Active Flows Cluster Cell Surface Proteins

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The plasma membrane of cells is a dynamic mixture of different lipids, proteins, and sugars. In a recent issue of *Cell*, Gowrishankar et al. (2012) propose a model for how the actin cortex may generate and regulate lateral heterogeneity in the plasma membrane by actively clustering cell surface molecules.

Lipid bilayers define compartment and cell boundaries fundamental to life. The plasma membrane is a laterally structured (Kusumi et al., 2005) lipid bilayer that mediates the interactions of the cell with the environment. The lateral heterogeneity of the plasma membrane can be maintained by anchoring membrane proteins to the cytoskeleton or by transient stabilization of local fluctuations in lipid composition in microdomains called "lipid rafts" (Lingwood and Simons, 2010). Outer leaflet components like GPI-anchored proteins can cluster in an actin- and cholesterol-dependent manner (Goswami et al., 2008) and may exhibit periods of actomyosin-dependent directed motion (Burckhardt et al., 2011). The formation of microdomains relies on plasma membrane components dynamically segregating into two phases, probably in concert with the cytoskeleton. While a role of actin in membrane organization is generally accepted, such nanoscale, dynamic processes are difficult to observe, and direct experimental evidence is scarce. Reporting in a recent issue of *Cell*, Gowrishankar et al. (2012) now present a model to explain actomyosin-dependent clustering of cell surface proteins, and they experimentally test the key predictions of their model.

Models for actin-membrane interactions have previously been proposed, but precisely how cells control and organize the clustering of plasma membrane molecules is not understood. One proposal to explain clustering of GPI-anchored proteins based on equilibrium thermodynamics alone is that these proteins cluster in rafts formed by critical lipid-composition fluctuations concentrated along cortical actin filaments (Machta et al., 2011). Other equilibrium models include scaffolding, diffusion barriers, and picket fences in the plasma membrane (Kusumi et al., 2005). None of these equilibrium models, however, explain the observed involvement of active actomyosin in the clustering of GPI-anchored proteins (Goswami et al., 2008).

Gowrishankar et al. (2012) use fluorescence resonance energy transfer (FRET) measurements and live-cell microscopy to study GPI-anchored proteins, specifically folate receptor and GPI-anchored GFP, which form clusters even at low concentration. They find that the clustering fraction is independent of protein concentration, suggesting an active interaction. Consistent with this idea, clustering is sensitive to perturbations of cholesterol, actin, and—importantly—myosin. Moreover, the existence of clusters depends on the presence of an active cortical actin meshwork. Clusters constantly break and form with fast aggregation-fragmentation kinetics, but their net amount remains constant, indicating a nonequilibrium steady state that requires energy input to persist. These results call for an "active" model of the plasma membrane and the actin cortex, in which mechanical forces are generated within the actin-membrane composite in an energy-dependent manner.

Gowrishankar et al. (2012) formulate a hypothesis to account for these ideas. They suggest that short actin filament "snippets" of about 250 nm in length may be dispersed among the long, stable filaments of the cortical actin meshwork and crosslinked to them by myosin motors (see Figure 1). In this hypothesis, the myosin-dependent active motion of the snippets causes them to transiently arrange in asters in a process that is counteracted by thermal diffusion. Any transmembrane molecule attached to the moving snippets induces flows in the membrane. These flows "drag along" GPI-anchored proteins in the outer leaflet by interactions involving cholesterol, sphingolipids, and PIPs, concentrating them in small, raft-like domains at the aster centers ("L_O" in Figure 1).

Would such a mechanism explain the experimental observations? Gowrishankar et al. (2012) address this question by formulating a predictive biophysical model to generate falsifiable predictions. A key prediction of this model is that molecules subject to these active flows should experience anomalously large density fluctuations that can be described by a mathematical power law with exponent 4/5. This is much larger than the exponent 1/2 expected in an equilibrium system. Gowrishankar et al. (2012) confirm this prediction using fluorescence intensity fluctuation measurements, showing an exponent of 0.8. Moreover, they experimentally verify that the dynamics of clustering is sensitive to myosin perturbations and relaxes to equilibrium dynamics when actin and myosin are inhibited or when the putative transbilayer coupling agent cholesterol is sequestered. In a telling experiment, the authors induce clustering in inert transmembrane proteins by incorporating an actin-binding domain into their cytosolic tail. The authors also provide direct evidence for the existence of short, highly mobile actin snippets using fluorescence correlation spectroscopy (FCS) and single-molecule tracking experiments.

The model is intriguing for several reasons: (1) it suggests a means for the cell to actively regulate membrane organization and receptor clustering, because

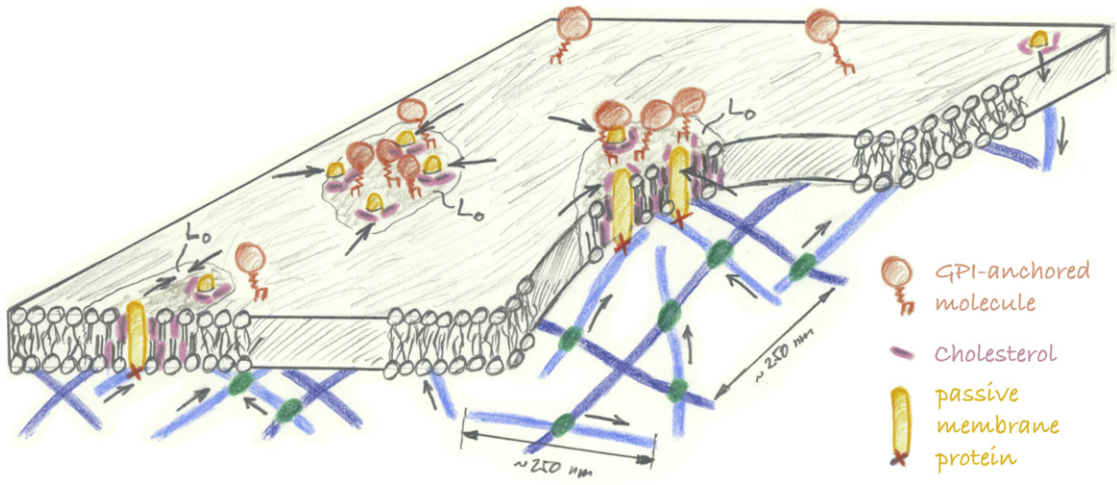

Figure 1. Sketch of the Proposed Mechanism
Short actin filaments (light blue) are dispersed among the cortical actin meshwork (dark blue) and crosslinked by myosin motors (green). They move as driven by the motors and drag along passive transmembrane proteins (orange) with a cytosolic actin-binding domain (red crosses). This movement induces flows in the membrane (arrows), dragging along GPI-anchored proteins in the outer leaflet (red) in a cholesterol (purple)-dependent manner.

myosin motors depend on ATP, and (2) production of the actin snippets may be subject to local regulation. The model is only as complicated as needed to explain the data, and it incorporates the main hallmarks of biological systems: nonequilibrium, nonlinearity, and coupling.

Nevertheless, an important issue pertains to the mobility of the clusters. While the model of Gowrishankar et al. (2012) predicts immobile clusters, experiments using single-molecule tracking have been able to detect only mobile clusters (Brameshuber et al., 2010). Small (<20 nm) low-mobility clusters have been observed by FCS and stimulated emission depletion microscopy (Eggeling et al., 2009). It will be insightful to reconcile these results.

Like any good study, the work of Gowrishankar et al. (2012) raises several new questions. It should be interesting, for example, to test whether their model also holds for other cluster-forming outer-leaflet molecules, such as gangliosides. In addition, the work of Gowrishankar et al. (2012) does not elucidate the molecular properties a GPI anchor has to have in order for the proposed mechanism to work. The study also does not explain how clustering varies spatially, something that has been observed earlier (Goswami et al., 2008) and could, in principle, be captured by the present biophysical model. Certainly, the properties of the cortical actin near the leading edge of migrating cells differ from those in other areas. Is there a resulting net flow of clustered GPI-anchored molecules in the plasma membrane of migrating cells? Finally, how does the local hydrodynamic mixing described here tie in with vesicles constantly bringing in and taking out material, hence globally mixing the plasma membrane?

On a functional level, one could ask whether the model by Gowrishankar et al. (2012) has implications for intracellular membrane transport, what it entails for regulatory receptor clustering in signaling pathways (Plowman et al., 2005), and what alternative mechanisms may exist in *C. elegans*, which lacks sterols in the plasma membrane. It would also be instructive to investigate how the clustering kinetics of membrane molecules relates to the size of the lipid domains. The size of the areas within which molecules can freely diffuse alters the aggregation kinetics and can lead to inversions of the steady state (Ramaswamy et al., 2012).

Taken together, the study by Gowrishankar et al. (2012) presents a testable, quantitative model that integrates actin cortex dynamics with membrane organization. The work adds actomyosin-driven flows to the list of plasma membrane organizing processes and advocates a concept of active membrane rafts.

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