New and Notable

Zebrafish Get Ordered: New Doors Open for Imaging Membrane Organization

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Understanding how the complex physical organization of the cell membrane impacts cellular function is a central problem in the biophysical and biomedical sciences. Membrane order, which reflects the degree of lipid packing in a membrane, is widely used to characterize the physical organization of membranes. Historically, the term was used interchangeably with membrane fluidity and to some extent, still is in the literature. More recently, membrane order has emerged to have greater meaning in the context of the lipid raft hypothesis. Membrane rafts are recognized as sphingolipid/cholesterol/protein-rich nanoscale structures that coalesce into larger, more stable microdomains that serve distinct functional roles (1). Membrane rafts are proposed to be tightly packed and ordered, which promotes phase separation from more fluid-disordered domains enriched in unsaturated lipids. Model membrane studies have established the formation of liquid-ordered rafts and more-recent cellular studies validate this notion (2). However, studies of membrane order and domains have been restricted to model membranes, immortal, or primary cells. In this issue of the *Biophysical Journal*, Owen et al. (3) open the door to studying cell membrane organization in vivo, an important step forward in the study of membrane organization. This has profound implications toward understanding the fundamental organization of membranes in a physiologically relevant system.

Owen et al. (2) used the membrane dye Laurdan to image membrane order in zebrafish embryos. The probe, which is documented for measuring membrane order in cells, is sensitive to membrane phase properties and undergoes a spectral shift when the polarity of the bilayer changes in response to water penetration (4). Owen et al. (2) injected the embryos with specific constructs to label their region of interest followed by soaking the embryos in Laurdan. Using multiphoton confocal microscopy, they imaged several tissues of live embryos and quantified membrane order in terms of the generalized polarization. As a proof of principle, they showed that epithelial cells in the kidney and gut have increased lipid order in the apical relative to the basolateral membrane, consistent with data from cell lines.

The new approach will allow investigators to study the impact of varying types of intervention on membrane order. However, several challenges lie ahead before in vivo imaging of membrane order can become a routine measurement. One challenge will be to image membrane order in mammals, which more effectively model the membranes of humans. The advantage of the zebrafish model is that, during their development, the embryos are transparent—which allows for relatively easy visualization of internal anatomy. Another challenge will be to image membrane order in a dynamic setting on a smaller size-scale. This becomes relevant when considering the organization of membrane domains, which are increasingly accepted to exist on a nanometer scale (1). One important step could be to couple this new method with a recently described in vivo microscopy approach that measured membrane dynamics of tumor cells with a spatial resolution of 7–9 nanometers using quantum dots (5).

The biological implications of this study are vast, especially if some of the aforementioned challenges are overcome. One can speculate that changes in membrane order could be linked with physiological responses at the whole-animal level in the future. Measuring the effects of specific pharmaceuticals on disrupting the order of membranes and possibly rafts has therapeutic value and utility as a biomarker. For instance, loss of caveolin-1 (which supports caveolae formation) promotes the degradation of insulin receptors and GLUT-4 in adipocytes (6).

Measurements of changes in membrane order of adipose tissue in response to drug intervention could yield new targets for improving insulin sensitivity. Similarly, immunomodulation of lipid rafts of lymphocytes with nutritional intervention is of therapeutic value for suppressing inflammation and also has implications for viral and bacterial infectivity (7). The T-cell side of the immunological synapse was shown by Gaus et al. (8), the senior author of the study in this issue, to be highly ordered, presumably due to the accumulation of rafts. Direct visualization of modifying membrane order of the synapse in vivo will validate in vitro and ex vivo findings and furthermore yield new mechanisms.

Overall, this study has opened a new range of possibilities for the study of a complex problem, which is elucidating the organization of biological membranes, and thereby, function in vivo. Of course, with the opening of any new door, more questions emerge and the need is magnified for more rigorous technique development and experimentation.

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