Type of presentation: Invited

LS-12-IN-1756 Distribution of membrane lipids observed by quick-freezing/freeze-fracture replica labeling electron microscopy

Fujimoto T.[1]

[1]Nagoya University Graduate School of medicine

Email of the presenting author: tfujimot@med.nagoya-u.ac.jp

The biological membrane is a highly dynamic two-dimensional structure. Lipids not only form the structural basis of the membrane, but also play many functional roles. In comparison to proteins, however, relatively little is known about how lipids distribute in the membrane. This is mainly because most microscopic methods used for proteins are not appropriate for determining lipid distribution at a small scale. Proteins can be immobilized with chemical fixatives like aldehydes so that various immunohistochemical techniques can be applied. In contrast, most membrane lipids do not react with aldehydes and remain mobile even in fixed cells. This ‘unfixability’ makes it difficult to determine lipid distribution even though specific probes are available for many membrane lipids.

We have been working on an electron microscopic method using freeze-fracture replica labeling combined with quick-freezing (QF-FRL). In this method, cells are fixed physically to avoid the problem of chemical ‘unfixability’ of lipids. Briefly, cells are subjected to quick-freezing to stop molecular motion instantaneously; then a membrane is split into two leaflets by freeze-fracturing, after which membrane molecules are immobilized by vacuum evaporation of platinum and carbon; to this physically-stabilized membrane preparation (i.e., freeze-fracture replica), specific probes are applied to label target molecules (Figure 1).

By using QF-FRL, we can observe not only the two dimensional distribution of membrane lipids (and proteins as well) but also the asymmetry that lipids show between the outer and inner leaflets. Because membranes are retained in a stable form in the freeze-fracture replica, they can be subjected to various chemical treatments that may perturb membrane structures when applied to native membranes. I would like to present several results obtained using QF-FRL and discuss the strength and weakness of this technique in comparison with other methods available today.

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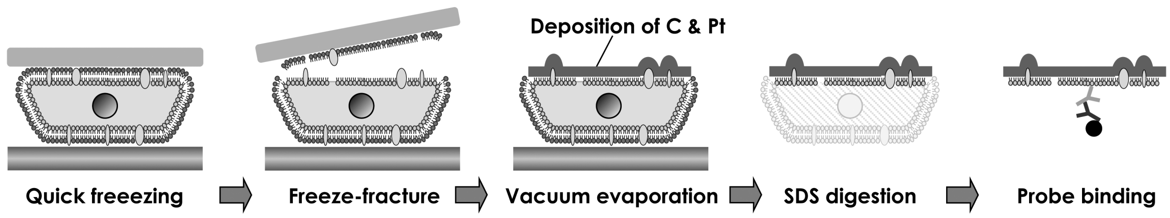

Fig. 1: The outline of quick-freezing/freeze-fracture replica labeling