Ultrastructural analysis of caveolae and lipid raft domains

R.G. Parton, J.F. Hancock, IMB, University of Queensland, St Lucia, Qld, Australia

The plasma membrane of mammalian cells is made up of a complex mosaic of different functional microdomains. Spatial segregation of plasma membrane components is essential for diverse cellular functions such as signal transduction and cell-cell interactions. We have used a novel method to examine microdomains involved in Ras signalling. EM grids are adhered to the surface of cultured cells which have been transfected with the proteins of interest. The grid is then removed with the entire dorsal plasma membrane of the cell attached. The plasma membrane sheet is then rapidly fixed. The sheet is then immunogold labelled for the cytoplasmically orientated antigens. Antibodies are directly conjugated to gold to improve the resolution of the technique. Images of the labeled plasma membrane sheets are digitized and processed to remove background and the (x, y) coordinates of all of the gold particles in a given area are determined. Ripley's K-function analysis is then used to determine whether the observed gold pattern is random, clustered, or dispersed. A modification of the K-function can also formally assess the extent of colocalisation of two proteins, each labeled with a different sized gold, for example, 2 nm and 4 nm. The results can be expressed graphically to provide a statistically significant measure of the clustering of a single marker protein or the colocalisation of two different markers. Analysis of the distribution of a GFP-tagged lipid raft-localised Ras isoform showed significant clustering in domains with a diameter of 44nm. Mathematical modelling estimated that the area of the plasma membrane occupied by these domains is 35%. The clusters are completely dispersed upon cholesterol depletion: there is no change in total plasma membrane labeling density, but the pattern becomes random. Further experiments suggest that H-ras has dynamic interactions with both raft and non-raft domains depending on the GTP-bound state. WT-H-ras showed strong colocalisation with a raft marker in serum-starved cells that decreased on serum stimulated GTP-loading. Constitutively active H-rasG12V showed negligible colocalisation with a lipid raft marker. Thus, microdomain localisation can be regulated by the GTP-bound state of the Ras protein.