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Active organization of membrane constituents in living cells

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A search for organizing principles underlying molecular patterning at the cell surface and its regulation over different scales is necessary. This is important for understanding how the cell builds membrane bound organelles that emanate from it and for how the cell interacts with its physical and chemical milieu. This requires a broad framework to rationalize the mass of accumulated data about the spatial localization and dynamics of its constituents, and their physical and chemical environment. Lateral heterogeneities in the organization of membrane components of a living cell appear to be a hallmark of how a cell addresses sorting and signaling functions. Here we explore two classes of mechanisms of segregation of membrane components in the plasma membrane. We suggest that viewing the membrane as a passive, thermally equilibrated system is unlikely to provide an adequate framework to understand the mechanisms of membrane component segregation *in vivo*. Instead the surface of living cells behaves as an active membrane composite.

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Introduction

The main characteristic of animal cells appears to be a reproducible organization of molecular constituents at multiple scales. The surface of any living cell, either prokaryotic or eukaryotic, is a complex assembly of a variety of molecular components, an interface that delineates the outside from the inside, and serves to control the exchange of chemicals and information across it, and of course all endocytic processes emanate from it. To fulfill these fundamental tasks, the cell surface has to be both robust and pliable to specific perturbations.

It is no exaggeration to say that researchers have studied the cell membrane for over a century [1•], and have provided valuable information regarding the molecular composition of this cellular compartment. Over recent years many researchers have focused their attention on the structural and functional organization of this multicomponent assembly in an attempt to understand the complex distribution and dynamics of its main constituents, proteins and lipids.

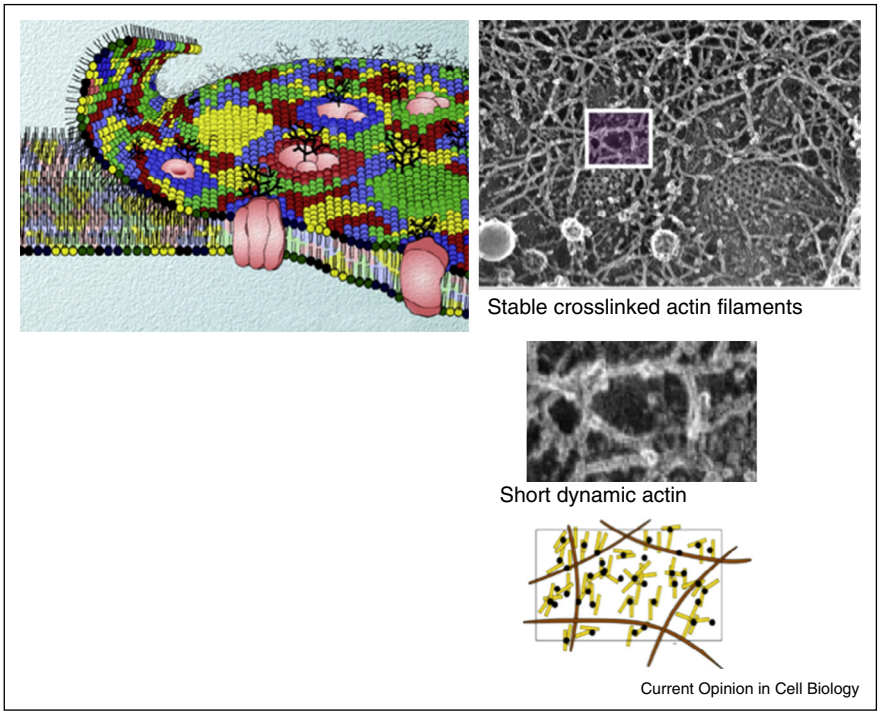
Even before we address how the cell builds its variety of cellular organelles (the subject of this volume), we must focus on the organization of the components of this cell membrane. Beginning at the exterior shell, the outer leaflet of the cell membrane is complex and comprises specific lipids and embedded proteins, that are coupled to the inner leaflet with its own set of distinct lipids and proteins, with transmembrane proteins straddling the two leaflets. In most instances, the cell membrane is supported by a complex layer of the cytoskeletal meshwork attached to the plasma membrane via anchoring proteins. These layers are structurally and dynamically coupled to one another at different spatio-temporal scales in ways that we are just beginning to understand [2] (Figure 1). Here we review current understanding of how organization at the molecular and mesoscopic scale may be understood in a combination of passive and active mechanisms of molecular interactions.

Some of the ideas that we propose here are likely to be applicable to how the cell can effect numerous endocytic processes at the cell surface [3] or mold membranes of organelles inside the cell.

Models which regard the cell membrane as a closed equilibrium system

The two models of the cell membrane that have dominated our thinking, one the fluid mosaic model and the other the raft model, both start out by assuming that the cell surface is a multicomponent equilibrium thermodynamic system. There are variants of these models which include proteins which engage in specific protein–protein (e.g. proteins that engage in multivalent interactions) or protein–lipid interactions (e.g. lipids that form a wetting layer surrounding specific proteins).

Figure 1



Cartoon depicting membrane bilayer exhibits a patchwork mosaic of the distribution of lipids in the cell membrane and captures the lateral heterogeneity of the organization of membrane components in live cells (reproduced with permission from ref. [1•]). This bilayer is anchored to the cortical actin meshwork as visualized by rapid-freeze deep etch tomographic renderings of the cortical surface closest to the membrane (Image reproduced from ref. [51]). A model for understanding the active organization of membrane components shows short actin filaments (yellow lines) within and between the actin meshwork (magnified view of a single mesh element, orange lines) and the membrane bilayer. The short actin filaments are proposed to interact with motors (black circles connecting the short filaments)

The 'fluid-mosaic' model

The 'fluid-mosaic' model proposed by Singer and Nicholson over 40 years ago [4••] represents a milestone in synthesizing a variety of information about the cell membrane, consequently arriving at an understanding of how molecular constituents may be arranged in the cell membrane, regarded as a two dimensional equilibrated fluid. Fundamentally, the fluid mosaic model posits that the multicomponent cell membrane is in a mixed, homogeneous phase. Within this model, the multi-component lipid nature of the plasma membrane facilitates the 'solvation' of a variety of membrane proteins, via specific interactions such as hydrophobic shielding, electrostatics, hydrogen bonding or Van der Waals.

Since the fluid-mosaic proposal, numerous studies have examined the architecture of the plasma membrane at different length and time scales, and have provided a slightly more complex picture of cell surface organization [1•,5]. The membrane appears to be partitioned into assemblies of specific components with defined protein compositions over long and short spatial and temporal scales. For example the cell builds uniquely differentiated regions of its membrane for specific functional purposes; focal adhesions [6], T-cell synapses [7,8], endocytosis [9,10], and many other functional structures such as the cilia and microvilli. Here a hierarchical process of assembly of protein complexes has been used to explain the construction of these huge macromolecular membrane systems [11].

The 'raft' model

In many instances, however, differentiated regions of the cell surface are not simply explained by the building up of hierarchical assemblies of protein complexes; the axonal hillock [12], the lamellipodium and other protrusive structures, filopodia and blebs of a migrating cell [13], or the generation of distinct cellular domains, apical and basolateral with distinct composition in polarized epithelia [14] demand a different type of explanation.

In the multi-component chemical milieu of the cell membrane, it may appear reasonable to expect some level of compositional heterogeneity either as a result of macroscopic phase segregation, or transient, short-scale, heterogeneities induced by thermal fluctuations in the mixed state (especially when close to a phase boundary). Fluctuations of lipid compositions are bound to arise due to the propensity of lipids to exhibit different miscibility properties [5,15].

The 'raft' hypothesis posits that the multicomponent cell membrane is in a heterogeneous phase [16••]. This hypothesis postulates that lipids such as cholesterol and sphingolipids, spontaneously associate with each other to form platforms for the segregation of proteins such as GPI-anchored proteins or others that may partition into the these phases. These segregated domains are then expected to have a role in membrane protein sorting and the construction of signaling complexes [17,18]. The basis for this partitioning is derived from theoretical and experimental studies of liquid–liquid phase coexistence in artificial membranes, where membranes exhibit liquid disordered (ld) and liquid-ordered (lo) phases, and has been addressed in several reviews [15]. Phase segregation depends on temperature, relative composition of lipids in both symmetric and the asymmetric bilayers, and membrane tension. Specifically, in artificial membranes consisting of equimolar amounts of cholesterol, sphingolipid and phosphatidylcholine, the ld phase correlates with a cholesterol-poor composition and a lo phase is enriched in sphingolipids and cholesterol [19]. This has led to the idea that the cell membrane is similarly partitioned [5,20]. In artificial membranes the lo phase is insoluble in non-ionic detergents at low temperatures, suggesting that 'rafts' are detergent resistant. This has spawned an enormous but misguided literature on this subject [21,22], bringing into question the whole premise of the role of phase segregation in cell membranes [23].

Condensed complexes and lipid shell model:

A modification of the 'raft' hypothesis is that the raft domain is a liquid rich in condensed complexes; a chemical complex of cholesterol and sphingolipids formed in the reversible reaction $pC + qS \leftrightarrow (CS)$ [24]. Even in the absence of macroscopic phase segregation, equilibrium thermal fluctuations in the mixed phase of a multi-component system may give rise to transient, small scale lo domains or condensed complexes whose life-time could be enhanced by proximity to a phase boundary [25]. This could also help in the solvation of proteins embedded in the membrane, especially if these molecules are surrounded by lipid shells due to their potential to interact specifically with certain classes of lipids. [26]. Manipulating the distribution of these proteins could create specific lipidic environments by the phenomenon known as 'wetting'. This produces a thin 'wetting layer' surrounding the proteins or protein complexes, which may be brought about by nonspecific physical interactions (van der Wal or packing forces) or specific chemical interactions. At high enough protein concentration, these wetting layers can *percolate* to generate lipid segregation at large scales.

Both these equilibrium pictures have promoted the artificial multi-component membrane as a good model system to describe the physical properties of the cell surface.

New quantitative experiments reveal issues with simple equilibrium models

However, a growing body of evidence shows that there are many constituents of the membrane, such as lipid-linked proteins and glycolipids, where the steady state organization is not in chemical equilibrium. For example, lipid tethered proteins at the inner leaflet (isoforms of Ras; [27]), and at the outer leaflet (GPI-anchored proteins; [28]), form a nanoclustered organization but not the large scale segregation into lo-like phases, expected from the conventional raft model. This does not mean that large scale phase segregated regions of specific lipid composition never arise in cell membranes; in fact when the cell membrane is detached from its underlying cytoskeletal cortex, the membrane segregates into lo and ld like phases at 20 °C but not at the physiological temperature of 37 °C [29].

The formation of nanoclusters of GPI-anchored proteins cannot be described by an equilibrium chemical clustering mechanism [30], despite the clusters forming and breaking up and exchanging with a large monomer pool [30,31••]. The spatial distribution of these clusters is also quite unique, showing a high population of regions of extremely high (low) enrichment [31••]; GPI-anchored protein clusters form as actin dependent 'hotspots' [32•] which are dynamic. For the GPI-anchored proteins, myosin activity and the presence of a dynamic cortical actin meshwork is necessary for its formation [31••] and dynamics and is regulated by inputs from signaling receptors such as the integrin receptor [32•]. The organization of Ras proteins is also dependent on the exact configuration of the actin cortex [33•], and the nucleotide status of the Ras GTPase [34••]. These observations have demanded a more intimate link between the fluid membrane bilayer and the actin cortex beneath to effect molecular organization.

The actin meshwork beneath the membrane bilayer is connected at a number of anchoring points [2]. The nature of the meshwork consequently has a major influence on the mobility of many membrane proteins and lipids, resulting in a kind of 'cage-hopping' [11], driven by thermal kicks. However, there are several exceptions to this. For example, the dynamics of oxidized LDL receptor, CD36 is spatially structured by active processes involving the cytoskeleton [35], and quantitative measurements of local density distribution and fluctuations of GPI-anchored proteins reveal striking anomalies inconsistent with the behavior of Brownian particles subject to thermal fluctuations [36••]. These anomalies are a clear consequence of the coupling of GPI-anchored proteins to the dynamic cortical actin driven by myosin activity [31••]. Simple equilibrium based models are inadequate in providing an explanation for these anomalous behaviors; a new model of the membrane is indicated.

There have been many attempts at reconciling equilibrium-based models with specific observations described above. One approach has been to provide an explanation for the small size of segregating domains, by invoking physical mechanisms that prevent the growth of domains. For instance, the growth of domains could be halted, and small domains stabilized, by having specific lipids or proteins which find it energetically favorable to sit at the domain boundary, thus reducing interfacial tension. Examples of such lipids are those which have one long saturated chain and one unsaturated chain [37]. Other possible explanations, invoke coupling between membrane tension, curvature and composition, which can also provide a length scale beyond which domains cannot grow. At a phenomenological level, such possibilities could arise from pulling forces [38], possibly from (de)polymerizing cortical actin. Alternatively, curvature generating proteins such as BAR domain proteins [39], could induce local membrane curvature over a short scale, and in the process could recruit specific lipids and proteins to these regions due to spontaneous curvature [40].

The other major approach has been to posit that the cell membrane is above but poised close to a critical point associated with the equilibrium lo–ld phase transition [41••]. This would allow for a broad distribution of domain sizes, and long-lived lo-domain fluctuations over a small spatial scale, as well as anomalous density fluctuations described above. Proximity to a critical point also ensures sensitive response to a slight perturbation which could drive the system across the phase boundary, inducing large scale segregation of specific lipid components. For example, depletion of cholesterol in living cells gave rise to large scale segregation of probes preferring the ld phase [42] and coupling with cortical actin could provide a field (conjugate to the lo-composition) which holds the membrane away from the critical point and suppresses phase segregation [43].

A major criticism of these 'close-to-criticality' models is that at physiological temperatures, the cell membrane is well above the lo–ld phase boundary, and thus suggestions that coupling to cortical actin suppresses phase segregation, simply do not work. Indeed, in artificially reconstituted multicomponent membrane systems attached to an actin shell, the coupling of actin with membrane composition drives phase segregation of membrane components [44]. Moreover, as detailed above, anomalous density fluctuations are a consequence of cortical actomyosin dynamics and are not suppressed by it. Proximity to a critical point requires a degree of fine tuning that is hard to engineer. For instance, since membrane tension is a relevant parameter affecting the lo–ld phase transition, one needs to invoke a homeostatic mechanism wherein the system adjusts itself to always be close to the critical point when it is subject to changes in tension. Alternatively, if the cell membrane is not in the vicinity of a phase transition, then one needs to provide mechanisms whereby the effects of the critical point are felt far from the transition.

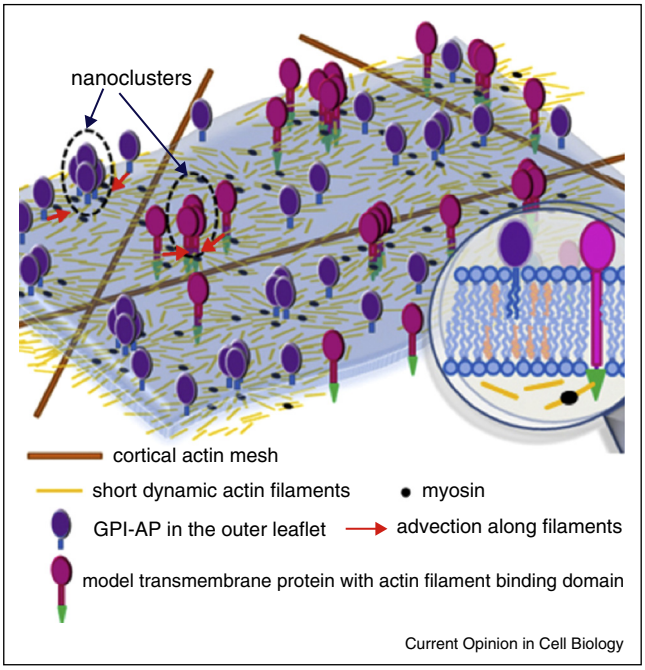
Cell surface as an open system driven out of equilibrium

A radically new approach to the physico-chemical nature of the cell surface has been to suggest that the cell surface composition is maintained out-of-equilibrium by active, energy-dissipating processes arising from an interaction with dynamic cortical actin and myosin. This has lead to the *Active Composite Model* of the cell surface [36••]. The starting point of the active composite model is the composite cell surface–a juxtaposition of a multicomponent membrane bilayer with a multicomponent cortical actin layer. Actin filaments at the cortex have a distribution of lengths, the longer ones form the static actin mesh, while the shorter ones are dynamic and transiently attach to the cell membrane via linker proteins. Both the actin mesh and fluid interact with components of the membrane. This gives rise to active stresses and currents which can affect both local membrane composition and shape [36••]. The active composite model differs fundamentally from the equilibrium-based models, in recognizing that the cortical layer of actin and myosin can drive the cell membrane out-of-equilibrium by the consumption of energy.

The active composite model suggests a major shift in perspective in terms of *chemical processes*, since it gives equal importance to inter-molecular interactions as it does to the interaction of cell membrane molecules with the cortical actin. Thus the localization, movement and environment of cell membrane molecules is dictated not only by their interactions with each other, but their engagement with cortical actin. This immediately leads to a broad classification of all cell surface molecules as *inert*, *passive* and *active*, based on their coupling to this active cytoskeleton. Inert molecules, such as short-chain lipids do not interact with cortical actin, while passive (e.g. GPI-APs) and active molecules interact with actin at the cortex. In addition, active molecules (e.g. Integrin receptors) can influence cortical actin in a variety of ways such as nucleation, (de)polymerization, cross-linking etc.

The active composite cell surface model predicts that passive molecules can be transiently clustered in localized and dynamically remodeling platforms created by actomyosin contractility. These platforms, called asters, are a result of the active hydrodynamics of the short filaments driven by treadmilling and motor elements (Figure 2). By engaging with the dynamic cortical actomyosin, nanoclustering is a consequence of active advection of membrane components, and not protein–protein interactions. The dynamics and fluctuations of such an active clustering accounts for all the observations on anomalous density distribution and fluctuations of GPI-anchored proteins. It successfully predicts that transmembrane proteins with

Figure 2



Short filaments organize into aster like configurations which template the organization of membrane proteins that have actin-binding capacity, and lipid anchored components that couple across the bilayer, connecting to the actin filaments at the inner leaflet. The consequences of this active actin engagement is key for the active composite model of the cell surface.
Reproduced with permission from Ref. [36••].

actin-binding motifs should also exhibit an actomyosin dependent dynamic nanoclustering [36••].

While the detailed chemistry behind transbilayer coupling of GPI-anchored proteins and the cortical actin filaments requires further elucidation, it is likely to involve long-acyl chain containing lipids which couple across the bilayer in the presence of cholesterol. Thus dynamic actin filament driven clustering of GPI-anchored proteins could create lo-like domains, thereby creating specialized regulatable platforms of distinct chemical composition. These dynamic asters could also provide a mechanism to spatio-temporally regulate the reactions of membrane constituents that are coupled to these structures [45].

Membrane trafficking is another agency of nonequilibrium behavior, which can curtail the size of domains undergoing aggregation-fragmentation kinetics. While the aggregation-fragmentation could be passive or active, the endocytic and recycling processes are active [46]. There is recent indication that this mechanism might be at work in maintaining the cluster size distribution of E-cadherins in cell–cell junctions in epithelial tissues [47].

Given the significance of active control of compositional heterogeneity on the surface of living cells, the choice of *in vitro* model systems to explore the influence of a dynamic actin-myosin cortex on a multicomponent membrane, requires taking a different strategy, and studies along these lines are currently underway.

Conclusion and perspectives

The living cell surface is an organized state of matter, highly regulated in space and time, where molecules are localized, clustered, transported and transformed. This regulation needs energy, usually available in the form of ATP. Indeed one of the most satisfying features of the active composite model is the natural manner in which it accounts for cellular regulation, by the local engagement of the cortical actin machinery. The underlying mechanics of the active composite model, also leads to a classification of molecules as inert, passive and active. In addition, the model brings forth an emergent property, namely localized focusing platforms, which can draw in other molecules and transiently focus them within its core. This sets the stage for asking how chemical transformation of molecules and the processing of chemical information might be affected by this underlying active mechanics. The principles underlying the active composite cell surface model could be relevant to other internal membranal contexts such as in endosomal membranes or the trans Golgi network (TGN). There is some indication of compositional segregation in both endosomes and the TGN involving cholesterol enriched patches and actin dynamics [48–50], and must be the subject of future investigation.

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