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"WHAT IS THE CURRENT STATE OF RESEARCH ON THE FUNCTIONS OF FORMATION OF LIPID RAFT DOMAIN IN CELLULAR MEMBRANE"

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ABSTRACT:

Compositional lipid micro domains ("Lipid drafts") in plasma membranes are believed to be important components of many cellular processes. Lipid drafts are plasma membrane micro domains rich in cholesterol, Sphingolipids and cell surface receptors. Lipid rafts containing a given set of proteins can change their size and composition in response to intra or extra cellular stimuli. This favours specific protein-protein interactions, resulting in activation of signaling cascades. The biophysical and the kinetic mechanisms by which cells regulate the size, lifetime and apatial localization of these domains are rather poorly understood at the moment. Over the years, experimental studies of raft formation have inspired several phenomenological theories and speculations incorporating a wide variety of thermodynamic assumptions regarding the lipid- lipid and lipid- protein interactions, and the potential role of active cellular processes on membrane structure. The kinetic regimes are conveniently catalogued with power law of form P) K_t R where P is a (measured) property related to domain composition. Here we critically review and discussed these theories, models, speculations, on the kinetics of formation of lipid raft domain in cellular membrane and present our view on future direction.

1. INTRODUCTION

The plasma membrane (PM) enveloping mammalian cells are a lipid bilayer composed primarily of thousands of types of lipids and membrane proteins. Besides being the physical boundary of cell, the PM also functions as a

selective sieve through which matter and information pass. It is now well-established that the PM is far from featureless and has a ‘‘patchy” microstructure with spatially organized regions of structure and function, both in terms of lipids and proteins. In terms of lipids, the heterogeneous membrane is believed to consist of a mixture of a dispersed ‘‘lipid raft” phase, enriched in cholesterol, raft-associated proteins, and saturated lipids(such as sphingolipids), and the ‘‘non-raft” matrix phase ;in model membranes, the lipid raft phase is typically associated with a so-called ‘‘liquid-ordered” phase, while the non-raft phase has been identified as the ‘‘liquid-disordered” phase based on differences in the short-ranged lipid translational and conformational order (1). The rafts have been implicated in a number of important cellular processes including signal transduction, membrane trafficking, and protein sorting; in addition, viral entry, assembly, and budding are also facilitated by the raft domains. These micro domains often contain proteins, and their spatial distribution may depend on the coupling of these proteins to the cytoskeleton (2).

Very recently, advances in experimental techniques have allowed for the first time non-invasive, in vivo imaging of single diffusing lipid molecules and proteins with unprecedented spatial resolution within the membrane. Most notably, in comparison to *in vivo* membranes, the raft domain size in model membranes is much larger, typically comparable to the vesicle size. One important difference between model membrane systems and a living cell is that model membranes are not affected by active cellular processes, such as vesicle trafficking or rapid lipid flip-flopping between two leaflets, which are critical in maintaining the asymmetric lipid distribution across the bilayer (3). Furthermore, model membranes typically neither contain proteins nor are supported by a cytoskeleton. Therefore, the corresponding molecular interactions are also absent. Significant differences exist between the structure of the domains observed in model membranes and the less-ordered structure of the raft domains found in multi component plasma membranes (4).However, the challenges in developing physically and biologically-based models for the membrane microstructure and its dynamics are multifaceted. First, molecular scale interactions between lipids and proteins are fundamentally responsible for microstructure formation, and the collective dynamics of the lipids and proteins, coupled to exterior solvent flow fields, facilitate large-scale reorganization of the membrane (5). Thus, while the raft domain organization fundamentally originates from molecular level phenomena.

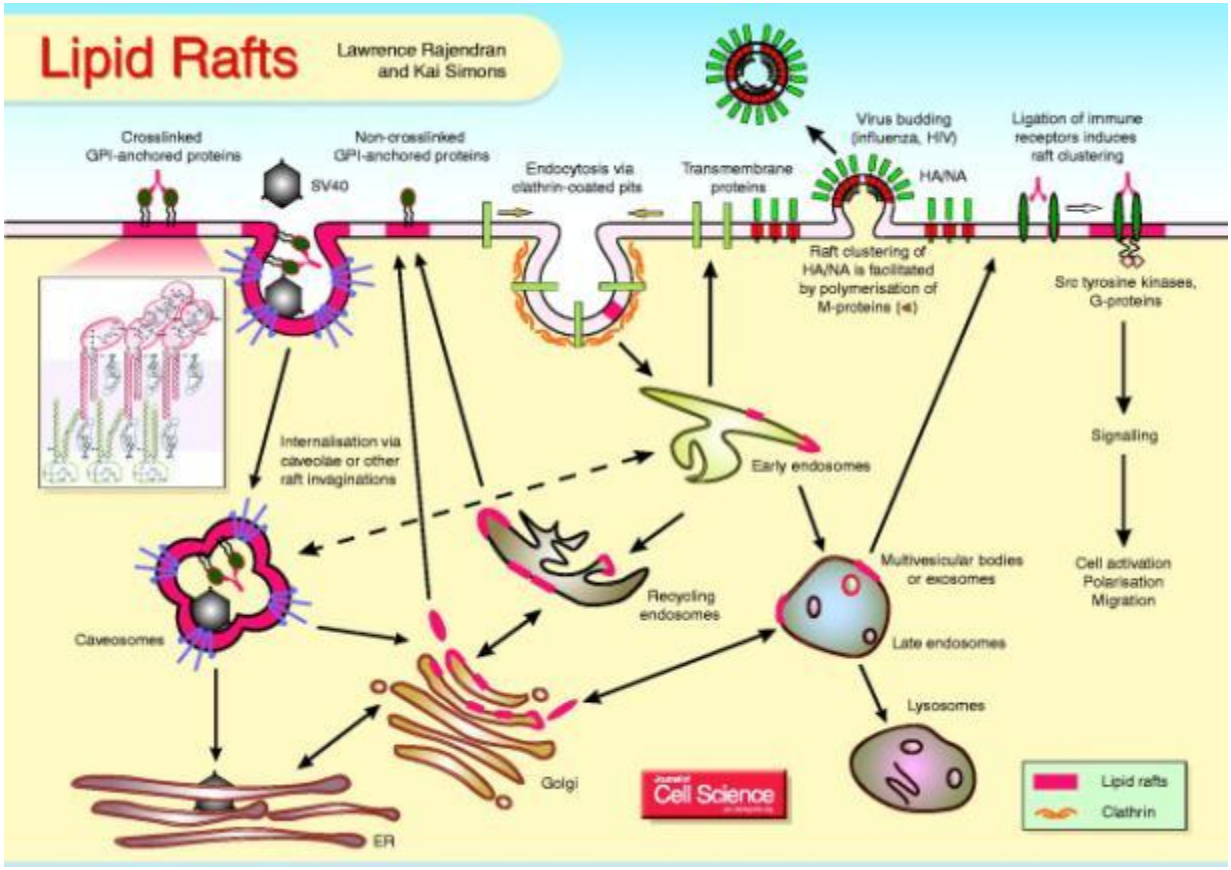

Figure 1: Schematic representation of Lipid rafts

The existing models for membrane microstructure formation are phenomenological by nature, and attempt to incorporate simple-yet-generic biophysical mechanisms for raft formation and regulation in the form of mathematical descriptions of the membrane patchiness. In more technical terms, these models are not tailored to resolve the properties of the system at the single molecule level; instead, they provide mathematically and computationally tractable, effective descriptions of the system behavior across length and time scales (6). The appealing feature of such models is that they provide a means to understand general membrane properties, among them membrane microstructure formation, for a broad class of cell membranes. Once the general physical mechanisms governing raft formation has been established, more microscopic models can be employed to assess and quantify system-specific properties (7).

Furthermore, there exists an attractive interaction between the proteins, mediated by the lipids, which extends across the width of the lipid band; that is, two proteins whose bands overlap spatially attract each other (8). The implication of this line of reasoning is that local variations in the spatial correlation length via, e.g., variations in the local lipid composition, could lead to spatially targeted protein (and thus lipid) aggregation (9).

2. THEORETICAL MODELS FOR LIPID MICRO DOMAIN FORMATION: NON EQUILIBRIUM PHENOMENA

The fundamental hypothesis underlying all of these models is that cells maintain a non-equilibrium lipid composition via lipid recycling to and from the membrane either by coupling to a lipid reservoir or by vesicular and non-vesicular lipid transports events. The main differences reside in the details of active lipid transport processes are modeled. Within this general framework, it is the interplay between active lipid transport processes and lipid thermodynamics, which endows the raft domains with their mechanical and biophysical properties. The membrane as a mixture of multiple species, which phase separate at temperatures below the critical temperature, several non-equilibrium phenomena based theories for micro domain formation in PM formulated (10).

More precisely, the competition between phase separation and lipid recycling in a multiple species system, at a temperature below the critical temperature, has been found to dominate the microstructure formation Currently, two fundamentally non-equilibrium models have been proposed: first, Turner et al. formulated a model to describe the distribution of rafts with different sizes in the cell membrane via a master equation approach (11). The main assumption of this model is that lipid patches with raft domain composition are constantly transported into the cell membrane. The smaller raft domains can associate into larger ones, and larger domains can either dissociate into smaller ones or be removed from the membrane altogether.

The rates of raft generation, removal, association, and dissociation determine the size distribution of rafts. Rafts have been proposed as lipid platforms of a special chemical composition (rich in Sphingolipids and cholesterol in the outer leaflet of the cell membrane) that function to segregate membrane components within the cell membrane (12). An estimate of the number of rafts in a cell plasma membrane (based upon lipid composition, total membrane size and size of the raft domains) would be on the order of 105 to 106. Their specificity with regard to lipid composition is reminiscent of phase separation behavior in heterogeneous model membrane systems (13). In fact, many of their properties with regard to chemical composition and detergent solubility are similar to what is observed in model systems composed of ternary mixtures. Lipid rafts could be considered domains of heterogeneous *l* phase lipid bilayer composing the plasma membrane. At this point it would appear most correct to equate rafts with a liquid-ordered phase and refer to the rest of the membrane as the non raft liquid phase. Although there is increasing consensus that lipid rafts do exist in cell membrane, this field of research is still in its infancy (14).

There is an ongoing debate on the size and lifetime of rafts. This is not understood even in model systems, but there is evidence that domain sizes of phases may depend on their composition. When proteins are incorporated into these domains, still another level of complexity is introduced. One view maintains that raft proteins act as nucleation sites

for raft domains. Raft proteins organize "shells" composed of maximally 80 molecules of cholesterol and sphingolipid around themselves. Assuming a GPI anchor for the raft protein, the shell would have about 4 to 5 layers of lipid around the anchor. The shells are postulated to be what facilitates the proteins to eventually integrate into largerafts (14).

"Boundary" lipids hardly indicate that such a strong interaction is probable. Boundary lipid-protein interaction, limited on the average to less than one millionth of a second, has not been demonstrated, with the exception of a few cases, to show any significant specificity (15). Formation of the succeeding layers in the shell would require that the lipids of the shell have a rather strong tendency to associate. Raft organization maintains that lipid rafts are manifestations of the thermodynamic properties of the lipid bilayer of the membrane, namely, that this lipid bilayer is a heterogeneous system. The use of different techniques has given variable size estimates (<700 nm) for lipid rafts in membranes. However, the different size determinations, it becomes obvious that they reflect different states of rafts. The large, 700-nm size was obtained from measurements by single molecule tracking on the plasma membrane of human aortic smooth muscle cells. An estimate of 200-nm raft size was obtained by single particle tracking by using beads to which multiple antibodies LIPID RAFTS **281** were bound and multiple contacts with GPI-anchored proteins on the cell surface could have caused raft aggregation.

The specificity of raft clustering is nicely illustrated by experiments in which raft clustering is induced by cross-linking with specific antibodies on the surface of living cells. If two raft proteins are cross-linked by antibodies, they will form overlapping patches in the plasma membrane. However, simultaneous patching of a raft protein and a non raft protein leads to the formation of segregated patches. Co-patching of the two raft components depends on the simultaneous addition of antibodies to both raft proteins to the cells. Lipid rafts cluster to perform functions in membrane trafficking, signal transduction, and cell polarization (16).

3. WHAT IS DETERGENT RESISTANCE OF LIPID DOMAINS

One of the issues in the lipid domain field is the issue of detergent resistance can be used to define lipid raft composition (16). The detergent-resistant membrane fraction (DRM) floated to low density and was enriched in sphingolipids and cholesterol. The property of detergent resistance to liquid-ordered domains, while liquid-disordered bilayers were solubilized by Triton X-100. This set in motion a wave of research in which DRMs were used to define raft association. This was coupled with cholesterol depletion using methyl β cyclo dextrin (17).

Solubilization of raft proteins after this treatment became a standard tool for specifying whether a protein was involved in raft processes. The usefulness of this methodology is attested to by the fact that most proteins that have a

substantial fraction in DRMs are shown to associate with rafts also when analyzed by other methods. A number of other mild detergents have been introduced to extract lipid domains from cell membranes (17). Several of these detergents were compared in a recent study that demonstrated that detergents of the Lubrol and the Brij series extracted DRMs with different lipid and protein composition when compared with that of Triton X-100-DRMs.

4. BIOLOGICAL ROLES OF RAFT HETEROGENEITY

The recent advances of high-resolution microscopic methodology together with observations of large-scale phase separation in plasma membranes have confirmed the potential for lipid phase separation. To illuminate the possible roles of rafts in cellular functions, we focus on four areas of cell biology —TCR signaling, HIV assembly, endoplasmic reticulum (ER)-to-Golgi and post- Golgi trafficking to the cell surface and glycosphingolipid-mediated endocytosis — and review progress made in the past decade (18).

5. T -CELL SIGNALLING

The rafts might affect T cell signalling was the observation that antibody-mediated cross-linking of GPI-anchored proteins (which do not span the membrane) could stimulate signalling58. Later, DRM analysis showed that factors important for T cell signalling were detergent-insoluble, whereas engineered palmitoylation-deficient proteins became detergent-soluble and impaired T cell activation. Cholesterol depletion inhibited T cell activation, whereas co-patching experiments using cholera toxin induced part of the T cell-activation programme and lead to microscopically observable domains containing essential T cell-activation proteins. Taken together, these data suggested that lipid rafts are involved in T cell signaling (19).

6. VIRUS BUDDING

Many viruses acquire a membrane envelope when budding off from the host cell plasma membrane. Some viruses, including HIV76 and influenza77, seem to do this by organizing a lipid raft domain around their nucleocapsid that includes viral glycoproteins and excludes most host cell surface proteins from the budding viral envelope. The Gag protein of HIV, the matrix domain of which assembles with the Env glycoprotein in the plasma membrane, becomes detergent-resistant while driving the budding process (20); furthermore, budding is cholesterol-and sphingolipid-dependent. These data suggested that the assembly of the virus envelope at the host cell plasma membrane involves the clustering of rafts.

7. RAFTS IN MEMBRANE TRAFFICKING (ER-TO-GOLGI TRAFFIC IN YEAST)

Secretory proteins are inserted into the ER and transported by coat protein II (COPII) vesicles to the Golgi and subsequently to their final destinations. In the ER of yeast, secretory proteins are sorted into at least three types of ER exit site85. Two of these sites concentrate mainly soluble or trans-membrane cargo, and early COPII machinery was found to be necessary for these functions. The third exit site also produces COPII vesicles, but COPII machinery is not required to concentrate its predominantly GPI-anchored cargo85. This concentration depends on the remodelling of GPI anchors with a saturated, long-chain fatty acid or a ceramide that confers detergent resistance. Defects in GPI-anchor synthesis dramatically reduce the total sphingolipid levels88, and ER exit of GPI-anchored proteins depends on ceramide synthases (21).

Therefore, the sorting mechanism for this type of exit site might be a concentration of ceramides that attracts GPI-anchored proteins but tends to exclude most trans-membrane proteins.

8. MOVING FORWARD WITH RAFTS

Although many cell functions take place in membranes where proteins and lipids are intimately mixing, research in this field has long avoided the study of how lipids and proteins function together. This neglect is now being remedied by the realization that membranes are made functional by lipid–lipid, lipid–protein and protein– protein interactions. For example, it has been recently shown that proteins adjust their trans-membrane length and composition to the specific physical properties of the different sub-cellular membranes in which they reside, which themselves have evolved highly specific lipid compositions (22).

Cell membranes are lipid bilayers, crowded with proteins occupying around 20% of the bilayer area. This means that lipids and proteins in membranes should really be studied as collectives. Membrane proteins alter their lipid environment not only by binding specific lipids but also by influencing their surrounding lipid environment.

Therefore, one area of increasing importance will be the study of lipid–protein interactions that regulate the nanoscale raft protein assemblies and how they can coalesce to form functional platforms. This work will require sophisticated and difficult image technology, as well as biochemistry that must overcome the technical hurdles that plague work with hydrophobic proteins and lipids. The well-known asymmetry of the bilayer and the proteins spanning the membrane add additional barriers to reconstituting membrane organization and function *in vitro*(22).

9. SUMMARY

Model membrane studies provide many clues into the possible nature of lipid rafts in cells. However, cell membranes are more complex than model membranes. In cells, ordered lipid domains may exist at the borderline of conditions at which they are stable, and/or in the form of almost infinitesimal nano-domains (23). If rafts only exist asnano-domains that are inaccessible to detection by experimental techniques then it can be questioned whether the raft concept is useful. However, even nano-domain-sized rafts could modulate the ability of proteins to interact with one another, and thus can be of functional significance (23).

Thus, the importance of developing methods to study nano domain-sized rafts must be emphasized. Future progress in the raft field will depend both upon methodological advances that can be applied to cellular studies, and model membrane experiments employing increasingly realistic lipid and protein compositions (24). Biochemical manipulations to isolate and/or modify ordered domain formation in cells off era powerful, albeit indirect, way to examine rafts and their functional influence whatever their size. However, model membrane studies show that, as used at present, the most commonly used methods of this type have limitations that prevent them from providing definitive answers to questions of when (or even whether) lipid rafts exist (25). Further improvement in these techniques is also critical. However, it is difficult to envision how raft domains could be spatially targeted within this framework, apart from condensation around immobile membrane proteins (25). On the long term, it is expected that such an endeavor would facilitate the emergence of an improved, quantitative understanding of membrane structure and kinetics over multiple length and time scales.

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