Defining and Imaging Membrane Domains

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Abstract

Though commonly conceived of as "fluid mosaics" cell plasma membranes are in fact patchy on many size and time scales. These patches, or domains, are detected by many different experimental techniques. They are as likely to arise as the consequence of vesicle traffic and barriers to lateral diffusion as they are to arise as a consequence of specific lateral interactions between their constiutent molecules.

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Biol. Skr. Dan. Vid. Selsk. 1998, **49**:19–21

Background

Membrane domains broadly speaking are defined as lateral heterogeneities in the composition or state of a liposome or cell membrane. These heterogeneities are usually detected by measuring the behavior of some probe, for example the fluorescence of a lipid analog, mobility of an ESR probe, or the lateral diffusion of antibody-labeled membrane proteins. Evidence for membrane lipid domains has been recently reviewed (Tocanne et al., 1994; Edidin, 1997), as well as evidence for membrane proteins in domains (Edidin, 1996). All these reviews make it clear that the definition of a domain is operational, depending on the time and spatial scales of the techniques used to probe membranes. Indeed, the resolution of the technique used to detect lateral heterogeneities may give a false impression of the size of the domains detected. When discussing membrane domains we need to make sure that all involved are describing domains on the same scale.

Different views of membrane domains also arise from differences in the membranes studied. From the biologist's point of view liposomes are static membranes with well-defined compositions, and constant physical properties; cell plasma membranes are complex mixtures which in many (though not all) cells change rapidly as membrane vesicles arrive at, or leave, the cell surface. Most work on domains in liposomes emphasizes lateral heterogeneity of lipid distribution, lipid domains. Much of the work on cell plasma membranes emphasizes lateral heterogeneity of protein distribution, protein-enriched membrane domains. Again, the sizes of domains and mechanisms of their for-

mation may be quite different in the two types of membrane. The energetics of lipid/lipid interactions may dominate lipid domain formation in liposomes, while the organization of the membrane skeleton seems to be most important for domain formation in cell plasma membranes. Indeed, I have recently argued that lipid domains in the cell surface and endomembranes of actively metabolizing cells are stabilized by proteins (Edidin, 1997).

Experiments

Most of our experiments measure lateral diffusion in order to detect lateral heterogeneities in the membranes. The diffusion measurement is made by labeling the membrane of interest with a fluorescent tag, then bleaching a small spot, $\sim 1\ \mu m^2$, in the fluorescence and following recovery of fluorescence after photobleaching, FRAP. A lateral diffusion coefficient, D, is estimated from the halftime for recovery of fluorescence, and the fraction of mobile label, R, is estimated from the extent of recovery. We expect that the halftime of recovery will depend upon the area bleached, but R is expected to be independent of this area, for areas << than that of the entire labeled surface. Instead, we find that both D and R depend upon on the area bleached. R falls as this area increases over a 50-fold range and D increases. This is the case for both membrane proteins labeled with antibody fragments, and for a lipid analog NBD-PC, but not for another lipid analog, diI (Yechiel and Edidin, 1987). It is likely that the NBD lipid, whose rather polar fluorophore is on an acyl chain, associates with membrane proteins, while the diI lipid analog, whose hydrocarbon chains are unmodified, more directly reports on lipid organization in the membrane.

We interpret the results for R to indicate the confinement of proteins and lipids to domains which are often smaller than the area bleached in our experiment. The increase in D with increasing spot size is probably due to the fact that we are sampling different populations of labeled molecules with large bleaching spots than with small. Rapidly diffusing molecules may not be detected with small spots since the half-time for their recovery is not resolved. Increasing the spot size increases the half-time for recovery of fluorescence and so resolves these species, while slowly diffusing molecules do not contribute to the recovery and instead contribute to the immobile fraction. We have modeled this effect in 3D using mixtures of free fluorescein (mw $\sim$ 300) and fluorescein IgM (mw $\sim$ 900,000) in glycerol. D for the mixture depends on the size of the bleaching spot used in the FRAP measurement, and on the proportions of low mw and high mw molecules in the sample.

This raises the possibility that the domains inferred from the FRAP measurements are artifacts of the resolution of the method; low mobile fractions would simply be due to small D. However, low D in turn suggests that the diffusing species is interacting with some other molecules on the surface and we are led back to the idea that transient lateral associations or confinements contribute to the observed D and R in the FRAP experiment. Indeed, measurements of lateral confinement of membrane proteins, using a laser trap to drag small groups of labeled molecules across the cell surface, define the spatial frequency of obstacles to unhindered lateral mobility (Edidin et al., 1991). These obstacles occur on about the same scale as we use for FRAP measurements and appear to be located in the cytoplasm (the membrane skeleton) rather than in the membrane bilayer or extracellular domain (Edidin et al., 1994).

As just noted, the results from laser trap experiments speak to the statistics of barriers to lateral mobility. They imply, but do not define, the scale of lateral heterogeneities. We have used near-field scanning optical microscopy to image these heterogeneities, resolving membrane organization on a scale of 10's to 100's of nm. At this stage most of our images are of fixed and dried cell membranes,

but the patchiness of fluorescence seen in the images is of the same scale as we found in all of our other experiments. Recently we have imaged proteins in wet cell membranes and these also appear to be distributed in patches.

Even the heterogeneities that we see in cell membranes must be transient. We know from a number of studies tracking Brownian motion of membrane proteins (labeled with nm-size gold beads) that the barriers to lateral mobility are fluctuating (reviewed by Saxton and Jacobson, 1997). If this is so, then in a static membrane, neither budding, nor receiving membrane vesicles, diffusion should randomize the distribution of membrane proteins. However, continued membrane traffic, for example endocytosis of surface membrane and fusion of transport vesicles with this membrane, will constantly create new lateral inhomogeneities, domains, and disrupt existing domains. Indeed, this traffic must be an important factor in disrupting lipid domains which have segregated due to weak interactions between lipid molecules. It seems significant to me that the only good examples of lipid domains detected by FRAP or other methods are in gametes, sperm and eggs, cells whose surface membranes are quiescent for relatively long times (Wolf, 1992).

Of course, our model of membrane domains as transients created by vesicle fusion or vesicle budding begs the question, where in biosynthesis and transport of membranes to the surface are local concentrations of proteins and lipids created? We know very little about the lateral organization of endomembranes, the Golgi complex and the endoplasmic reticulum. However, recent work on the lateral diffusion of endogenous proteins of the Golgi complex (genetically labeled with the green fluorescent protein, GFP) suggests that there are no barriers to lateral diffusion in Golgi membranes (Cole et al., 1996). This observation limits models for selective retention of proteins in the Golgi complex, as well as limiting mechanisms for segregation of proteins into transport vesicles budding from the complex.

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