Progress in Lipid Research 49 (2010) 390–406

Contents lists available at ScienceDirect

Progress in Lipid Research

journal homepage: www.elsevier.com/locate/plipres

Review

A lipid matrix model of membrane raft structure

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ARTICLE INFO

Article history:
Received 7 April 2010
Accepted 6 May 2010

Keywords:
Membrane rafts
Detergent-resistant membranes
Signalosomes
Lipid-anchored proteins
Rafts

ABSTRACT

Domains in cell membranes are created by lipid–lipid interactions and are referred to as membrane rafts. Reliable isolation methods have been developed which have shown that rafts from the same membranes have different proteins and can be sub-fractionated by immunoaffinity methods. Analysis of these raft subfractions shows that they are also comprised of different molecular species of lipids. The major lipid classes present are phospholipids, glycosphingolipids and cholesterol. Model studies show that mixtures of phospholipids, particularly sphingomyelin, and cholesterol form liquid-ordered phase with properties intermediate between a gel and fluid phase. This type of liquid-ordered phase dominates theories of domain formation and raft structure in biological membranes. Recently it has been shown that sphingolipids with long (22–26C) N-acyl fatty acids form quasi-crystalline bilayer structures with diacylphospholipids that have well-defined stoichiometries. A two tier heuristic model of membrane raft structure is proposed in which liquid-ordered phase created by a molecular complex between sphingolipids with hydrocarbon chains of approximately equal length and cholesterol acts as a primary staging area for selecting raft proteins. Tailoring of the lipid anchors of raft proteins takes place at this site. Assembly of lipid-anchored proteins on a scaffold of sphingolipids with asymmetric hydrocarbon chains and phospholipids arranged in a quasi-crystalline bilayer structure serves to concentrate and orient the proteins in a manner that couples them functionally within the membrane. Specificity is inherent in the quasi-crystalline lipid structure of liquid-ordered matrices formed by both types of complex into which protein lipid anchors are interpolated. An interaction between the sugar residues of the glycolipids and the raft proteins provides an additional level of specificity that distinguishes one raft from another.

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doi:10.1016/j.plipres.2010.05.002

1. Introduction

The segregation of membrane components is known to be required for biogenesis and differentiation of membranes and in the performance of a variety of specific physiological processes. Several models have been proposed to explain different types of clustering of proteins and lipids that are observed in biological membranes. The models differ in whether lipid–lipid, lipid–protein or protein–protein interactions provide the primary driving force responsible for creation of domains. The notion of membrane domains has origins in capping, patching and diffusional motion of membrane antigens on the surface of lymphocytes cited in evidence to support the fluid mosaic model of membrane structure [1]. The involvement of the underlying cytoskeletal network in membrane domain formation in many cases [2] including the creation of membrane junctions [3] underscores the need to factor interactions extrinsic to the membrane into formulation of a plausible model.

The aim of this review is to examine the evidence used to support proposed models of membrane domain formation with the objective of reconciling apparently conflicting views on the subject. Conjectures have arisen mainly because two fundamentally different methodologies have been used to characterise membrane domains. One has been to isolate membrane domains and to determine their composition. The other has been to monitor the motion of components of the cell membrane and make deductions about the causes of motional constraints.

The central argument in the hypothesis I propose is the hitherto unrecognised importance of the N-acyl chain length of the sphingolipids in the formation of different liquid-ordered structures. Thus specific interactions between membrane glycerophospholipids and molecular species of sphingolipids, some with a 4-hydroxysphinganine base, with long (C_{22-26}) and sometimes hydroxylated N-acyl fatty acid substituents to form quasi-crystalline bilayer structures under physiological conditions. By contrast, sphingomyelin with symmetric hydrocarbon chains (C_{16-18}) forms a stoichiometric complex in molar proportions 1.7:1 with cholesterol that also has the properties of a quasi-crystalline liquid-ordered phase. The complex excludes glycerophospholipids and cholesterol in excess of that required to form the structure. The putative function of these quasi-crystalline structures in cell membranes in selecting and assembling particular membrane protein components and how these ordered domains might be coupled on either side of the membrane will be considered.

2. Current models of domain formation

The refinement of models to explain domain formation in cell membranes has been considerably advanced over the past few years by the development of more reliable methods of domain isolation [4,5], analysis [6] and methods of monitoring domain structure and dynamics [7]. Application of these methods has helped to more accurately define structure–function relationships particularly relevant to signalling processes. The main differences between the various models of membrane domain formation centres around the primary forces said to be responsible for creating the domain and the process of selection of domain components from the surrounding membrane. These differences may simply reflect the way different cell types employ proteins to organize membrane lipids or the manner in which different lipids interact to create nanoclusters that amalgamate to form larger domains. Undoubtedly the manner of sorting membrane proteins and lipids in the conduct of cellular business is a complex process. Whether a single model can account for the details associated with each process may turn out to be improbable. Nevertheless, it should be possible to establish some principles that govern domain formation and to propose a heuristic model that can usefully serve as a test bed for experimental examination.

2.1. Domains created by protein–protein networks

Models of domains created by protein–protein interactions without reliance on ordered lipid domains or anchoring to cytoskeletal elements have been proposed on the basis of ultra microscopic observation and measurements of molecular motion. The principles of formation of macroscopic membrane domains reliant only on membrane protein–protein interactions have been well established in the case of the purple membrane of primitive halophylic micro-organisms [8,9]. Thus trimers of bacteriorhodopsin are formed with tightly bound lipids in the fluid lipid bilayer and associate/dissociate at the hexagonal crystal lattice interface of assembled purple membrane. The organism does not have sterols or a cytoskeleton and coupling of light-driven proton transport, mediated by bacteriorhodopsin, with ATP synthesis is indirect via a proton gradient. Protein–protein interactions also appear to dominate the creation of chemotaxis receptor complexes in bacterial membranes [10].

Microscopic membrane domains or clusters, as opposed to phase-separated membrane protein structures, created by protein–protein interactions without reliance on ordered lipid domains or anchoring to cytoskeletal elements have been described [11]. In these models specific lipids and proteins constituting signalling complexes are believed to be trapped within a network created by protein–protein interactions, possibly involving scaffolding proteins. The association with signalling components is said to be mediated by protein phosphorylation. The components of the signalling clusters are exchangeable on different time-scales, some static over several minutes while others exist in two distinct populations, one relatively mobile the other immobile. It is argued that marked differences in mobility of raft-associated proteins indicate that they are not components of a single signalling complex. Moreover, some raft-associated lipid-anchored proteins and mutant proteins unable to interact through phosphorylation are not associated with signalling clusters. Furthermore, it is claimed that the degree of immobility of membrane proteins is poorly correlated with proteins associated with detergent-resistant membrane fractions.

2.2. Protein corralling domains

The direct involvement of the cytoskeletal network has been invoked to explain the constrained lateral diffusion of membrane proteins [12]. According to this model membrane domains are created by the erection of barriers to lateral diffusion of lipids

and proteins. The barriers are portrayed as a picket fence composed of intrinsic membrane proteins anchored to the cytoskeleton. Evidence cited in support of these models includes the particular motion of lipids and proteins in biological membranes that are consistent with a coral-like environment which is lost on disruption of the underlying cytoskeleton. Filtering components between domains is governed by the size of the diffusing molecular complexes. Thus the formation of oligomeric conformers representing signalling complexes prevents their passage through the barrier and they are physically constrained to a raft platform. The signalling complexes are comprised not only of proteins but there is evidence that phospholipids like phosphatidylethanolamine undergo motion that appears consistent with tethering to elements of the cytoskeleton. Typical of such studies is the apparent confinement of the GPI-anchored protein, CD59 [13]. It was demonstrated that aggregated CD59 molecules undergo a temporary confinement that is dependent on the presence of cholesterol in the membrane, an intact actin-filament network and Gαi2-dependent Src-family kinase activation.

The participation of cytoskeletal proteins in organizing cell surface receptors has been defined in the case of cell adhesion processes [14]. Clustering of proximal nanodomains of integrin LFA-1 and GPI-anchored proteins in the unstimulated cell are induced to combine to form supramolecular platforms competent in facilitating cell adhesion. It was found that clustering of integrin LFA-1 on the surface of monocytes did not depend on cholesterol in the membrane but the receptors were only able to bind to ICAM ligands when incorporated into lipid rafts. The formation of the cell adhesion platform required participation of the cytoplasmic network. Other examples of the participation of cytoplasmic proteins in assembling cell membrane components include the participation of clathrin in the formation of coated pits [15] and the role of matrix protein, MA, in the assembly of viral envelope components at the budding site [16,17].

2.3. Lipid shell model

The principle underlying this model is that individual receptor molecules are surrounded by a shell of cholesterol and sphingolipids that serve to target the protein they are associated with to pre-existing domains of similar lipoprotein complexes. The rafts formed by association of lipoprotein complexes are said to reach diameters in the region 50–200 nm. In this model specific protein–lipid interactions determine the lipid environment of the signalling platform [18,19]. Accordingly, rafts are formed by self assembly of sphingolipids and cholesterol around individual GPI-anchored protein by a combination of physical interactions including specific protein–sphingolipid binding, glycan–sphingolipid interactions, hydrophobic mismatch and water exclusion from cholesterol. Connection to the underlying cytoskeleton occurs via incorporation of transmembrane proteins into the aggregated condensed lipid complexes. The condensed complexes on the cytoplasmic leaflet of the membrane bilayer are comprised of cholesterol and acidic phospholipids like phosphatidylserine that are able to interact with basic adaptor proteins such as lipid-anchored MARCKS that provide a link to the cytoplasmic network.

Evidence of the involvement of the transmembrane protein, Csk-binding protein, in the recognition and immobilization of GPI-anchored Thy-1 clusters on fibroblast membranes has been obtained [20]. Thy-1 co-localizes with Csk-binding protein which provides a transient anchorage of the Thy-1 raft. The anchorage is said to involve phosphorylation of Csk-binding protein and possibly other Src-family kinase substrates in reactions mediated by Src-family kinases. The phosphorylated protein is then able to bind to actin filaments via an adapter protein, EBP50, that is believed to associate with GPI-anchored protein clusters. Endocytosis of Thy-1, in common with many other GPI-anchored proteins [21], is accomplished independently of clathrin coated pits.

2.4. Lipid raft model

The basic tenet of lipid raft models is that lipid domains are created in membranes that act as molecular filters to select particular proteins and exclude others. Raft structure is envisaged as an asymmetric bilayer with sphingolipids located on the exoplasmic leaflet and glycerophospholipids, like phosphatidylserine and phosphatidylethanolamine located in the cytoplasmic leaflet with cholesterol distributing between the two leaflets [22]. The rafts are surrounded by fluid bilayers comprised mainly of unsaturated phosphatidylcholines. Specific transmembrane proteins are interpolated into the raft bilayer together with GPI-anchored receptor proteins located on the exoplasmic side and fatty acid-tethered effector proteins on the opposite side. The two sides of the membrane are said to be coupled by interdigitation of the long N-acyl chains of sphingolipids with cholesterol intercalated with glycerophospholipids in the cytoplasmic leaflet. Trafficking of rafts is mediated by the interaction of caveolin, preferentially with cholesterol on the cytoplasmic surface of the raft which shepherds the rafts into caveolae.

Lateral diffusion of lipids in multi-component bilayer membranes is known to take place through complex concerted, dynamically-correlated motion with surrounding lipids in the form of small clusters [23]. The driving force for creation of larger clusters or domains according to the lipid raft model is the preferential interaction between cholesterol and sphingolipids. Such interactions are known to form a bilayer phase with molecular motion and hydrocarbon chain order intermediate between liquid-disordered or fluid phase and a gel [24]. The clustering of GPI-anchored proteins in the raft takes place by intermolecular hydrogen bonding and voids created by sterically-hindered bulky head groups are largely occupied by cholesterol. The inclusion of intrinsic, membrane-spanning proteins in the raft is as yet unclear in this model [25].

3. What defines a membrane raft?

The existence of membrane rafts and their purported functions in membrane signalling and related processes has been and continues to remain conjectural. The original definition of membrane rafts was operational and comprised a membrane fraction enriched in cholesterol and glycosphingolipids that remain insoluble in the presence of detergent. The detergent-resistant membrane fractions could be distinguished from the parent membrane by their lighter buoyant density as they had a relatively greater mass of lipid relative to protein than the membrane from which they were derived. The definition relies on fidelity of the detergent-resistant membrane fraction to some functional entity in the parent membrane. This criterion has proved to be problematic because the standard detergent isolation method currently employed has serious limitations when it comes to relating the components to their original location in membrane rafts.

3.1. Standard detergent method for isolation of membrane rafts

The standard method of isolation of membrane rafts is to treat cell membranes with Triton X-100 at 4 °C and recover the detergent-resistant membranes from a light fraction in a density gradient centrifugation [26–28]. It is recognised that this procedure results in remodelling of the original membrane such that cholesterol and glycosphingolipids are re-located into new structures that do not exist in the parent membrane [29]. While other

detergents such as sodium cholate, CHAPS, Tween-20, Pluronic F-127, Brij 96V, Brij 98, Lubrol WX and Triton X-110 have been used to prepare detergent-resistant membrane fractions Triton X-100 appears to generate more discrete bands in density gradients that are more amenable to recovery and analysis [30,31]. The contentious issue centres on the relationship between the composition and structure of detergent-resistant membrane fractions prepared by the standard method and domains that are functional components of the membranes of living cells.

There are two aspects to the standard method that give rise to concern. Firstly, the method of isolation involves treatment of the biological membranes at 4 °C for relatively long periods of time. Temperature is known to be a major factor in lipid dynamics and cooling to temperatures below that optimum for growth is known to bring about associations between the lipids and between the lipids and proteins that do not take place *in vivo* at the growth temperature. Secondly, it is possible that by their association with the detergent the membrane lipids and proteins become scrambled in the bilayer so that although putative raft components are present they are not in an arrangement that exists in the living organism [32]. Indeed, examples of instances where proteins from completely different cells are found to be associated with detergent-resistant membrane fractions have been reported [33]. Examination of the structure of detergent-resistant membranes from erythrocytes by freeze-fracture electron microscopy indicates that the original membrane undergoes considerable rearrangement as a result of treatment with Triton X-100 [34]. Thirdly, there is a marked tendency for Triton X-100 to selectively solubilise phospholipids present on the cytoplasmic leaflet of cell membranes which is likely to be responsible for the merger of sphingolipid- and cholesterol-enriched domains [35–37]. It is now becoming obvious that the structural relationship between membranes isolated using the standard method of Triton X-100 treatment at 4 °C and the biological membrane from which they originated is of doubtful significance [38,39].

3.2. Improved strategies for membrane raft isolation

In devising more reliable methods of isolating membrane domains it is necessary to establish agreed criteria for measuring success. These must include: (1) the ability to sub-fractionate membranes into different sets of raft components by immunoaffinity purification methods, (2) demonstration that the different raft receptors or antigens are associated with appropriate transducer elements, (3) evidence that asymmetry of the cell membrane with respect to location of receptors and transducers is preserved, and (4) the size distribution of vesicles prepared by these methods should be consistent with the domain size of ordered lipid raft domains observed in living cells.

In satisfying these criteria the use of mild detergents such as Brij 98 is preferable to Triton X-100 because it has a reduced tendency to solubilise phospholipids located in the cytoplasmic leaflet [39], cause fusion of membrane fragments from different sources to form an amalgamated detergent-resistant membrane [33] or scramble membrane asymmetry [32]. An often neglected factor is the buffer used to perform the detergent treatment. Buffers generally employed have an electrolyte composition designed specifically to mimic the extracellular environment. This ignores the fact that stabilization of the phase properties of phospholipids like phosphatidylethanolamine and phosphatidylserine, which dominate the cytoplasmic leaflet, are quite different from those required for neutral or zwitterionic polar lipids that reside in the protoplasmic leaflet. Indeed, the molecular species of phosphatidylethanolamine found in membranes relies on acidic lipids to maintain a lamellar structure and stabilize the inner leaflet during membrane solubilisation [40–42]. Calcium ions have a particularly pernicious effect on the stability of inner leaflet phospholipids causing delamination of phosphatidylethanolamine into non-bilayer phases by binding to and phase separating phosphatidylserine [43–48]. It is not sufficient to remove calcium ions by incorporating chelating agents in the buffer as many membrane-dependent processes performed by isolated subcellular organelles are known to rely on relatively high Mg^{2+} and K^{+} concentrations in reducing conditions [49] to promote order in the lipid bilayer [50].

Other strategies that can be usefully adopted are to perform the detergent treatment at the growth temperature of the organism. As noted above this avoids temperature-dependent phase changes in the membranes that are invariably associated with cooling the system to 4 °C. Prolonged treatment with detergent is also unnecessary and potentially detrimental for treatments performed at higher temperatures. In most cases solubilisation can be achieved in a matter of minutes.

Methods have been reported that avoid the use of detergents altogether. They are not, however, relieved of all the problems associated with low-temperature detergent treatments. Methods that rely on ultrasonic irradiation, for example, are invariably performed at low temperatures and the ultrasonic treatment itself has untested consequences on the arrangement of membrane components [51–53]. Exposure of fibroblasts to temperatures of 4 °C has also been shown to cause an increase in the size of membrane raft domains compared to nanoclusters that exist at 37 °C and to result in redistribution of raft markers [54]. Similar conclusions have been reported in membrane rafts isolated from synaptic vesicles in studies comparing treatments at 4° and 37 °C [55]. An efficient method of isolation of T-cell receptor domains at 37 °C involves the incubation of lymphocytes with dynabeads coated with anti-T-cell receptor antibodies and after adsorption to the antigen the cell membranes are fragmented by nitrogen cavitation [56]. The method relies on the rafts containing the signalling complexes surviving the mechanical disruption process.

3.3. Composition of membrane rafts

The relationship between raft domains in the plasma membrane of cells and their isolated raft progeny is a critical factor in establishing the veracity of the raft hypothesis. Membrane rafts are usually stated to be enriched in cholesterol and sphingolipids and rationalized as domain forming lipids on the basis of their tendency to interact to form ordered phases that, in model systems, phase separate from domains of liquid-disordered structure. The lipids associated with detergent-resistant membranes have been extensively used to model the behaviour of detergent treatments [57,58]. The general conclusions are that the structures formed by appropriate mixtures of phospholipids and cholesterol survive dissolution by detergents because of an interaction between them that is manifest by an ordering of the hydrocarbon domain of the bilayer matrix. The ordering effect results in a phase that exhibits properties intermediate between a gel and a liquid-disordered phase.

Notwithstanding the difficulties already described in the use of detergents to isolate membrane fractions representing membrane raft domains a number of studies have been reported of the detailed lipid and protein content of these membranes. Detergent-resistant membranes from brain have been sub-fractionated by immunoprecipitation methods into membranes enriched in GPI-anchored Thy-1 and PrP antigens and comparative lipid analyses performed [59]. The two raft populations were found to have lipid contents that are significantly different in some important respects from each other and from the total detergent-resistant membrane fraction from which they were prepared. The main lipid classes present in the membrane rafts were phospholipids, glycosphingolipids and cholesterol (Table 1). The most conspicuous fea-

Table 1
Lipid composition (mol%) of membrane raft preparations and related membranes.

Lipid	Brain DRM[a]		HIV-1 envelope[b]		TCR[c]	Yeast TGN[d]
	Thy-1	PrP	MT-4 cells	293T cells	Jurkat cells	
Cholesterol	48	48	44	47	49	23[f]
PC	34	28	10	22	19	18
SM	16	15	13	9	8	0
Glycosphingolipids	0.2	10	0.2	1	0.5	18
Acidic phospholipids[e]	nd	nd	33	22	23	37
Ceramide	0.1	0.1	–	–	0.03	–

[a] Ref. [59].
[b] Ref. [62].
[c] TCR, T-cell receptor. Ref. [66].
[d] TGN, trans-Golgi network. Ref. [4]; nd, not determined.
[e] Phosphatidic acid and digycerides are excluded as possible phospholipid metabolites.
[f] Ergosterol.

tures of the molecular species of raft lipids were that 75% of the N-acyl fatty acids associated with the glycosphingolipids were 24C in length the majority of which were hydroxylated fatty acids. The proportion of these long N-acyl molecular species of sphingolipids was an order of magnitude greater in the PrP membranes that those associated with Thy-1. This is related to the relative residence times of the two antigens on the cell surface; PrP is recycled through the endosome compartment in a clathrin-independent process at a half time in the region of 60 min with a residence time on the cell surface of about 20 min [60] whereas Thy-1 is resident for a duration of days [61]. Differences between raft populations were seen in the proportions of unsaturated, longer-chain molecular species of phospholipids and glycosphingolipids as well as the proportion of cholesterol. It was noteworthy that the content of cholesterol was significantly less in the sub-fractionated vesicles than the detergent-resistant membrane pool from which they were prepared.

Another approach to relate the composition of membranes to detergent-resistant membrane fractions has been to compare the envelope of retrovirus with detergent-resistant membrane rafts prepared from the plasma membrane of the host cell [62,63]. Viral envelopes were considered as large raft-like membrane domains that are assembled with the assistance of the envelope protein, Gag. Raft marker proteins of the viral envelope were found to be associated with the detergent-resistant membrane raft fraction prepared from membranes of infected cells from which the virus had budded. Furthermore, the lipid composition of the viral envelope and membrane rafts were characterised by enrichment in sphingomyelins, particularly dihydrosphingomyelin, ethanolamine plasmalogens and phosphatidylserines compared to the parent membrane thus confirming their common origin. The particular lipids found in the envelope membrane appears also to depend on the T-cell line from which the envelope is derived (Table 1) and this is reflected in the extent to which the envelope lipids are ordered [64]. The lipid composition of other enveloped viruses such as vesicular stomatitis virus and Semliki Forest virus have lipid envelopes that are similar and closely resemble that of the host plasma membrane [65]. Clearly in these examples sequestering particular membrane lipids into the viral envelope does not take place as is the case with the HIV-1 viral envelope.

An immunoaffinity absorption method has been used to isolate T-cell receptor membrane fragments from Jurkat cells activated by adsorption to dynabeads coated with αCD3 antibodies [66]. The lipid composition of the membrane isolated fragments (Table 1) showed enrichment with respect to the plasma membrane of the Jurkat cells in cholesterol, sphingomyelin and phosphatidylserine and depleted in phosphatidylcholine.

A different approach has been to isolate vesicle populations from the Golgi that are destined to be incorporated into the plasma membrane. Raft proteins are delivered from the trans-Golgi network to the plasma membrane by a population of light-density vesicles. Preparations of these vesicles from yeast have been reported using immunoprecipitation of a raft-specific protein antigen and the lipid composition determined (Table 1) [4]. As with other raft glycosphingolipids the preponderance of N-acyl fatty acids are long and, in the case of yeast, they are almost exclusively C-26 fatty acids. The sphingolipids are present in equimolar amounts with phosphatidylcholine together representing about 36% of the total membrane lipids. The remaining lipids were comprised of acidic phospholipids (37 mol%) and ergosterol (22.5 mol%) giving a molar ratio of about 1.7:1 phospholipid:sterol. Given that the glycosphingolipids and phosphatidylcholines are located in the protoplasmic leaflet of the membrane and the acidic phospholipids in the exoplasmic leaflet the distribution of sterol between the two membrane leaflets must be of critical importance in determining the phase structure of the membrane.

The noteworthy features of the lipid composition of membrane preparations presented in Table 1 is that cholesterol represents the most abundant lipid and is surprisingly constant at almost 50 mol% of total membrane lipid. In terms of mass this represents about 25% of the overall weight of membrane lipid. The next most abundant lipid is phosphatidylcholine which, with the exception of envelope membranes of HIV-1 virus budded from MT-4 cells, is about equal to the combined acidic phospholipid fraction and twice that of sphingomyelin. Glycosphingolipids are relatively minor components of the membranes but the dominant molecular species of this lipid class have very long N-acyl fatty acids.

3.4. Structure of membrane rafts

One aspect of membrane rafts that needs to be reconciled is the apparent disparity of domain size of rafts on the cell surface and the properties of isolated raft preparations. The size of domains indicated from surface labeling of raft proteins and glycolipids is that they are relatively small and have dimensions of only a few tens of nanometres. The implications from such labeling experiments are that raft components are assembled into tightly

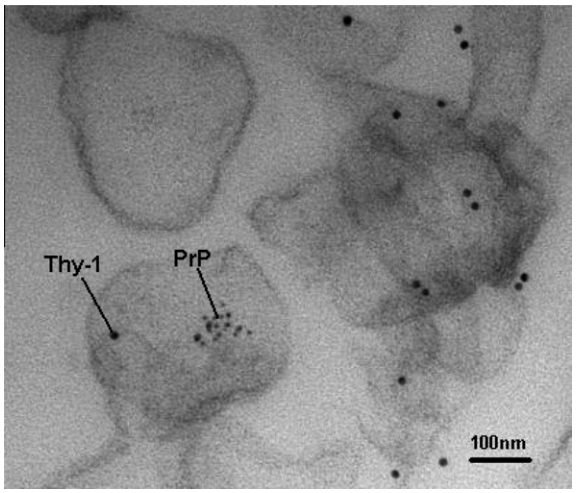

Fig. 1. Electron micrographs of detergent-resistant membranes isolated from rat brain by solubilization in Brij 96 at 37 °C and immunolabeled with 5 nm gold-Fab for Prion protein (PrP) and 10 nm gold-Fab for Thy-1. PrP is invariably clustered into small domains in the raft membrane as it is seen on the surface of cultured neurons whereas Thy-1 is observed at relatively low density in the raft membrane and is only occasionally seen in linear arrays. Electron micrograph by Dr. X. Chen.

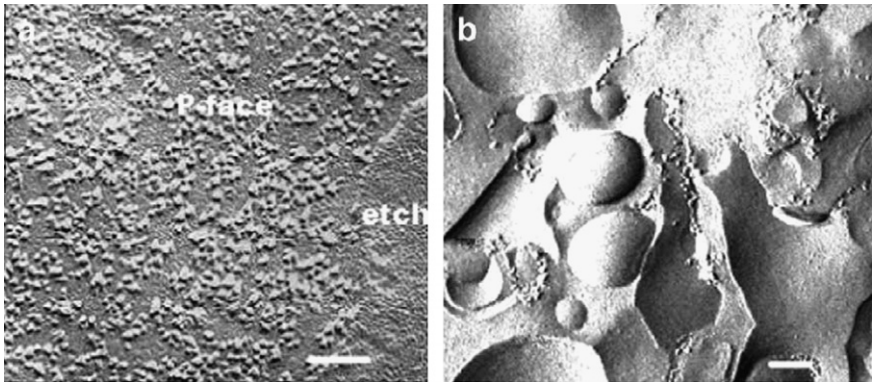

Fig. 2. Electron micrographs of freeze-fracture replicas prepared from etched human erythrocyte ghost membranes (a) and detergent-resistant membranes prepared from ghost membranes by the standard Triton X-100 detergent treatment protocol (b). Bar = 100 nm. Reprinted from Ref. [34] with permission from Elsevier.

organized clusters on the cell surface. Such clusters would be expected to have a relatively high protein:lipid ratio despite the fact that raft proteins tend to be somewhat smaller than other intrinsic membrane proteins. Structures of this type are not consistent with the buoyant density observed for raft preparations isolated by detergent treatments or other methods (1.05 g/ml) [67]. This suggests that methods of isolating membrane rafts incorporate additional lipids so as to reduce their buoyant density or clusters of raft components are formed within the lipid raft itself.

Some evidence that raft proteins are clustered within a larger lipid raft structure is obtained from visualizing the raft proteins within isolated detergent-resistant membrane preparations [5]. This is seen in Fig. 1 which shows electron micrographs of raft preparations isolated from whole rat brain by detergent treatment and immunogold labeled with anti-Thy-1 (10 nm gold particles) and anti-Prion protein (PrP^c; 5 nm gold particles). The antigens can be separated by immunoadsorption methods into separate populations of rafts carrying Thy-1 and PrP^c, respectively. PrP^c is almost invariably clustered into small domains within the raft vesicle which is predominantly comprised of lipid bilayer. Clustering of PrP^c may be a requirement for the relatively rapid endocytosis and recycling of this protein to the cell surface. Thy-1, by contrast, appears randomly distributed and occasionally associated with one or two other molecules in a large lipid bilayer domain. The association of Thy-1 with actin on the cytoplasmic surface of the raft membrane is possibly a mechanism for stabilizing the location of the protein on the cell surface where the residence time is orders of magnitude greater than PrP^c.

Another factor that distinguishes membrane raft preparations from cell membranes is that they are devoid of large intrinsic oligomeric protein complexes [34]. This is seen from freeze-fracture electron micrographs of detergent-resistant membrane preparations which show smooth membrane fracture planes without membrane-associated particles as exemplified by rafts isolated from erythrocyte membranes shown in Fig. 2. This observation is consistent with the finding that many raft proteins are anchored to the bilayer with GPI anchors as in the case of proteins like Thy-1 and PrP^c of neuronal cells rather than intrinsic membrane proteins that require a rearrangement of membrane lipids to package them into the lipid matrix.

Membrane raft preparations using detergent treatment methods that avoid solubilisation of acidic phospholipids are also of relatively uniform size. Dynamic light scattering methods employed on neuronal raft preparations indicate a size range of 120–180 nm in diameter suggesting an area of bilayer in the order of 0.7 μm^2 [68]. This size is somewhat greater than is generally accepted to be the size of raft domains on a cell surface. The fact that the detergent-resistant membrane fraction can be sub-fractionated into Thy-1 and PrP^c-containing vesicle populations by immunoadsorbent methods rules out extensive fusion of raft domains during the isolation procedure but leaves open the possibility of local fusion events with rafts that do not carry either Thy-1 or PrP^c [69].

The thickness of membrane raft preparations has been derived from neutron scattering experiments and values of bilayer thickness ranged from 4.6 to 5.0 nm, varying only slightly with the different isolation conditions. Comparison of membrane thicknesses of raft membrane isolated under optimal conditions with the membranes from which they were derived showed only small differences (4.64 vs 4.53 nm, respectively) and this is likely to be due to the fact that the rafts consist primarily of lipids in a liquid-ordered phase whereas the parental membrane contains lipids both in an *Lo* and a fluid phase. This observation is supported by studies investigating model membrane systems which have reported that the liquid-ordered phase, as a consequence of the longer, more saturated hydrocarbon chains present, has a greater thickness than fluid phase bilayers [70]. Nevertheless, because biomembranes contain a complex assortment of components and the fact that both the liquid-ordered and the liquid-disordered phases are composed of many molecular species of lipids, the actual difference between the two phases may not be as obvious as that seen in simple model systems. While the similarity in thickness between membrane rafts and cell membranes may not support the notion that hydrophobic mismatch between lipid bilayers and transmembrane proteins is responsible for sorting proteins in membranes there is abundant evidence that transmembrane helices may simply flex their conformation to accommodate differences in bilayer thickness [71].

4. Phase behaviour and structure of raft lipids

Models proposed for formation of domains in cell membranes have focussed on liquid-ordered phases that are formed between sphingomyelin and cholesterol. This has stemmed from model membrane studies characterising the motional constraints on the lipids in dispersions of the phospholipid with cholesterol and resistance of such phases to solubilisation by Triton X-100 at 4 °C. Examination of the presently available data on the lipid composition of membrane rafts (Table 1) indicates that a liquid-ordered phase of sphingomyelin and cholesterol represents less that 25% of the raft lipids. To reconcile this apparent discrepancy it is necessary to look more closely at the structure and phase characteristics of other lipids that are present in raft membranes.

One of the most conspicuous features of the lipid composition of membrane rafts is the presence of a significant proportion of acidic phospholipids which are not usually associated with ordered

lipid phases. Such lipids predominate on the cytoplasmic leaflet of cell membranes and their presence in membrane rafts is not unexpected. As noted above, care is required in providing a balanced electrolyte composition in buffers used for detergent treatment in the isolation of membrane rafts so as to retain acid phospholipids in a stable bilayer structure. If ordered phases of phospholipid and cholesterol are not created in the cytoplasmic leaflet of the raft the question arises as to how the proteins associated with the rafts on the cytoplasmic surface are sorted and assembled into complexes with the receptors on the exoplasmic leaflet? Several models have been considered that include the role of adaptor proteins in clustering receptors and transducer proteins on opposite sides of the membrane [72] but the role of lipids has not been clearly defined.

Another exceptional feature of the lipid composition of membrane rafts is the high proportion of molecular species of sphingolipids with long (22–26C) N-acyl fatty acids many of which are hydroxylated fatty acids. It has been suggested that these lipids are involved in coupling the protoplasmic and cytoplasmic leaflets of the raft membrane by interdigitation of the long-chain fatty acids across the bilayer mid-plane [4].

4.1. Phase diagrams of putative raft lipids

Investigations of the phase behaviour of lipids used to model the structure of membrane rafts have relied largely on mixtures of pure synthetic lipids with the aim of characterising the underlying principles of lateral phase separation in bilayers [73–77]. Ternary mixtures of these chemically-defined lipids with cholesterol are found to exhibit liquid–liquid immiscibility over certain regions of the composition phase diagram. The temperature at which critical phase separations are observed, however, are usually below physiological values because of technical limitations of the methods used to establish the phase boundaries and three phase coexistence including gel phases are often observed [78,79].

In ternary mixtures of phosphatidylcholine/sphingomyelin/cholesterol it is generally believed that a preferential interaction of cholesterol with sphingomyelin is responsible for phase separation of a liquid-ordered phase composed mainly of sphingomyelin and cholesterol and a liquid-disordered phase composed largely of phosphatidylcholine [80]. The underlying premise upon which these phase diagrams are interpreted is that cholesterol partitions between sphingomyelin-rich and phosphatidylcholine-rich phases [81–86].

A number of studies have reported partition coefficients of cholesterol in ternary mixtures of phospholipids and sphingomyelin. Cyclodextrin binding to cholesterol has been used to determine the partition coefficients of cholesterol between C-16-sphingomyelin and palmitoyl-oleoyl-phosphatidylcholine and bilayers comprised of an equimolar mixture of glycerophospholipid and C-16-sphingomyelin; a value of 2.56:1 at 37 °C was reported [87]. Cholesterol exchange between egg-phosphatidylcholine and brain-sphingomyelin is found to take place with a partition coefficient of 2.0 whereas that between egg-phosphatidylcholine and C-16-sphingomyelin is reported to be 1.9 [81]. Partition of 20 mol% cholesterol in bilayers comprised of equimolar proportions of egg-sphingomyelin and palmitoyl-oleoyl-phosphatidylcholine using β-cyclodextrin binding and isothermal titration calorimetry gave a value of 4.3:1 at 37 °C [80]. This decreases to 2.3:1 in mixtures containing 30 mol% cholesterol. This dependence of measured partition coefficient on the composition of the mixture between 20 and 30 mol% cholesterol was explained by a tendency to avoid cholesterol–cholesterol contacts in arranging the egg-sphingomyelin and cholesterol molecules in the form of a superlattice that maximizes the number of mixed phospholipid–cholesterol contacts [80]. No evidence of domain formation or specific complex formation between cholesterol and egg-sphingomyelin in bilayers containing the diacyl phospholipid was observed rather there was a progressive ordering of the lipids in a largely homogeneous membrane with increasing cholesterol.

Interpretation of these data, as we shall see below, is not as straight forward as it appears. Indeed, in ternary mixtures of cholesterol with symmetric molecular species of sphingomyelin and phosphatidylcholines the lipids are not free to partition between a sphingomyelin-rich and a phosphatidylcholine-rich phase. This is in notable contrast with the behaviour of cholesterol in mixtures of glycerophospholipids with high and low transition temperatures, respectively. The distribution of cholesterol in equimolar mixtures of dipalmitoyl- and dioleoyl-phosphatidylcholines at 20 °C, for example, is found to be 10% in each of coexisting liquid-disordered and gel phases and 30% in liquid-ordered phase [75]. Specific interactions between cholesterol and sphingolipids clearly distinguish those between glycerophospholipids and cholesterol and measurements of "partition coefficients" of cholesterol in lipid mixtures containing sphingomyelin must be regarded with caution.

4.2. Interaction of sphingolipids and glycerophospholipids

The miscibility of phosphatidylcholines and sphingolipids has been the subject of considerable interest because of the marked difference in the main order–disorder phase transitions of molecular species of these phospholipids found in cell membranes. Phosphatidylcholines tend to have unsaturated fatty acids acylated to the C-2 of the glycerol whereas sphingolipids have more saturated N-acylated fatty acids. The phase transition temperatures of glycerophospholipids are therefore much lower than the sphingolipids which undergo phase transitions in the physiological range of temperatures.

An impressive body of evidence has purported to show that at temperatures above the main transition temperature, Tm, of mixtures of molecular species of sphingomyelin and phosphatidylcholine with similar hydrocarbon chain lengths the two phospholipids are completely miscible in all proportions. Such studies using synthetic chemically-defined mixtures have included miscibility of palmitoyl–sphingomyelin with dipalmitoylphosphatidylcholine [88] and with dimyristoylphosphatidylcholine [89,90] and N-stearoyl-SM with dipalmitoylphosphatidylcholine [91]. Similar conclusions have been reported for mixtures containing natural sphingomyelins from egg-yolk and dioleoylphosphatidylcholine [92], palmitoyl-oleoyl-phosphatidylcholine [93], dipalmitoylphosphatidylcholine [94] and a variety of phospholipids and sphingolipids with similar hydrocarbon chain lengths [95].

More recent synchrotron X-ray results are inconsistent with these earlier studies and indicate that mixtures of sphingomyelins and phosphatidylcholines from egg-yolk, where the hydrocarbon chains are of comparable lengths, are largely immiscible not only at temperatures below the Tm of egg-SM but also at higher temperatures [96].

The immiscibility of egg-sphingomyelin and egg-phosphatidylcholine is illustrated in Fig. 3. This shows detail of the changes in the second-order Bragg reflections from a synchrotron X-ray experiment in which a dispersion of an equimolar mixture of the two phospholipids are heated from 20 to 50 °C. The structural assignments and scattering intensities of the respective structures indicate an almost complete phase separation of the two phospholipids at temperatures both below and above the main structural transition of the sphingomyelin from gel to liquid-disordered structure (~40 °C).

The reason why the two phospholipids are immiscible at temperatures above the Tm of egg-sphingomyelin is most likely to be due to creation of a network of intermolecular hydrogen bonds

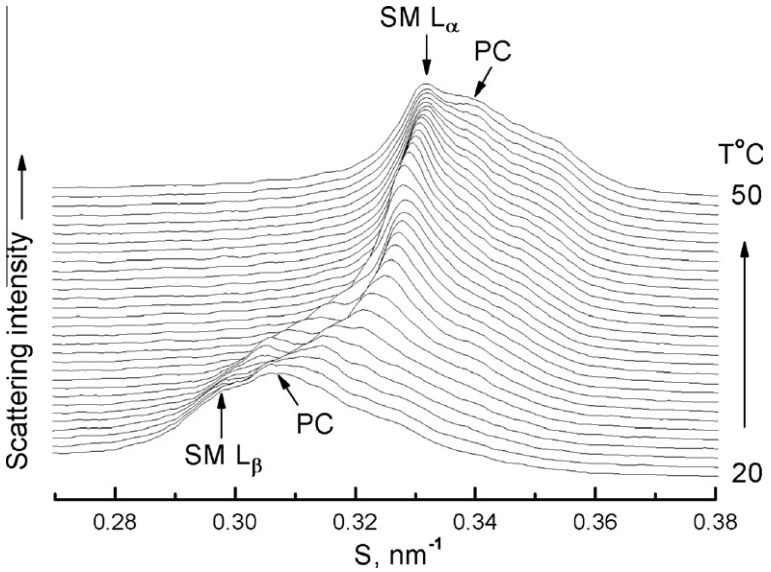

Fig. 3. X-ray scattering intensity profiles in the region of second-order lamellar reflections recorded from an aqueous dispersion of a binary mixture of egg-phosphatidylcholine:egg-sphingomyelin in equimolar proportions recorded at 1° intervals during a heating scan from 20 to 50 °C at 2°/min. Assignment of lamellar structures is shown and indicates a transition of gel phase sphingomyelin (SM L_β) to the fluid phase (SM L_α) takes place in the temperature region of 37–40 °C. Egg-phosphatidylcholine is in the fluid phase (PC) throughout the temperature scan [96].

between the sphingomyelin molecules. Evidence for this comes from molecular dynamic simulations of equimolar mixtures of C-18-sphingomyelin and dioleoylphosphatidylcholine in which increasing order is observed in the sphingomyelin molecules as they form clusters distinct from the more disordered diacylphospholipid molecules [97]. The intermolecular hydrogen bonds are shown to comprise a dynamic network characterized by the creation of hydrogen-bonded clusters of up to nine sphingomyelin molecules [98]. Experimental evidence has been provided from FTIR studies of the amide-I band of egg-sphingomyelin in equimolar mixtures with egg-phosphatidylcholine [99] or dipalmitoylphosphatidylcholine [94]. The band frequency recorded from pure egg-SM bilayers undergoes a decrease with a midpoint at a temperature about 38 °C on transition from a gel to liquid–crystalline bilayer. The temperature-dependence of the amide-1 band frequency in the mixed dispersions with egg-sphingomyelin does not undergo a marked change over the temperature range 15–68 °C and was located at a frequency intermediate between that observed for gel and liquid–crystal phases of the pure egg-sphingomyelin. In mixtures with dipalmitoylphosphatidylcholine some perturbation of hydrogen bonding was observed in the region of the phase transitions of the phospholipids but this was said to be due to minor conformational changes in the head group region of the diacyl phospholipid rather than a change in the intermolecular hydrogen bonding between egg-sphingomyelin molecules.

The miscibility of sphingolipids with asymmetric hydrocarbon chains, i.e. N-acyl fatty acids of 22–26 carbons in length, and molecular species of glycerophospholipids of the type found in cell membranes is fundamentally different from symmetric molecular species of sphingolipids. Earlier attempts to distinguish differences in phase behaviour between cerebrosides with symmetric and asymmetric hydrocarbon chains in mixtures with diacylphospholipids using ^{2}H NMR [100], calorimetry [101] and spin-label [102] methods have not been definitive. There are, however, clear differences in the different molecular species evident from synchrotron X-ray diffraction studies [96,103]. This can be seen in mixed aqueous dispersions of egg-phosphatidylcholine and asymmetric molecular species of bovine-brain sphingomyelin which are miscible at temperatures above the Tm of the sphingomyelin and, as judged from the acyl chain packing parameter ($d = 0.44 - 0.45$ nm), forms a liquid-ordered phase.

A partial phase diagram of dipalmitoylphosphatidylcholine and asymmetric glucosylceramide in the temperature range 25–85 °C is presented in Fig. 4. This shows that the asymmetric molecular species of glucosylceramide forms a liquid-ordered phase over the entire region of the phase diagram investigated which coexists with excess dipalmitoylphosphatidylcholine. The liquid-ordered phase is formed from a stoichiometric complex of equimolar proportions of the two lipids. The phospholipid in excess of that required to form the complex undergoes $L_\beta \rightarrow P_\beta \rightarrow L_\alpha$ phase

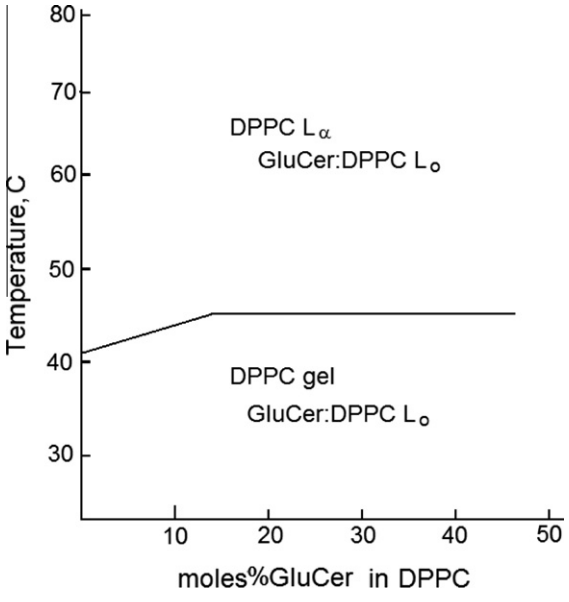

Fig. 4. Partial phase diagram of dipalmitoylphosphatidylcholine (DPPC) and asymmetric glucosylceramide dispersed in excess water. The liquid-ordered phase of DPPC-GlcCer, Lo, consists of a molecular complex of equimolar proportions of the phospholipid and sphingolipid. Reprinted from Ref. [103] with permission from Elsevier.

transitions at temperatures close to that of pure dipalmitoylphosphatidylcholine. The phase behaviour of binary mixtures of nervonylceramide and palmitoyl-oleoyl-phosphatidylcholine has also been investigated by a number of biophysical methods and the results can be interpreted as the formation of a stoichiometric complex between the two lipids that has the properties of a liquid-ordered phase [104].

The miscibility of dipalmitoylphosphatidylcholine with asymmetric cerebroside contrasts markedly with that of symmetric C-16 cerebroside [105]. Mixtures of the phospholipid with the symmetric molecular species in proportions less than about 23 mol% are completely miscible and a gel phase is observed that undergoes a transition to the liquid-crystal phase at a temperature close to that of the pure phospholipid. With proportions of cerebroside greater than 22 mol% a phase-separated lamellar crystal phase is detected that coexists with the gel and liquid-crystal phases comprised of 23 mol% cerebroside in dipalmitoylphosphatidylcholine.

The miscibility of phosphatidylethanolamine with asymmetric cerebrosides has also been examined [106]. Like phosphatidylcholine, palmitoyl-oleoyl-phosphatidylethanolamine forms a quasi-crystalline lamellar phase comprised of a stoichiometric complex of molar proportions 2:1, phospholipid:cerebroside. The structure is stable at temperatures above 70 °C suggesting that the structure will form at physiological temperatures whenever the two lipids are in the same leaflet of a lipid bilayer.

Clearly, to obtain a more accurate perspective of membrane lipid phase behaviour it is necessary to examine the properties of different molecular species of lipids from biological sources. In particular, the phase behaviour of molecular species of lipids which may represent relatively small proportions of the total complement of lipids in the membrane could nevertheless be essential for raft structure.

4.3. Interaction of sphingolipids with sterols

Cholesterol has long been known to affect the structure and properties of phospholipids. The, so called, condensing effect of cholesterol on polar lipids was observed early in the last century [107] and the dynamic basis of the interaction with phospholipids was characterised by electron spin resonance spectroscopy more than 25 years ago [108–110]. The liquid-ordered phase is central to the concept of lipid domain formation in membranes and sterols are one of the primary ingredients of this phase [24].

The interaction of cholesterol with sphingolipids depends on the molecular species as seen from studies of binary mixtures of the two lipids [111]. Cholesterol forms a stable liquid-ordered phase with C-16/C-18 molecular species of sphingomyelin in a proportion of about 27 mol% cholesterol. Molecular species of sphingomyelin with predominantly C-22/C-24 N-acyl chains appear to pack less tightly than their shorter symmetric counterparts and form a stable liquid-ordered bilayer structure with a proportion of 33 mol% cholesterol [111].

Clearly an important factor that determines the miscibility of sphingolipids with phospholipids and cholesterol is the length of the N-acyl fatty acid attached to the sphingolipid. A clue as to why C-16/C-18 molecular species of sphingolipids are not miscible with phospholipids of comparable chain lengths as demonstrated in Fig. 3 is provided from calorimetric studies of the hydration of the polar groups of pure sphingomyelin bilayers [112]. It was found that C-16 sphingomyelin binds only 5.5 molecules of H_2O/lipid compared with 9 molecules of H_2O/molecule of C-22/C24 sphingomyelin at comparable levels of hydration. This remarkable difference is due to the number of intermolecular hydrogen bonds between the sphingomyelin molecules as observed above from molecular dynamic simulation studies and FTIR spectroscopy. Intermolecular hydrogen bonds form between sphingomyelin molecules when the interacting dipoles are in close proximity and in collinear alignment. This condition is favoured if the polar groups of sphingomyelin are located at a planar lipid/water interface and is satisfied if the hydrocarbon chain lengths are equivalent to the C-16/C-18 chain lengths as are most of the molecular species of glycerophospholipids.

The greater hydration of the polar group of asymmetric sphingolipids indicates that the long N-acyl chain acts to elevate the head group of the lipid above the plane of the lipid–water interface. This effect is augmented in molecular species of sphingolipids which possess an additional hydroxyl group attached to the long-chain base at the carbon-4 position. This is the case for dihydrosphingosine hydroxylated to phytosphingosine and hydroxylation of sphingosine to form dehydrophytosphingosine. The presence of the additional hydroxyl group on these molecular species serves to increase the amphiphilic properties of the lipids.

It is generally believed that molecular species of sphingolipids with long N-acyl fatty acid chains have a polar group anchored in the same interfacial plane as molecular species with C-16/C-18 molecular species and the additional hydrocarbon chain length is accommodated within the hydrophobic domain of the bilayer. The asymmetry in acyl chain length of natural sphingolipids has been largely considered as a device to couple the two leaflets of the bilayer by partial interdigitation of the longer N-acyl chain into the opposing monolayer. There is convincing evidence that interdigitation does occur in lipid bilayers in gel phase but a function in coupling the bilayers particularly in the fluid state requires more careful consideration. Direct evidence for interdigitation has been obtained from X-ray diffraction studies [113,114] and inferred from electron density calculations of sphingolipid bilayers [115]. Spin-label [116], infrared [117] and ^{2}H NMR spectroscopic studies [118] of asymmetric sphingolipid bilayers have also led to conclusions that the terminal methyl groups of the long-chain substituents are localized away from the central plane of the bilayer. Nevertheless, the terminal methyl group of C-24 glycolipids retains a remarkable degree of disorder [119] and exists in an environment that is distinct from the interchain region expected if the terminal methyl group extended into this domain of the opposing monolayer at temperatures where the bilayer is not in the gel phase [106]. Indeed it has been proposed on the basis of spin-label probe studies that the additional hydrocarbon of the long N-acyl chain is localized in the central domain of fluid bilayers and the asymmetric lipid is accommodated by greater exposure of the polar group to the aqueous interface [120].

4.4. Ternary mixtures of sphingolipids, phospholipids and cholesterol

The construction of ternary phase diagrams of lipids that form putative raft structures have been established using a wide variety of biophysical methods. Some methods of defining phase boundaries are indirect and rely in some cases on tenuous assumptions. Others are unreliable at physiological temperatures and at lower temperatures reveal regions of the phase diagram where gel phase can be detected. Another factor that has hitherto been largely neglected is the fact that biological membranes are comprised of a plethora of lipid molecular species. Establishing principles of lipid phase behaviour of molecularly-defined systems have tended to ignore the importance that minor lipid molecular species may have in the structure of membrane rafts.

A direct method of characterising ternary lipid phase diagrams that avoids many of the problems referred to above is X-ray powder diffraction. The advantages of the method include the fact that reporter probe molecules are not required with inherent problems associated with their distribution in the lipid mixture. Mixtures can be examined at any temperature and coexisting structures present can be identified by their characteristic small- and wide-

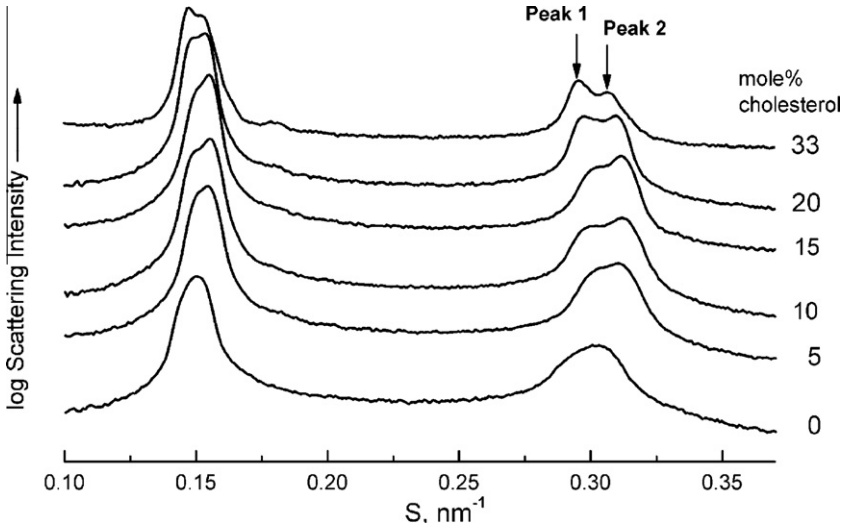

Fig. 5. Static X-ray diffraction profiles of the first two orders of lamellar repeats recorded at 37 °C from ternary mixtures consisting of equimolar proportions of symmetric egg-sphingomyelin and egg-phosphatidylcholine containing the designated mole% cholesterol. The first-order Bragg peaks are seen at $S \approx 0.15\ nm^{-1}$ and the second-order reflections at $S \approx 0.30\ nm^{-1}$. Peak 1 is assigned to bilayers of sphingomyelin–cholesterol and Peak 2 is assigned to bilayers enriched in egg-phosphatidylcholine.

angle dimensions as well as the relative proportions in which they are present in the mixture. These advantages have been exploited in the discovery of the influence of N-acyl chain length of sphingolipids in their competition for binding to phospholipids and cholesterol as described above.

4.4.1. Ternary mixtures with symmetric sphingolipids

Sphingolipids with N-acyl fatty acids 16–18C in length have hydrocarbon chains that extend to approximately the same distance as glycerophospholipids into the lipid bilayer. As already noted they interact via intermolecular hydrogen bonds that prevent them from mixing with glycerophospholipids even at temperatures above the main transition temperature of the lipids when they are both in the liquid-disordered phase. The effect of the presence of cholesterol in mixtures of symmetric sphingomyelins and glycerophospholipids has been the subject of numerous studies aimed to establish the principles of domain structure in membranes.

The effect of cholesterol on the structure of equimolar mixtures of egg-phosphatidylcholine and egg-sphingomyelin has been examined by X-ray diffraction methods and scattering intensity profiles of mixtures containing different amounts of cholesterol are shown in Fig. 5. The presence of two coexisting lamellar structures is clearly seen in the mixtures which are designated Peak 1 and Peak 2. Peak 1 is assigned on the basis of lamellar *d*-spacing and electron spin resonance spectroscopy as liquid-ordered bilayer phase comprised of egg-sphingomyelin and cholesterol and Peak 2 as liquid-disordered phase enriched in egg-phosphatidylcholine [113,121].

The relative scattering intensities from the egg-SM-cholesterol structure (Peak 1) as a function of relative mass of cholesterol in the mixture is presented in Fig. 6. Also shown in the figure is the contribution to the total scattering intensity due to cholesterol, egg-sphingomyelin and egg-sphingomyelin + cholesterol present in the ternary mixtures. In the absence of cholesterol the relative scattering intensity of Peak 1 is 0.456. This compares with a value of 0.477 expected from the egg-sphingomyelin in the equimolar binary mixture of the two phospholipids (X-rays are scattered in proportion to the number of electrons in the molecule). This indicates that approximately 7 mol% of the egg-sphingomyelin is mixed with egg-phosphatidylcholine and contributes to Peak 2 in the absence of cholesterol. The likely reason for this is that egg-sphingomyelin contains about 6 mol% of molecular species with long (C-22, C-24) N-acyl fatty acids [111] and such sphingolipids, as described above, mix with phospholipids to form a stoichiometric complex and do not form coexisting bilayer phases in ternary mixtures with cholesterol and phosphatidylcholine that can be detected by pulsed field gradient NMR spectroscopic methods [92]. The scattering intensity of Peak 1 recorded from the ternary mixture containing 5 mol% cholesterol decreases to values less than expected if all the egg-sphingomyelin remains in a separate phase indicating that some sphingomyelin has partitioned into the phosphatidylcholine phase. This can be explained by the presence of small proportions of cholesterol which disrupt the intermolecular hydrogen bond network between the egg-sphingomyelin molecules that is responsible for their phase separation from the egg-

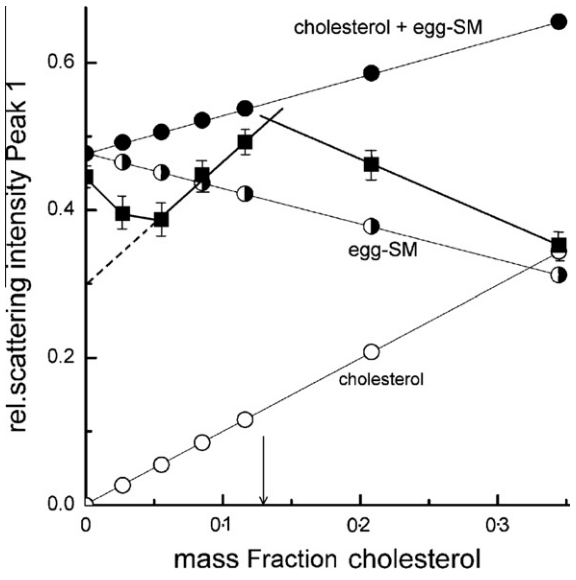

Fig. 6. Relationship between mass fraction of cholesterol and the proportion of the total scattering intensity observed in Peak 1 (■) recorded from dispersions of equimolar mixtures of egg-phosphatidylcholine and egg-sphingomyelin containing varying molar fractions of cholesterol. The scattering intensities expected from all the cholesterol (○) and sphingomyelin (◑) in the mixture are indicated together with the total intensity of sphingomyelin + cholesterol (●).

phosphatidylcholine. A linear regression function of the form $y = 0.017x + 0.297$ can be fitted to data obtained from ternary mixtures containing 10, 15 and 20 mol% cholesterol (mass fractions 0.055, 0.085 and 0.116, respectively). From the intercept of the regression line with the *y*-axis it can be inferred that cholesterol at low concentrations induces mixing of 38% of the sphingomyelin with the phosphatidylcholine. The relative intensity of Peak 1 increases linearly in the range 10–20 mol% cholesterol because cholesterol forms structures with sphingomyelin by recruiting sphingomyelin molecules that have partitioned into the phosphatidylcholine-rich phase (contributing to Peak 2 at cholesterol concentrations less than 20 mol%). The relative intensity of Peak 1 decreases in ternary mixtures containing proportions of cholesterol greater than about 20 mol% because cholesterol is in excess of the amount of required to form a stoichiometric structure with all the sphingomyelin in the mixture. The excess cholesterol partitions into the phosphatidylcholine-rich phase. In mixtures containing more than about 50 mol% cholesterol the excess cholesterol forms crystals that are signified by a sharp Bragg peak at 3.4 nm and a number of characteristic peaks in the wide-angle scattering region [122].

The stoichiometry of symmetric sphingomyelin and cholesterol in the structure comprising Peak 1 can be obtained from Fig. 6 as the relative intensity of the peak at the intersection between the regression line fitted to mixtures containing 10–20 mol% cholesterol and the line passing through scattering intensities recorded from ternary mixtures containing 33 and 40 mol% cholesterol. The *x*-axis value at this point indicates that the mass fraction of cholesterol in the ternary mixture is 0.13 giving proportions in the ternary mixture of egg-phosphatidylcholine:egg-sphingomyelin:cholesterol of approximately 38.5:38.5:22.5 expressed as mol%. Since all the scattering in Peak 1 can be accounted for by the symmetric molecular species of sphingomyelin + cholesterol in the mixture the stoichiometry of the complex is 1.7:1.

A ternary phase diagram of the symmetric egg-sphingomyelin mixed with egg-phosphatidylcholine and cholesterol in which the phase boundary is defined by ternary mixtures of varying proportions is shown in Fig. 7. The phase diagram is dominated by coexistence of liquid-ordered and liquid-disordered bilayer structure at 37 °C. Indeed, the coexistence of these two phases throughout most of the composition phase diagram appears to be limited only by the ability to detect a liquid-ordered phase in mixtures containing less than 0.2 mol fraction of sphingomyelin. The dashed line shown on the diagram delineates a stoichiometric complex in molar proportions 1.7:1 of sphingomyelin and cholesterol which coexists with liquid-disordered bilayers of almost pure phosphatidylcholine. Cholesterol in the region of the phase diagram < 0.2 mol fraction causes disruption of the intermolecular hydrogen bond network that prevents mixture of the sphingomyelin with phosphatidylcholine resulting in mixing of some of the sphingomyelin with phosphatidylcholine. With increasing proportions of cholesterol this sphingomyelin is retrieved from the phosphatidylcholine until all the sphingomyelin and cholesterol are combined in a molecular complex identified by the dashed line in the phase diagram. Above the line excess cholesterol is excluded from the complex and, together with the phosphatidylcholine, forms a coexisting liquid-disordered bilayer structure. When the proportion of cholesterol is relatively high compared to the two phospholipids crystals of pure cholesterol can be identified (designated C in Fig. 7) in the phase separated mixture.

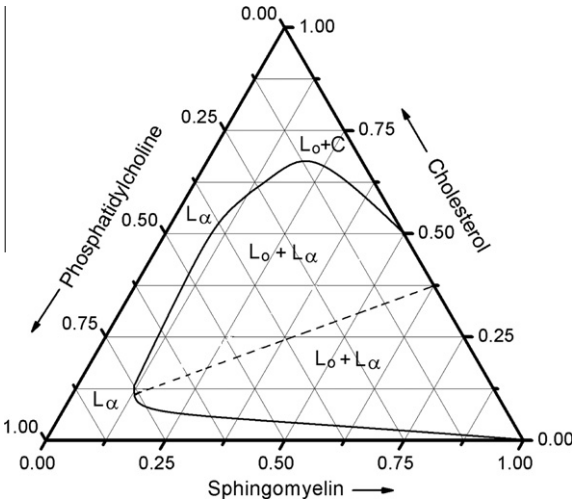

Fig. 7. Ternary phase diagram of egg-phosphatidylcholine:egg-sphingomyelin:cholesterol at 37 °C. The diagram is dominated by coexistence of liquid-ordered (L_o) structure consisting of a complex of sphingomyelin and cholesterol in a stoichiometry of 1.7:1 and a liquid-disordered phase (L_α) of mainly phosphatidylcholine. The dashed line represents coexistence of the pure complex with bilayers of mostly phosphatidylcholine. Below the line some sphingomyelin partitions into the phosphatidylcholine bilayer; above the line cholesterol in excess of that required to form the complex partitions into the phosphatidylcholine phase.

The stoichiometry of sphingomyelin and cholesterol in the complex forming liquid-ordered phase is of considerable interest in view of the supposed function of the complex in membrane rafts. It is well known that cholesterol occupies a goldilocks position in mammalian membranes in that its properties in membranes cannot be duplicated by other sterols, not even by its closely related metabolic precursor, dehydrocholesterol [123]. Thus the dimensions and architecture of cholesterol fit precisely into a bilayer of sphingomyelin to create liquid-ordered structure.

The fit of cholesterol into sphingomyelin bilayers can be appreciated from the cross-sectional areas of the respective molecules illustrated in Fig. 8a. It can be seen that the cross-sectional dimensions of the cholesterol molecule which is roughly rectangular in shape and measuring about 0.43×0.84 nm is too large to be accommodated into sphingomyelin molecules with hydrocarbon chains packed in hexagonal array. A precise fit can, however, be accomplished by interaction of four molecules of sphingomyelin with cholesterol. Seven hydrocarbon chains of the sphingomyelins are in direct contact with a particular cholesterol molecule and one chain is not. One possible arrangement of molecules into liquid-ordered structure is illustrated in Fig. 8b. The rectangles represent cholesterol molecules and the circles are of relative dimension of hydrocarbon chains of sphingomyelin molecules derived from the wide-angle reflection of liquid-ordered phase ($d = 0.44$ nm). From the stoichiometry of the complex each cholesterol molecule must be in direct contact with 1.7 sphingomyelin molecules or 3.4 hydrocarbon chains. To achieve this arrangement all hydrocarbon chains must, on average, be in contact with two cholesterol molecules. This stoichiometry is shown within the array bounded by the solid line in Fig. 8b. The transition of the structure shown in the cartoon into a two-dimensional phase must proceed by the amalgamation of smaller units which are coupled on either side of the bilayer. Clusters of complexes of the type illustrated in Fig. 8b may act as nucleation centres for the attraction of other raft-associated lipids or retention of raft lipids within a complex [124]. Other thermodynamic arguments have been proposed to infer that a reduction in entropy of mixing is a consequence of lipid clustering which serves to extend the boundaries of fluid phase coexistence [125]. The complex illustrated in Fig. 8b shows 4 hydrocarbon chains with three cholesterol contacts, 10 chains with two contacts and 2 chains (black circles) with only one contact with a cholesterol molecule. Irregularities in packing that occur when the chain is in contact with only one cholesterol molecule may provide a mechanism for intercalation of GPI anchors of raft proteins into the complex.

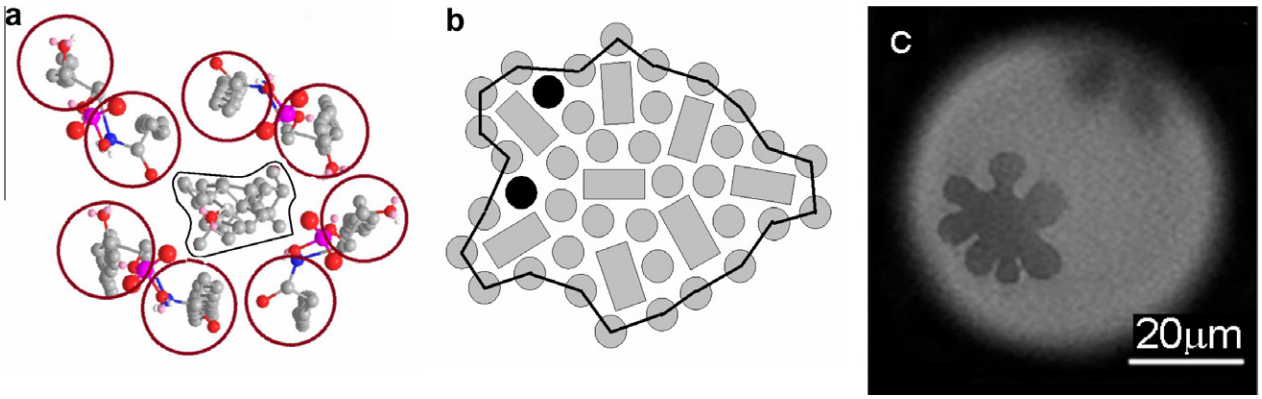

Fig. 8. Characteristics of liquid-ordered phase formed by symmetric sphingolipids and cholesterol. a. Cross-sectional molecular model of cholesterol embedded with four molecules of sphingomyelin. (b) Cartoon of a liquid-ordered complex of symmetric sphingolipid and cholesterol with a stoichiometry 1.7:1 bounded by the solid line. Each hydrocarbon chain is in contact with, on average, two cholesterol molecules. The black circles show hydrocarbon chains in contact with only one cholesterol molecule and may represent sites for interpolation of hydrocarbon chains of lipid-anchored raft proteins. There are no cholesterol–cholesterol contacts. (c) Liquid-ordered domain shown as dark shading in a giant unilamellar vesicle at 20 °C formed from a quaternary mixture comprised of egg-phosphatidylcholine:egg-sphingomyelin:egg-ceramide:cholesterol in molar proportions 75:5:10:10. Reprinted from Ref. [126] with permission from Elsevier.

The nucleation and growth of liquid-ordered phase has been documented in giant unilamellar vesicles using fluorescence microscopy. A close correlation between the packing geometry shown in Fig. 8a and liquid-ordered phase is shown in Fig. 8c. This presents a characteristic feature of liquid-ordered phase of ceramide/sphingomyelin and cholesterol in a fluid phospholipid bilayer which consists of a flower-like arrangement with seven petals [126]. The modulation by ceramides of the liquid-ordered structure formed by symmetric sphingomyelins and cholesterol has been proposed as a molecular mechanism of signalling by ceramides in a range of physiological functions [127].

4.4.2. Asymmetric sphingolipids

Asymmetric sphingolipids are characterised by N-acyl fatty acid substituents 22–26C in length and are found predominantly in brain tissue and as relatively minor membrane components of most mammalian plasma membranes [128]. The behaviour of asymmetric sphingolipids in mixtures with glycerophospholipids and cholesterol is quite different from that described above for ternary mixtures containing symmetric sphingolipids. As already noted asymmetric sphingolipids form stoichiometric complexes with glycerophospholipids that, in the case of phosphatidylethanolamines and phosphatidylcholines, are stable at temperatures greater than about 70 °C.

Ternary mixtures of asymmetric sphingolipids, glycerophospholipids and cholesterol have been examined by a number of biophysical methods and the results can be interpreted as formation of a binary complex of asymmetric sphingolipid and glycerophospholipid that excludes cholesterol. Pulsed field gradient NMR spectroscopy and differential scanning calorimetry have been used to investigate the phase behaviour of symmetric and asymmetric molecular species of sphingomyelin in ternary mixtures with dioleoylphosphatidylcholine and cholesterol [92,129]. Symmetric sphingomyelins (from egg and porcine brain) apparently formed coexisting liquid-ordered and liquid-disordered phase comprised predominantly of sphingomyelin and cholesterol and liquid-disordered phase enriched in glycerophospholipid. Asymmetric sphingomyelin isolated from cow's milk, however, did not exhibit biphasic diffusional modes at temperatures in the range 20–40 °C to indicate coexistence of liquid-ordered and liquid-disordered phases. The most likely explanation for this result is that a complex forms between asymmetric sphingomyelin and dioleoylphosphatidylcholine that has diffusional characteristics more akin to a gel than a liquid-ordered phase formed by symmetric sphingomyelin and cholesterol. Such a phase would not be detectable by the pulsed field gradient NMR method. The single component spectra observed in the mixture was probably generated from a disordered bilayer structure comprised of dioleoylphosphatidylcholine and cholesterol.

Fluorescence quenching methods have been used to assess the effect of asymmetric sphingolipids on domain formation in ternary mixtures with cholesterol [130]. Probes were employed to define overall coexistence of ordered and disordered phases and to identify how cholesterol partitioned in the mixtures. It was reported that sphingolipids with C24:0 N-acyl fatty acids either alone or together with C16:0 sphingomyelin form stable ordered domains in fluid bilayers of glycerophospholipids. Interestingly, these domains excluded cholesterol particularly if the asymmetric sphingolipid was glycosylated. Consistent with the conclusions stated in the previous section symmetric glycosphingolipids incorporated sterols into ordered domains [131].

Direct evidence of the phase properties of ternary mixtures of asymmetric sphingolipids, glycerophospholipids and cholesterol has been obtained from synchrotron X-ray powder diffraction studies undertaken by the author. Fig. 9 shows the result of a deconvolution of the first-order Bragg reflection from a ternary mixture in which three bilayer structures can be identified. The mixture examined in this figure is egg-phosphatidylcholine:bovine

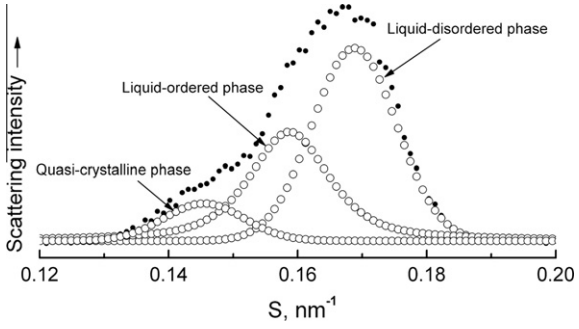

Fig. 9. Deconvolution of a first-order Bragg small-angle scattering intensity peak recorded from an aqueous dispersion of a ternary mixture of egg-phosphatidylcholine:brain-sphingomyelin:cholesterol in molar proportions 80:10:10 at 37 °C into 3 coexisting lamellar phases. The assignment of the phases are fluid bilayers of mainly egg-phosphatidylcholine containing less than 10 mol% cholesterol, liquid-ordered phase of phosphatidylcholine and cholesterol and quasi-crystalline phase of brain-sphingomyelin and egg-phosphatidylcholine.

brain-sphingomyelin:cholesterol dispersed in molar proportions 80:10:10 in which three coexisting bilayer structures can be identified at 37 °C. The structure of greatest *d*-spacing is assigned to a quasi-crystalline phase comprised of equimolar proportions of asymmetric sphingomyelin and egg-phosphatidylcholine, a liquid-ordered structure formed from saturated molecular species of glycerophospholipid and cholesterol and a disordered bilayer phase of glycerophospholipid into which some cholesterol has partitioned. While it is not possible to distinguish the relationship between the three phases with regard to their lateral distribution in the plane of the bilayers it can be stated with certainty that each of the bilayer phases are coupled across the bilayer otherwise only a single broad reflection would be observed.

4.5. Role of asymmetric sphingolipids in raft functions

The lipid composition of membrane rafts is characterised by a variety of asymmetric sphingolipids which poses the question as to their role in raft function. The significance of the N-acyl chain length has been implicated in membrane trafficking in yeast. The delivery of raft-associated proteins to the surface plasma membrane has been examined in strains that are defective in complex lipid synthesis and specifically *elo2Δ* and *elo3Δ* which are responsible for biosynthesis of long-chain fatty acids [132,133]. Lipidomic analysis of the mutant strains showed that these strains indeed had glycosphingolipids which had shortened N-acyl fatty acids [134]. Such mutants appear to bypass the known v-SNARE functions in secretion and participate only in selective protein cargo trafficking and secretion [135]. Moreover, there is evidence that assembly of a functional vacuolar membrane is impaired and proteins destined for the cell surface are missorted to the vacuole or retained in the trans-Golgi network. [136]. Other mutants defective in hydroxylation of the sphingosine base [137,138] or glycosylation of the sphingolipids [139] also appear to exhibit aberrant raft function. It has been suggested that the long-chain molecular species of sphingolipids are required for the assembly of lipid rafts and yeast mutants deficient in elongation of fatty acids have defective raft functions [140].

The reliance of long N-acyl molecular species of lactosylceramide in coupling external stimuli such as phagocytosis *via* glycolipid pattern recognition receptors, superoxide generation in the respiratory burst and chemotaxis for β-glucan to the Src-family kinase, Lyn, on the cytoplasmic surface of the plasma membrane of human neutrophils has been convincingly demonstrated [141]. The strategy adopted in these studies was to duplicate transmembrane signalling triggered by ligand binding to lactosylceramide on the cell surface of a neutrophil differentiated cell line possessing only C-16–18 molecular species of lactosylceramide, thereby lacking functionally-coupled membrane rafts, by supplementation with asymmetric molecular species of the glycosphingolipid. Thus Lyn was shown to co-localize with lactosylceramide clustered by polyvalent anti-lactosylceramide antibody and be converted to an auto-phosphorylated form only in cells supplemented with C-24:0 or C-24:1 but not with C-16:0 or C-20:0 molecular species of lactosylceramide. Likewise, migration of cells by chemotaxis towards β-glucan or anti-lactosylceramide antibodies depended on supplementation with C-24:1 lactosylceramide and was blocked by interference with activation of Lyn. Finally, it was shown that αMβ2 integrin CD11b/CD18 and lactosylceramide binding to β-glucan in mediation of phagocytosis of non-opsonized micro-organisms required asymmetric molecular species of the glycosphingolipid. The asymmetric lactosylceramide was essential for activation of Src-family kinases, initiation of the respiratory burst of superoxide and co-localization with CD11b in the actin-enriched phagocytic cup regions of the membrane in formation of the phagosome.

5. The lipid matrix model of raft structure

Most emphasis to date in assigning the role of lipids in domain formation in membranes has been on the creation of liquid-ordered phases mediated by the interaction of cholesterol with sphingolipids and principally, sphingomyelin. It can be seen from the discussion above that the particular properties conferred by N-acyl chain asymmetry of sphingolipids has implications for the formation of lipid domains in cell membranes. In particular, glycerophospholipids compete more effectively for binding to asymmetric sphingolipids than does cholesterol whereas the reverse is true for sphingolipids comprised of symmetric hydrocarbon substituents. Another problem with models that emphasize interactions between cholesterol and glycerophospholipids, albeit saturated molecular species, in creating liquid-ordered phase is that it is difficult to conceptualize how liquid-ordered phases formed by these lipids could provide the level of detailed molecular organization and stability obviously required to execute the observed structural discrimination between individual membrane rafts. Symmetric molecular species of sphingomyelin, by contrast, form a stoichiometric complex into which specific hydrocarbons may intercalate and are more likely to form the basis of selectivity that raft functions demand.

In considering the mechanisms of lateral domain formation in membranes four main types of structure can be recognised in cell membranes. These include:

1. Ordered structures in which membrane proteins are the principle architects. These consist of structures like membrane junctions in which specific intrinsic membrane proteins are responsible for organizing the structure and coated pits in which extrinsic proteins also fulfil an organizing role.
2. Fluid domains in which oligomeric protein structures are packaged into the fluid bilayer matrix by lipids that typically form non-bilayer structures.
3. Liquid-ordered domains formed by interaction of sterols with polar membrane lipids. In mammalian membranes the phase consists of a stoichiometric complex formed between symmetric molecular species of sphingolipids and cholesterol in molar proportions 1.7:1. Intrinsic membrane proteins are largely excluded from these domains which are favoured by lipid-anchored proteins or transmembrane proteins that have binding domains for particular raft lipids.
4. Quasi-crystalline domains that form between asymmetric sphingolipids and phospholipids. Such structures have properties of a liquid-ordered phase in that the packing of the hydrocarbon chains is intermediate between those of a gel and a liquid-disordered phase. It is argued that the polar group of asymmetric sphingolipids which are often characterised by complex carbohydrate structures provides the basis for specific interactions with raft proteins. Furthermore, intercalation of GPI anchors into a quasi-crystalline structure with asymmetric sphingolipids provides another potential mechanism for specific interaction with raft proteins.

A cartoon of the lipid matrix model I propose is presented in Fig. 10. The model envisages that liquid-ordered structure formed from symmetric sphingolipids and cholesterol or other sterols of plant or microbial origin with appropriate polar lipids fulfils a primary role in display of raft proteins on the cell surface. Accordingly, the structure may provide a matrix for supporting raft proteins that are resident for relatively long periods on the cell surface such a Thy-1 on neuronal cells. These proteins show little tendency to form clusters on the cell surface and appear to be coupled to the cytoskeleton on the cytoplasmic membrane surface. The do-

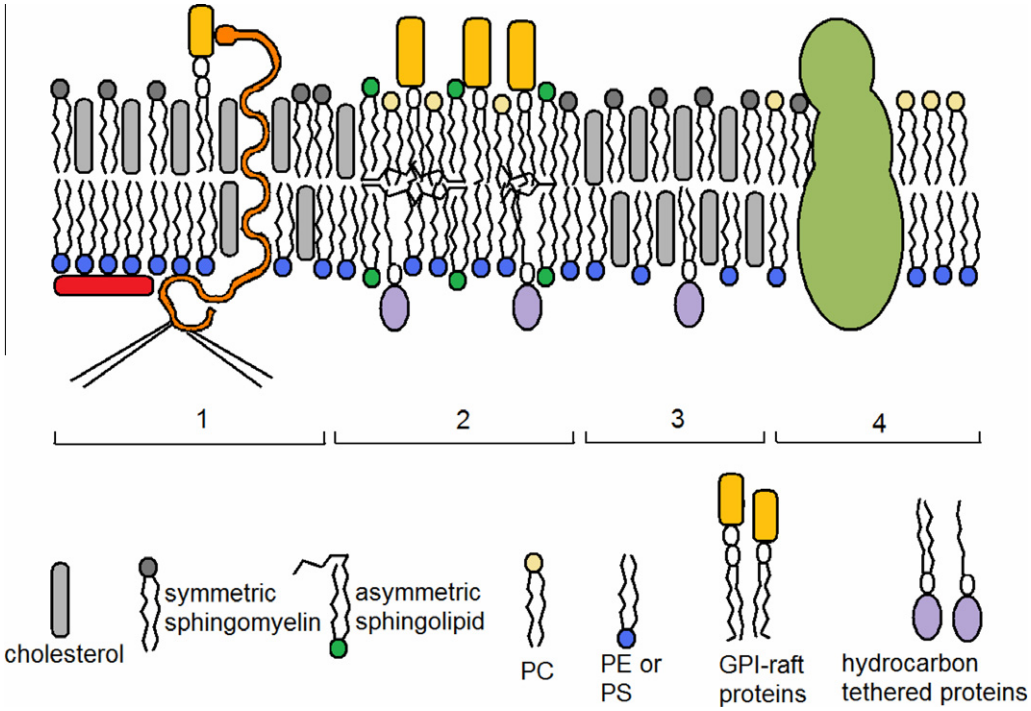

Fig. 10. The lipid matrix model: the cartoon illustrates four membrane domains. (1) Raft domain comprised of complexes of symmetric molecular species of sphingomyelin and cholesterol in proportions 1.7:1 in the protoplasmic leaflet of the membrane into which GPI-anchored raft proteins are interpolated. Special raft adaptor proteins may be components of the raft domain either as transmembrane proteins specially adapted to interact with the sphingomyelin–cholesterol complex or extrinsic proteins associating with the polar groups of the lipids on the cytoplasmic leaflet of the membrane. The cytoplasmic domains of such proteins may be associated with elements of the cytoskeleton. (2) Signaling platforms formed from quasi-crystalline structure comprised of 1:1 complex of asymmetric molecular species of galactosylsphingolipid or sphingomyelin and phosphatidylcholine into which GPI-anchored receptor proteins are interpolated into the protoplasmic membrane leaflet. The outer leaflet of the platform is coupled to the cytoplasmic leaflet which is comprised of a quasi-crystalline structure of a 1:2 stoichiometric complex of glucosylsphingolipid and phosphatidylethanolamine. Appropriate effecter proteins tethered to the raft by fatty acid or prenyl substituents and required to transduce the binding signal on the cell surface are assembled in the structure. (3) This raft domain is a liquid-ordered structure comprised of sphingomyelin/cholesterol in the protoplasmic leaflet and acidic phospholipid/cholesterol in the cytoplasmic leaflet. The domain acts as a staging area for retailoring hydrocarbon tethers of effector proteins where modifications needed to enter domain 2 are accomplished. Domain 3 excludes non-raft membrane proteins and lipid components of domain 4.

main may fulfil a subsidiary role in signalling processes associated with membrane raft functions by acting as a staging area for biochemical modifications of effecter proteins anchored to the cytoplasmic leaflet by hydrocarbon chains.

The principal raft domain in the model concerned with trans membrane signalling functions consists of stoichiometric complexes of asymmetric sphingolipids and phospholipids. It is likely but not necessary for these complexes to be associated with liquid-ordered structure formed from symmetric sphingolipids and cholesterol. Complexes comprised of equimolar proportions of galactosylsphingolipids and phosphatidylcholine form the matrix into which the GPI anchors of cell surface raft proteins are interpolated. This process is responsible for clustering the proteins and is exemplified by PrP^c shown in Fig. 1. Coupling of the clustered GPI-anchored proteins on the cell surface with effecter proteins on the opposite side of the membrane occurs through a quasi-crystalline matrix of phosphatidylethanolamine and asymmetric molecular species of glucosylceramide in molar proportion 2:1 located in the cytoplasmic leaflet of the plasma membrane. This matrix serves as a specific platform for interpolation of fatty acid or prenyl groups of raft proteins located in the cytoplasmic leaflet. To supplement the specificity associated with the lipid lattices of the respective complexes on opposite sides of the membrane additional interactions between the lipids of the complex, such as carbohydrate–protein interactions, may provide further means of refining the targeting of particular raft proteins. In this way receptors on the cell surface can be aligned with appropriate executer proteins on the cytoplasmic surface. In this process alignment must be such that an efficient coupling is achieved to bring about the conduction of molecular signals across the membrane.

6. Conclusions

The lipid matrix model provides a plausible molecular mechanism to explain how membrane rafts are formed and perform their various functions. Specificity in creation of particular raft platforms resides in the N-acyl fatty acid substituents of the sphingolipids and the sugar residues of glycosphingolipids. Organization into signal transducing elements is bought about by the molecular lattice formed by the precise arrangement of glycosphingolipid and phospholipids in the quasi-crystalline arrays. Thus the stoichiometry of the lattice itself creates the framework for efficient coupling between the two halves of the bilayer.

Liquid-ordered structure formed by the complex between symmetric molecular species of sphingolipids and cholesterol in molar proportion 1.7:1 provides a matrix for interpolation of particular GPI-anchored raft proteins on the cell surface. The structure acts as a device for sorting raft proteins because only raft-associated proteins are able to integrate into the matrix formed by the complex. The model can be subject to experimental scrutiny and in particular the role of acidic phospholipids that represent significant components of raft lipids needs to be clarified.

It is implicit in the model that regulation of raft formation shifts from the creation of liquid-ordered phase comprised of sphingomyelin and cholesterol on the exoplasmic side of the membrane

to the molecular architecture on the cytoplasmic leaflet. It is after all on the cytoplasmic side of the membrane where the biochemical business of membrane differentiation and retailoring of lipid molecular species largely takes place.

Acknowledgements

Professor Y. Barenholz and Dr. Claude Wolf are thanked for helpful advice.

References

[1] Singer SJ, Nicolson GL. The fluid mosaic model on the structure of cell membranes. Science NY 1972;175:720–31.
[2] Garcia GG, Akha AAS, Miller RA. Age-related defects in moesin/ezrin cytoskeletal signals in mouse CD4 T cells. J Immunol 2007;179:6403–9.
[3] Olk S, Zoidl G, Dermietzel R. Connexins, cell motility, and the cytoskeleton. Cell Motil Cyotskel 2009;66:1000–16.
[4] Klemm RW, Ejsing CS, Surma MA, Kaiser HJ, Gerl MJ, Sampaio JL, et al. Segregation of sphingolipids and sterols during formation of secretory vesicles at the trans-Golgi network. J Cell Biol 2009;185:601–12.
[5] Chen X, Jen A, Warley A, Lawrence MJ, Quinn PJ, Morris RJ. Isolation at physiological temperature of detergent-resistant membranes with properties expected of lipid rafts: influence of buffer composition. Biochem J 2009;417:525–33.
[6] Proschogo N, Gaus K, Jessup W. Taking aim at cell lipids: shotgun lipidomics and imaging mass spectrometry push the boundaries. Curr Opin Lipidol 2009;20:522–3.
[7] Owen DM, Williamson D, Rentero C, Gaus K. Quantitative microscopy: protein dynamics and membrane organization. Traffic 2009;10:962–71.
[8] Casuso I, Kodera N, Le Grimellec C, Ando T, Scheuring S. Contact-mode high-resolution high-speed atomic force microscopy movies of the purple membrane. Biophys J 2009;97:1354–61.
[9] Yamashita H, Voitchovsky K, Uchihashi T, Contera SA, Ryan JF, Ando T. Dynamics of bactriorhodopsin 2D crystal observed by high-speed atomic force microscopy. J Struct Biol 2009;167:153–8.
[10] Weiss RM, Hirai T, Chalah A, Kessel M, Peters PJ, Subramaniam S. Electron microscopic analysis of membrane assemblies formed by the bacterial chemotaxis receptor Tsr. J Bact 2003;185:3636–43.
[11] Douglass AD, Vale RD. Single-molecule microscopy reveals plasma membrane microdomains created by protein–protein networks that exclude or trap signalling molecules in T cells. Cell 2005;121:937–50.
[12] Kusumi A, Nakada C, Ritchie K, Murase K, Suzuki K, Murakoshi H, et al. Paradigm shift of the plasma membrane concept from the two-dimensional continuum fluid to the partitioned fluid: high-speed single-molecule tracking of membrane molecules. Ann Rev Biophys Biomol Struct 2005;34:351–78.
[13] Suzuki KG, Fujiwara TK, Sanematsu F, Iino R, Edidin M, Kusumi A. GPI-anchored receptor clusters transiently recruit Lyn and G alpha for temporary cluster immobilization and Lyn activation: single-molecule tracking study 1. J Cell Biol 2007;177:717–30.
[14] van Zanten TS, Cambi A, Koopman M, Joosten B, Figdor CG, Garcia-Parajo MF. Hotspots of GPI-anchored proteins and integrin nanoclusters function as nucleation sites for cell adhesion. Proc Nat Acad Sci USA 2009;106:18557–62.
[15] Kirchhausen T. Imaging endocytotic clathrin structures in living cells. Trends Cell Biol 2009;19:596–605.
[16] Murakami T. Roles of the interactions between Env and Gag proteins in the HIV-1 replication cycle. Microbiol Immunol 2008;52:287–95.
[17] Yang P, Ai LS, Huang SC, Li HF, Chan WE, Chang CW, et al. The cytoplasmic domain of human immunodeficiency virus type 1 transmembrane protein gp41 harbours lipid raft association determinants. J Virol 2010;84:59–75.
[18] Anderson RGW, Jacobson K. A role for lipid shells in targeting proteins to caveolae, rafts, and other lipid domains. Science 2002;296:1821–5.
[19] Jacobson K, Mouritsen OG, Anderson GW. Lipid rafts: at a crossroad between cell biology and physics. Nat Cell Biol 2007;9:7–14.
[20] Chen Y, Veracini L, Benistant C, Jacobson K. The transmembrane protein CBP plays a role in transiently anchoring small clusters of Thy-1, a GPI-anchored protein, to the cytoskeleton. J Cell Sci 2009;122:3966–72.
[21] Lakhan SE, Sabharanjak S, De A. Endocytosis of glycerylphosphatylinositol-anchored proteins. J Biomed Sci 2009;16:93.
[22] Simons K, Ikonen E. Functional rafts in cell membranes. Nature 1997;387:569–72.
[23] Apajalahti A, Niemela P, Govindan PN, Miettinen MS, Salonen E, Marrink S-J, et al. Concerted diffusion of lipids in raft-like membranes. Faraday Disc. 2010;144:411–30.
[24] Quinn PJ, Wolf C. The liquid-ordered phase in membranes. Biochim Biophys Acta 2009;1788:33–46.
[25] Lingwood D, Kaiser H-J, Levental I, Simons K. Lipid rafts as functional heterogeneity in cell membranes. Biochem Soc Trans 2009;37:955–60.
[26] Brown DA, Rose KJ. Sorting of GPI-anchored proteins to glycolipid enriched membrane subdomains during transport to the apical cell surface. Cell 1992;68:533–44.
[27] Hooper NM, Bashir A. Glycosyl-phosphatidylinositol-anchored membrane proteins can be distinguished from transmembrane polypeptide-anchored proteins by differential solubilization and temperature-induced phase separation in Triton X-114. Biochem J 1991;280:745–51.
[28] London E, Brown DA. Insolubility of lipids in Triton X-100: physical origin and relationship to sphingolipid/cholesterol membrane domains (rafts). Biochim Biophys Acta 2000;1508:182–95.
[29] Lingwood D, Simons K. Detergent resistance as a tool in membrane research. Nat Protocol 2007;2:2159–65.
[30] Jakop U, Fuchs B, Suess B, Wibbelt G, Braun B, Muller K, et al. The solubilisation of boar sperm membranes by different detergents – a microscopic, MALDI-TOF MS, P-31 NMR and PAGE study of membrane lysis, extraction efficiency, lipid and protein composition. Lipid Health Dis 2009;8:49.
[31] Waugh MG, Hsuan JJ. Preparation of membrane rafts. Meth Mol Biol 2009;462:403–14.
[32] Radeva G, Sharom FJ. Isolation and characterization of lipid rafts with different properties from RBL-2H3 (rat basophilic leukaemia) cells. Biochem J 2004;380:219–30.
[33] Madore N, Smith KL, Graham CH, Jen A, Brady K, Hall S, et al. Functionally different GPI proteins are organised in different domains on the neuronal surface. EMBO J 1999;18:6917–26.
[34] Quinn PJ, Tessier C, Rainteau D, Koumanov KS, Wolf C. Structure and thermotropic phase behaviour of detergent-resistant membrane raft fractions isolated from human and ruminant erythrocytes. Biochim Biophys Acta 2005;1713:5–14.
[35] Lichtenberg D, Goni FM, Heerklotz H. Detergent-resistant membranes should not be identified with membrane rafts. Trends Biochem Sci 2005;30:430–6.
[36] Ingelmo-Torres M, Gaus K, Herms A, Gonzalez-Moreno E, Kassan A, Bosch M, et al. Triton X-100 promotes a cholesterol-dependent condensation of the plasma membrane. Biochem J 2009;420:373–81.
[37] Koumanov KS, Tessier C, Momchilova AB, Rainteau D, Wolf C, Quinn PJ. Comparative lipid analysis and structure of detergent-resistant membrane raft fractions isolated from human and ruminant erythrocytes. Arch Biochem Biophys 2005;434:150–8.
[38] Pike LJ, Han X, Chung KN, Gross RW. Lipid rafts are enriched in arachidonic acid and plasmenylethanolamine and their composition is independent of caveolin-1 expression: a quantitative electrospray ionization/mass spectrometric analysis. Biochemistry 2002;41:2075–88.
[39] Schuck S, Honsho M, Ekroos K, Shevchenko A, Simons K. Resistance of cell membranes to different detergents. Proc Natl Acad Sci USA 2003;100:5795–800.
[40] Lewis RN, McElhaney RN. Surface charge markedly attenuates the nonlamellar phase-forming propensities of lipid bilayer membranes: calorimetric and 31P-nuclear magnetic resonance studies of mixtures of cationic, anionic, and zwitterionic lipids. Biophys J 2000;79:1455–64.
[41] Li X, Schick M. Theory of lipid polymorphism: application to phosphatidylethanolamine and phosphatidylserine. Biophys J 2000;78:34–46.
[42] Papahadjopoulos D, Poste G, Schaeffer BE, Vail WJ. Membrane fusion and molecular segregation in phospholipid vesicles. Biochim Biophys Acta 1974;352:10–28.
[43] Cullis PR, Verkleij AJ. Modulation of membrane structure by Ca^{2+} and dibucaine as detected by ^{31}P NMR. Biochim Biophys Acta 1979;552:546–51.
[44] Duzgunes N, Wilschut J, Fraley R, Papahadjopoulos D. Studies on the mechanism of membrane fusion: role of head-group composition in calcium- and magnesium-induced fusion of mixed phospholipid vesicles. Biochim Biophys Acta 1981;642:182–95.
[45] Tilcock CP, Cullis PR. The polymorphic phase behaviour of mixed phosphatidylserine–phosphatidylethanolamine model systems as detected by ^{31}P-NMR. Biochim Biophys Acta 1981;641:189–201.
[46] Tokutomi S, Lew R, Ohnishi S. Ca^{2+}-induced phase separation in phosphatidylserine, phosphatidylethanolamine and phosphatidylcholine mixed membranes. Biochim Biophys Acta 1981;643:276–82.
[47] Bakht O, Pathak P, London E. Effect of the structure of lipids favouring disordered domain formation on the stability of cholesterol-containing ordered domains (lipid rafts): identification of multiple raft-stabilization mechanisms. Biophys J 2007;93:4307–18.
[48] Bally MB, Tilcock CP, Hope MJ, Cullis PR. Polymorphism of phosphatidylethanolamine–phosphatidylserine model systems: influence of cholesterol and Mg^{2+} on Ca^{2+}-triggered bilayer to hexagonal (HII) transitions. Can J Biochem Cell Biol 1983;61:346–52.
[49] Martoglio B, Hauser S, Dobberstein B [Co-translation of proteins into microsomes derived from the rough endoplasmic reticulum of mammalian cells]. In: Cellis JE, editor. Cell biology: a laboratory handbook. Aarhus: Academic Press; 1998. p. 265–73.
[50] Trauble H, Eibl H. Electrostatic effects on lipid phase transitions: membrane structure and ionic environment. Proc Natl Acad Sci USA 1974;71:214–9.
[51] Smart EJ, Ying YS, Mineo C, Anderson RG. A detergent-free method for purifying caveolae membrane from tissue culture cells. Proc Natl Acad Sci USA 1995;92:10104–8.
[52] Song KS, Li S, Okamoto T, Quilliam LA, Sargiacomo M, Lisanti M. Co-purification and direct interaction of Ras with caveolin, an integral membrane protein of caveolae microdomains. Detergent-free purification of caceolae membranes. J Biol Chem 1996;271:9690–7.

[53] Kim S-y, Wang T-k, Singh RD, Wheatley CL, Marks DL, Pagano RE. Proteomic identification of proteins translocated to membrane microdomains upon treatment of fibroblasts with the glycosphingolipids, C8-β-D-lactosylceramide. Proteomics 2009;9:4321–8.
[54] Chen Y, Qin J, Cai J, Chen ZW. Cold induces micro- and nano-scale reorganization of lipid raft markers at mounds of T-call membrane fluctuations. PloS One 2009;4:e5386.
[55] Gil C, Cubi R, Blasi J, Aguilera J. Synaptic proteins associate with a sub-set of lipid rafts when isolated from nerve endings at physiological temperature. Biochem Biopbys Res Commun 2006;348:1334–42.
[56] Harder T, Kuhn M. Selective accumulation of raft-associated membrane protein LAT in T cell receptor signalling assemblies. J Cell Biol 2000;151:199–208.
[57] Heerklotz H, Szadkowska H, Anderson T, Seelig J. The sensitivity of lipid domains to small perturbations demonstrated by the effect of Triton. J Mol Biol 2003;329:793–9.
[58] Heerklotz H. Interactions of surfactants with lipid membranes. Q Rev Biphys 2008;41:205–64.
[59] Brugger B, Graham C, Leibrecht I, Mombelli E, Jen A, Wieland F, et al. The membrane domains occupied by glycosylphosphatidylinositol–anchored prion protein and Thy-1 differ in lipid composition. J Biol Chem 2004;279:7530–6.
[60] Shyng S-L, Huber MT, Harris DA. A prion protein cycles between the cell surface and an endocytic compartment in cultured neuroblastoma cells. J Biol Chem 1993;268:15922–8.
[61] Lemansky P, Fatemi SH, Gorican B, Meyale S, Rossero R, Tartakoff AM. Dynamics and longevity of the glycolipid-anchored membrane protein, Thy-1. J Cell Biol 1990;110:1525–31.
[62] Brugger B, Glass B, Haberkant P, Leibrecht I, Wieland FT, Krausslich H-G. The HIV lipidome: a raft with an unusual composition. Proc Nat Acad Sci USA 2006;103:2641–6.
[63] Kawabata A, Tang H, Huang H, Yamanishi K, Mori Y. Human herpesvirus 6 envelope components enriched in lipid rafts: evidence for virion-associated lipid rafts. Virol J 2009. doi:10.1186/1743-422X-6-127.
[64] Lorizate M, Brugger B, Akiyama H, Glass B, Muller B, Anderluh G, et al. Probing HIV-1 membrane liquid order by laurdan staining reveals producer cell-dependent differences. J Biol Chem 2009;284:22238–47.
[65] Kalvodova L, Sampaio JL, Cordo S, Ejsing CS, Shevchenko A, Simons K. The lipidomes of vesicular stomatitis virus, Semliki Forest virus, and the host plasma membrane analysed by quantitative shotgun mass spectrometry. J Virol 2009;83:7996–8003.
[66] Zech T, Ejsing CS, Gaus K, de Wet B, Shevchenko A, Simons K, et al. Accumulation of raft lipids in T-cell plasma membrane domains engaged in TCR signaling. EMBO J 2009;28:466–76.
[67] Chen X, Morris R, Lawrence MJ, Quinn PJ. The isolation and structure of membrane lipid rafts from rat brain. Biochimie 2007;89:192–6.
[68] Chen X, Lawrence MJ, Barlow DJ, Morris RJ, Heenan RK, Quinn PJ. The structure of detergent-resistant membrane vesicles from rat brain cells. Biochim Biophys Acta 2009;1788:477–83.
[69] Morris RJ. Ionic control of the metastable inner leaflet of the plasma membrane: fusions natural and artefactual. FEBS Lett 2010;584:1665–9.
[70] Kiselev MA, Zemlyanaya EV, Aswal VK, Neubert RHH. What can we learn about the lipid vesicle structure from the small-angle neutron scattering experiment. Eur Biophys J Biophys let 2006;35:447–93.
[71] Yeagle PL, Bennett M, Lemaitre V, Watts A. Transmembrane helices of membrane proteins may flex to satisfy hydrophobic mismatch. Biochim Biophys Acta 2007;1768:530–7.
[72] Duke T, Graham I. Equilibrium mechanisms of receptor clustering. Prog Biophys Mol Biol 2009;100:18–24.
[73] Marsh D. Cholesterol-induced fluid membrane domains: a compendium of lipid-raft ternary phase diagrams. Biochim Biophys Acta 2009;1788:2114–23.
[74] Juhasz J, Sharom FJ, Davis JH. Quantitative characterization of coexisting phases in DOPC/DPPC/cholesterol mixtures: comparing confocal fluorescence microscopy and deuterium magnetic resonance. Biochim Biophys Acta 2009;1788:2541–52.
[75] Chen L, Yu ZW, Quinn PJ. The partition of cholesterol between ordered and fluid bilayers of phosphatidylcholine: a synchrotron X-ray diffraction study. Biochim Biophys Acta 2007;1788:2873–81.
[76] Feigenson GW. Phase boundaries and biological membranes. Annu Rev Biophys Biomol Struct 2007;36:63–77.
[77] Feigenson GW. Phase diagrams and lipid domains in multicomponent lipid bilayer mixtures. Biochim Biophys Acta 2009;1788:47–52.
[78] Veatch SL, Keller SL. Seeing spots: complex phase behavior in simple membranes. Biochim Biophys Acta 2005;1746:172–85.
[79] Veatch SL, Cicuta P, Sengupta P, Honerkamp-Smith A, Holowka D, Baird B. Critical fluctuations in plasma membrane vesicles. Am Chem Soc Chem Biol 2008;3:287–93.
[80] Tsamaloukas A, Szadkowska H, Heerklotz H. Thermodynamic comparison of the interactions of cholesterol with unsaturated phospholipid and sphingomyelins. Biophys J 2006;90:4479–87.
[81] Lange Y, D'Alessandro JS, Small DM. The affinity of cholesterol for phosphatidylcholine and sphingomyelin. Biochim Biophys Acta 1979;556:388–98.
[82] Tsamaloukas A, Szadkowska H, Heerklotz H. Nonideal mixing in multicomponent lipid/detergent systems. J Phys: Condens Mat 2006;18:S1125–38.
[83] McIntosh TJ, Vidal A, Simon SA. Sorting lipids and transmembrane peptides between detergent-soluble and detergent-resistant rafts. Biophys J 2003;85:1656–66.
[84] Melzak KA, Gizeli E. Relative activity of cholesterol in OPPC/cholesterol/sphingomyelin mixtures measured with an acoustic sensor. Analyst 2009;134:609–14.
[85] Lindblom G, Oradd G. Lipid lateral diffusion and membrane heterogeneity. Biochim Biophys Acta 2009;1788:234–44.
[86] Halling KK, Ramstedt B, Nystrom JH, Slotte JP, Nyholm TKM. Cholesterol interactions with fluid-phase phospholipids: effect on the lateral organization of the bilayer. Biophys J 2008;95:3861–71.
[87] Niu SL, Litman BJ. Determination of membrane cholesterol partition coefficient using a lipid vesicle–cyclodextrin binary system: effect of phospholipid acyl chain unsaturation and headgroup composition. Biophys J 2002;83:3408–15.
[88] Maulik PR, Shipley GG. N-palmitoyl sphingomyelin bilayers: structure and interactions with cholesterol and dipalmitoylphosphatidylcholine. Biochemistry 1996;35:8025–34.
[89] Calhoun WI, Shipley GG. Sphingomyelin–lecithin bilayers and their interactions with cholesterol. Biochemistry 1979;18:1717–22.
[90] Bar LK, Barenholz Y, Thompson TE. Effect of sphingomyelin composition on the phase structure of phosphatidylcholine–sphingomyelin bilayers. Biochemistry 1997:2507–16.
[91] Maulik PR, Shipley GG. Interactions of N-stearoyl sphingomyelin with cholesterol and dipalmitoylphosphatidylcholine in bilayer membranes. Biophys J 1996;70:2256–65.
[92] Filippov A, Oradd G, Lindblom G. Sphingomyelin structure influences the lateral diffusion and raft formation in lipid bilayers. Biophys J 2006;90:2086–92.
[93] Degovics G, Latal A, Prenner E, Kriechbaum M, Lohner K. Structure and thermotropic behaviour of mixed choline phospholipid membranes. J Appl Cryst 1997;30:776–80.
[94] Villalain J, Ortiz A, Gomez-Fernandez JC. Molecular interactions between sphingomyelin and phosphatidylcholine in phospholipid vesicles. Biochim Biophys Acta 1988;941:55–62.
[95] Wang T-Y, Silvius JR. Sphingolipid partitioning into ordered domains in cholesterol-free and cholesterol-containing lipid bilayers. Biophys J 2003;84:367–78.
[96] Quinn PJ, Wolf C. Hydrocarbon chains dominate coupling and phase coexistence in bilayers of natural phosphatidylcholines and sphingomyelins. Biochim Biophys Acta 2009;1788:33–46.
[97] Pandit SA, Jakobsson E, Scott HL. Simulation of the early stages of nano domain formation in mixed bilayers of sphingomyelin, cholesterol and dioleoylphosphatidylcholine. Biophys J 2004;87:3312–22.
[98] Mombelli E, Morris R, Taylor W, Fraternali F. Hydrogen-bonding propensities of sphingomyelin, cholesterol and dioleoylphosphatidylcholine. Biophys J 2003;84:1507–17.
[99] Veiga MP, Arrondo JL, Goni FM, Alonso A, Marsh D. Interaction of cholesterol with sphingomyelin in mixed membranes containing phosphatidylcholine, studied by spin-label ESR and IR spectroscopies: a possible stabilization of gel-phase sphingolipid domains by cholesterol. Biochemistry 2001;29:451–64.
[100] Lu D, Singh D, Morrow MR, Grant CWM. Effect of glycosphingolipids fatty acid chain length on behaviour in unsaturated phosphatidylcholine bilayers: a ^{2}H NMR study. Biochemistry 1993;32:290–7.
[101] Gardam M, Silvius JR. Intermixing of dipalmitoylphosphatidylcholine with phospholipids and sphingolipids bearing highly asymmetric hydrocarbon chains. Biochim Biophys Acta 1989;980:319–25.
[102] Mehlhorn IE, Barber KR, Florio E, Grant CWM. A comparison of physical behaviour amongst four glycosphingolipid families. Biochim Biophys Acta 1989;986:281–9.
[103] Quinn PJ. Long N-acyl fatty acids on sphingolipids are responsible for miscibility with phospholipids to form liquid-ordered phase. Biochim Biophys Acta 2009;1788:2267–76.
[104] Pinto SN, Silva LC, de Almeida RFM, Prieto M. Membrane domain formation, interdigitation, and morphological alterations induced by very long chain asymmetric C24:1 ceramide. Biophys J 2008;95:2867–79.
[105] Ruocco MJ, Shipley GG, Oldfield E. Galactosylcerebroside–phospholipid interactions in bilayer membranes. Biophys J 1983;43:91–101.
[106] Feng Y, Rainteau D, Chachaty C, Yu Z-W, Wolf C, Quinn PJ. Characterisation of a quasi-crystalline phase in codispersions of phosphatidylethanolamine and glucocerebroside. Biophys J 2004;86:2208–17.
[107] Leathes JB. Role of fats in vital phenomena. Lancet 1925;1:853–6.
[108] Recktenwald DJ, McConnell HM. Lateral phase separations in binary mixtures of phosphatidylcholine and cholesterol. Biophys J 1981;33:191a.
[109] Ipsen JH, Karlstrom G, Mouritsen OG, Wennerstrom H, Zuckermann MJ. Phase equilibria in the phosphatidylcholine–cholesterol system. Biochim Biophys Acta 1987;905:162–72.
[110] Sperotto MM, Ipsen JH, Mouritsen OG. Theory of protein-induced lateral phase separation in lipid membranes. Cell Biophys 1989;14:79–95.
[111] Quinn PJ, Wolf C. Thermotropic and structural evaluation of the interaction of natural sphingomyelins with cholesterol. Biochim Biophys Acta 2009;1778:1877–89.
[112] Kodama M, Abe M, Kowasaki Y, Ohira S, Nozaki H, Katagiri C, et al. Estimation of interlamellar water molecules in sphingomyelin bilayer systems studied by DSC and X-ray diffraction. Thermochim Acta 2004;416:105–11.

[113] Chachaty C, Rainteau D, Tessier C, Quinn PJ, Wolf C. Building up of the liquid ordered phase formed by sphingomyelin and cholesterol. Biophys J 2005;8:4032–44.
[114] Grant CWM, Mehlhorn IE, Florio E, Barber K. A long-chain spin label for glycosphingolipids studies: transbilayer fatty acid interdigitation of lactosylceramide. Biochim Biophys Acta 1987;902:160–77.
[115] Kulkarni K, Snyder DS, McIntosh TJ. Adhesion between cerebroside bilayers. Biochemistry 1999;38:15264–71.
[116] Mehlhorn IE, Florio E, Barber KR, Lordo C, Grant CMW. Evidence that transbilayer interdigitation of glycolipid long-chain fatty acids may be a general phenomenon. Biochim Biophys Acta 1988;939:151–9.
[117] Nabet A, Boggs JM, Pezolet M. Study by infrared spectroscopy of the interdigitation of C26:0 cerebroside sulphate into phosphatidylcholine bilayers. Biochemistry 1996;35:6674–83.
[118] Lu D, Singh D, Morrow MR, Grant CWM. Effect of glycosphingolipids fatty acid chain length on behaviour in unsaturated phosphatidylcholine bilayers: a ^{2}H NMR study. Biochemistry 1993;32:290–7.
[119] Boggs JM, Koshy KM. Do the long fatty acid chains of sphingolipids interdigitate across the center of a bilayer of shorter chain symmetric phospholipid. Biochim Biophys Acta 1994;1189:233–41.
[120] Denic V, Weissman JS. A molecular caliper mechanism for determining very long-chain fatty acid length. Cell 2007;130:663–77.
[121] Tessier C, Staneva G, Trugnan G, Wolf C, Nus P. Liquid–liquid immiscibility under non-equilibrium conditions in a model membrane: an X-ray synchrotron study. Colloid Surf B – Biointerfaces 2009;74:293–7.
[122] Self-Medlin Y, Byun J, Jacob RF, Mizuno Y, Mason RP. Glucose promotes membrane cholesterol crystalline domain formation by lipid peroxidation. Biochim Biophys Acta 2009;1788:1398–403.
[123] Staneva G, Chachaty C, Wolf C, Quinn PJ. Comparison of the liquid-ordered bilayer phases containing cholesterol or 7-dehydrocholesterol in modelling the Smith-Lemli-Opitz syndrome. J Lipid Res 2010; doi:10.1194/jlr.M003467.
[124] Kusumi A, Koyama-Honda I, Suzuki K. Molecular dynamics and interactions for creation of stimulation-induced stabilized rafts from small unstable steady-state rafts. Traffic 2004;5:213–30.
[125] Putzel GG, Schick M. Theory of raft formation by cross-linking of saturated or unsaturated lipids in model lipid bilayers. Biophys J 2009;96:4935–40.
[126] Staneva G, Momchhilova A, Wolf C, Quinn PJ, Koumanov K. Membrane domains: role of ceramides in the maintenance of their structure and functions. Biochim Biophys Acta 2009;1788:666–75.
[127] Staneva G, Chachaty C, Wolf C, Koumanov K, Quinn PJ. The role of sphingomyelin in regulating the coexistence in complex lipid model membranes: competition between ceramide and cholesterol. Biochim Biophys Acta 2008;1778:2727–39.
[128] Weber N, Kiewitt I, Mukherjee KD. Modulation of brain lipids of rats by various dietry oils: sunflower, high-oleic sunflower, olive, rapeseed or coriander oil. Nut Res 1999;19:997–1007.
[129] Filippov A, Oradd G, Lindblom G. Lipid lateral diffusion in ordered and disordered phases in raft mixtures. Biophys J 2004;86:891–6.
[130] Bjorkqvist YJE, Brewer J, Bagatolli LA, Slotte JP, Westerlund B. Thermotropic behavior and lateral distribution of very long chain sphingolipids. Biochim Biophys Acta 2009;1788:1310–20.
[131] Manula S, Bjorkqvist YJ, Slotte JP, Ramstedt B. Differences in the domain forming properties of N-palmitoylated neutral glycosphingolipids in bilayer membranes. Biochim Biophys Acta 2007;1768:336–45.
[132] Oh CS, Toke DA, Mandala S, Martin CE. ELO2 and ELO3, homologues of the *Saccharomyces cerevisiae* ELO1 gene, function in fatty acid elongation and are required for sphingolipid formation. J Biol Chem 1997;272:17376–84.
[133] Schneiter R, Tatzer V, Gogg G, Leitner E, Kohlwein SD. Elo1p-dependent carboxyterminal elongation of C14:1Delta(9) to C16:1Delta(11) fatty acids in *Saccharomyces cerevisiae*. J Bacteriol 2000;182:3655–60.
[134] Ejsing CS, Sampaio JL, Surendranath V, Duchoslav E, Ekroos K, Klemm RW, et al. Global analysis of the yeast lipidome by quantitative shotgum mass spectrometry. Proc Nat Acad Sci USA 2009;106:2136–41.
[135] David D, Sundarababu S, Gerst JE, Gerst JE. Involvement of long chain fatty acid elongation in the trafficking of secretory vesicles in yeast. J Cell Biol 1998;143:1167–82.
[136] Proszynski TJ, Klemm RW, Gravert M, Hsu PP, Gloor Y, Wagner J, et al. A genome-wide visual screen reveals a role for sphingolipids and ergosterol in cell surface delivery in yeast. Proc Nat Acad Sci USA 2005;102:17981–6.
[137] Haak D, Gable K, Beeler T, Dunn T. Hydroxylation of *Saccharomyces cerevisiae* ceramides requires Sur2p and Scs7p. J Biol Chem 1997;272:29704–10.
[138] Kuikka M, Ramstedt B, Ohvo-Rekila H, Tuuf T, Slotte JP. Membrane properties of D-erythro-N-acyl sphingomyelins and their corresponding dihydro species. Biophys. J. 2001;80:2327–37.
[139] Rockwell NC, Wolfger H, Kuchler K, Thorner J. ABC transporter Pdr10 regulates the membrane microenvironment in *Saccharomyces cerevisiae*. J Membr Biol 2009;229:27–52.
[140] Kohlwein SD, Eder S, Oh C-S, Martin CE, Gable K, Bacikova D, et al. Tsc13p is required for fatty acid elongation and localizes to a novel structure at the nuclear-vacuolar interface in *Saccharomyces cerevisiae*. Mol Cell Biol 2001;21:109–25.
[141] Iwabuchi K, Nakayama H, Iwahara C, Takamori K. Significance of glycosphingolipid fatty acid chain length on membrane microdomain-mediated signal transduction. FEBS Lett 2010;584:1642–52.