Lipid Rafts and Apical Membrane Traffic

JOACHIM FÜLLEKRUG AND KAI SIMONS
Max-Planck-Institute of Molecular Cell Biology and Genetics, Dresden, Germany

ABSTRACT: Lipid rafts are dynamic assemblies floating freely in the surrounding membranes of living cells. This membrane heterogeneity provides a useful concept for understanding processes as diverse as cell polarity, signal transduction, and membrane sorting. Individual rafts are small entities containing thousands of lipids but only a few proteins. Regulation of raft association and size is an elementary feature of interactions at the molecular level. By clustering small rafts into a bigger platform, proteins are brought together for modification. Oligomerization might transform a monomeric weakly raft-associated protein into an assembly with higher raft affinity. Lectins are multivalent glycoprotein-binding proteins and are likely to be key players in mediating the clustering of rafts in vivo. Glycosylation-dependent surface delivery in a polarized fashion is a feature conserved across evolution, and we expect lectins to be at the heart of the molecular machinery responsible for lipid raft delivery to the cell surface. Currently, we are evaluating candidate proteins by affinity chromatography, proteomics, and RNA interference.

KEYWORDS: polarity; rafts; epithelia; glycosylation; MDCK

LIPID RAFTS

Lipid rafts are dynamic microdomains in the membranes of living cells, composed of cholesterol and sphingolipids. Cholesterol and sphingolipids carrying saturated hydrocarbon chains assemble to form tightly packed subdomains corresponding to liquid-ordered phases biophysically characterized in model membranes.1,2 These lipid rafts float freely in the surrounding membrane which is more fluid and analogous to the liquid-disordered phase. At the molecular level, the higher fluidity is the consequence of the high surface area occupied by unsaturated phospholipids compared to the dense packing of the sphingolipid-cholesterol assemblies. Cholesterol also condenses monounsaturated phospholipids but leaves them in the liquid-disordered phase.3

Proteins strongly associating with lipid rafts include glycosylphosphatidylinositol (GPI)-anchored proteins, doubly acylated proteins, and several transmembrane proteins, especially palmitoylated ones.
CELL POLARITY

Epithelial cells form sheets that line surfaces, especially in tissues specialized for absorption and secretion. Two different plasma membrane domains are separated by tight junctions: the basolateral surface is responsible for cell-cell and cell-matrix interactions, and the apical membrane faces the lumen. The protein and lipid composition of these two membranes is strikingly different, or “polarized.” The apical plasma membrane is highly enriched in glycosphingolipids and GPI-anchored proteins.

Cholesterol and ceramide, the precursor for sphingolipids, are synthesized in the endoplasmic reticulum (ER), but the steady-state concentration of these compounds is too low to allow formation of rafts. Sphingomyelin and glycosphingolipids are synthesized in the Golgi apparatus, and it is here that raft assembly takes place. Cholesterol and other raft lipids are excluded from retrograde traffic between the Golgi apparatus and ER, and the overall consequence is an increasing concentration of cholesterol and sphingolipids from the ER to the plasma membrane.

Biochemically, this corresponds to an increased resistance of membranes to non-ionic detergents in the cold. This method is a good first approach to investigate raft association of proteins, although not without pitfalls. Raft proteins acquire detergent resistance while transiting the Golgi apparatus, concomitantly with the enrichment of raft lipids (Fig. 1).

FIGURE 1. Floatation analysis. Resistance to non-ionic detergents in the cold is a hallmark of lipid microdomains enriched in sphingolipids and cholesterol and is widely used in assessing raft association of a given protein. Raft proteins would still be associated with lipids after detergent extraction and have a lower density than that of solubilized membrane proteins not associated with rafts. The different densities are exploited by ultracentrifugation, leading to floatation of DRMs (fractions 2–4) versus solubilized proteins (fractions 7,8). Two raft markers for MDCK cells, VIP17 and caveolin-1, localize to detergent-resistant membranes (DRMs) at the top of the gradient. Note that although gp114 is an apical marker protein derived from a membrane rich in glycolipids and cholesterol, it is solubilized under these conditions. However, crosslinking of proteins often strengthens a weak affinity for rafts, and the same has been observed for gp114.
At the level of the trans-Golgi network (TGN), a sorting process leads to transport containers with different protein and lipid composition. Apical containers are derived from sorting platforms enriched in lipid rafts, whereas basolateral sorting relies on the binding of adaptor proteins to short amino acid sequences present in the cytoplasmic tails of proteins. This TGN-mediated sorting ensures the maintenance of the polarized phenotype not only in epithelial cells but also in other types of polarized cells such as neurons. Cognate pathways retaining some characteristics of polarized sorting are also present in fibroblasts.\textsuperscript{8}

**CLUSTERING OF RAFTS**

There is agreement that individual rafts are small, with a diameter of 50 nm\textsuperscript{9} (see Ref. 10 for a different view). This size corresponds to several thousands of lipid molecules but, based on fibroblast measurements, only to about 10–20 protein molecules. This means that regarding protein content there are many different kinds of rafts with a unique protein composition. Consequently, for efficient interaction of different raft-associated proteins, rafts need to form larger platforms. This process can be manipulated experimentally by the use of antibodies in an approach referred to as clustering or crosslinking.

Lateral crosslinking of membrane proteins leads to multimerization of protein-lipid interactions. Weak interactions are amplified and cause stronger attracting or repelling forces. Raft domains coalesce and segregate from non-raft lipids and proteins.

Experimentally, if two raft-associated proteins expressed in fibroblasts are subjected to antibody clustering, they colocalize in large patches readily observed by light microscopy. By contrast, antibody clustering of a non-raft protein and a raft protein leads to segregated patches. The crosslinking approach works only on intact rafts as evidenced by inhibition after cholesterol depletion.\textsuperscript{11}

Some proteins are only weakly associated with lipid rafts in the monomeric state, but oligomerization, either by ligands in vivo or by antibodies in vitro, will increase raft affinity. This is the case for the IgE receptor in the allergic immune response of mast cells. In the resting state, the IgE receptor is outside rafts, but moves into rafts after crosslinking by multivalent IgE-antigen complexes. The doubly acylated Lyn tyrosine kinase is raft associated and is thought to initiate the signaling response by phosphorylating the subunits of the IgE receptor.\textsuperscript{12}

**GLYCOXYLATION AND APICAL SORTING**

Glycosylation was shown to be crucial for apical delivery for a variety of different proteins in mammalian cells, both soluble and membrane associated.\textsuperscript{13,14} Recent results from our lab suggest that glycosylation-dependent polarized delivery is also a feature of yeast cells, underscoring the importance of glycosylation in this process across evolution. The simplest model to integrate the findings on lipid microdomains and glycosylation is the postulation of lectins binding specifically to glycoproteins (FIG. 2). Multivalent lectins would bind to several glycoproteins at the same time and, by crosslinking, enhance low intrinsic raft affinities. We think that several
lectins (which could be raft associated themselves) will account for different kinds of cargo glycoproteins. Small rafts would be brought together into larger sorting platforms to allow polarized sorting in the TGN. This would fit well with the observation that at least in some cases, raft association is not sufficient for apical delivery of GPI-anchored proteins, but that glycosylation has to be present to make the process more efficient.15

**MDCK CELLS AND GLYCOSYLATION**

MDCK cells have served as a model system in epithelial cell biology for years. The main reason is that these cells differentiate into fully polarized cells when grown on semipermeable filter supports. The cells form a monolayer, with tight junctions separating a basolateral domain (facing the filter support and neighboring cells) from an apical surface. Protein sorting, raft formation, and glycosylation have been studied extensively in MDCK cells. Our current focus is the identification and characterization of lectins in this epithelial polarity model system. Using glycoproteins purified from MDCK cells, we identified an endogenous lectin by affinity chromatography, which is described elsewhere. Another approach focuses on marker glycoproteins of MDCK cells. Monoclonal antibodies derived from earlier studies16 have served to identify apical or basolateral membranes in immunocytochemistry and biochemical analysis (Fig. 3). However, the molecular identity of these proteins is unknown in most cases. Experimental findings relating to the marker proteins themselves have remained isolated within the model system of MDCK cells. We have identified a frequently used apical marker protein, the sialoglycoprotein gp114, as a member of the carcinoembryonic antigen (CEA) family. Previous results on endocytosis and transcytosis of gp114 in MDCK cells17 should now lead to new insights
FIGURE 3. MDCK cell polarity. MDCK cells were grown on permeable filters for 4 days. (a) Indirect immunofluorescence using antibodies directed against gp114 shows granular staining of the apical surface that is especially prominent towards neighboring cells. (b) A vertical section demonstrates the apical confinement of gp114. (c) A midsection through an MDCK cell shows the localization of p58 to the lateral domain. In addition, there is a pool of intracellular granules containing p58. (d) The vertical view depicts lateral and basal staining.

FIGURE 4. Lectin-mediated clustering of rafts. Individual rafts are small, containing only a few proteins. Clustering these rafts into larger assemblies enables molecular interactions between proteins. Lectins are major candidates for regulating raft size at the plasma membrane.
regarding CEA biology and function. In addition, the p58 basolateral glycoprotein was identified as the β subunit of Na⁺K⁺-ATPase. This identification of marker proteins is embedded in a proteomics effort on glycoproteins/lectins of MDCK cells. Candidate proteins are being tested by RNA interference to evaluate their role in apical sorting and epithelial traffic routes.

**SUMMARY**

The concept of lipid raft microdomains builds a framework to incorporate seemingly unrelated processes such as cell polarity, signal transduction, and membrane sorting in a coherent fashion. Crucial to this concept is the regulation of raft association and size. By clustering small individual rafts together into a larger platform, efficient interaction of raft-associated proteins is realized. Weak raft affinities of individual proteins are strengthened by oligomerization. Apart from classic ligands and cytoskeletal scaffolds, we believe that lectin-mediated clustering of glycoproteins is an important way for cells to regulate lipid rafts in vivo (FIG. 4).

**REFERENCES**