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Biomembrane Liquid-liquid Phase Separation and Detergent Resistance: A Relationship Strengthened

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Abstract

Since evidence first appeared for “detergent-resistant membranes” in the early to mid 1990s, cell biologists from a wide spectrum of biological sciences have been intrigued by the functional relevance of this indication of membrane heterogeneity, commonly referred to as “lipid rafts”. Model membrane studies revealed that these lipid rafts are related to the more ordered liquid phase that forms in a ternary mixture of cholesterol with a phospholipid containing saturated acyl chains and one with unsaturated acyl chains. Giant plasma membrane vesicles that pinch off from cells undergo similar liquid-liquid phase separation as ternary model membranes and have provided an experimental bridge between these and intact cells. The study by Levental et al. in the current issue of *Biochemical Journal* provides new insights into the relationship between liquid-liquid phase separation in these plasma membrane vesicles and detergent resistance of cellular lipid rafts.

Keywords

lipid rafts; cholesterol; GUVs; plasma membrane vesicles; critical point; cytoskeleton

A Google Scholar (<http://scholar.google.com/>) search for “detergent-resistant membranes” identifies over 3000 publications using this phrase during the last 15 years, and a search for “lipid rafts” identifies over 30,000 publications in this same time period! Clearly, these terms have entered the mainstream of modern cell biology vernacular, and the general paradigm of lipid-based membrane heterogeneity, often referred to as “lipid rafts”, is now a commonly accepted property of eukaryotic cell membranes. Among the earliest experimental evidence for this heterogeneity is the study by Brown and Rose describing the characterization of cell-derived, non-ionic detergent-insoluble membrane vesicles containing sphingolipids and glycosylphosphatidylinositol (GPI)-linked proteins [1], as well as studies from the Simons’ laboratory describing selective biosynthetic trafficking of these and related components to the apical side of polarized epithelial cells [2]. These and other studies inspired systematic investigations of sphingolipid and cholesterol-dependent phase separation in fluid model membranes, which established properties of liquid-ordered lipids in these three-component membranes, including the physical basis for their detergent-resistance [3,4].

Giant unilamellar vesicles (GUVs) have been a useful experimental preparation for these model membrane studies, as fluorescently labeled lipids can be used to determine relative

abundance and properties of liquid-ordered (Lo) or liquid-disordered (Ld) phases using confocal imaging. Several years ago it became apparent that large membrane vesicles that form and pinch off from the plasma membrane of intact cells also undergo liquid-liquid phase separation when cooled to ambient temperature or below, and these phases have properties similar to those in GUVs previously characterized [5]. Importantly, these giant plasma membrane vesicles (GPMVs) contain a nearly full complement of plasma membrane proteins as well as lipids, so that the relative partitioning of different classes of these proteins can be compared. In these studies, transmembrane and outer leaflet lipid-anchored proteins are generally found to partition according to expectations based on their detergent resistance in cell assays, but lipid-anchored proteins that localize to the inner leaflet in cells are most frequently found to partition with the Ld phase in GPMVs, despite preferential fractionation with detergent-resistant membranes from cells. This apparent inconsistency could be due to changes in lipid asymmetry during the formation of GPMVs [6].

An important question is why these GPMVs undergo robust liquid-liquid phase separation at lowered temperatures, whereas the plasma membrane of intact cells does not normally undergo this large-scale phase separation under similar conditions. Simons and colleagues have shown that large-scale Lo/Ld phase separation can be detected in the plasma membrane of intact cells that have been stressed by energy depletion [7], indicating that this phase separation is not limited to isolated membrane vesicles that may have altered lipid or protein compositions. One clear difference between GPMVs and cells is the presence of the actin-based cortical cytoskeleton that underlies the plasma membrane of intact cells and its absence from GPMVs [5]. A recent study highlights the high sensitivity of the lipid composition of isolated detergent-resistant membranes to perturbations of the actin cytoskeleton in the cells from which these are derived, both by pharmacological and signaling-dependent means [8]. This study and several others point to the regulation of lipid heterogeneity by the actin cytoskeleton.

The study by Levental et al. in the current issue of *Biochemical Journal* [9] provides an important new link between the imaging-detected, large-scale phase separation in GPMVs and the biochemical-based assay for Lo-preferring proteins and lipids that depends on detergent lysis and sucrose gradient fractionation. In addition to demonstrating the role of cholesterol in Lo/Ld phase separation in GPMVs, these authors confirm expected differences in the lateral diffusion coefficient between these phases, and they determine that the Lo phase predominates in GPMVs, consistent with previous biochemical and biophysical measurements in live cells. Most notable, Levental et al. show a strong correlation between the temperature dependence for Lo/Ld phase separation in GPMVs and the temperature dependence of detergent insolubility for both cholesterol and labeled ganglioside GM₁ in cells. A significant implication of these observations is that detergent resistance of Lo-preferring proteins and lipids depends not only on the incapacity of detergent micelles to penetrate and solubilize these membrane domains, but also on the extent to which Lo/Ld segregation has occurred in the plasma membrane of intact cells prior to exposure to micelles of Triton X-100 or other mild detergents.

These observations illuminate the relationship between large-scale Lo/Ld phase separation that is observed in GPMVs and the biochemical assay for detergent-resistant “lipid rafts”

that are derived from intact cells. They further support a relationship between large-scale phase separation in GPMVs and smaller scale membrane heterogeneity in intact cells. How may these different size scales of heterogeneity be related? In a recent study, Veatch et al. described the surprising finding that lipids and proteins in GPMVs undergo fluctuations in domain size and lifetime as these membranes are cooled towards their phase transition temperature. These fluctuations are remarkably robust, occurring in most of the GPMVs, regardless of their specific transition temperature that is variable due to compositional heterogeneity [10]. Interestingly, this and other behavior near the transition temperature were found to be well-fit by the two-dimensional Ising model for physical processes that are near a critical point. In contrast, critical behavior in three component model membranes occurs over a much more limited range of lipid compositions and temperatures, suggesting that evolutionary pressures have selected this property in biological membranes. Extrapolation to 37°C of the correlation length for fluctuation size that is calculated from the Ising model analysis yields a value of ~20 nm, which is close to some experimental estimates for the average size of “lipid rafts” on intact cells at this temperature [11].

Although it is likely that the size and dynamics of lipid-based membrane domains on intact cells are determined by several factors that await further characterization, including cytoskeletal regulation, it is exciting to think that we are beginning to learn the fundamental principles that govern the complex behavior of biological membranes, and the study by Levental et al. provides a significant step in this quest.

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