



Lipid rafts are localized regions of elevated cholesterol and glycosphingolipid content within cell membranes. Caveolae, small plasma membrane invaginations that are coated with the cholesterol-binding protein caveolin, are a subset of lipid rafts. The acyl groups of the phospholipids present in lipid rafts and caveolae are more highly saturated than those in the surrounding membrane. This allows close packing of these phospholipid side chains with the saturated acyl chains of sphingolipids and probably leads to phase separation. Due to the presence of cholesterol, a liquid ordered domain is formed that exhibits less fluidity than the surrounding plasma membrane. The presence within lipid rafts and caveolae of a variety of membrane proteins involved in cell signaling, including the EGF receptor, has led to the consensus that these lipid domains play an important role in the process of signal transduction. My laboratory is interested in the structure and function of the EGF receptor and the role of lipid rafts in modulating signaling via this receptor.

I. EGF Receptor Structure/Function Studies

In all cell types examined thus far, the EGF receptor selectively partitions into lipid rafts. We are interested in defining the sequences within the EGF receptor that cause it to partition into lipid rafts. Mutational analysis has been used to demonstrate that the most membrane proximal segment of the extracellular domain of the EGF receptor is involved in targeting the EGF receptor to lipid rafts. Additional studies are in progress to further define the critical residues and determine how those sequences function in localizing the receptor to lipid rafts. We are also examining the effects of mutations in the extracellular domain of the EGF receptor on the ability of the receptor to bind, dimerize and transduce a signal.

II. Effect of Lipid Rafts on EGF Receptor Signaling

EGF receptors partition into lipid rafts and this localization of the receptor appears to affect its signaling capacity. We have shown that when lipid rafts are disrupted by cholesterol depletion, both the binding and tyrosine kinase activity of the EGF receptor is increased.

Cholesterol depletion also leads to the activation of MAP kinase—both basal and EGF-stimulated. These data suggest that incorporation of the EGF receptor into lipid rafts suppresses the activity of this receptor and is probably important in maintaining the EGF receptor and the pathways that it stimulates in a basal state of activity.

Unlike MAP kinase activation, EGF-stimulated PI turnover is inhibited by raft disruption. This indicates that intact rafts are necessary for proper functioning of this downstream signaling pathway. We have shown that much of the cellular phosphatidylinositol 4,5-bisphosphate (PtdIns 4,5-P₂) is present in lipid rafts and that it is this raft-localized pool that is turned over in response to hormone. It seems likely that clustering of the receptor, phospholipase C- γ , and PtdIns 4,5-P₂ are necessary for effective PI turnover.

Future experiments will further examine the effects of cholesterol and lipid rafts on EGF receptor function both *in vivo* and *in vitro*. In particular, the function of EGF receptors present in isolated lipid raft fractions will be compared with those of EGF receptors present in non-raft membranes. In addition, the role of cholesterol will be probed by exchanging natural cholesterol with its enantiomeric form to determine whether the effects of cholesterol depletion are due to direct binding of cholesterol to the receptor or are the result of the effects of cholesterol on membrane properties.