Membrane Lipid Rafts and Their Role in Axon Guidance

Carmine Guirland and James Q. Zheng*

Abstract

The plasma membrane of cells contains a variety of lipid and protein molecules that are often segregated and heterogeneously distributed in microdomains. Lipid rafts represent a generalized concept of membrane microdomains that are enriched in cholesterol and sphingolipids and, characteristically, resistant to cold detergent extraction. Lipid rafts have recently received considerable attention because they are thought to be involved in many cellular functions, in particular, signal transduction for extracellular stimuli. Many of these functions are also intimately related to the processes involved in neural development, including neurotrophic factor signaling and synaptic plasticity. Recent studies from our lab and others have indicated an important role for lipid rafts in axonal growth and guidance. Specifically, our data show that lipid rafts on the plasma membrane provide platforms for spatial and temporal control of guidance signaling by extracellular cues. In addition, lipid rafts may also function in other aspects of axonal growth and guidance, including spatial and temporal regulation of adhesion, cytoskeletal dynamics, and growth cone motility. Further elucidating how membrane rafts are involved in guided axonal growth would provide important insights into the intricate signaling mechanisms underlying neuronal wiring, which is fundamental for normal brain development and functional recovery after injury and diseases.

Introduction

In the fluid mosaic model of the plasma membrane posited by Singer and Nichols, the membrane is a bilayer composed of a relatively continuous and homogenous fluid of amphipathic lipids that is interspersed with a mosaic of proteins. Most eukaryotic cells are mainly composed of lipids belonging to three major lipid classes: glycerophospholipids, sphingolipids, and sterols. Various membrane proteins, including receptors, can associate with the plasma membrane by virtue of hydrophobic and electrostatic forces, covalently attached lipid anchors, and membrane-spanning domains. However, this picture of cell membranes has since been evolving steadily. For example, it is known that the lipid and protein constituents of membranes are distributed asymmetrically. Different lipid classes of the membrane have been found in ratios that vary across each leaflet of the membrane, different cell types, and different cell compartments. The diversity of lipids and their distinct spatial distribution suggest that they may be involved in a variety of cellular functions. Arguably the most significant modification of the original fluid mosaic model is the existence of lipid domains of different lipid composition.
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and physical state from the rest of the lipid bilayer. The initial notion of lipid domains was suggested by studies in model membranes, but it was the observation of caveolae, flask-shaped plasmalemmal invaginations of the cell membrane that led to extensive studies on membrane microdomains. Caveolae exhibit several distinct features including a special lipid composition rich in cholesterol and sphingolipids, a striped coat formed by caveolin proteins on the cytoplasmic surface, and in addition to their characteristic flask shape, they can also have vesicular and tubular morphologies. Caveolae were initially thought to mainly function in clathrin-independent endocytosis. Subsequent biochemical analyses of the molecular composition of caveolae, based on the findings that caveolae are low-density membranes and resistant to cold detergent extraction, suggested other possible functions. Most notably, these studies have found the presence of multiple signaling components in caveolae preparations, indicating that caveolae may also play a role in signal transduction.

Later studies have pointed out that membrane domains lacking caveolin proteins are also present on the plasma membrane, suggesting the existence of other types of detergent-resistant microdomains (DRMs) that do not involve caveolins. As such the term "lipid rafts" was later used to describe dynamic membrane domains in a broader sense. Before exploring the functions of rafts, it is helpful to consider some of their characteristics. Lipid rafts are small and dynamic: they can be as little as several nanometers in diameter and their transient existence is in the msec range. Both lipid raft size and half-life are flexible parameters that are altered in live cells, which may be involved in lipid raft functions. Rafts are thought to be a liquid ordered phase of membrane, which consists of saturated sphingolipids. The rafts "float" in a liquid disordered phase, which mainly consists of unsaturated glycerophospholipids. Cholesterol is thought to stabilize the sphingolipids in this liquid ordered phase since cholesterol interacts more favorably with sphingolipids over unsaturated phospholipids. This partitioning of the membrane into laterally heterogeneous domains can therefore provide an organized membrane environment for protein interactions or other cellular functions.

Approaches to Study Lipid Rafts and Their Functions

Many studies have relied on two experimental approaches involving detergent resistance and cholesterol dependence to study lipid rafts and their functions. Lipid rafts are biochemically defined on the basis that they remain resistant to cold nonionic detergent treatment and/or are low-density membranes, thus float to the top of a buoyant density gradient. The so-named detergent resistant membranes (DRMs) are also known as detergent-insoluble glycolipid-enriched complexes (DIGs). Proteins that associate with lipid rafts are defined as those that cofractionate with DRM fractions and typically have some lipid modification such as glycosylphosphatidylinositol (GPI) or acyl anchors. Therefore, cold-detergent extraction and membrane fractionation have been extensively used to identify proteins associated with lipid rafts. Using this approach, numerous proteins, including GPI-anchored proteins, caveolins, src-family kinases, and G-proteins, have been shown to associate with lipid raft fractions. Since lipid raft integrity depends on cholesterol, cellular functions that require lipid rafts could be affected by manipulating membrane cholesterol. Using various means to manipulate the synthesis or plasma member distribution of cholesterol has been instrumental in investigating the role of lipid rafts in cell functions beyond protein associations. While such experimental approaches have inherent flaws, they have proved to be useful methods for identifying many of the constituents and functions involving lipid raft membrane microdomains.

One challenge in studying lipid rafts is the direct visualization of these dynamic microdomains on the native membrane of living cells. While DRMs have been biochemically isolated and analyzed, the dynamics and spatial properties of lipid rafts remain to be directly examined. Detergent extraction does remove some lipids and proteins from rafts, which in combination with methodological differences, may account for the considerable degree of variability in analyses of raft components. However, it is the lack of visual evidence that is fueling the continuing