Minireview

Detergent-resistant membranes and the protein composition of lipid rafts

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Abstract

The plasma membrane of eukaryotic cells contains lipid rafts with protein and lipid compositions differing from the bulk plasma membrane. Several recent proteomic studies have addressed the composition of lipid rafts, but the different definitions used for lipid rafts need scrutinizing before results can be evaluated.

The plasma membrane of mammalian cells includes several types of microdomain. Caveolae - flask-shaped invaginations - were described fifty years ago [1] and are now known to depend on the caveolin family of proteins and on cholesterol for their formation [2]. Lipid rafts are suggested to form by the self-aggregation and tight packing of cholesterol and sphingolipids, thus forming domains with lower fluidity and a higher degree of saturation than the bulk membrane [3]. This 'lipid-raft hypothesis' provides an attractive explanation for processes as diverse as the differential sorting of proteins in epithelial cells, immunological signaling and the entry of pathogens into host cells.

The existence of lipid rafts is gradually gaining acceptance, and they have been implicated in many membrane processes [4]. Their existence in intact cells is supported by both biochemical analyses [5] and microscopy [6-8], but they have been very elusive, and there are examples of investigators finding no evidence for their existence [9]. Estimates of raft size vary from a few to several hundred nanometers (nm) in diameter [10]. Aggregation of rafts, for instance by ligands binding to receptors that reside in them, would be expected to create superior sites for signaling events, as the smaller circumference-to-area ratio of the aggregated rafts would slow down interactions with deactivating molecules outside the rafts. Several recent proteomic studies have identified proteins found in detergent-isolated lipid rafts [11-14]. Although these studies have given useful insights into the lipid-raft proteins, the methods used by the authors [11-14] to isolate the rafts leave some uncertainty about the conclusions.

Isolation of lipid rafts

One biochemical definition of lipid rafts is their insolubility in non-ionic detergents at 4°C - a condition that yields detergent-resistant membranes (DRMs). Because of their high lipid-to-protein ratio, DRMs have a low density and can thus be isolated by flotation on sucrose-density gradients. It is a commonly held view in the field, as recently discussed extensively at the EURESCO Conference on Microdomains, Lipid Rafts and Caveolae [15], that progress has been hindered by the use of numerous detergents under different solubilization conditions. A recent report [16] stresses that the use of most detergents can result in the transferrin receptor, the archetypal non-lipid-raft marker, appearing in the DRM fraction; the only two detergents among those tested that demonstrated specificity for the lipid-raft markers cholesterol and sphingomyelin were CHAPS and Triton X-100. Extraction with any detergent results in a low-density fraction, however, so this criterion alone cannot be used as a definition of a lipid raft. Frequently, DRMs are isolated at the 5%-30% sucrose interface, to which any lipid-rich

membrane material would float, even if the cells from which they are derived have never been subjected to any detergents. This also means that non-detergent methods for preparing 'lipid rafts' are likely to generate a floating fraction containing membranes in general, from a number of subcellular sites, but not necessarily one enriched specifically in lipid rafts.

It is unlikely that the addition of detergents to cells will faithfully preserve particular membrane structures. For instance, concentrations of Triton X-100 that are too low to lyse cells cause cell membranes to vesiculate and fuse [10]. DRMs prepared after Triton X-100 extraction of Jurkat cells (TX-DRMs) are a mixture of vesicles ranging in diameter between 50 nm and several micrometers (Figure 1). Considering that individual lipid rafts on the cell surface are estimated to be under a few hundred nanometers in diameter [10], it is clear that TX-DRMs are substantially aggregated structures and are, therefore, likely to contain molecules that would not be in close proximity in an intact cell. Consequently, it is not valid to say that proteins are associated in the same domain in the cell solely because of their appearance in DRMs. Also, Triton X-100 can itself promote the formation of distinct lipid domains [17,18]. On the other hand, a failure to recover a protein in DRMs does not necessarily mean that that protein is not found in lipid rafts in the intact cell. This seems to be the case for the T-cell receptor [19] and the epidermal growth factor receptor [20], which have been shown to be in lipid rafts by means of fluorescence microscopy codistribution or biochemical isolation, respectively, although they are not always present in DRMs.

Lipids in lipid rafts

Despite the above reservations, Triton X-100 insolubility and subsequent flotation on sucrose density gradients is a very useful tool and is the best available biochemical indication of partitioning into lipid rafts. TX-DRMs are enriched in cholesterol, glycosphingolipids, sphingomyelin and saturated glycerophospholipids [2,21]. These DRM lipids can by themselves form a liquid-ordered-like state in which acyl chains are tightly packed, highly ordered and extended, supporting the raft hypothesis [22]. Extensive lipid analyses have revealed that, in TX-DRMs from resting mast cells, 60% of the phospholipids are either saturated or mono-unsaturated compared with 50% of the phospholipids in the total plasma membrane [23]. Interestingly, after cross-linking of the immunoglobulin E receptor FcɛRI, the proportion of polyunsaturated phospholipids in the TX-DRM fraction increased by 12-22%. The reason for this is unclear. It has been shown that TX-DRMs from Jurkat T cells are also enriched in saturated and mono-unsaturated phospholipids [24] and that the production of diacylglycerol from phosphatidylinositols occurs in response to lipid-raft aggregation [25]. In contrast, a floating fraction from epidermal cells prepared without detergents does not have the enrichment of
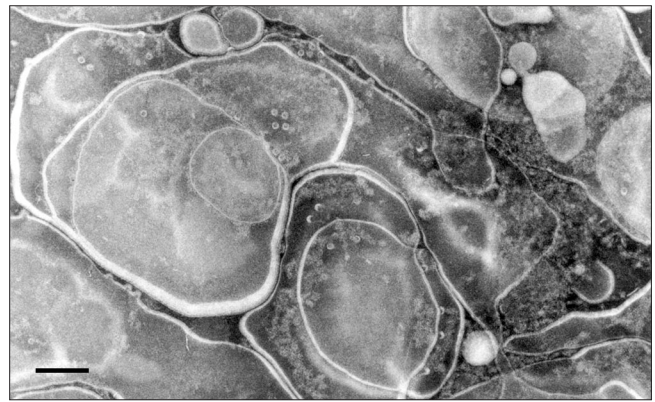
Figure 1
Electron micrograph of detergent-resistant membranes from Jurkat T cells prepared using 1% Triton X-100 on ice for 15 min (TX-DRMs). Vesicles of varying sizes can be seen. DNA was sheared, the lysate was mixed with 1 ml 80% sucrose and overlaid with 30% and 5% sucrose followed by centrifugation at 200,000 x g for 16 h. The DRM fraction was collected from the 5%-30% sucrose interface and samples were adsorbed to carbon films and negatively stained with 1% sodium silicotungstate. The scale bar represents 200 nm.

saturated and mono-unsaturated phospholipids [20], although it does have more cholesterol and sphingomyelin than does a mixed membrane fraction.

Proteins in lipid rafts

There are many examples of proteins that have been found in TX-DRMs without the use of proteomics, including various proteins anchored by glycosylphosphatidylinositol (GPI) in the exoplasmic leaflet of the membrane, and acylated intracellular protein tyrosine kinases of the Src family [21,26]. Both these kinds of protein carry largely saturated, unbranched lipid modifications that would easily partition into a liquid-ordered domain. Recently, several groups have taken a proteomic approach to identify proteins in DRMs [11-14]. Using the detergent Brij-58 to prepare DRMs, Bini *et al.* [11] found that 11 of 17 proteins identified by mass spectrometry were of mitochondrial origin, and plasma-membrane lipid-raft proteins were detectable only by western blotting, suggesting that the latter were of very low abundance in the Brij-58 DRMs. As mitochondrial membranes are not expected to contain lipid rafts and Brij-58 is a poor detergent for making DRMs enriched in lipid raft proteins, the relationship between the identified markers [16] and lipid rafts is unclear.

In another study [12], a subset of heavier TX-DRMs - those with a higher protein-to-lipid ratio - was found to contain numerous proteins of the membrane cytoskeleton [12]. This result emphasizes the link between lipid rafts and intracellular structures, which has been touched on by several other studies reporting an enrichment of cytoskeletal proteins in

DRMs (see, for example, [27]). The link is not surprising, however, because Triton X-100 insolubility was originally a method for preparing the cytoskeleton.

Given the difficulty in solubilizing hydrophobic proteins for two-dimensional electrophoresis, various tricks have been used to identify proteins of low abundance and/or high hydrophobicity. Often these tricks lead to identification on the basis of only one peptide per protein, which does not always allow unambiguous identification by mass spectroscopy, but in most cases proteins can be successfully identified. Using a cysteine-specific biotinylation agent in combination with in-gel digestion, von Haller *et al.* [13] identified 70 proteins from Jurkat T-cell TX-DRMs. These could mainly be grouped into signaling and cytoskeletal proteins. This study [13] also identified some proteins from cellular locations not expected to contain lipid rafts, but there have been an increasing number of reports of 'moonlighting' proteins that have different functions at different locations, so it is advisable to keep an open mind when using protein location to assess preparation purity.

The most recent proteomic study of DRMs is that of Matthias Mann and co-workers [14]. This work is technically impressive: the ratios of isotopes from cells labeled with either leucine or trideuterated leucine were measured by mass spectrometry and used to group TX-DRM proteins into three categories on the basis of the sensitivity of their presence in the TX-DRMs to acute cholesterol depletion: raft proteins, raft-associated proteins and 'nonspecific' proteins. The cell type used (HeLa cells) contains caveolae as well as lipid rafts, and the methods used for lipid-raft purification do not distinguish between the two. The paper [14] makes the assumption that the association of genuine raft proteins with TX-DRMs should be sensitive to cholesterol depletion whereas that of contaminating proteins should not, but this has not been universally established. Although cholesterol depletion does make some components of lipid rafts sensitive to detergent extraction, both lipid and protein markers of lipid rafts can still be purified in TX-DRMs after such treatment [28]. There is also evidence that cholesterol depletion causes coalescence rather than dispersion of lipid domains in living cells [29]. Mann and co-workers [14] treated cells with methyl-β-cyclodextrin until no less than 96% of the cells' cholesterol had been removed. During this one-hour treatment the cells almost certainly lose viability, probably undergo extensive intracellular reorganization and degradation, and lose many proteins through membrane blebbing. The authors found that disorganization of the cholesterol in rafts using the agents nystatin and filipin did not identify any specific grouping of proteins sensitive to this, and nor did any of the three cholesterol-disrupting agents give rise to a useful discrimination when applied to floating membranes prepared in the absence of detergent, reinforcing the questionable utility of non-detergent methods for preparing 'lipid rafts'.

The supplementary information (Tables 3-5) to the study by Mann and colleagues [30] gives complete and informative data on the proteins they identified. The 'nonspecific' category, showing low sensitivity to cholesterol depletion, reassuringly contains the transferrin receptor, the classical non-raft marker, as well as many other proteins not expected to be in rafts. In general, the authors find the expected proteins such as small and heterotrimeric G-proteins, Src-family tyrosine kinases and cytoskeletal proteins in the 'raft' category, with the addition of several glycolytic enzymes, ribosomal proteins and nuclear proteins. These non-traditional raft proteins may be examples of proteins with multiple functions; they do not necessarily reflect contamination. It is less straightforward to know where to draw the line between 'raft' proteins (defined as showing high dependence on cholesterol for association with DRMs) and 'raft-associated' proteins (showing intermediate dependence on cholesterol for DRM association). The cut-off values are set somewhat arbitrarily where there are minor discontinuities in the graph of proteins plotted in order of their dependence on cholesterol (see Figure 3 of Foster *et al.* [14]). This results in the classical caveolar or lipid-raft protein caveolin-1 being classified as only 'raft-associated' whereas by all other criteria this is a highly raft-enriched protein. In fact, caveolin-1 is very close to the cut-off between raft-associated and non-specific proteins. The failure of caveolin-1 to show strong dependence on cholesterol for DRM association could be because the protein can itself bind cholesterol, perhaps making it less susceptible to the general loss of cholesterol from cell membranes, and this could also apply to other cholesterol-binding proteins.

Mann and co-workers [14] conclude that lipid rafts are rich in signaling molecules, membrane-skeletal and cytoskeletal proteins, consistent with studies showing that lipid-raft components can associate with actin filaments, either directly or through adaptors, temporarily anchoring them to intracellular structures. Many of the proteins identified have not previously been reported to partition to lipid rafts, and they extend the list of identified raft proteins to 241. This is an impressive number given that TX-DRMs are estimated to contain only 0.3-2% of total cellular protein. Mann and co-workers [14] also characterize a large number of hypothetical raft or raft-associated proteins, which may turn out to be the most useful aspect of this study because it points to new raft proteins as well as new raft functions. No doubt we will learn more about the organization of the plasma membrane once these proteins have been functionally assigned; the raft and raft-associated proteins identified so far suggest an involvement of lipid rafts in ubiquitinylation and endocytosis.

It is clear that the dynamic plasma membrane of cells is far from homogenous, and we are still only just scraping the surface of its complexity. With careful isolation of lipid rafts, proteomics may be a useful tool for understanding this complexity.

Acknowledgements

References

1. Palade GE: **Fine structure of blood capillaries.** *J Appl Phys* 1953, **24:**1424-1436.
2. Parton RG, Simons K: **Digging into caveolae.** *Science* 1995, **269:**1398-1399.
3. Simons K, Ikonen E: **Functional rafts in cell membranes.** *Nature* 1997, **387:**569-572.
4. Horejsi V: **Membrane rafts in immunoreceptor signaling: new doubts, new proofs?** *Trends Immunol* 2002, **23:**562-564.
5. Friedrichson T, Kurzchalia TV: **Microdomains of GPI-anchored proteins in living cells revealed by crosslinking.** *Nature* 1998, **394:**802-805.
6. Varma R, Mayor S: **GPI-anchored proteins are organized in submicron domains at the cell surface.** *Nature* 1998, **394:**798-801.
7. Pralle A, Keller P, Florin EL, Simons K, Horber JK: **Sphingolipid-cholesterol rafts diffuse as small entities in the plasma membrane of mammalian cells.** *J Cell Biol* 2000, **148:**997-1008.
8. Schutz GJ, Kada G, Pastushenko VP, Schindler H: **Properties of lipid microdomains in a muscle cell membrane visualized by single molecule microscopy.** *EMBO J* 2000, **19:**892-901.
9. Kenworthy AK, Petranova N, Edidin M: **High-resolution FRET microscopy of cholera toxin B-subunit and GPI- anchored proteins in cell plasma membranes.** *Mol Biol Cell* 2000, **11:**1645-1655.
10. Edidin M: **The state of lipid rafts: from model membranes to cells.** *Annu Rev Biophys Biomol Struct* 2003, **32:**257-283.
11. Bini L, Pacini S, Liberatori S, Valensin S, Pellegrini M, Raggiaschi R, Pallini V, Baldari CT: **Extensive temporally regulated reorganization of the lipid raft proteome following T-cell antigen receptor triggering.** *Biochem J* 2003, **369:**301-309.
12. Nebl T, Pestonjamasp KN, Leszyk JD, Crowley JL, Oh SW, Luna EJ: **Proteomic analysis of a detergent-resistant membrane skeleton from neutrophil plasma membranes.** *J Biol Chem* 2002, **277:**43399-43409.
13. von Haller PD, Donohoe S, Goodlett DR, Aebersold R, Watts JD: **Mass spectrometric characterization of proteins extracted from Jurkat T cell detergent-resistant membrane domains.** *Proteomics* 2001, **1:**1010-1021.
14. Foster LJ, De Hoog CL, Mann M: **Unbiased quantitative proteomics of lipid rafts reveals high specificity for signaling factors.** *Proc Natl Acad Sci USA* 2003, **100:**5813-5818.
15. Lai EC: **Lipid rafts make for slippery platforms.** *J Cell Biol* 2003, **162:**365-370.
16. Schuck S, Honsho M, Ekroos K, Shevchenko A, Simons K: **Resistance of cell membranes to different detergents.** *Proc Natl Acad Sci USA* 2003, **100:**5795-5800.
17. Heerklotz H: **Triton promotes domain formation in lipid raft mixtures.** *Biophys J* 2002, **83:**2693-2701.
18. 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:**793-799.
19. Janes PW, Ley SC, Magee AI: **Aggregation of lipid rafts accompanies signaling via the T cell antigen receptor.** *J Cell Biol* 1999, **147:**447-461.
20. 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:**2075-2088.
21. Brown D, Rose JK: **Sorting of GPI-anchored proteins to glycolipid-enriched membrane subdomains during transport to the apical cell surface.** *Cell* 1992, **68:**533-544.
22. Brown DA, London E: **Functions of lipid rafts in biological membranes.** *Annu Rev Cell Dev Biol* 1998, **14:**111-136.
23. Fridriksson EK, Shipkova PA, Sheets ED, Holowka D, Baird B, McLafferty FW: **Quantitative analysis of phospholipids in functionally important membrane domains from RBL-2H3 mast cells using tandem high-resolution mass spectrometry.** *Biochemistry* 1999, **38:**8056-8063.
24. Rouquette-Jazdanian AK, Pelassy C, Breittmayer JP, Cousin JL, Aussel C: **Metabolic labelling of membrane microdomains/rafts in Jurkat cells indicates the presence of glycerophospholipids implicated in signal transduction by the CD3 T-cell receptor.** *Biochem J* 2002, **363:**645-655.
25. Parmryd I, Adler J, Patel R, Magee AI: **Imaging metabolism of phosphatidylinositol 4,5-bisphosphate in T-cell GM1-enriched domains containing Ras proteins.** *Exp Cell Res* 2003, **285:**27-38.
26. Rodgers W, Crise B, Rose JK: **Signals determining protein tyrosine kinase and glycosyl-phosphatidylinositol-anchored protein targeting to a glycolipid-enriched membrane fraction.** *Mol Cell Biol* 1994, **14:**5384-5391.
27. Harder T and Simons K: **Clusters of glycolipid and glycosylphosphatidylinositol-anchored proteins in lymphoid cells: accumulation of actin regulated by local tyrosine phosphorylation.** *Eur J Immunol* 1999, **29:**556-562.
28. Ilangumaran S, Hoessli DC: **Effects of cholesterol depletion by cyclodextrin on the sphingolipid microdomains of the plasma membrane.** *Biochem J* 1998, **335:**433-440.
29. Hao M, Mukherjee S, Maxfield FR: **Cholesterol depletion induces large scale domain segregation in living cell membranes.** *Proc Natl Acad Sci USA* 2001, **98:**13072-13077.
30. **Data Collection for Foster et al. (2003)** *Proc Natl Acad Sci USA*, **10.1073/pnas.0631608100** [http://www.pnas.org/cgi/content/full/0631608100/DC1]