Nanoscale analysis of membrane lipids by electron microscopy

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Lateral heterogeneities of cell membranes are envisaged as microdomains, and one such microdomain, or 'raft', is thought to consist of glycosphingolipids, cholesterol, and membrane proteins. Recent analyses have suggested that rafts in normal unstimulated cells are extremely small and may last only for a short time. Electron microscopy (EM) is expected to have sufficient resolving power to visualize rafts if they are in the nanometer size range, but chemical fixatives that need to be used for conventional sample preparations are unlikely to preserve the in situ localization of membrane molecules, particularly lipids, and may even cause artifactual redistribution. To minimize the possibility of perturbation, we utilized quick-freezing and freeze-fracture techniques to physically immobilize molecules in situ, and labeled them on the replicas. Using this 'physical fixation' method, the distribution of gangliosides GM1 and GM3, putative raft molecules in the cell membrane, could be visualized in the nanometer size range. The gangliosides formed clusters in normal mouse fibroblasts, and those clusters were modified by either cholesterol depletion or chilling（Mol. Biol. Cell, 18, 2112-2122, 2007). The above technique can be used for the analysis of other membrane lipids, and should elucidate the lateral heterogeneity of the cell membrane at the nanometer scale.