3.6: Rafts

The traditional fluid mosaic model of biological membranes stated that lipids are homogeneously mixed. However, biological membranes are actually composed of a complex mixture of lipid domains that exist in different degrees of order, ranging from the high order gel phase to low order liquid phase. Lipid “rafts” are membrane microdomains composed of higher order lipids that have tight interaction with each other relative to the “sea” of liquid phase lipids known as bulk lipids. These rafts have been estimated to be 10-200 nm in size. Small rafts can merge to form large rafts through protein-protein and protein-lipid interactions. The rafts are important because certain membrane proteins preferentially localize to these domains and biological processes such as signal transduction occur there as a result. Proteins are often targeted to rafts by palmitoylation and myristoylation, which are covalent attachments of fatty acids. Glycosylphosphatidylinositol (GPI)-anchors also localize proteins to rafts.

Structure and Formation

Rafts exist as planar domains or invaginated structures known as **caveolae**[1]. Caveole type rafts contain the cholesterol binding protein cavin, which are located in the inner membrane leaflet and are needed for membrane invagination. Cavins are peripheral membrane proteins that bind to caveolar phosphatidylserines. Caveolin is another caveolar protein that is involved in signaling processes. Caveolin contains three palmitoylated cysteines and a cholesterol binding sequence (VTKYWFYR) that facilitate localization to rafts.

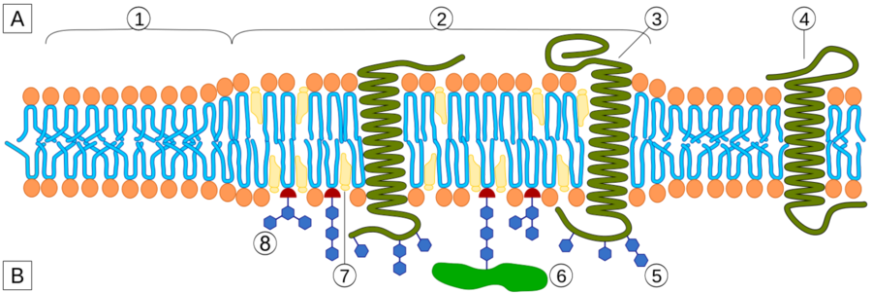

Figure 3.6.1: Lipid raft organization, region (1) is standard lipid bilayer, while region (2) is a lipid raft. (Public Domain; Artur Jan Fijałkowski.)

Lipid rafts are also found in the endomembrane system in addition to the plasma membrane[1]. Sphingolipids and cholesterol are synthesized in the ER. They are then trafficked to the Golgi, where they associate to form rafts. Raft containing vesicles are then sent to the plasma membrane through the trans Golgi network, which is involved in raft recycling as well. Rafts also participate in sorting and membrane targeting of lipids in the trans Golgi network.

Lipid Composition and Biophysical Properties

Rafts are composed of sphingolipids such as sphingomyelin, cholesterol, and phospholipids.[2] Sphingomyelin is composed of a sphingosine, a fatty acid and a phosphocholine head group. Sphingomyelin has a high T_m that makes rafts resistant to detergent at low temperatures. Rafts are often therefore referred to as detergent resistant membranes (DRMs).

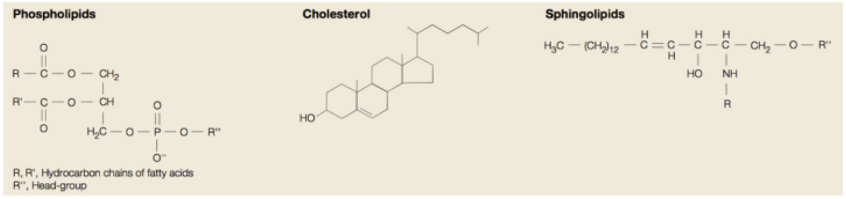

Figure 3.6.1: Raft component structures. Reprinted by permission of Macmillan Publishers Ltd: Nature Reviews[2], copyright 2000.

Phase separation caused by higher order rafts and lower order bulk lipids is the driving force for raft formation. Raft associated lipids are more saturated than bulk lipids and therefore are more tightly packed. Cholesterol preferentially localizes to rafts because it has a rigid structure that prefers the higher order state of the raft. The hydroxyl group in cholesterol interacts with the sphingosine amide, which contributes to the higher ordering. In fact, the DRM rafts disappear if cholesterol is extracted from the membrane.

Another driving force for raft formation is phase separation caused by differential hydrophobic acyl chain length in raft associated and non-associated lipids. The acyl chains of shpingomyelin are longer than that of typical phospholipids found in the bulk lipid of biological membranes. A homogenous mixure of long and short chain lipids would result in higher exposure of the hydrophobic

region of the long chain lipids to the surrounding water than if phase separation were to occur. Therefore, phase separation of long and short chain lipids decreases the free energy of the membrane. The inner and outer leaflet of the raft may be held together by interdigitation of the long chain acyl chains and/or integral protein-lipid interactions. Hydrophobic mismatch, or the difference between protein hydrophobic transmembrane domain lengths and hydrophobic membrane widths, can cause proteins to associate or not associate with rafts. Hydrophobic mismatch can also effect protein conformation and therefore protein activity.

Discovery and Evidence

The Singer and Nicolson fluid mosaic model of biological membranes was proposed in 1972. By the early 1980s it became clear that membranes lipids preferentially segregated into different phases under physiological conditions and therefore existed as a heterogeneous mixture in membranes[3]. Rafts were first discovered in the 1990s when a significant fraction of membrane remained resistant to Triton X-100 detergent at 4˚C[4]. However, there was still a question of whether the DRM was an artifact of the cold treatment. To answer this question, resonance energy transfer (RET) was measured in cells expressing two types of membrane associated fluorescent folate analogs, GPI anchored folate receptors and transmembrane anchored folate receptors[5]. The GPI anchored probe was predicted to localize to the DRM domains, while the transmembrane anchored probe was not. The GPI anchored probe's RET did not increase with increased density of the probe, suggesting that the probes were non-randomly clustered at a spatial distribution below the resolution of the microscope. The transmembrane anchored probe's RET increased linearly with increased density, suggesting that they were randomly distributed throughout the membrane. Also, when cholesterol was extracted from the membrane, the GPI anchored probes were found to be randomly distributed. This data suggests that the DRM rafts are in fact real.

Another key piece of evidence for the existence of rafts came from membrane protein cross-linking experiments[6]. Membrane proteins were cross-linked by aggregating a certain protein with an antibody and cross-linking nearby proteins with aldehyde fixatives. Membrane proteins associated with the DRM rafts were colocalized, while proteins not associated with the DRM rafts did not colocalize with raft proteins. The close proximity of raft-associated proteins suggests that the DRM rafts are real membrane microdomains.

Signal Transduction

Many proteins involved in signal transduction have been found to colocalize with the raft microdomains. Rafts can be important for signal transduction because they provide a microenvironment where specific signal responses can occur upon ligand binding to the receptor. There are two models for raft mediated signal transduction[2]. One model is that ligand bound receptors migrate to rafts where the downstream signal transduction occurs. The other model is that ligand binding causes several rafts with different signaling components to merge and produce the downstream signal response. A combination of the two models where receptor migration to a raft causes different rafts to merge is also possible.

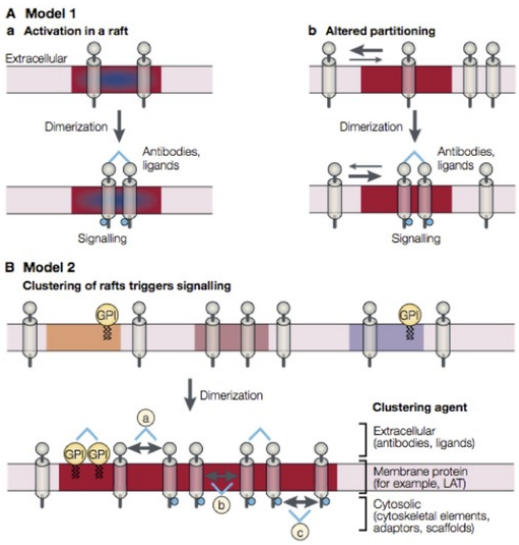

Figure 3.6.2: Models for raft mediated signal transduction involving single rafts (A) and multiple rafts (B). Reprinted by permission of Macmillan Publishers Ltd: Nature Reviews[2], copyright 2000.

The first discovered example of raft associated signal transduction was the tyrosine kinase signal transduction of immunoglobulin E (IgE) signaling in the allergic immune response[2]. In this case, IgE binds to the FceRI receptor, then oligomeric antigens bind IgE, thereby crosslinking receptor complexes. The crosslinked receptor complexes are recruited to rafts where Lyn, a tyrosine kinase, phosphorylates the receptor complex, which starts the phosphorylation signaling cascade. Rafts have also been shown to be involved in other types of signal transduction such as G-protein coupled signal transduction[2].

A new and exciting aspect of the lipid raft mediated signal transduction is the interaction of rafts with the cytoskeleton. Actin binds to caveolins in caveoles and tetraspanins in planar rafts. Through this interaction with the cytoskeleton, rafts have been shown to be involved in cellular polarity, cell migration, neuronal signaling, neuronal membrane repair, and T-cell activation[1].

Viral Interactions

Another major role for lipid rafts is in entry and shedding of certain viruses[7]. Both envelope lacking and enveloped viruses can depend on lipid rafts for cell entry. The mechanism of attachment and entry can depend on lipid-lipid, lipid-protein, and protein-protein interactions. Many viral proteins have been shown to interact with cholesterol and sphingolipids in the rafts. Caveoles can also be involved in endocytosis of certain viruses.

When certain viruses are released from the host cell, they bud off and their membrane is formed from the host cell plasma membrane. The similarity of certain viral and raft lipid composition indicates that these raft-dependent viruses bud off at raft sites. Since a single raft is not large enough to form a full viral membrane, multiple lipid rafts are likely recruited before virus budding. HIV is an example of a virus that has raft like lipid composition[7]. HIV Gag proteins have positively charged residues and a myristate group that targets it to phophatidylinositol (PI(4,5)P2) in the plasma membrane inner leaflet. Gag aggregation in the plasma membrane creates a saturated lipid environment that recruits raft microdomains. HIV budding then proceeds at the raft microdomain.

A study on the flu virus has shown that removing cholesterol from the host membrane increases viral budding, but produces viruses with very low infectivity[8]. This suggests that rafts are more important for cell entry than for viral shedding.

Prevalence of Lipid Rafts

Rafts have historically been studied in animal membranes, but plant membrane rafts have more recently been studied as well. Plants do not contain cholesterol, but other sterols contribute to plant lipid rafts. As with animal rafts, plant rafts also mediate signal transduction events. One well studied case is **Pathogen-Associated Molecular Patterns (PAMPs)**-induced signaling events[9]. Another recent study has revealed an association of lipid rafts to plasmadesmata, which are direct cytosolic connections between plant cells[10]. The full implications of this finding are yet to be elucidated. Rafts have also been described in other eukaryotes such as fungi[11].

Rafts may not be exclusive to eukaryotes. Recent work in *Bacillus subtilus* has shown differential protein localization in DRMs[12]. Some of these proteins have homology to eukaryotic raft associated proteins and many are involved in bacterial signaling and transport processes.

Non-Raft Microdomains

Other microdomains that are not cholesterol-associated rafts also exist in biological membranes. These microdomains are sometimes called “non-raft” domains, a term which was coined at the 2006 Keystone Symposium of Lipid Rafts and Cell Function[13]. These non-raft domains are likely quite diverse in composition and function, but have not been well characterized. Many non-raft microdomains are made up of poly-unsaturated lipids, which exclude cholesterol. Some membrane proteins are thought to have differential activity when localized to raft or non-raft microdomains.[14]

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