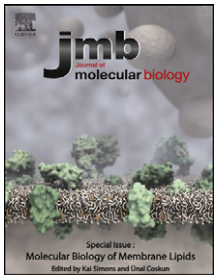

Compartmentalization of the Cell Membrane

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Abstract

Many cell-membrane-associated processes require transient spatiotemporal separation of components on scales ranging from a couple of molecules to micrometers in size. Understanding these processes mechanistically involves understanding how lipids and proteins self-organize and interact with the cell cortex. Here, we review recent advances in dissecting the mechanisms of cell membrane compartmentalization. We introduce the challenges in studying cell membrane organization, the current understanding of how complex membranes self-organize to form transient domains, and the role of protein scaffolds in membrane organization. We discuss the formation of signaling domains as an important example of transient membrane compartmentalization. We conclude by pointing to the current limitations of measuring membrane organization in living cells and the steps that are required to advance the field.

Introduction: Challenges to Studies of the Cell Membrane Organization

Understanding plasma membrane organization has been a major aim in cell biology and biophysics for almost half a century. Despite knowing the majority of the molecular building blocks (lipids, proteins, and sugars) and most of the organization principles, we lack a quantitative understanding of how molecular interactions between membrane components and its coupling to the cell cortex give rise to structure and function in cell membranes. There are several fundamental reasons for this knowledge gap: first, the relevant length scales of compartmentalization in the membrane–cytoskeleton system are too small to be resolved by diffraction-limited light microscopy, and their transient nature complicates direct imaging with super-resolution microscopy [1,2]. Second, the molecular composition of the cell membrane is very complex (>500 different lipid species, >1 k sugar species, and >400 membrane proteins). In contrast to proteins, we lack methods to tag and detect most of the endogenous sugar and lipid species under live-cell conditions. Consequently, we lack molecular distribution information for many components of the cell membrane. Third, living cells are far from equilibrium, energy-driven processes constantly stir and reorganize the system. These active processes involve membrane transport and metabolism and the polymerization and motor-driven constriction of the cell cortex. How these processes couple to the composition and structure of the cell membrane is only partly understood. Lastly, molecular dynamics simulations of membranes are still far too small and short to predict bottom-up membrane behavior on a scale relevant for membrane compartmentalization, which would be at least 1 μm and 1 s [3,4]. All these facts make it very challenging to directly measure many of the relevant processes of cell membranes and to translate the knowledge gained from minimal lipid model systems or MD simulations to the membrane of intact cells.

Still, we argue that there are strong indications for the existence of compartments in the cell membrane. We present the arguments in a hierarchical order starting with the complexity of molecular interactions and the self-organization defining the “ground state” of the plasma membrane and we move up to the formation of transient signaling domains and more stable, larger membrane domains in the context of cell functions.

Self-organization of Simple and Complex Membranes

Cells are compartmentalized from the molecular to macroscopic scale to allow for the spatiotemporal control of biochemical reactions. Cellular organelles separated from the cytoplasm by dedicated lipid membranes are the most obvious form of compartmentalization. However, due to their two-dimensional (2D) and fluid nature, membranes itself are ideal structures to organize and control biochemical reactions. In fact, many important cellular processes take place at membranes, for example, signaling and sensing, energy conversion, and metabolism. The simple reduction of dimensionality upon binding to 2D membranes results in an effective increase in concentration and therefore increases the reaction rates by orders of magnitude, which is, for example, used as a switch to control some cellular signaling reactions [5]. Preferential orientation of membrane-bound molecules can additionally modulate affinities, either by blocking or exposing interaction sites [6]. In addition, Pólya's recurrence theorem states that 2D diffusion covers the entire surface [7].

Besides these obvious differences between the reaction diffusion systems in three and two dimensions, biological membranes provide more qualities for compartmentalizing and controlling reactions. Membranes assemble via hydrophobic interactions of lipids without the need for attractive interactions between neighboring components. Depending on lipid composition, the emergent bilayer structure can be in a solid, liquid-ordered, or liquid-disordered state. In multicomponent membranes, even weak interactions between components (charge, propensity for hydrogen bonding, etc.) can lead to segregation of components and result in domains of specific composition. In addition, matching molecular characteristics of membranes components to local membrane features like thickness, curvature, and surface charge further influences the sorting of molecules in membranes [8,9]. These self-organization properties of lipid membranes have been worked out in simple model membrane systems, and all of these principles have been suggested to be important for the function of cell membranes. The cell membrane has a very complex lipid composition with a large variety of proteins, as one-third of our genes encode membrane proteins [10]. Depending on cell type and measurement method, these proteins occupy 23% to 40% of the membrane surface [11,12].

With the high cholesterol and high protein content, the overall state of the cell membrane is liquid with relatively high lipid order [13,14], and in non-polarized cells, the distribution of membrane proteins such as receptors and fluorescent lipid tracers appears to be homogenous on scales larger than 200 nm. However, intricate high spatiotemporal resolution measurements of fluorescent lipid and protein tracers in living cells have revealed anomalous diffusion characteristics or clustering on scales below 200 nm, which indicate the transient confinement of membrane components in cytoskeletal compartments and/or lipid–protein domains on the nanoscale [15–27]. The characterizations of nanoscale lipid–protein domains in intact cells come from indirect measurements because these domains are small and dynamic and their structure is easily perturbed by fixation processes required for higher resolution techniques [16,28–30]. Diffusion measurements of lipids or proteins interacting with nanoclusters require high spatiotemporal resolution, as the residence time of individual molecules in the nanodomains is likely shorter than 100 ms and the domains are smaller than 60 nm. Förster resonance energy transfer (FRET) measurements have also been applied to determine whether a protein interacts with nanodomains. As there are few proteins within each cluster, homo-FRET of the same species has provided more consistent results than dual-color FRET. The short interaction time of diffusing molecules with the nanodomains indicates binding energies not much larger than the thermal energy $k_B T$. [31]. As we can only measure the interaction of individual molecules with nanodomains, but we cannot image the domains, we currently have no information on the lifetime and shape dynamics of the domains [32].

Nanoscale domains or heterogeneities in the cell membrane can coalesce into larger domains. Cross-linking lipid or protein components by multivalent toxins or antibodies induces micrometer-scale domains in the cell membrane, which are reminiscent of liquid-ordered/liquid-disordered domains in model membranes [33]. Similar domains appear in vesicles extracted from the plasma membrane when they are cooled below room temperature [34,35]. These experiments indicate that the complex composition of the plasma membrane has evolved to be close to a transition into a state where some components spontaneously separate. The high lipid diversity found in cell membranes may be one way to be close to this point and yet avoid spontaneous demixing, because the diverse lipids reduce interface energies and act as buffer. In addition, anchoring some cell membrane components to the cytoskeleton or via adhesion to the extracellular matrix has been shown to quench segregation [36]. We will discuss this phenomenon in more detail below.

Another important structural aspect of cell membrane is their asymmetry. Most of the sphingolipids and gangliosides are located on the outer membrane leaflet, while negatively charged lipids like phosphatidylserine and phosphatidylinositol species (PIPs) are found on the inner leaflet. This lipid asymmetry is actively established and maintained by flippases and lipid transport proteins [37,38]. In the inner leaflet, protein and lipid clustering has been shown to be mediated by electrostatic interactions between divalent