Defining lipid raft structure and function with proximity imaging

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THE PRESENCE OF LIPID MICRODOMAINS in eukaryotic plasma membranes, later termed lipid rafts, was first postulated almost two decades ago (2, 23, 25). The existence of these domains has profoundly changed our view of the plasma membrane, as proposed by Singer and Nicolson (26). Since then, many important cellular functions, including signal transduction (24), pathogen invasion/uptake (13), intracellular trafficking (8), and secretion and endocytosis (10, 20), have been attributed to lipid rafts. Lipid rafts and caveolae, a subset of lipid rafts characterized by the presence of the protein caveolin, have been implicated in numerous signaling pathways that regulate the proper functioning of the cardiovascular system (4, 5, 7, 9, 11, 15, 19). In fact, many signaling molecules have been shown to be associated with lipid rafts. These molecules may be permanently localized in lipid rafts, where they assemble into preformed signaling complexes, as for caveolae (14). This system would facilitate and accelerate the response to extracellular stimuli. It is also possible that molecules of the same pathway may be segregated into different subsets of lipid rafts and that they would interact only upon clustering of these microdomains mediated by ligand-induced stimulation.

The regulation of lipid raft size may play an important role in controlling their function (22). Depending on the method and cell type used, it has been estimated that lipid raft size may vary greatly (between 50 and 700 nm) (1). In the resting state, rafts may be small (∼50 nm); but upon activation, however, their clustering may lead to the formation of much larger entities, which could allow interaction between functionally related proteins (17, 22).

The functional organization of lipid rafts may therefore play an important role in the regulation of various signaling pathways. As a consequence, it will be important to develop novel systems capable of analyzing the distribution and clustering of lipid rafts under various conditions in live cells. In this issue of the American Journal of Physiology-Heart and Circulatory Physiology, Patschan et al. (16) used a glycosylphosphatidylinositol (GPI)-anchored thermostolerant green fluorescent protein (GPI-ttGFP) to examine the relative area and plasma membrane distribution of lipid rafts under several conditions. Their method is based on the change in the relative fluorescence intensities emitted upon excitation at 395 vs. 475 nm when two molecules of green fluorescent protein are brought into close proximity (3). Examining the changes in this ratio allows one to quantitatively determine the levels of oligomerization in live cells. Using this technique, termed proximity imaging (PRIM), Patschan et al. coupled green fluorescent protein with a GPI anchor, which allowed examination of the distribution of lipid rafts and their clustering upon treatment with certain stimuli.

Using human umbilical vein endothelial cells as a model cell system and urokinase-type plasminogen activator receptor as the marker, Patschan et al. (16) first showed that the fluorescent probe indeed colocalized with lipid rafts. Importantly, in the presence of inhibitors of raft formation (cholesterol oxidase and methyl-β-cyclodextrin), the area occupied by lipid rafts was reduced, as expected (21). These observations are similar to those reported for caveolae formation (6, 27). By themselves, oxidized LDL and LDL did not affect the distribution of lipid rafts. However, when combined with N,N-dimethyl-L-arginine dihydrochloride [an inhibitor of endothelial nitric oxide (NO) synthase activity], oxidized LDL, but not LDL, could reduce GPI-ttGFP clustering at the surface of the cells. As a consequence, lipid raft association of endothelial NO synthase was reduced. NO donors could also increase the distance between GPI-ttGFP molecules. Interestingly, these data suggest that stimulation of human umbilical vein endothelial cells results in “unclustering” of GPI-ttGFP. NO-mediated unclustering of GPI-ttGFP was not due to a prooxidant effect; rather, NO appears to induce dissociation of the F-actin cytoskeleton from lipid rafts. This finding highlights the important relationship between raft formation and an intact cytoskeleton (18).

Interestingly, in a previous study, Li et al. (12) showed that NO could attenuate signal transduction via caveolae in endothelial cells. Taken together, these findings suggest that the unclustering of lipid rafts may affect the ability of endothelial cells to respond to external stimuli.

This study (16) shows that lipid raft organization and clustering can be regulated through two important mechanisms. 1) Because cholesterol and sphingolipids are major constituents of lipid rafts, their involvement in lipid raft formation is not surprising. 2) Lipid rafts also appear to interact with and require the presence of an intact cytoskeleton, which would provide the framework for the organization of the signaling complexes.

These data suggest that various mediators can affect lipid raft properties and can, in turn, affect important signaling pathways regulated by these structures. This novel technique will allow the investigation of various lipid raft effectors that may have important roles in the regulation of cardiovascular function. Also, it will permit one to distinguish between direct lipid raft effectors and regulators of downstream signaling pathways.

Future studies are required to address this aspect in vivo, inasmuch as it remains to be demonstrated that lipid rafts in...
isolated cells are similar in size and function to those in whole organisms, as in the case of caveolae.

REFERENCES


