FEBS Letters 584 (2010) 1685–1693

FEBS Letters

journal homepage: www.FEBSLetters.org

Review

Membrane rafting: From apical sorting to phase segregation

Ünal Coskun, Kai Simons *

Max Planck Institute for Molecular Cell Biology and Genetics, Pfotenhauerstraße 108 Dresden, Germany

ARTICLE INFO

Article history:
Received 11 November 2009
Accepted 10 December 2009
Available online 28 December 2009

Edited by Wilhelm Just

Keywords:
Lipid rafts
Membrane heterogeneity
Resting state
Phase separation
Apical transport

ABSTRACT

In this review we describe the history of the development of the raft concept for membrane subcompartmentalization. From its early beginnings as a mechanism for apical sorting in epithelial cells the concept has evolved to a general principle for membrane organisation. After a shaky start with crude methodology based on detergent extraction the field has become increasingly sophisticated, employing a host of different methods that support the existence of dynamic raft domains in membranes. These are composed of fluctuating nanoscale assemblies of sphingolipid, cholesterol and proteins that can be stabilized to coalesce, forming platforms that function in membrane signalling and trafficking.

1. Introduction

In 19th century Agnes Pockels observed that fat/oil washed from dishes by detergents formed a layer on water surfaces. She invented the first apparatus to measure surface pressure of *contaminated water* [1], which was developed further by Irving Langmuir into the so-called Langmuir trough [2]. Langmuir proposed that membranes are formed by fatty acids, which sort themselves as a monolayer at the water/air interface by orienting their polar head groups towards, and their acyl groups away from, the water surface. Only 8 years later, Gorter and Grendel [3] calculated that lipids of red blood cells are capable of forming bilayers in aqueous solutions, and this basic organizing principle of lipid self-aggregation remains valid for all cell membranes. However, the integration of proteins in the bilayer remained enigmatic. The membrane model of Dawson–Danielli–Robertson postulated that the membrane proteins were restricted to either side of the membrane with none penetrating into the bilayer [4,5]. Mark Bretscher was the first to demonstrate (by formylmethionyl sulphone methyl phosphate (FMMP), a membrane impermeable compound) that erythrocyte membrane proteins span the bilayer [6]. He labeled a major protein component of red blood cells from one or both side of the membrane showing that parts of the protein resides on both side of the membrane, and clearly demonstrating that the protein was spanning the membrane with a fixed orientation [7]. FMMP was later used together with the chemical crosslinker dimethylsuberimidate to show that the spike glycoproteins of Semliki Forest virus extend through the viral membrane [8]. In 1972, Singer and Nicolson [9] published their classical view of membrane structure. In their model, mainly monomeric and amphiphilic membrane proteins diffuse in a two-dimensional fluid lipid bilayer, in line with the discovery that proteins mix in the plane of the plasma-membrane after two cells have been fused with each other [10]. Indeed, Henderson and Unwin later showed by 2D-crystallography that the purple membrane protein bacteriorhodopsin has seven transmembrane helices that span the membrane [11] and that these transmembrane helices were of hydrophobic nature [12]. The first three dimensional atomic structure of the photosynthetic reaction center of a membrane protein [13] and the advances in recombinant DNA technology directed most of the efforts in membrane research towards proteins. While the scientific community focused on the proteins, the lipid bilayer turned into its stepchild. Although lateral membrane heterogeneity and the capacity of lipids to form membrane domains were described early [14–16], the lipid bilayer itself was simply considered as a component of a secondary importance on protein bioactivity, regulation or cell biological processes. What was lacking was the functional significance for the lipid organization. In our early work on epithelial cells, which polarize their cell surface into apical and basolateral plasma membrane domains, lipids were included in the analysis. The two epithelial surface domains not only have distinct protein composition but also different lipids apically and basolaterally. In the 1970s, Pascher and Karlsson emphasized the differences in structure and behaviour between glycosphingolipids and glycerolipids, the former having the capacity for hydrogen bonding at the

* Corresponding author. Fax: +49 (0)351 210 1209.
E-mail addresses: coskun@mpi-cbg.de (Ü. Coskun), simons@mpi-cbg.de (K. Simons).

0014-5793/$36.00
doi:10.1016/j.febslet.2009.12.043

hydrophobic–hydrophilic interface of the lipid backbone, while the latter could only be a hydrogen bond donor. The first clues to glycolipid sorting at the Golgi complex came from studies using NBD–ceramide, which was metabolized into NBD–glucosylceramide in the Golgi and then delivered apically and basolaterally. We could demonstrate that the fluorescently labeled glycolipid displayed a higher apical delivery from the Golgi as compared to the basolateral direction [17].

On the basis of these data, we postulated in 1988 that sphingolipids and apical proteins are linked to each other in the trans-Golgi network to form transport carriers for delivery to the apical membrane, forming the biochemical basis for the raft concept [18]. Later, novel insights from biophysical studies on model membranes and biochemical studies on sphingolipid–cholesterol–protein assemblies led the way to further advances, resulting in the lipid raft concept [19] wherein physicochemical membrane properties were linked to biological functions. The raft hypothesis proposed a new dimension of membrane organization, that is lateral heterogeneity driven by preferential association of sterols, glycosphingolipids, and a subset of membrane proteins that was thought to function in a number of cellular processes, particularly in the context of cell signalling and membrane traffic [20].

2. Detergent resistance of membranes and rafts

The first biochemical advance of lateral heterogeneity in cell membranes came from detergent solubilisation studies. The differential resistance of membrane proteins towards detergents has been known long before the raft hypothesis was formulated. In 1973, Yu and colleagues had shown the selective solubilisation of proteins and phospholipids by Triton X-100 treatment of human erythrocyte membrane ghosts at low ionic strengths and temperatures [21]. The remaining insoluble fraction appeared by electron microscopy to be a filamentous reticulum with adherent lipoid sheets and vesicles that they called detergent-insoluble ghosts. Quantification revealed that the majority of lysolecithin, phosphatidic acid, phosphatidylcholine, phosphatidyl–ethanolamine and phosphatidylserine were recovered in the supernatant, while 83% of the recovered sphingomyelin was in the insoluble membrane fraction, as well as the vast majority of glycosphingolipids. Moreover, at 37 °C the detergent tended to disperse the membranes completely. Sucrose density centrifugation showed a dense and a light zone, corresponding to the soluble and insoluble membrane fractions. After having been forgotten for years, detergent resistance turned out to be a helpful tool in membrane research. The first important application came from Brown and Rose who showed that newly synthesized glycophosphatidylinositol (GPI) anchored proteins are sorted into a detergent resistant, sphingolipid-enriched membrane fraction while passing through the Golgi apparatus on their way to the apical surface [22]. Interestingly, the basolateral marker protein remained soluble, biochemically demonstrating a potential association of apical membrane proteins with glycosphingolipids. Similar to erythrocytes, the soluble membrane fraction contained most of the phospholipids, while the detergent resistant membranes (DRM) contained the bulk of the sphingolipids. Together, these observations formed the first biochemical support of our proposed model of glycolipid sorting from the TGN to the apical membrane. Additional support came from Hanada and colleagues, who showed that the detergent resistance of the GPI anchored PLAP is decreased when cholesterol and/or sphingomyelin are deprived from cells by genetic manipulations, while insolubility could be restored by metabolic complementation [23].

The second finding came from our lab and demonstrated that the proteins found in DRMs from MDCK contained a subset that we had observed by 2-D gel electrophoresis of immuno-isolated apical and basolateral carrier vesicles. Thus this subset could represent apical sorting machinery. One of these proteins was of 21 kDa size, localized to the Golgi-apparatus, the plasma membrane and vesicular structures, making it a candidate for vesicular transport machinery, cycling between the plasma membrane and the Golgi. Therefore the protein was termed vesicular integral protein VIP21 [24]. Unexpectedly, VIP21 was very abundant in the non-clathrin-coated plasma membrane invaginations called caveolae [25], uncoated plasma membrane pits into which the GPI anchored folate receptor had been reported to partition in a cholesterol-dependent manner [26,27]. The DNA sequence encoding for caveolin then cleared the mystery when it was found that canine VIP21 shared 86% sequence identity to chicken caveolin [28], which was later found to be a cholesterol binding protein [29]. These and other findings led to an upsurge of studies in which detergent-insolubility was used as a criterion to define protein localization to caveolae [30,31] through their affinity for membrane domains/rafts. However, it had been shown previously that detergent insoluble membranes with GPI anchored proteins as constituents can also be isolated from cells lacking caveolae [32,33] and that potentially raft-associating GPI-anchored proteins can be separated from caveolin, suggesting that these proteins may exist side by side or together in the plasma membrane to organize signalling molecules and process surface-bound ligands differentially [34]. Concurrently, pitfalls in the biochemical interpretation of differential detergent extraction began to be discussed. Kurzchalia et al. stated in 1995 that detergent insolubility cannot be related for microdomain association per se, also pointing out that DRMs give no clue as to where DRM constituents are localized in the cell [35]. Despite such disclaimers, DRM association continued to be misconstrued, and although no longer being equated to caveolae, DRMs became synonymous with lipid rafts. We have suggested that DRMs are most useful in assigning potential raft association when changes in the composition are induced by physically/biochemically meaningful events (e.g. ligand binding, receptor oligomerizaion, mutagenesis of protein structure) [36]. However, the self-evident fact that detergent resistant membranes represent the artifactual coalescence of insoluble raft proteins and lipids into a residue that does not exist in living cells [36–39] remained overlooked in the overzealous aim of assigning raft-association and functionality to a wide variety of membrane proteins.

3. Cholesterol removal by cyclodextrin

In cell culture studies, 2-hydroxypropyl-cyclodextrin was described to be a helpful agent for preparation and delivery of stable aqueous solutions of various forms of sterols and bile acids [40]. Fahrenholz et al. introduced ß-cyclodextrin to deplete or deliver cholesterol to the myometrial plasma membrane in order to modulate the oxytocin receptor, with the removal of cholesterol from the membrane leading to conversion of the receptor from a high to a low affinity state, and vice-versa [41]. We used cholesterol depletion by cyclodextrin to demonstrate the direct involvement of cholesterol in the DRM association of the apical marker protein hemagglutinin (HA) [42], while also showing that TGN-to-surface transport of the apical marker could be impaired by decreasing cholesterol levels without perturbing the transport of the basolateral marker vesicular stomatitis virus glycoprotein (VSVG) [43]. Sensitivity to cholesterol depletion became a routine method in the cell biological community to assign raft involvement to cellular processes, often used side-by-side with detergent resistant membranes. However similar to the case of DRMs, several possible pitfalls resulting from acute cholesterol depletion had to be considered. Pike and Miller noted that depletion of cellular cholesterol leads to the inhibition of epidermal growth factor- and bradykinin

stimulated PtdIns turnover in A431 cells and therefore a loss of compartmentalization of $PI(4,5)P_2$ [44], one of the key cofactors in signalling to the actin cytoskeleton and in vesicle trafficking [45]. Additionally, it was found that cholesterol depletion of membranes by methyl-ß-cyclodextrin (MBCD) results in tight packing of sphingolipids hydrocarbon chains to form a rigid gel-like phase [46].

It was becoming clear that the tools used to study the properties of membrane domains and protein partitioning were inherently pleiotropic. Nevertheless, detergent insolubility of specific components and cholesterol dependence of specific cellular processes suggested functional lateral heterogeneities of membrane proteins, dependent on sphingolipids and cholesterol. The next challenge was to visualize and analyze how raft and non-raft domains are organized in the plasma membrane.

4. Membrane clustering by toxins and antibodies I: rafts are dynamic

Locomotory animal cells, except sperms, show a patchy distribution at the plasma membrane upon clustering with polyvalent agents such as antibodies, which move to one end of the cell to form a "cap" [47,48] best studied in lymphocytes. The same capping principle was observed when the ganglioside GM1 was clustered by antibodies [49,50]. These redistribution events were dynamic and depended on the extent of cross-linking by toxins and antibodies. This was also shown to be true for receptors. To observe the native distribution of the folate receptor Mayor et al. labeled a monoclonal antibody with the fluorophore Cy3 [51]. At 37 °C the fluorescence was diffusely distributed over the cell surface, whereas the addition of a polyclonal unlabeled secondary antibody drove receptor redistribution to caveolae, suggesting that multimerization/cross-linking of the GPI-anchored protein regulates its sequestration into caveolae. The same sequestering effect upon clustering was then reported for glycosphingolipids and sphingomyelin [52].

Similarly, we showed that simultaneous cross-linking of raft markers (GM1, GPI-anchored PLAP, and HA) led to their co-localization into distinct patches, segregating away from the transferrin receptor as a non-raft marker [53]. Furthermore cyclodextrin extraction of cellular cholesterol inhibited the segregation, highlighting the role of cholesterol in raft coalescence [42]. Using a cell system lacking caveolae, it was demonstrated that raft and non-raft markers segregated in the same cholesterol-dependent way in the absence of caveolae [53]. These results definitively demonstrated that clustered raft markers segregate away from non-raft proteins in a cholesterol dependent, but caveolae independent, manner.

5. Membrane clustering by toxins and antibodies II: role in signalling

Capping after cross-linking describes the redistribution of lymphocyte membrane constituents not only into a patchy pattern, but more importantly into a signalling hotspot with an important biological function: the formation of the immunological synapse. Upon antigen presentation by a neighboring cell, T cells show selected non-random large-scale molecular rearrangement of their plasma membrane, which results in the formation of an immunological synapse [54], forming a supramolecular activation cluster [55]. The formation of the immunological synapse is the prime example of functional membrane segregation *in vivo* suggesting a potential involvement of the raft concept of membrane subcompartmentalization. Evidence for this involvement was the finding that cross-linking of a GPI anchored protein stimulated T cell activation [56] The corresponding immunoprecipitation showed that the clustering effect involved the palmitoylated protein tyrosine kinase (PTK) Lck. Moreover, this stimulating effect of raft cross-linking on T cell activation was cholesterol dependent [57]. Interestingly, in cells that are defective for GPI anchor synthesis the activation of T cells through the T cell receptor (TCR) was reported to be impaired [58]. The molecular basis for selective coalescence behavior during immunological synapse formation was recently investigated by lipidomics. These signalling foci were immuno-isolated and a comprehensive mass spectrometric analysis revealed specific enrichment of membrane order-potentiating sphingolipids, saturated/longchain glycerophospholipids and cholesterol that is concordance with the involvement of raft coalescence as means for lateral sorting of membrane proteins into signalling domains [59].

Also the transmembrane IgE receptor FcεRI could be activated by antibodies or by polyvalent ligand, and also in this case, the surface clustering of FcεRI recruited a palmitoylated PTK, Lyn [60,61]. In contrast to the transmembrane FcεRI, it was unclear how a GPI anchored protein that is linked to the membrane only by the lipid moiety can communicate with intracellular tyrosine kinases. Our co-patching experiments of GPI- anchored Thy-1 showed that the PTK Fyn co-localized cytosolic leaflet with the clustered raft markers, segregating away from the non-raft transferrin receptor clusters [53]. We later reported a strong accumulation of actin to clusters of GPI-anchored CD59 and GM1 depending on the recruitment of PTKs [62], in accord with the finding that the clustering of GM1 accompanied signalling via the T cell receptor [63].

Taken together, these results suggested that selective communication across the bilayer is possible by microdomain association. Additional support for this hypothesis came from Viola et al., showing that the co-stimulatory effect of the co-receptor CD28 in T cells facilitates the activation of the naïve T lymphocytes based on raft association [64]. The homogenous distribution of GM1 in unstimulated resting T cells was switched after stimulation of T cells by anti-CD28-coated beads. The stimulation led to the redistribution of GM1 to a cap, contacting the stimulating bead, and a concomitant increase in life-time of early tyrosine phosphorylation events. Importantly, clustering of molecules that are thought not to be raft associated did not result in the lateral reorganization of lipidated cytosolic effector proteins [65]. Overall, these findings strongly supported the view that raft clusters can function as centers of signal transduction [62].

6. The cellular membrane and its complexity

The redistribution/coalescence of raft lipids and proteins upon cross-linking and their subsequent engagement in signalling brought to the forefront the question of the size of unperturbed raft domains. Because raft lipids and proteins appeared microscopically homogeneously dispersed in the plasma membrane before cross-linking, the native size was obviously smaller than the resolution of light microscopy. We tried to meet this technical challenge by a new technique called photonic force microscopy. Employing a laser trap to measure the local viscous drag of single membrane proteins [66] yielded a mean radius of 26 ± 13 nm for raft assemblies, which seemed stable for minutes. However, the antibodies immobilized on the beads required for the technique likely biased both the size and particularly the lifetime, of the observed domains. The size of rafts as well as their lifetime has continued to be investigated, and particularly the life-time value has remained controversial, while the size seems to vary depending on the method employed to measure them [67]. However, all findings seemed to point to the same result: rafts are small and

dynamic, could change their composition upon intra- or extracellular stimuli and could be clustered to larger domains with consequences for cell signalling [20,68].

Important advances came from an outpouring of data from biophysicists, exploring model membranes to get insight into lipid behaviour in bilayers. This area has been reviewed elsewhere [69,70] and will not be further pursued here, except to emphasize the fundamental disconnect between simple mixtures of purified lipids and the tremendous protein and lipid complexity of biological membranes, which can host up to 9600 species of glycerophospholipids, thousands of mono/di/triacyl glycerol variants, in addition to numerous fatty acids and sterol-based structures [71]. Considering that this lipid diversity is augmented by the density of protein in the plasma membranes in animal cells [72], it becomes clear that proteins must contribute to the behaviour and heterogeneity of cellular membranes. Assuming that the protein entities have varying transmembrane domain size and dimensions, with some consisting of large ecto- and cytosolic domains, one can conclude that the massive amount of protein will cover most of the lipid surface and produce steric restrictions [73,74], not to mention the additional contribution by ectodomain glycosylation. Furthermore, membrane proteins are rather oligomeric than monomeric [73], adding another level of complexity to membrane–protein interactions. The interplay of the different protein properties would be expected to create membrane regions of varying thickness and composition, since lipids will be forced to rearrange to match the rather rigid protein [73,75,76]. Considering all this, it is tempting to intuitively conclude that the lipid bilayer might indeed be a passive solvent for protein as it had been surmised. However, two issues are rarely considered. First, the amount of various lipids and proteins in the biological membrane makes it unlikely that all interaction energies of the different molecular species are within thermal energies. Therefore, the protein and lipid distribution cannot be laterally random [73]. Additionally, not only specific lipid–lipid interactions, but also lipid–protein interactions, bring in an additional level of specificity and heterogeneity. Second, the massive amount of membrane proteins does not necessarily reflect their effective transmembrane occupancy within the bilayer. Protease "shaving" of red blood cell membrane resulted in a protein occupancy of the bilayer of at least 23% [72]. This value is strikingly similar to the transmembrane occupancy of densely packed systems; in synaptic vesicles the total protein:lipid ratio of 1.94 (w/w) results in a effective transmembrane domains occupancy of 20% [77]. Similarly, the Semliki Forest virus (SFV) was estimated to have a transmembrane protein occupancy of about 20% [78]. It is also possible to estimate the transmembrane protein occupancy of the virus bilayer by observing the cross-section through 9 Å 3D structure [79] of the Sindbis virus, which belongs as SFV to the alphaviruses. From the cross-sectional projection, it is obvious that the transmembrane domains are occupying a minority of the space within the bilayer (Fig. 1), and our calculations resulted in an average value of ~25%. Higher protein contents within the bilayer are tolerable. For example, the *Halobacterium halobium* tolerates 75% bacteriorhodopsin in its plasma membrane [80], however it must be noted that this protein is concentrated to a semicrystalline lattice in the bilayer. Clearly proteins contribute to a significant fraction of membrane heterogeneity. It would seem that a reduction in lateral membrane dimensionality through the formation of functional complexes is a theme that has been favoured during evolution. Consequently it is likely that cells unite protein- and lipid-species into self-assembling complexes to effect this organization. As such, we do not define membrane heterogeneity as either lipid or protein, but rather as a co-operation between both.

7. The need for novel tools and methodology

In 2007, Ken Jacobson, Ole Mouritsen and Richard Anderson published a review entitled "Lipid rafts: at a crossroad between cell biology and physics" [67]. They stated that "*...the lipid raft field is now at a technical impasse since the physical tools to study biological membranes as a liquid in space and time are still being developed...*" and that "*...further application of existing tools and the development of new tools are needed to understand the dynamic heterogeneity of biological membranes...*". Indeed, membrane research has advanced through a number of new high-resolution imaging methods and quantitative mass spectrometry, paving the way for quantitative evaluation of preferential lipid–lipid and lipid–protein associations in the cell bilayer [59,81–85]. The exciting recent achievements as well as the current view on rafts are extensively described in another recent review [86], and will not be covered in detail here, except to note that most results point to a unifying principle: in uncrosslinked conditions, rafts are small and highly dynamic in terms of size and stability. The size and lifetime distributions are clearly cholesterol dependent, while the involvement of the membrane-underlying actin cytoskeleton is being actively investigated [81,83,87,88].

8. Biologically-complex membrane model systems

In parallel to recent technical developments that have allowed observation of membrane properties at submicroscopic levels, new plasma membrane model systems have been established that allow studies of raft clustering events and membrane phase separation in biologically complex membranes. The systems bypass metabolic constraints, such as endo- and exocytotic turnover of the plasma membrane, as well as cellular constraints such as membrane-cytoskeleton interaction. Baumgart et al. demonstrated that chemically induced giant plasma membrane vesicles (GPMVs) have a temperature-dependent capacity to separate into phases analogous to the Lo (liquid-ordered) and Ld (liquid-disordered) states seen in model systems [89]. It is important to note that the temperature dependence of this process highlights collective behaviour of the lipids as underlying the phase separation phenomenon. However, both in model membrane systems such as GUV's with controlled lipid composition, as well as in the GPMV system, transmembrane raft proteins are typically excluded from the ordered and more packed membrane phase [90–93]. GPMV blebbing is induced by a combination of paraformaldehyde (PFA) and dithiothreitol (DTT) treatment of cells for 8–12 h, both being a pivotal issue. Protein cross-linking due to the PFA treatment will certainly induce constraints in membrane and protein mobility. Additionally, treatment with DTT leads to the removal of palmitoyl modifications on proteins [94] and an altered partitioning behaviour of raft marker proteins into membrane domains [95].

Recently we introduced a new plasma membrane system, in which reactive chemicals such as PFA or DTT were avoided. Plasma membrane spheres (PMS) were produced by swelling of cells in phosphate buffered saline [96]. Common to the Baumgart method, cytoskeletal constraints as well as exocytic/endocytic turnover are removed. The surprising feature of the PMS system is that the incubation with the pentavalent cholera toxin induces cholesterol-dependent large-scale phase separation at physiological 37 °C. This finding is in accord with another report where osmotic swelling of cells was reported to form large membrane domains [97]. Measuring the membrane order by the order-sensing dye C-laurdan has shown that the Lo phase in phase separated GUV's and GPMV's is more ordered than in the GM1 "phases" in the PMS [98].

This suggests that raft coalescence employs chemical specificity in addition to membrane order, likely selective protein interaction,

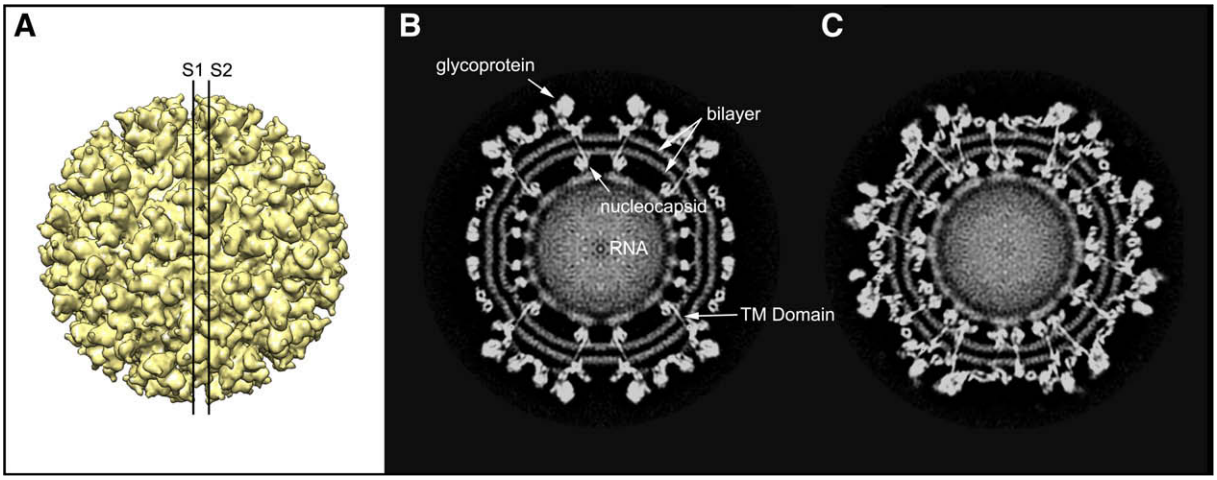

Fig. 1. The transmembrane-domain occupancy of Sindbis virus spike proteins within the virus bilayer. (A) Surface representation of the Sindbis virus (EMDB Entry EMD-1121) with cross-sections S1 (B) and S2 (C) through the 9 Å [79] map. Images were produced using the UCSF Chimera package from the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco [124].

to laterally sort and regulate membrane constituents. Another novelty of the PMS system is that in contrast to GUVs and GPMVs, transmembrane raft proteins are enriched and non-raft proteins are excluded from the GM1 phase after cholera toxin clustering [96]. The PMS system highlights that the plasma membrane has an inherent capability to separate microscopic raft domains at physiological temperatures when cellular constraints are released without chemical perturbation. This demonstrates the existence of selective connectivity between certain components of the plasma membrane; the challenge is to now ascertain how these principles operate in the living cell. We believe that these new systems open the possibility to follow biological membrane heterogeneity from the resting state of fluctuating nanoscale sphingolipid–sterol–protein assemblies to functional raft platforms in living cells all the way to phase separation in isolated plasma membranes (Fig. 2).

9. Lipid–protein interactions

Many membrane proteins are lipid modified by GPI-anchors, myristoyl- or palmitoyl groups equipping them with membrane binding specificity. Additionally, an increasing number of proteins are reported to exhibit specific lipid binding and/or interaction capacities, with possible structural and regulatory functions. Several proteins interact with cholesterol by direct binding, with caveolin as the prime example [29]. The influenza virus ion channel and maturation cofactor M2 is a palmitoylated and cholesterol-binding protein with a possible function in raft dependent virus budding [99]. An increasing number of X-ray structures show that specific lipids are selected as integral components of the quaternary structure of many membrane protein complexes [100–105]. As an example, the ß-adrenergic receptor forms a dimer whose dimeric interface is formed 70% from lipids, consisting of two cholesterol molecules and six palmitates [103,106]. In fact a strict cholesterol consensus motif (CCM) for cholesterol interaction within the A class of GPCRs was defined, where 21% of human class A receptors are predicted to bind cholesterol at the same site as β2AR [104]. In addition to cholesterol-binding proteins, several proteins are reported to either bind specifically, or to be regulated by, gangliosides [107]. For example, the TNF receptor Fas has a regulatory glycosphingolipid binding motif in the extracellular domain [108] which shares some sequence similarity with the V3 loop of HIV-1 gp120, which has been reported as a (glyco)sphingolipid-binding domain in Alzheimer, prion, and HIV-1 proteins [109], and a structural protein motif has been identified for binding to sphingolipids [110]. Protein regulation by glycosphingolipids has been shown for the human EGF receptor ectodomain, which has a capacity to bind GM3 [111] with the consequence of kinase domain inhibition [112]. A similar sensitivity has been described for the insulin receptor, which becomes insulin resistant in the presence of GM3 [113,114] with relevance in diseases such as diabetes and Gaucher disease [115]. Finally, it has been shown in proteoliposomal systems that amyloid precursor protein cleavage by the beta-secretase BACE can be modulated by cholesterol, glycosphingolipids and anionic phospholipids [90].

The number of proteins reported to be regulated by specific lipid interaction is increasing steadily, but the precise structural mechanisms behind specific binding and receptor regulation in membranes remain uncharacterized. Some questions are obvious: e.g. a liquid-ordered bilayer is thicker than a liquid-disordered, does thus the length of the transmembrane domain of the proteins carry not only preferential sorting [116] but also regulatory function? How do lipid–protein interactions dynamically change protein function? Do transmembrane proteins become raftophilic by being wetted or lubricated by binding raft lipids [86,107]? The perspective that lipids can modulate membrane protein function promises to bridge the long-standing gap between membrane protein biochemistry and crystallography on one side and lipid research on the other.

10. Outlook

Due to recent tremendous technical achievements, we now start to understand and cope with the complexity of dynamic membrane heterogeneity at the molecular level and its relevance in cell biology. The technical development seems just to have started. Recently van Zenten et al. presented a combined confocal/NSOM approach, allowing nanoscopic co-localization studies that showed integrin nanoclusters residing proximal to GPI anchored protein nanodomains, forming hotspots, which function as nucleation sites for adhesion on the cell surface [117]. Another example of a technical leap forward is the recent quantification of the lipid content of biological membranes in absolute amounts by mass spectrometry [84,118,119]. This highly sophisticated method together with new improvements in protein tagging and organelle purification protocols, allowed us to show that sphingolipids

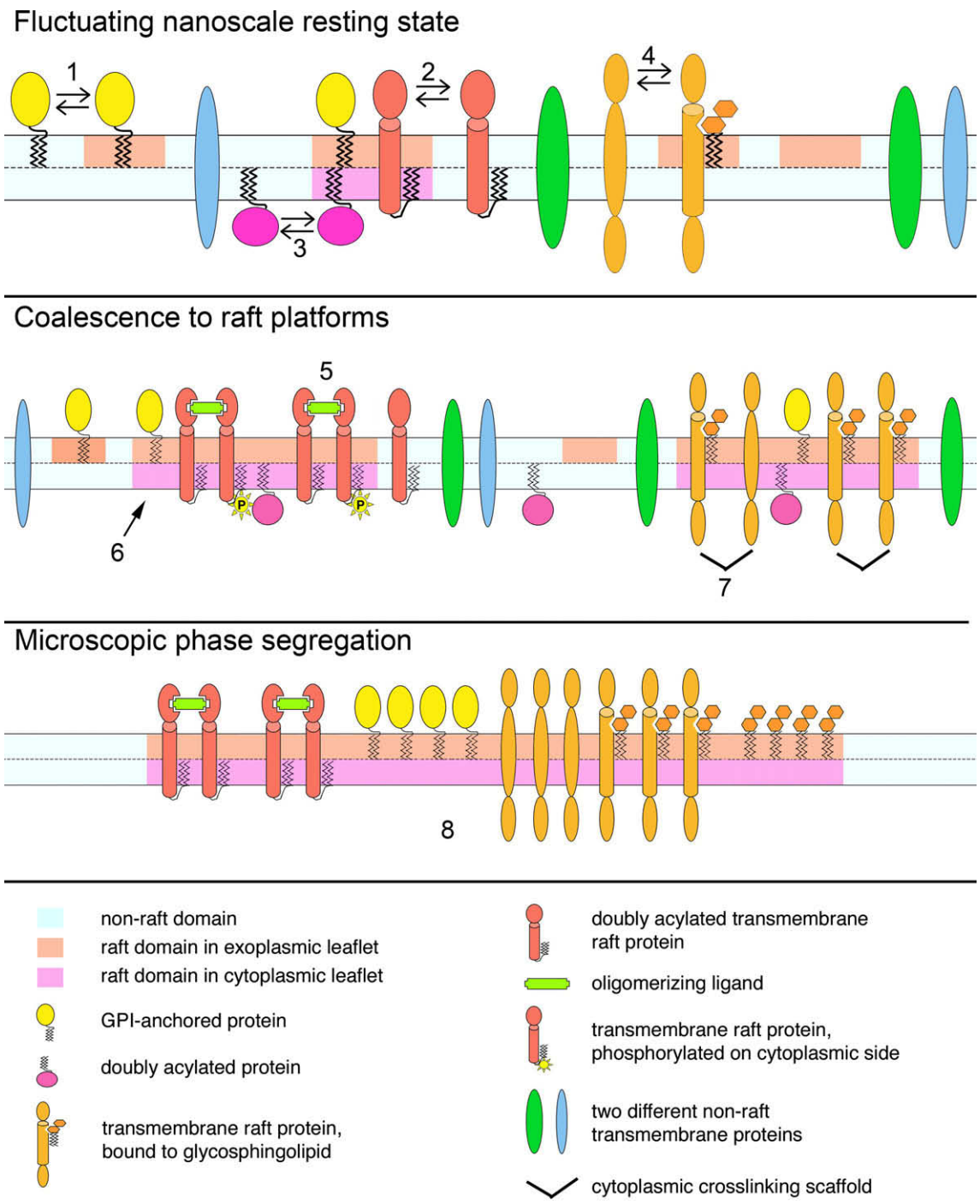

Fig. 2. Schematic representation of the current view on raft properties and behaviour. Rafts are small and dynamic in uncrosslinked resting state, but can change their composition upon intra- or extracellular stimuli and clustered to larger domains with consequences for cell signalling. So far GPI-anchored proteins (1), acyl chain containing transmembrane proteins (2) and doubly acylated PTKs (3) are clearly reported to be raft associated. A fourth group of transmembrane proteins is reported to be raft associated by raft lipid binding (4). Upon oligomerization of raft proteins by multivalent ligands (5) raft associated proteins/lipids can coalesce into larger domains. The role and involvement of the membrane-underlying actin cytoskeleton (7) in raft coalescence is being investigated. It is not known how cytoplasmic leaflet lipids will coalescence to a raft phase upon extracellular domain formation (6), but recruitment of doubly acylated proteins like the Src-family kinases to the cytoplasmic leaflet has been already documented. Large-scale domain/phase formation can be induced by clustering of raft lipids and/or proteins in the absence of cellular constrains, such as in plasma membrane spheres (8).

and sterols are sorted into secretory vesicles at the trans-Golgi network carrying raft protein cargo [85]. For the first time, lipidomes of carriers involved in raft cargo transport were quantified and indeed demonstrated that sphingolipids and sterols are enriched in these carriers. Based on a genomic screen [120] as well as classical yeast genetics combined with mass spectrometry [121] both ergosterol and sphingolipids could be functionally implicated and synergistically interacting. Thus there is no doubt in this case that lipids are directly involved in the sorting process as postulated.

Despite all achievements of the last 20 years, we are still far away from understanding membrane heterogeneity at the level

of lipids and proteins and its functional consequences. However, the multidisciplinary efforts of scientists from different backgrounds and the critical dialogue that characterizes the field have decisively promoted substantial progress in this fascinating research area. The controversies about the raft concept have stimulated the efforts required to meet the challenge of studying collective behaviour of lipids and proteins in membranes. Rafts were introduced as a metaphor for dynamic membrane platforms that have different functions and are built up of sphingolipids, sterols and proteins. Their size can vary as also logs in a river can form platforms of different sizes and can jam up into giant rafts, like a separated GM1 domain in PMS or an apical membrane in an epithelial cell [122]. Principles of collective physiochemical behaviour are properties not exclusive to membrane research. Recently, phase separation was introduced to understand how RNA–protein complexes form P-granules during the asymmetric division of *Caenorhabditis elegans* [123]. The perspective that we can start to understand how assemblies of molecules perform functions together is fascinating and opens new vistas in cellular research.

Acknowledgments

We thank the entire members of the laboratory for helpful comments on the manuscript and stimulating discussions. We like to thank Michael Rossmann and Andrei Fokine (Purdue University, West Lafayette) for advice on Sindbis Virus Cryo-EM structure and Doris Meder (CRG, Barcelona) for providing us the template for Fig. 2. This work was supported by grants from Deutsche Forschungsgemeinschaft (SFB/TR13-04 & SP1175) and the EU FP6 "PRISM" project.

References

[1] Pockels, A. (1891) Surface tension. Nature 43, 437–439.
[2] Langmuir, I. (1917) The constitution and fundamental properties of solids and liquids. II. Liquids. 1. J. Am. Chem. Soc. 39, 1848–1906.
[3] Gorter, E. and Grendel, F. (1925) On biomolecular layers of lipoids on the chromocytes of the blood. J. Exp. Med. 41, 439–443.
[4] Robertson, J.D. (1957) New observations on the ultrastructure of the membranes of frog peripheral nerve fibers. J. Biophys. Biochem. Cytol. 3, 1043–1048.
[5] Danielli, J.F. and Davson, H. (1935) A contribution to the theory of permeability of thin films. J. Cell. Comparat. Physiol. 5, 495–508.
[6] Bretscher, M.S. (1971) Human erythrocyte membranes: specific labelling of surface proteins. J. Mol. Biol. 58, 775–781.
[7] Bretscher, M.S. (1971) A major protein which spans the human erythrocyte membrane. J. Mol. Biol. 59, 351–357.
[8] Garoff, H. and Simons, K. (1974) Location of the spike glycoproteins in the Semliki Forest virus membrane. Proc. Natl. Acad. Sci. USA 71, 3988–3992.
[9] Singer, S.J. and Nicolson, G.L. (1972) The fluid mosaic model of the structure of cell membranes. Science 175, 720–731.
[10] Frye, L.D. and Edidin, M. (1970) The rapid intermixing of cell surface antigens after formation of mouse–human heterokaryons. J. Cell Sci. 7, 319–335.
[11] Henderson, R. and Unwin, P.N. (1975) Three-dimensional model of purple membrane obtained by electron microscopy. Nature 257, 28–32.
[12] Unwin, N. and Henderson, R. (1984) The structure of proteins in biological membranes. Sci. Am. 250, 78–94.
[13] Deisenhofer, J., Epp, O., Miki, K., Huber, R. and Michel, H. (1984) X-ray structure analysis of a membrane protein complex. Electron density map at 3 Å resolution and a model of the chromophores of the photosynthetic reaction center from *Rhodopseudomonas viridis*. J. Mol. Biol. 180, 385–398.
[14] Karnovsky, M.J., Kleinfeld, A.M., Hoover, R.L. and Klausner, R.D. (1982) The concept of lipid domains in membranes. J. Cell Biol. 94, 1–6.
[15] Thompson, T.E. and Tillack, T.W. (1985) Organization of glycosphingolipids in bilayers and plasma membranes of mammalian cells. Annu. Rev. Biophys. Biophys. Chem. 14, 361–386.
[16] Stier, A. and Sackmann, E. (1973) Spin labels as enzyme substrates, heterogeneous lipid distribution in liver microsomal membranes. Biochim. Biophys. Acta 311, 400–408.
[17] van Meer, G., Stelzer, E.H., Wijnaendts-van-Resandt, R.W. and Simons, K. (1987) Sorting of sphingolipids in epithelial (Madin–Darby canine kidney) cells. J. Cell Biol. 105, 1623–1635.
[18] Simons, K. and van Meer, G. (1988) Lipid sorting in epithelial cells. Biochemistry 27, 6197–6202.
[19] Simons, K. and Ikonen, E. (1997) Functional rafts in cell membranes. Nature 387, 569–572.
[20] Simons, K. and Toomre, D. (2000) Lipid rafts and signal transduction. Natl. Rev. Mol. Cell Biol. 1, 31–39.
[21] Yu, J., Fischman, D.A. and Steck, T.L. (1973) Selective solubilization of proteins and phospholipids from red blood cell membranes by nonionic detergents. J. Supramol. Struct. 1, 233–248.
[22] Brown, D.A. and Rose, J.K. (1992) Sorting of GPI-anchored proteins to glycolipid-enriched membrane subdomains during transport to the apical cell surface. Cell 68, 533–544.
[23] Hanada, K., Nishijima, M., Akamatsu, Y. and Pagano, R.E. (1995) Both sphingolipids and cholesterol participate in the detergent insolubility of alkaline phosphatase, a glycosylphosphatidylinositol-anchored protein, in mammalian membranes. J. Biol. Chem. 270, 6254–6260.
[24] Kurzchalia, T.V., Dupree, P., Parton, R.G., Kellner, R., Virta, H., Lehnert, M. and Simons, K. (1992) VIP21, a 21-kD membrane protein is an integral component of trans-Golgi-network-derived transport vesicles. J. Cell Biol. 118, 1003–1014.
[25] Dupree, P., Parton, R.G., Raposo, G., Kurzchalia, T.V. and Simons, K. (1993) Caveolae and sorting in the trans-Golgi network of epithelial cells. EMBO J. 12, 1597–1605.
[26] Rothberg, K.G., Heuser, J.E., Donzell, W.C., Ying, Y.S., Glenney, J.R. and Anderson, R.G. (1992) Caveolin, a protein component of caveolae membrane coats. Cell 68, 673–682.
[27] Rothberg, K.G., Ying, Y.S., Kamen, B.A. and Anderson, R.G. (1990) Cholesterol controls the clustering of the glycophospholipid-anchored membrane receptor for 5-methyltetrahydrofolate. J. Cell Biol. 111, 2931–2938.
[28] Glenney Jr., J.R. and Soppet, D. (1992) Sequence and expression of caveolin, a protein component of caveolae plasma membrane domains phosphorylated on tyrosine in *Rous sarcoma* virus-transformed fibroblasts. Proc. Natl. Acad. Sci. USA 89, 10517–10521.
[29] Murata, M., Peranen, J., Schreiner, R., Wieland, F., Kurzchalia, T.V. and Simons, K. (1995) VIP21/caveolin is a cholesterol-binding protein. Proc. Natl. Acad. Sci. USA 92, 10339–10343.
[30] Anderson, R.G. (1998) The caveolae membrane system. Annu. Rev. Biochem. 67, 199–225.
[31] Smart, E.J., Graf, G.A., McNiven, M.A., Sessa, W.C., Engelman, J.A., Scherer, P.E., Okamoto, T. and Lisanti, M.P. (1999) Caveolins, liquid-ordered domains, and signal transduction. Mol. Cell Biol. 19, 7289–7304.
[32] Fra, A.M., Williamson, E., Simons, K. and Parton, R.G. (1994) Detergent-insoluble glycolipid microdomains in lymphocytes in the absence of caveolae. J. Biol. Chem. 269, 30745–30748.
[33] Gorodinsky, A. and Harris, D.A. (1995) Glycolipid-anchored proteins in neuroblastoma cells form detergent-resistant complexes without caveolin. J. Cell Biol. 129, 619–627.
[34] Schnitzer, J.E., McIntosh, D.P., Dvorak, A.M., Liu, J. and Oh, P. (1995) Separation of caveolae from associated microdomains of GPI-anchored proteins. Science 269, 1435–1439.
[35] Kurzchalia, T.V., Hartmann, E. and Dupree, P. (1995) Guilty by insolubility – does a protein's detergent insolubility reflect a caveolar location? Trends Cell Biol. 5, 187–189.
[36] Lingwood, D. and Simons, K. (2007) Detergent resistance as a tool in membrane research. Natl. Protoc. 2, 2159–2165.
[37] Lichtenberg, D., Goni, F.M. and Heerklotz, H. (2005) Detergent-resistant membranes should not be identified with membrane rafts. Trends Biochem. Sci. 30, 430–436.
[38] Shogomori, H. and Brown, D.A. (2003) Use of detergents to study membrane rafts: the good, the bad, and the ugly. Biol. Chem. 384, 1259–1263.
[39] Schuck, S., Honsho, M., Ekroos, K., Shevchenko, A. and Simons, K. (2003) Resistance of cell membranes to different detergents. Proc. Natl. Acad. Sci. USA 100, 5795–5800.
[40] De Caprio, J., Yun, J. and Javitt, N.B. (1992) Bile acid and sterol solubilization in 2-hydroxypropyl-beta-cyclodextrin. J. Lipid Res. 33, 441–443.
[41] Fahrenholz, F., Klein, U. and Gimpl, G. (1995) Conversion of the myometrial oxytocin receptor from low to high affinity state by cholesterol. Adv. Exp. Med. Biol. 395, 311–319.
[42] Scheiffele, P., Roth, M.G. and Simons, K. (1997) Interaction of influenza virus haemagglutinin with sphingolipid–cholesterol membrane domains via its transmembrane domain. Embo. J. 16, 5501–5508.
[43] Keller, P. and Simons, K. (1998) Cholesterol is required for surface transport of influenza virus hemagglutinin. J. Cell Biol. 140, 1357–1367.
[44] Pike, L. and Miller, J. (1998) Cholesterol depletion delocalizes phosphatidylinositol bisphosphate and inhibits hormone-stimulated phosphatidylinositol turnover. J. Biol. Chem. 273, 22298.
[45] Caroni, P. (2001) New embo member's review: actin cytoskeleton regulation through modulation of PI(4, 5)P2 rafts. EMBO J. 20, 4332.
[46] Ilangumaran, S. and Hoessli, D.C. (1998) Effects of cholesterol depletion by cyclodextrin on the sphingolipid microdomains of the plasma membrane. Biochem. J. 335 (Pt 2), 433–440.
[47] Cerottini, J.C. and Brunner, K.T. (1967) Localization of mouse isoantigens on the cell surface as revealed by immunofluorescence. Immunology 13, 395–403.
[48] Taylor, R.B., Duffus, W.P.H., Raff, M.C. and Depetris, S. (1971) Redistribution and pinocytosis of lymphocyte surface imminoglobulin molecules induced by anti-immunoglobulin antibody. Nat. New Biol. 233, 225–229.
[49] Révész, T. and Greaves, M. (1975) Ligand-induced redistribution of lymphocyte membrane ganglioside GM1. Nature 257, 103–106.

[50] Spiegel, S., Kassis, S., Wilchek, M. and Fishman, P.H. (1984) Direct visualization of redistribution and capping of fluorescent gangliosides on lymphocytes. J. Cell Biol. 99, 1575–1581.
[51] Mayor, S., Rothberg, K.G. and Maxfield, F.R. (1994) Sequestration of GPI-anchored proteins in caveolae triggered by cross-linking. Science 264, 1948–1951.
[52] Fujimoto, T. (1996) GPI-anchored proteins, glycosphingolipids, and sphingomyelin are sequestered to caveolae only after crosslinking. J. Histochem. Cytochem. 44, 929–941.
[53] Harder, T., Scheiffele, P., Verkade, P. and Simons, K. (1998) Lipid domain structure of the plasma membrane revealed by patching of membrane components. J. Cell Biol. 141, 929–942.
[54] Grakoui, A., Bromley, S.K., Sumen, C., Davis, M.M., Shaw, A.S., Allen, P.M. and Dustin, M.L. (1999) The immunological synapse: a molecular machine controlling T cell activation. Science 285, 221–227.
[55] Monks, C.R., Freiberg, B.A., Kupfer, H., Sciaky, N. and Kupfer, A. (1998) Three-dimensional segregation of supramolecular activation clusters in T cells. Nature 395, 82–86.
[56] Stefanova, I., Horejsi, V., Ansotegui, I.J., Knapp, W. and Stockinger, H. (1991) GPI-anchored cell-surface molecules complexed to protein tyrosine kinases. Science 254, 1016–1019.
[57] Stulnig, T.M., Berger, M., Sigmund, T., Stockinger, H., Horejsi, V. and Waldhausl, W. (1997) Signal transduction via glycosyl phosphatidylinositol-anchored proteins in T cells is inhibited by lowering cellular cholesterol. J. Biol. Chem. 272, 19242–19247.
[58] Romagnoli, P. and Bron, C. (1997) Phosphatidylinositol-based glycolipid-anchored proteins enhance proximal TCR signaling events. J. Immunol. 158, 5757–5764.
[59] Zech, T., Ejsing, C.S., Gaus, K., de Wet, B., Shevchenko, A., Simons, K. and Harder, T. (2009) Accumulation of raft lipids in T-cell plasma membrane domains engaged in TCR signalling. EMBO J. 28, 466–476.
[60] Field, K.A., Holowka, D. and Baird, B. (1995) Fc epsilon RI-mediated recruitment of p53/56lyn to detergent-resistant membrane domains accompanies cellular signaling. Proc. Natl. Acad. Sci. USA 92, 9201–9205.
[61] Field, K.A., Holowka, D. and Baird, B. (1997) Compartmentalized activation of the high affinity immunoglobulin E receptor within membrane domains. J. Biol. Chem. 272, 4276–4280.
[62] Harder, T. and Simons, K. (1999) Clusters of glycolipid and glycosylphosphatidylinositol-anchored proteins in lymphoid cells: accumulation of actin regulated by local tyrosine phosphorylation. Eur. J. Immunol. 29, 556–562.
[63] Janes, P.W., Ley, S.C. and Magee, A.I. (1999) Aggregation of lipid rafts accompanies signaling via the T cell antigen receptor. J. Cell Biol. 147, 447–461.
[64] Viola, A., Schroeder, S., Sakakibara, Y. and Lanzavecchia, A. (1999) T lymphocyte costimulation mediated by reorganization of membrane microdomains. Science 283, 680–682.
[65] Gri, G., Molon, B., Manes, S., Pozzan, T. and Viola, A. (2004) The inner side of T cell lipid rafts. Immunol. Lett. 94, 247–252.
[66] Pralle, A., Keller, P., Florin, E.L., Simons, K. and Horber, J.K. (2000) Sphingolipid–cholesterol rafts diffuse as small entities in the plasma membrane of mammalian cells. J. Cell Biol. 148, 997–1008.
[67] Jacobson, K., Mouritsen, O.G. and Anderson, R.G. (2007) Lipid rafts: at a crossroad between cell biology and physics. Nat. Cell Biol. 9, 7–14.
[68] Brown, D.A. and London, E. (1998) Functions of lipid rafts in biological membranes. Annu. Rev. Cell Dev. Biol. 14, 111–136.
[69] Feigenson, G.W. (2009) Phase diagrams and lipid domains in multicomponent lipid bilayer mixtures. Biochim. Biophys. Acta 1788, 47–52.
[70] Simons, K. and Vaz, W.L. (2004) Model systems, lipid rafts, and cell membranes. Annu. Rev. Biophys. Biomol. Struct. 33, 269–295.
[71] Yetukuri, L., Ekroos, K., Vidal-Puig, A. and Oresic, M. (2008) Informatics and computational strategies for the study of lipids. Mol. Biosyst. 4, 121–127.
[72] Dupuy, A.D. and Engelman, D.M. (2008) Protein area occupancy at the center of the red blood cell membrane. Proc. Natl. Acad. Sci. USA 105, 2848–2852.
[73] Engelman, D.M. (2005) Membranes are more mosaic than fluid. Nature 438, 578–580.
[74] Ryan, T.A., Myers, J., Holowka, D., Baird, B. and Webb, W.W. (1988) Molecular crowding on the cell surface. Science 239, 61–64.
[75] Mouritsen, O.G. and Bloom, M. (1993) Models of lipid–protein interactions in membranes. Annu. Rev. Biophys. Biomol. Struct. 22, 145–171.
[76] Mitra, K., Ubarretxena-Belandia, I., Taguchi, T., Warren, G. and Engelman, D.M. (2004) Modulation of the bilayer thickness of exocytic pathway membranes by membrane proteins rather than cholesterol. Proc. Natl. Acad. Sci. USA 101, 4083–4088.
[77] Takamori, S. et al. (2006) Molecular anatomy of a trafficking organelle. Cell 127, 831–846.
[78] Simons, K. and Warren, G. (1984) Semliki Forest virus: a probe for membrane traffic in the animal cell. Adv. Protein Chem. 36, 79–132.
[79] Mukhopadhyay, S. et al. (2006) Mapping the structure and function of the E1 and E2 glycoproteins in alphaviruses. Structure 14, 63–73.
[80] Oesterhelt, D. and Stoeckenius, W. (1971) Rhodopsin-like protein from the purple membrane of *Halobacterium halobium*. Nat. New Biol. 233, 149–152.
[81] Suzuki, K.G., Fujiwara, T.K., Sanematsu, F., Iino, R., Edidin, M. and Kusumi, A. (2007) 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. 177, 717–730.
[82] Eggeling, C. et al. (2009) Direct observation of the nanoscale dynamics of membrane lipids in a living cell. Nature 457, 1159–1162.
[83] Goswami, D. et al. (2008) Nanoclusters of GPI-anchored proteins are formed by cortical actin-driven activity. Cell 135, 1085–1097.
[84] Ejsing, C.S., Sampaio, J.L., Surendranath, V., Duchoslav, E., Ekroos, K., Klemm, R.W., Simons, K. and Shevchenko, A. (2009) Global analysis of the yeast lipidome by quantitative shotgun mass spectrometry. Proc. Natl. Acad. Sci. USA 106, 2136–2141.
[85] Klemm, R.W. et al. (2009) Segregation of sphingolipids and sterols during formation of secretory vesicles at the trans-Golgi network. J. Cell Biol. 185, 601–612.
[86] Lingwood, D. and Simons, K. (2010) Lipid rafts as a membrane-organizing principle. Science 327, 46–50.
[87] Lenne, P.F. et al. (2006) Dynamic molecular confinement in the plasma membrane by microdomains and the cytoskeleton meshwork. Embo. J. 25, 3245–3256.
[88] Lasserre, R. et al. (2008) Raft nanodomains contribute to Akt/PKB plasma membrane recruitment and activation. Natl. Chem. Biol. 4, 538–547.
[89] Baumgart, T., Hammond, A.T., Sengupta, P., Hess, S.T., Holowka, D.A., Baird, B.A. and Webb, W.W. (2007) Large-scale fluid/fluid phase separation of proteins and lipids in giant plasma membrane vesicles. Proc. Natl. Acad. Sci. USA 104, 3165–3170.
[90] Kalvodova, L., Kahya, N., Schwille, P., Ehehalt, R., Verkade, P., Drechsel, D. and Simons, K. (2005) Lipids as modulators of proteolytic activity of BACE: involvement of cholesterol, glycosphingolipids, and anionic phospholipids in vitro. J. Biol. Chem. 280, 36815–36823.
[91] Bacia, K., Schuette, C.G., Kahya, N., Jahn, R. and Schwille, P. (2004) SNAREs prefer liquid-disordered over "raft" (liquid-ordered) domains when reconstituted into giant unilamellar vesicles. J. Biol. Chem. 279, 37951–37955.
[92] Hammond, A.T., Heberle, F.A., Baumgart, T., Holowka, D., Baird, B. and Feigenson, G.W. (2005) Crosslinking a lipid raft component triggers liquid ordered-liquid disordered phase separation in model plasma membranes. Proc. Natl. Acad. Sci. USA 102, 6320–6325.
[93] Fastenberg, M.E., Shogomori, H., Xu, X., Brown, D.A. and London, E. (2003) Exclusion of a transmembrane-type peptide from ordered-lipid domains (rafts) detected by fluorescence quenching: extension of quenching analysis to account for the effects of domain size and domain boundaries. Biochemistry 42, 12376–12390.
[94] Tu, Y., Wang, J. and Ross, E.M. (1997) Inhibition of brain Gz GAP and other RGS proteins by palmitoylation of G protein alpha subunits. Science 278, 1132–1135.
[95] Shogomori, H., Hammond, A.T., Ostermeyer-Fay, A.G., Barr, D.J., Feigenson, G.W., London, E. and Brown, D.A. (2005) Palmitoylation and intracellular domain interactions both contribute to raft targeting of linker for activation of T cells. J. Biol. Chem. 280, 18931–18942.
[96] Lingwood, D., Ries, J., Schwille, P. and Simons, K. (2008) Plasma membranes are poised for activation of raft phase coalescence at physiological temperature. Proc. Natl. Acad. Sci. USA 105, 10005–10010.
[97] Ayuyan, A.G. and Cohen, F.S. (2008) Raft composition at physiological temperature and pH in the absence of detergents. Biophys. J. 94, 2654–2666.
[98] Kaiser, H.J., Lingwood, D., Levental, I., Sampaio, J.L., Kalvodova, L., Rajendran, L. and Simons, K. (2009) Order of lipid phases in model and plasma membranes. Proc. Natl. Acad. Sci. USA 106, 16645–16650.
[99] Schroeder, C., Heider, H., Moncke-Buchner, E. and Lin, T.I. (2005) The influenza virus ion channel and maturation cofactor M2 is a cholesterol-binding protein. Eur. Biophys. J. 34, 52–66.
[100] Belrhali, H., Nollert, P., Royant, A., Menzel, C., Rosenbusch, J.P., Landau, E.M. and Pebay-Peyroula, E. (1999) Protein, lipid and water organization in bacteriorhodopsin crystals: a molecular view of the purple membrane at 1.9 Å resolution. Structure 7, 909–917.
[101] Lee, A.G. (2003) Lipid–protein interactions in biological membranes: a structural perspective. Biochim. Biophys. Acta 1612, 1–40.
[102] Shinzawa-Itoh, K. et al. (2007) Structures and physiological roles of 13 integral lipids of bovine heart cytochrome c oxidase. EMBO J. 26, 1713–1725.
[103] Cherezov, V. et al. (2007) High-resolution crystal structure of an engineered human beta2-adrenergic G protein-coupled receptor. Science 318, 1258–1265.
[104] Hanson, M.A. et al. (2008) A specific cholesterol binding site is established by the 2.8 Å structure of the human beta2-adrenergic receptor. Structure 16, 897–905.
[105] Wenz, T., Hielscher, R., Hellwig, P., Schagger, H., Richers, S. and Hunte, C. (2009) Role of phospholipids in respiratory cytochrome bc(1) complex catalysis and supercomplex formation. Biochim. Biophys. Acta 1787, 609–616.
[106] Kobilka, B. and Schertler, G.F. (2008) New G-protein-coupled receptor crystal structures: insights and limitations. Trends Pharmacol. Sci. 29, 79–83.
[107] Allende, M.L. and Proia, R.L. (2002) Lubricating cell signaling pathways with gangliosides. Curr. Opin. Struct. Biol. 12, 587–592.
[108] Chakrabandhu, K., Huault, S., Garmy, N., Fantini, J., Stebe, E., Mailfert, S., Marguet, D. and Hueber, A.O. (2008) The extracellular glycosphingolipid-binding motif of Fas defines its internalization route, mode and outcome of signals upon activation by ligand. Cell Death Differ. 15, 1824–1837.
[109] Mahfoud, R., Garmy, N., Maresca, M., Yahi, N., Puigserver, A. and Fantini, J. (2002) Identification of a common sphingolipid-binding domain in Alzheimer, prion, and HIV-1 proteins. J. Biol. Chem. 277, 11292–11296.

[110] Fantini, J. (2003) How sphingolipids bind and shape proteins: molecular basis of lipid–protein interactions in lipid shells, rafts and related biomembrane domains. Cell Mol. Life Sci. 60, 1027–1032.
[111] Miljan, E.A., Meuillet, E.J., Mania-Farnell, B., George, D., Yamamoto, H., Simon, H.G. and Bremer, E.G. (2002) Interaction of the extracellular domain of the epidermal growth factor receptor with gangliosides. J. Biol. Chem. 277, 10108–10113.
[112] Yoon, S.J., Nakayama, K., Hikita, T., Handa, K. and Hakomori, S.I. (2006) Epidermal growth factor receptor tyrosine kinase is modulated by GM3 interaction with N-linked GlcNAc termini of the receptor. Proc. Natl. Acad. Sci. USA 103, 18987–18991.
[113] Inokuchi, J. (2006) Insulin resistance as a membrane microdomain disorder. Biol. Pharm. Bull. 29, 1532–1537.
[114] Yamashita, T. et al. (2003) Enhanced insulin sensitivity in mice lacking ganglioside GM3. Proc. Natl. Acad. Sci. USA 100, 3445–3449.
[115] Langeveld, M., Ghauharali, K.J., Sauerwein, H.P., Ackermans, M.T., Groener, J.E., Hollak, C.E., Aerts, J.M. and Serlie, M.J. (2008) Type I Gaucher disease, a glycosphingolipid storage disorder, is associated with insulin resistance. J. Clin. Endocrinol. Metab. 93, 845–851.
[116] Bretscher, M.S. and Munro, S. (1993) Cholesterol and the Golgi apparatus. Science 261, 1280–1281.
[117] van Zanten, T.S., Cambi, A., Koopman, M., Joosten, B., Figdor, C.G. and Garcia-Parajo, M.F. (2009) Hotspots of GPI-anchored proteins and integrin nanoclusters function as nucleation sites for cell adhesion. Proc. Natl. Acad. Sci. USA 106, 18557–18562.
[118] Ejsing, C.S., Duchoslav, E., Sampaio, J., Simons, K., Bonner, R., Thiele, C., Ekroos, K. and Shevchenko, A. (2006) Automated identification and quantification of glycerophospholipid molecular species by multiple precursor ion scanning. Anal. Chem. 78, 6202–6214.
[119] Brugger, B., Glass, B., Haberkant, P., Leibrecht, I., Wieland, F.T. and Krausslich, H.G. (2006) The HIV lipidome: a raft with an unusual composition. Proc. Natl. Acad. Sci. USA 103, 2641–2646.
[120] Proszynski, T.J. et al. (2005) A genome-wide visual screen reveals a role for sphingolipids and ergosterol in cell surface delivery in yeast. Proc. Natl. Acad. Sci. USA 102, 17981–17986.
[121] Guan, X.L. et al. (2009) Functional interactions between sphingolipids and sterols in biological membranes regulating cell physiology. Mol. Biol. Cell 20, 2083–2095.
[122] Meder, D., Moreno, M.J., Verkade, P., Vaz, W.L. and Simons, K. (2006) Phase coexistence and connectivity in the apical membrane of polarized epithelial cells. Proc. Natl. Acad. Sci. USA 103, 329–334.
[123] Brangwynne, C.P., Eckmann, C.R., Courson, D.S., Rybarska, A., Hoege, C., Gharakhani, J., Julicher, F. and Hyman, A.A. (2009) Germline P granules are liquid droplets that localize by controlled dissolution/condensation. Science 324, 1729–1732.
[124] Pettersen, E.F., Goddard, T.D., Huang, C.C., Couch, G.S., Greenblatt, D.M., Meng, E.C. and Ferrin, T.E. (2004) UCSF Chimera – a visualization system for exploratory research and analysis. J. Comput. Chem. 25, 1605–1612.