluorescence studies are difficult to reconcile with any model of raft structure that does not include pre-existing heterogeneity among the population of lipid rafts. Together with the data on differential detergent sensitivity of raft proteins, as well as the ability to separate rafts into subtypes that contain some

proteins, but not others, these data strongly support the view that rafts represent a collection of related domains that differ in both their protein and lipid constituents (model III in Figure 1).

DEVELOPING HETEROGENEITY THROUGH CROSS-TALK BETWEEN RAFT CONSTITUENTS

How is this heterogeneity in raft protein and lipid composition established and maintained? As discussed below, the answer seems to be that there is cross-talk between raft proteins and raft lipids that ultimately determines the composition of a lipid raft. Rafts appear to be dynamic structures that reflect the specific lipid and protein composition of a membrane, and respond to transient changes in the level of these constituents with changes in raft composition. This section describes the role of cholesterol and sphingolipids in the formation of lipid rafts and summarizes the evidence for cross-talk between raft lipids and raft proteins. These data provide additional support for the concept of pre-existing heterogeneity among the lipid raft population.

Cholesterol and lipid rafts

Phospholipids that contain saturated and unsaturated acyl chains of variable length tend to exist in membranes in a liquid crystalline state in which the acyl chains are fluid and disordered. In contrast, sphingolipids generally have long saturated acyl chains, which are capable of packing tightly together to form a gel phase. In the absence of other lipids, model membranes that contain only phospholipids and sphingolipids phase-separate into a highly ordered gel phase made up of the sphingolipids and a disordered phase that contains the phospholipids [18]. Addition of cholesterol to such a mixture permits the formation of the so-called liquid-ordered phase in which saturated acyl chains are highly organized, as in the gel phase, but exhibit lateral mobility more similar to that in the liquid crystalline phase [18,42]. Thus lipids in the liquid-ordered phase are less ordered than those in the gel phase, but are more ordered than those in the liquid-crystalline phase. Cholesterol is permissive for the formation of the liquid-ordered phase and hence determines the general physical properties of lipid rafts.

Although cholesterol appears to be critical for domain formation, the absolute configuration of the sterol molecule is not important for its ability to support lipid raft formation. Westover and Covey [43] synthesized the enantiomer of cholesterol in which the stereochemistry of cholesterol was reversed at each of its chiral centres. When cells were depleted of cholesterol and then repleted with either natural cholesterol or enantiomeric cholesterol, both forms of cholesterol showed a similar ability to reconstitute lipid rafts, as judged by the ability of raft proteins to partition into the low-density fractions of a membrane preparation [44]. These data suggest that the interaction of cholesterol with other lipids is not chiral in nature and demonstrate that it is the general structure, rather than the absolute configuration, of cholesterol that allows it to support the formation of the liquid-ordered phase.

Given that cholesterol is crucial for the generation of the liquid-ordered phase, it follows that alterations in the cholesterol content of cells should lead to changes in the properties of these domains. Indeed, many studies have shown that depletion of cholesterol from cells leads to the disruption of lipid rafts and the release of raft constituents into the bulk plasma membrane [45–51]. However, not all lipid rafts appear to be equally sensitive to cholesterol depletion. For example, depletion of cholesterol from enterocyte explants by treatment with methyl-β-cyclodextrin removed 70 % of the microvillar cholesterol, but did not affect the ability of a raft marker protein, galectin-4, to localize to the low-density Triton-X-100-insoluble membrane fraction [52]. Similarly, Rajendran

et al. [53] showed that, in Jurkat cells and U937 cells, several raft proteins including lck, lyn and LAT (linker for activation of T-cells) were released from rafts by treatment with methyl-β-cyclodextrin, but flotillins remained in low-density detergent-resistant domains. These findings suggest that some rafts require less cholesterol than others to maintain their integrity or that some rafts retain their cholesterol more effectively than others in the face of global cholesterol depletion. In either case, the findings suggest that there is heterogeneity in the lipid raft population in terms of its dependence on or interaction with cholesterol.

With respect to the 'cholesterol-independence' of lipid rafts, a cautionary tale is told by the data of Schuck et al. [25]. These workers found that extraction of >70% of the cholesterol from intact MDCK (Madin–Darby canine kidney) or Jurkat cells using methyl-β-cyclodextrin did not affect the detergent-insolubility of lipid rafts. However, extraction of approx. half of the cholesterol from membranes isolated from MDCK or Jurkat cells led to the disruption of lipid rafts as evidenced by a loss of raft marker proteins from the detergent-insoluble fraction [25]. Thus sensitivity to methyl-β-cyclodextrin apparently depends upon the exact conditions under which the depletion is carried out. Although this caveat applies to the observation of a generalized insensitivity of rafts to cholesterol depletion, findings, such as those of Rajendran et al. [53], of differential sensitivity to cholesterol depletion are consistent with the existence of heterogeneity in either the overall content of cholesterol in different rafts or the affinity of cholesterol for particular rafts.

Cross-talk involving sterols

Differences in the cholesterol dependence of rafts may derive from variations in the concentrations of other raft lipids. In model membrane systems, small amounts of ceramide were found to significantly stabilize domain formation in mixtures of sphingo-myelin, phosphatidylcholine and cholesterol [54]. Furthermore, cerebrosides were shown to support the formation of membrane domains in the absence of cholesterol, and the addition of sterols to such membranes did not significantly increase the ability of domains to form. Thus some combinations of lipids may indeed give rise to rafts that are less dependent on cholesterol for their stability.

The cholesterol content of membranes in turn modulates the ability of other raft lipids to become incorporated into these domains. In HL-60 cells, exogenously added ganglioside GM1 was shown to become incorporated into lipid rafts in control cells. Depletion of cholesterol did not affect the ability of GM1 species acylated with a fatty acid longer than 12 carbon atoms in length to partition into detergent-resistant domains. However, raft partitioning of GM1 species acylated with fatty acid chains shorter than 12 carbon atoms was significantly reduced by prior depletion of cholesterol from the cells [55]. This suggests that changes in cholesterol content lead to alterations in the physical environment of the remaining rafts and consequently change the kind of lipids that can partition into these domains.

In addition to being modulated by other lipids, raft cholesterol levels are also affected by raft protein content. For example, expression of caveolin-1 in cells that do not normally express the protein results in a 50% increase in the amount of cholesterol found in the isolated lipid raft fraction [16]. Similarly, blocking the synthesis of GPI-anchored proteins in cells that lacked caveolae caused an increase in cholesterol levels [56]. Although caveolin-1 is a known cholesterol-binding protein and could theoretically affect cholesterol levels through increased binding of this sterol, it is doubtful that enough caveolin could be expressed to achieve such a large increase in cellular cholesterol levels. Furthermore, this

mechanism cannot explain the rise in cholesterol levels that accompanied the loss of GPI-anchored proteins. Thus these data suggest that proteins directly or indirectly affect the cholesterol content of lipid rafts.

The reverse also seems to be true, namely that raft sterol content affects raft protein content. Keller et al. [57] treated rats with an inhibitor of 7-dehydrocholesterol reductase, the final step in cholesterol biosynthesis. This mimics the defect in Smith–Lemli–Opitz syndrome and results in an increase in the content of 7-dehydrocholesterol and a corresponding decrease in cellular cholesterol levels. These workers showed that the 7-dehydrocholesterol became incorporated into rat brain lipid rafts and that this was associated with specific differences in the protein composition of those rafts as judged by two-dimensional gel analyses. Thus changing the nature of the sterol in the rafts led to changes in the protein composition of these domains.

Sphingolipids in lipid rafts

Sphingolipids are derivatives of the long-chain amino alcohols, sphingosine and dihydrosphingosine. Attachment of a fatty acid to the amino group via an amide bond generates a ceramide. Because they contain a long alkyl chain from the sphingosine base, a fatty acid side chain (in an amide bond) and a free hydroxy group (from the alcohol), ceramides are functionally similar to diacyl-glycerol.

Like diacylglycerols that are converted into different glycero-phospholipids by the addition of different head groups, ceramides are converted into different sphingolipids by the addition of different head groups to the free hydroxy group. With the exception of sphingomyelin that contains a phosphocholine head group, all other sphingolipids bear sugar head groups and hence are glyco-sphingolipids. The cerebrosides contain a single sugar, typically glucose or galactose. Gangliosides contain multiple sugars in their head group.

The long alkyl chain of the sphingosine base is saturated and sphingolipids are frequently acylated with saturated fatty acids. Thus a noteworthy aspect of the structure of sphingolipids is the presence of two long saturated alkyl chains. It is these alkyl chains that can be organized and condensed by sterols to form the liquid-ordered phase of lipid rafts. The importance of the saturated acyl chains of sphingolipids in raft stability is demonstrated by the finding that ganglioside GM1 acylated with a saturated fatty acid could be recovered in detergent-resistant lipid rafts, whereas a similar GM1 species acylated with an unsaturated fatty acid did not partition into rafts [55].

Another characteristic of sphingolipids that is relevant to their role in raft formation is their unequal distribution across the lipid bilayer. Unlike cholesterol that seems to be present in both leaflets of the membrane bilayer, sphingolipids distribute preferentially into the exofacial leaflet of the membrane at a ratio of 6:1 [28]. As a result, sphingolipids are important in determining the properties of rafts on the outer leaflet of the membrane, but are likely to be much less important in the formation and properties of inner-leaflet rafts. Although there is evidence that rafts are bilayer structures, the asymmetric distribution of sphingolipids ensures that the composition of rafts of each leaflet is different. These differences probably affect the ability of the different leaflets to accommodate and interact with the various raft proteins.

Like cholesterol, sphingolipids are universally found to be enriched in lipid rafts as compared with bulk plasma membrane [4,16,17]. However, rafts are able to form in the absence of some classes of sphingolipids. A line of MEB-4 melanoma cells lacks ceramide glucosyltransferase, the first enzyme in the biosynthetic pathway for glycosphingolipids. As a result, these cells do
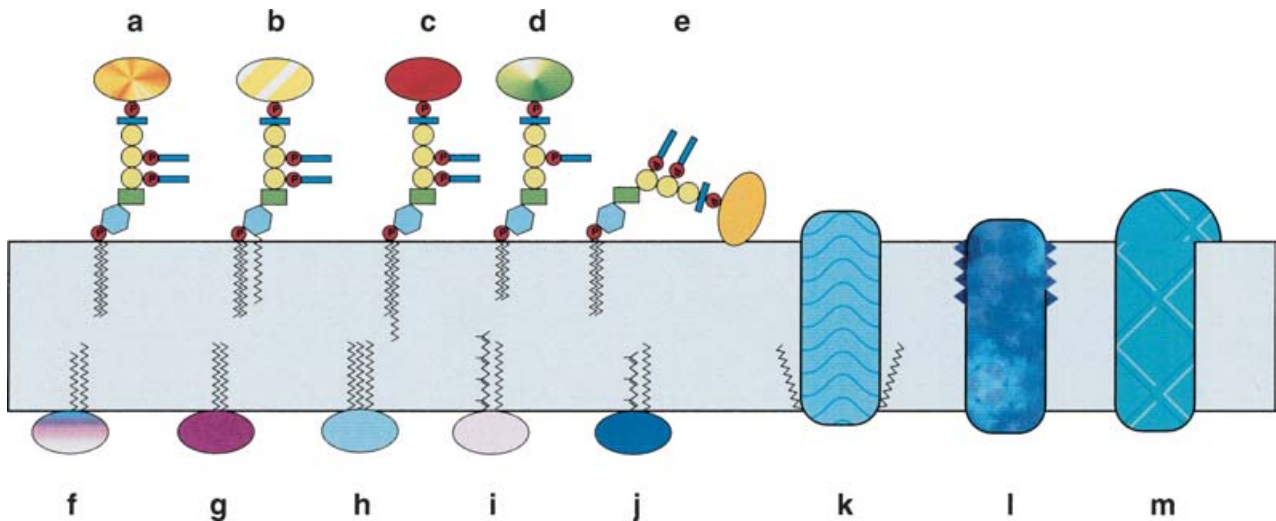
Figure 2   Heterogeneity in the mechanisms through which proteins are targeted to lipid rafts

(**a**) GPI-anchored protein in which the phosphatidylinositol moiety contains two $C_{18:0}$ acyl groups. (**b**) GPI-anchored protein in which the inositol head group is acylated. (**c**) GPI-anchored protein in which the glycerophospholipid moiety is replaced with a ceramide. (**d**) GPI-anchored protein in which the sn-1 and sn-2 acyl chains have been remodelled to contain myristate. (**e**) GPI-anchored protein in which an additional raft-targeting signal is present in the protein component of the molecule. (**f**) A protein modified by the addition of a myristate and a palmitate group. (**g**) A protein modified by the addition of two palmitate groups. (**h**) A protein modified by the addition of three palmitate groups. (**i**) A protein modified by the addition of a geranylgeranyl group and a palmitate. (**j**) A protein modified by the addition of a farnesyl group and a palmitate. (**k**) A transmembrane protein modified by the addition of two palmitate groups. (**l**) A transmembrane protein targeted to rafts via interaction of amino acid residues with the exoplasmic leaflet of the plasma membrane. (**m**) A transmembrane protein targeted to rafts by interaction of its extracellular domain with raft constituents. The list is not complete as other examples are known to exist.

not make glycosphingolipids [58]. Despite the absence of glyco-sphingolipids, Ostermeyer et al. [59] showed that detergent-resistant membranes could be isolated from these cells. The properties of these glycosphingolipid-deficient rafts were similar to those of rafts from wild-type cells, including the fluidity of the membrane and their sensitivity to cholesterol depletion by methyl-β-cyclodextrin. Analysis of the lipids present in the detergent-resistant domains confirmed the absence of glycosphingolipids, but demonstrated that the level of sphingomyelin had been increased so that the total level of sphingolipids in rafts from the gly-cosphingolipid-deficient cells was similar to that in rafts from wild-type cells. These data suggest that the sphingolipid requirement for the generation of lipid rafts is somewhat flexible and are consistent with the possibility that different rafts contain different complements of sphingolipids.

Cross-talk involving sphingolipids

It is clear from model membrane studies that sphingolipids are important in organizing the lipid platform of rafts [54]. However, these lipids also appear to be involved in determining the nature of the proteins that partition into these microdomains. In MDCK cells, addition of exogenous gangliosides induced the loss of a GPI-linked form of growth hormone decay-accelerating factor from the detergent-insoluble fraction of cells [60]. Conversely, degradation of cell-surface glycosphingolipids by treatment of cerebellar granule cells with exogenous endoglycoceramidase resulted in the shift of the GPI-anchored protein, TAG-1, from high-density membranes into the low-density lipid raft fraction of cells [61]. Overexpression of GM2/GD2 synthase and GM1 synthase in Swiss 3T3 cells resulted in the generation of cells in which the levels of ganglioside GM1 were markedly increased both in membranes and in detergent-resistant rafts [62]. This increase in GM1 was associated with a decrease in the propensity of the PDGF (platelet-derived growth factor) receptor to partition into the lipid raft fraction. Similarly, Wang et al. [63] used anti-

sense constructs to block the metabolism of ganglioside GM3 and succeeded in increasing the endogenous levels of GM3 in SCC12 cells approx. 2-fold. This elevation of GM3 was associated with a shift of caveolin-1 from the detergent-insoluble fraction to the detergent-soluble fraction of the cells. Together, these data suggest that alteration of the sphingolipid content of rafts leads to changes in the partitioning of proteins into these domains.

The interaction of proteins with rafts

The observation that raft protein composition can be affected by raft lipid content and vice versa suggests that raft proteins play a role in the determination of raft structure. In terms of heterogeneity, proteins represent the most significant source of diversity in rafts because of their overall structure and also because of how they interact with raft components. A plethora of mechanisms ranging from lipid modifications, such as GPI anchors, to protein-based signals are used to target proteins to lipid rafts. The different types of raft-targeting signals are shown schematically in Figure 2 and are described in detail below. It is apparent from Figure 2 that there are substantial structural differences even within a single class of targeting signal. Because each type of targeting signal would interact differently with raft constituents, each could alter the lipid content of these domains in a different way. Thus the mechanisms through which proteins are targeted to rafts may contribute significantly to the generation of heterogeneity in these domains. This section describes the different signals that are known to target proteins to lipid rafts and discusses the implications of these differences in terms of the ability of proteins to interact with and modify the composition of lipid rafts.

GPI-anchored proteins

One of the first classes of proteins shown to be targeted to lipid rafts was the GPI-anchored proteins [4,64–68]. GPI anchors are built on a phosphatidylinositol moiety that inserts into the exoplasmic leaflet of the membrane. A head group is constructed

on the inositol of the phospholipid and contains a glucosamine, three mannose residues and a phosphoethanolamine that is linked in an amide bond to the C-terminal residue of the protein. In different cell types and species, this core head group structure is modified by the addition of extra sugar residues and the acylation of the inositol group [69,70].

Substantial heterogeneity exists within the lipid moiety of the GPI anchor. GPI anchors typically contain a phosphatidylinositol that is a diacyl lipid. However, some GPI-anchored proteins contain alkyl-acyl groups [71,72] rather than acyl-acyl groups. Furthermore, the length of the acyl groups on the phosphatidylinositol species can differ significantly among anchors. For example, in *Leishmania*, a major type of GPI-anchor contains $C_{24:0}$ or $C_{26:0}$ alkylacyl-phosphatidylinositol species, while another form of anchor contains $C_{18:0}$ alkylacyl-phosphatidylinositol [73]. Additional heterogeneity in the lipid moiety is introduced by remodelling both after synthesis of the anchor and after its attachment to proteins. One type of lipid remodelling involves the sequential replacement of *sn*-2 and *sn*-1 fatty acids with myristate. A second type of remodelling, which occurs mainly in yeast, involves the exchange of the glycerophospholipid moiety of the phosphatidylinositol with a ceramide. This produces a more sphingolipid-like anchor containing inositolphosphoceramide, which tends to contain very long acyl chains (for review see [74]). Although not all modifications of GPI anchors take place in all cells, it is clear that within a given cell, the pool of GPI-anchored proteins can exhibit significant heterogeneity even when one considers only the lipid parts of this molecule. Obviously, proteins modified with GPI anchors that have vastly different acyl chain lengths or that contain an additional acyl group on the inositol ring would interact very differently with lipid rafts.

Further heterogeneity is introduced into GPI-anchored protein targeting signals by structural features of the protein itself. Removal of the GPI anchor either through mutation or enzymic digestion with a phosphatidylinositol-specific phospholipase C generally results in the production of a soluble protein, demonstrating that the GPI anchor is responsible for membrane localization, as well as raft targeting. However, mutation of the prion protein to remove its signal for attachment of a GPI anchor or to replace the signal with a transmembrane domain, resulted in prion proteins that still targeted to lipid rafts [75]. Additional studies localized the GPI-anchor-independent raft-targeting sequence to a short region in the N-terminus of the prion protein. Thus, although the prion protein is GPI-anchored, its ability to target to rafts does not depend on this targeting signal, but is instead due to a protein-based targeting motif.

These data indicate that, far from being a uniform targeting signal, GPI anchors are structurally heterogeneous and their targeting signal may be modified or superseded by sequences contributed by the protein moiety. If different combinations of anchor and protein structures alter the affinity of proteins for rafts, this could contribute to the partitioning of various GPI-anchored proteins into distinct subsets of rafts. Furthermore, because the anchors themselves contribute to the lipid content of rafts, the presence of a GPI-anchored protein in a raft could induce alterations in the lipid environment due to cross-talk between the anchor and other raft lipids.

Fatty acylation

A second type of modification that serves to target proteins to lipid rafts is fatty acylation [76–81]. Included in this group are modifications such as N-myristoylation and palmitoylation. In general, it has been found that at least two fatty acyl groups are required to target proteins to lipid rafts, either a myristate and a palmitate [78,79] or two palmitate groups [82,83]. However, some proteins contain three or more fatty acyl groups including combinations of palmitate and myristate or simply multiple palmitoylations [84]. Clearly, variations in the number and length of the acyl groups attached to proteins would generate differences in their affinities for lipid rafts.

As is the case for GPI-anchored proteins, it appears that acylation-based targeting signals can be modified by targeting motifs present in the protein moiety. Although many fatty acylated proteins are soluble proteins that are targeted to membranes as a result of the lipid modification, numerous transmembrane domain proteins have been shown to be palmitoylated [85–88]. In some cases, the palmitoylation appears to be important for localization to lipid rafts [85]. Thus palmitoylation may function in concert with a transmembrane domain anchor to promote raft localization.

McCabe and Berthiaume [89] reported that dually acylated GFPs co-localized with raft markers such as GM1, but were not recovered in a detergent-resistant membrane fraction [89]. This suggests that, in some cases, multiple acylations may not be sufficient to target a protein to detergent-insoluble lipid rafts. These authors hypothesized that additional protein-based interactions may be required to draw acylated proteins into the detergent-resistant core of lipid rafts. Similarly, protein-based signals may be important in the targeting of proteins modified by farnesyl or geranylgeranyl groups.

The presence of prenyl groups has been shown to reduce the tendency of proteins to partition into lipid rafts [79,81], possibly because the bulky branched structure of these groups may not fit well into the ordered structure of lipid rafts. However, Ras proteins that are both prenylated and palmitoylated [90] have been found to be localized to lipid rafts [91], suggesting that factors in addition to the lipid modifications may contribute to the raft localization of these proteins. As suggested by McCabe and Berthiaume [89], the lipid modifications may target the protein to the membrane but protein–protein interactions may be necessary to direct the molecule to lipid rafts.

Like GPI anchors, the raft-targeting signals provided by the acylation and prenylation of proteins are heterogeneous in terms of the general type of lipid modification, as well as the number and length of the lipid groups. The data suggest that the lipid modification is necessary, but, in some cases, may not be sufficient to direct proteins to lipid rafts. Additional protein-based motifs may co-operate with these lipid modifications to specifically target proteins to lipid rafts. Thus extensive heterogeneity exists within acylated proteins in terms of how they interact with rafts. These differences could be responsible for targeting acylated proteins to different subsets of rafts, possibly modifying the composition of the targeted rafts.

Protein-based raft-targeting motifs

In addition to the lipid–lipid interactions described above that serve to target proteins to lipid rafts, protein–lipid and protein–protein interactions also appear to be important in localizing some proteins to lipid rafts. The influenza virus haemagglutinin (HA) protein has been shown to be present in Triton-X-100-resistant membranes and this localization is dependent on cholesterol. Mutational analysis demonstrated that the ability of the HA protein to target to lipid rafts was dependent on the presence of amino acids in the transmembrane domain that contact the exoplasmic leaflet of the membrane [92]. Thus the transmembrane domain of HA appears to interact with a membrane lipid or protein thereby targeting this protein to lipid rafts.

The transmembrane domain of proteins may also target proteins to rafts based solely on the length of the transmembrane segment.
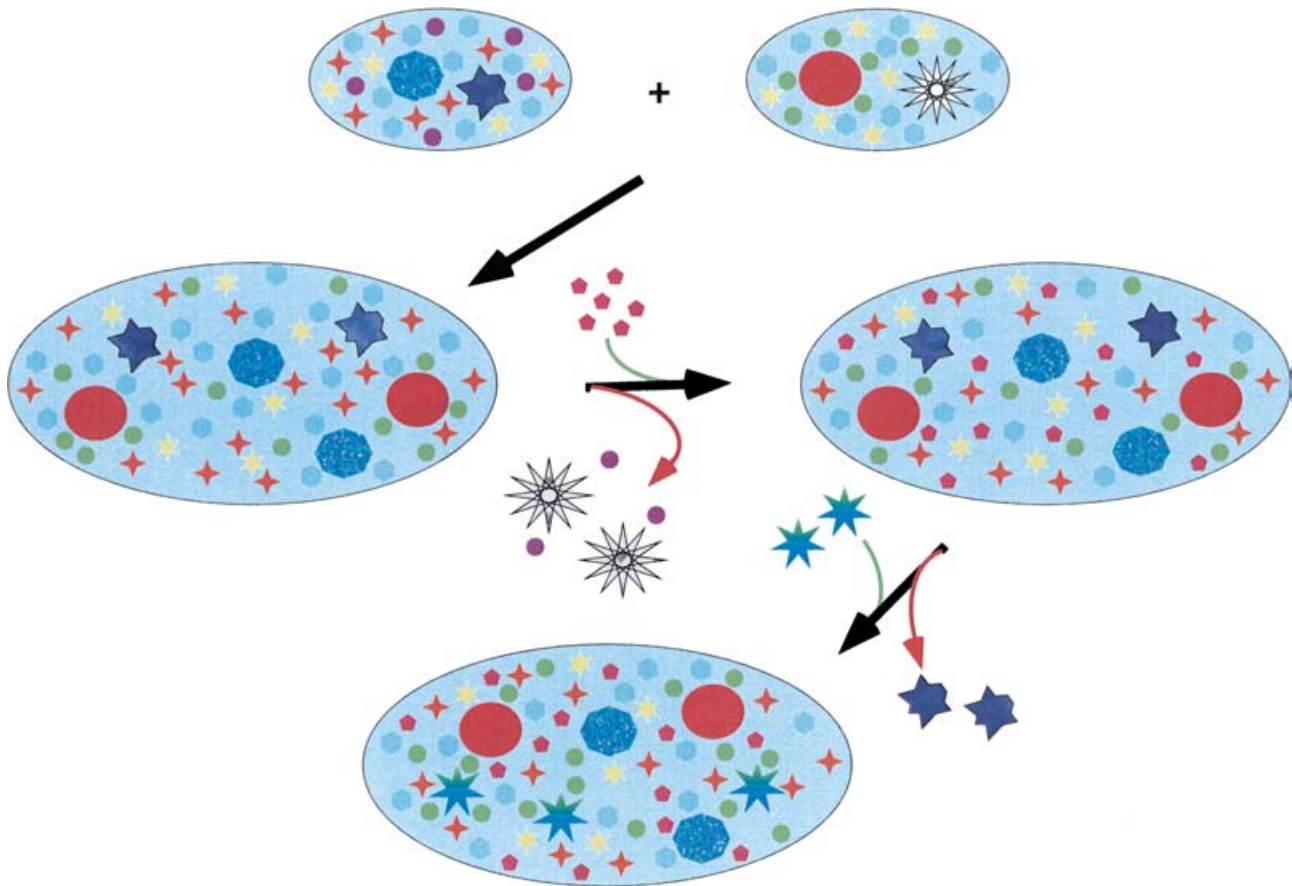
Figure 3   The Induced-Fit Model of Raft Heterogeneity

Small shapes represent lipid constituents of rafts. Larger textured shapes represent protein components of rafts. See the text for a complete description.

Membranes containing cholesterol tend to be thicker than membranes that do not contain the sterol [93]. Munro [94] showed that in single transmembrane domain proteins, the addition or removal of residues from the transmembrane domain altered the ability of the protein to target to rafts and be sorted to the plasma membrane. When the transmembrane domain was greater than 23 residues, the protein sorted to the cell surface, but it was retained in the Golgi when the transmembrane domain was less than 17 residues [94]. Thus the overall length of a transmembrane domain may predispose the protein to partition into the thicker membrane of lipid rafts.

Domains of proteins other than the transmembrane domain also appear to contain information that causes localization of proteins to rafts. Cbl-associated protein (CAP) is a cytosolic adapter protein that contains three C-terminal SH3 (Src homology 3) domains and an N-terminal region with homology with the gut peptide, sorbin. This sorbin homology (SoHo) domain has been shown to mediate the interaction of CAP with the resident raft protein, flotillin. This leads to the recruitment of CAP into lipid rafts [95]. Similar SoHo domains are also present in vinexin-α and ArgBP2, and, in the case of vinexin, have been shown to mediate raft localization via association with flotillin [95]. Yamabhai and Anderson [96] used mutagenesis to show that raft-targeting information is contained in the membrane proximal cysteine-rich region of the epidermal growth factor receptor. As noted above, the N-terminal region of the ectodomain of the prion protein, a GPI-linked protein, was found to contain a sequence that targeted the protein to rafts even in the absence of the GPI anchor [75]. These observ-

ations suggest that protein–protein interactions and possibly protein–lipid interactions can induce the partitioning of proteins into lipid rafts. Because of the tremendous flexibility available in protein-based targeting sequences, this mechanism offers the most opportunities to introduce heterogeneity into the distribution of proteins into rafts of differing protein and/or lipid composition, and to likewise induce changes in the other constituents of rafts.

THE INDUCED-FIT MODEL OF RAFT HETEROGENEITY

The results of a variety of studies strongly support the conclusion that lipid rafts are not a uniform population. Rather, rafts appear to comprise a mixture of domains that differ in their protein and lipid composition. Such heterogeneity is probably required for rafts to subserve the many different functions in a cell that have been attributed to them. But how is this heterogeneity generated using the proteins and lipids that are known to be constituents of lipid rafts?

Proteins are the most intrinsically heterogeneous components of lipid rafts in terms of the way in which they interact with the other raft lipids and proteins. Thus it seems logical that proteins would provide the organizing principle for the development of different types of rafts. The finding that there is cross-talk between raft proteins and raft lipids provides a mechanism through which the composition of a raft can respond to changes in the environment introduced by the partitioning of a specific protein into that domain. Based on these principles, the mechanism depicted in Figure 3 can be proposed to explain the generation of

heterogeneity among lipid rafts in a single cell. It is referred to as the 'Induced-Fit Model of Raft Heterogeneity'.

The central tenet of this model is that individual raft proteins interact differently with raft lipids and, as a consequence, induce a remodelling of the raft constituents to best 'fit' the structure of that protein or proteins. In this model, a raft starts out as a small cluster of glycosphingolipids and cholesterol surrounding one or a few protein molecules. The interaction/fusion of such a 'proto-raft' with a second proto-raft, possibly via protein–protein interactions, would produce a raft with a different physical environment than was present in either proto-raft. Because of cross-talk between the raft proteins and lipids, this would trigger a remodelling of the newly formed raft to optimize the stability of the new structure. This might be accomplished by excluding some proteins and lipids from the raft while recruiting others. The new proteins and lipids would in turn cause changes in the physical environment of the raft, which would result in the recruitment/exclusion of other proteins and/or lipids to maximize the stability of the raft of the remodelled composition. Continuing iterations would yield rafts of a unique composition, specifically tailored to the structure of its protein components, their interactions with each other and their interactions with lipids.

By relying on the diversity of interactions between raft lipids and raft proteins, this mechanism can explain readily how rafts of different protein and lipid composition can form within a single cell. The model also explains how changes in the lipid composition of a membrane could modify the ability of individual proteins to partition into rafts. Altering the availability of specific raft lipids would shift the normal equilibrium between the proteins and lipids in rafts, resulting in a change in the physical properties of the rafts. This would alter the affinity of some proteins for the modified rafts and lead to the recruitment to or release of proteins from those rafts. Differing physical environments that allow some rafts to retain their 'preferred' lipids in the face of global depletion of that lipid could explain why some rafts appear to be cholesterol-independent, whereas others are highly cholesterol-dependent. Since the model allows for multiple different signals on a single protein to influence raft localization and remodelled composition, it also explains how proteins, such as GPI-anchored proteins, that are seemingly similar in the way they interact with rafts, can come to reside in domains of different lipid composition and physical properties.

This model for the generation of heterogeneity in rafts shares some features of the 'lipid shell' model for the targeting of proteins to rafts that was proposed by Anderson and Jacobson [97]. In that model, membrane proteins that have an affinity for self-assembled condensed complexes of cholesterol and phospholipids become surrounded by a shell of these lipids. These shelled proteins are predisposed to interact with lipid rafts either through protein–protein or lipid–lipid interactions, and this serves to target the proteins to lipid rafts. Although the Induced-Fit Model of Raft Heterogeneity begins with the simple clusters of proteins and lipids proposed in the lipid shell model, it differs from the shell model in that it proposes a continuous remodelling of the protein and lipid composition of the raft, based on the accretion of additional compatible proteins and lipids, and the exclusion of incompatible constituents.

CONCLUSIONS AND FUTURE DIRECTIONS

Lipid rafts are membrane domains that have been implicated in diverse cellular processes, including signal transduction, endocytic events, such as viral entry, and cholesterol trafficking. This diversity in function has apparently given rise to a diversity in

structure, as recent evidence indicates that lipid rafts are heterogeneous in both their protein and their lipid composition. Data suggesting the existence of cross-talk between raft proteins and lipids provides the basis for understanding how changes in the level of one raft constituent could affect the levels of other components of these domains, resulting in rafts of different compositions.

The Induced-Fit Model of Raft Heterogeneity synthesizes the findings regarding raft heterogeneity and offers a model to explain how this heterogeneity is established and maintained. Future studies on raft biology will need to test this paradigm to determine whether it represents an accurate picture of the heterogeneity in lipid rafts and how it develops.

A second important question that is raised by the finding of raft heterogeneity is, why are rafts different? The logical answer is because they perform different tasks in the cell, but there is currently no evidence to support this conjecture. Addressing this question could be difficult and may require the development of additional tools to allow the visualization, purification and analysis of subsets of lipid rafts.

Singer and Nicholson [98] first proposed the fluid mosaic model of cell membranes in 1972, but it was not until some 20 years later that it was appreciated that cell membranes exhibit fine structure in the form of lipid rafts. The last decade has seen an explosion in our knowledge of these domains, including the recognition that not all rafts are alike. Given the rate of progress in this area, we will not have long to wait before we understand the why and the how of lipid raft heterogeneity.

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