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Compartmentalization of the plasma membrane

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The compartmentalization of the plasma membrane is essential for cells to perform specialized biochemical functions, in particular those responsible for intracellular and intercellular signaling pathways. Study of membrane compartmentalization requires state-of-the-art imaging tools that can reveal dynamics of individual molecules with high spatial and temporal resolution. In addition, quantitative analyses are employed to identify transient changes in molecule dynamics. In this review, membrane compartments are classified as stable domains, transient compartments, or nanodomains where proteins aggregate. Interestingly, in most cases, the cortical cytoskeleton plays important roles. Recent studies of the membrane–cytoskeleton interface are providing new insights about membrane organization involving a scale-free self-similar fractal structure and cytoskeleton active processes coupled to membrane dynamics.

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The organization of the plasma membrane is essential for many physiological processes. Importantly, the cell surface is organized into specific domains that are responsible for diverse functions such as cell-to-cell communication, initiation of intracellular signals, and transmission of electrical impulses. Furthermore, by concentrating membrane components into nanodomains, it is possible to surpass density thresholds needed for cooperative activity and, conversely, by segregating membrane components, cells can hinder reactions unless triggered by a specific signal. As a consequence, the compartmentalization of the cell membrane allows for highly specific biochemical functions. This review focuses on our current understanding of the mechanisms governing the compartmentalization of the plasma membrane and the tools employed to study the segregation of cell surface components.

Advances in imaging techniques and tools to identify compartmentalization

The last decade has witnessed tremendous progress in our understanding of the compartmentalization of the plasma membrane. This progress was mostly enabled by advances in imaging tools that allowed the observation of structures and dynamics within cells with unprecedented detail. In this section, I survey a non-exhaustive list of the tools employed in the study of membrane compartmentalization with the goal of placing these studies in perspective. The key techniques used to unravel the plasma membrane compartmentalization are single-particle tracking [1–3], fluorescence correlation spectroscopy (FCS [4–6]), and super-resolution imaging [7,8]. Single-particle tracking has long been a valuable tool to extract information on individual molecule dynamics with nanometer precision, where the molecule of interest is labeled with either gold nanoparticles [9], quantum dots (which enable particularly large signal-to-noise ratios) [10,11], fluorescent dyes, or fluorescent proteins. Complementary approaches include imaging the underlying cytoskeleton, which can introduce barriers to the motion of membrane molecules. These measurements are performed via electron microscopy [12,13] or, more recently, via super-resolution imaging [14–16]. Further, as imaging tools continue to progress, super-resolution imaging is pushing previous boundaries and is allowing investigations within living cells, providing experimental data with minimal processing of the cell [17]. An imaging frontier lies in the simultaneous combination of different techniques where, for example, super-resolution imaging in live cells can visualize underlying structures while single-particle tracking provides dynamic information of membrane molecules [18••].

One of the challenges to study membrane compartmentalization lies in the identification of changes in the diffusive behavior that can enable the reliable recognition of clustering and transient confinement within single-particle trajectories. Therefore, considerable efforts have been committed to these analyses. A few notable approaches used in the identification of diffusion changes include (i) system-level maximum-likelihood methods [19,20], (ii) classification parameters such as anomalous exponent [21] and convex hull [22] obtained using a temporal sliding window, (iii) recurrence analysis [23], (iv) fluctuation analysis of the time-averaged mean square displacement (MSD) [24], and (v) hidden Markov models [25,26]. In addition, variations of single-particle tracking

and super-resolution tools have been employed to probe diffusivity landscapes with high lateral spatial resolution [27–31].

Stable membrane domains

Stable membrane domains are typical in polarized cells where different cell regions are characterized by distinct functions. Examples of two such cells are spermatozoa and neurons. The former cells are comprised of a head and a flagellum, with the flagellum being divided into midpiece, principal piece and end piece (Figure 1a). The plasma membrane in each of these compartments has its own set of protein and lipid components. The membrane of the head is also subdivided into three compartments, known as anterior acrosome, equatorial segment, and postacrosome. Many proteins have been found to be sequestered to specific domains but the mechanisms by which membrane components are segregated remain elusive. The most appealing candidate mechanisms that govern segregation are physical barriers to diffusion and scaffolds that interact with specific proteins. Note that in spermatozoa, active protein trafficking is silent and the cells are devoid of organelles involved in protein delivery and synthesis, thus protein domains cannot be maintained by active delivery and retrieval at the membrane. Experimental evidence points to the existence of diffusion barriers. In particular, the principal piece and the midpiece are separated by the annulus, a ring-shaped septin cytoskeletal structure. Septins are GTP-binding proteins that form hetero-oligomeric complexes, filaments, bundles, and rings. Septin ring structures also appear to form membrane diffusion barriers in other eukaryotic cells. The most studied and well understood of these structures takes place in budding yeast where a barrier between mother cell and bud is formed at the neck (Figure 1b). In addition to yeast, septin rings are thought to be involved as diffusion barriers in at the base of primary cilia in mammalian cells (Figure 1c) and at the base of dendritic spines in neurons. In dendritic spines, cilia, and sperm flagella, it is becoming apparent that the restriction of membrane protein motion by septin cytoskeletal structures has important physiological roles. Mice null for septin SEPT4 lack the sperm annulus, the sperm membrane does not compartmentalize, and these mice are infertile [32]. In neurons, septins are required in dendritic spine morphology and AMPA receptor compartmentalization, where abnormalities are associated with neuropsychiatric disorders [33,34]. Thus, the biochemical compartmentalization of dendritic spines is essential for synaptic function and likely regulates human behavior [35].

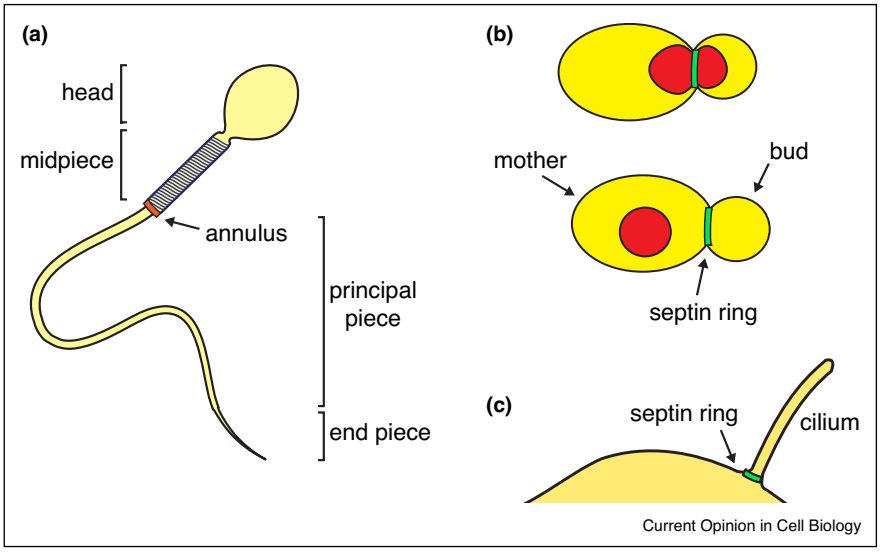
Another membrane domain in neurons, the axon initial segment (AIS), separates the neuron body (i.e., the soma) and the axon. These two compartments have very different cell functions, with the soma being responsible for the integration of electrical inputs and the axon transmitting information in the form of action potentials, that is, electrical impulses, to downstream neurons. The generation and propagation of these impulses is controlled by ion channels on the cell surface. So it is not surprising that the composition of the axonal membrane is completely different from the somatic membrane. Since the AIS is where action potentials are initiated [36], this region must maintain specialized membrane components. Three mechanisms act in concert to compartmentalize the soma and axon membranes, but substantial work yet needs to be done to properly understand them. On one level, a diffusion barrier exists at the AIS to block exchange between the soma and axon. Single-molecule tracking experiments suggest a barrier to the diffusion of both proteins and lipids is established in neurons at 7–10 days *in vitro* by the anchoring of a large number of membrane proteins to the cortical actin cytoskeleton [37]. A second level of membrane compartmentalization is provided by scaffolding proteins such as ankyrinG that have a high affinity for specific ion channels and can, thus, directly cluster molecules within the AIS, even before a diffusion barrier is formed [38,39]. At a third level, it has been recently shown that Nav1.6 sodium channel localization to the AIS is additionally achieved by direct vesicular delivery, where it is then immediately immobilized via interactions with ankyrinG [40•].

Actin-based compartmentalization

Many independent measurements have confirmed that the actin cytoskeleton modulates membrane dynamics and introduces barriers to the diffusion of membrane proteins (Figure 2a). Stimulated emission depletion (STED)-FCS measurements with a spatial resolution of 40 nm showed that the plasma membrane components in fibroblasts are transiently constrained to compartments with a mean compartment size of 80–150 nm, depending on cell type [6]. This compartmentalization was no longer observed upon treating the cells with CK-666, an inhibitor of the complex responsible for actin filament branching, Arp2/3 [41]. Pharmacological inhibition of actin polymerization with latrunculin B also disrupted this compartmentalization but in a milder way than CK-666 treatment. Similarly, several groups have reported that inhibition of actin polymerization with different drugs increased the mobility of membrane proteins and changed compartment sizes, as measured by single-molecule tracking [9,13,42–45].

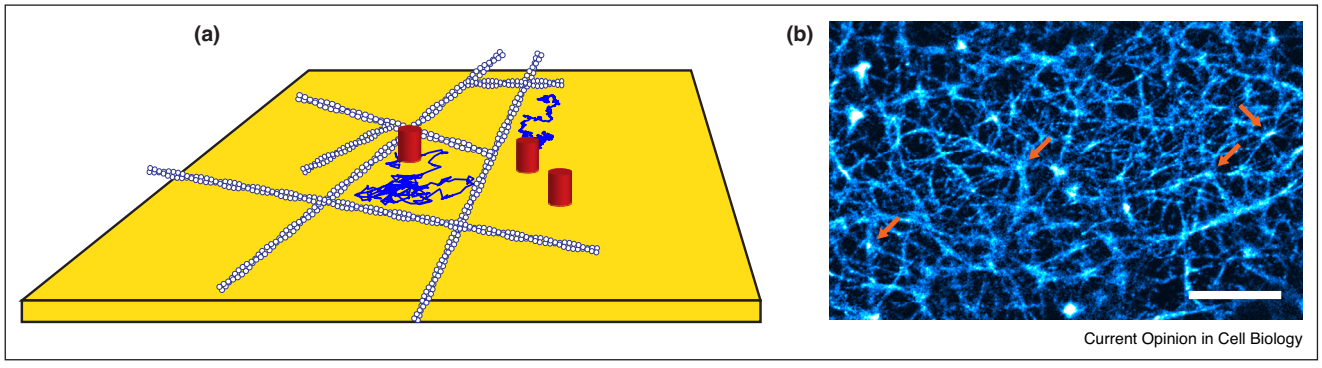
The visualization of the cortical actin cytoskeleton in live cells while simultaneously tracking membrane proteins has provided the most direct evidence for interactions of the actin cytoskeleton with membrane components. Quantum dot-tagged high-affinity IgE receptors were tracked while proximal actin bundles were imaged in live cells stably transfected with GFP-actin [42]. These measurements show that actin bundles create micrometer-sized compartments and that receptor motion remains limited to actin-poor regions. These actin-delimited

Figure 1



Membrane domains in cells. Often domains are delimited by a septin ring structure. **(a)** A septin ring separates the midpiece from the principal piece in the flagellum of sperm cells. **(b)** A septin ring forms in budding yeast at the neck between mother and bud. Two different phases in a dividing cell are shown. The lower sketch shows a mitotic cell at an earlier stage and the upper sketch shows a later stage in the cell cycle where the nucleus has migrated to the neck. **(c)** A septin ring at the base of a primary cilium.

Figure 2



Compartmentalization of the plasma membrane by the cortical actin. **(a)** Sketch of the actin cortex adjacent to the plasma membrane. Membrane molecules perform random walks in the membrane but their motion is transiently confined by the actin cytoskeleton. **(b)** Superresolution STORM image of the cortical actin in a HEK cell [18••]. The actin meshwork is clearly visible. Note the multiple aster-like structures, like the ones marked by orange arrows. Scale bar is 2 μm.

regions are highly dynamic and actin is observed to reorganize over time scales of 1–10 s. Using a toolset of particle tracking, nanocluster imaging via STED, and fluorescent imaging of the cortical actin, Garcia-Parajo and coworkers have recently reported striking effects on the regulation of CD1 nanoclusters in the membrane of antigen presenting cells by the actin cytoskeleton [46••], a mechanism employed by these cells to prevent autoimmunity. In these experiments, the actin cytoskeleton was found to segregate CD1 nanoclusters away from each other and, in turn, to limit their coalescence. As a consequence, upon pharmacological disruption of the actin cytoskeleton with cytochalasin D, more molecules became trapped into nanoclusters and, in turn, the mobility of CD1 decreased, in contrast to other compartmentalized membrane components. Control measurements with exactly the same pharmacological treatment showed an enhancement of CD71 mobility in THP-1 cells. A similar effect to that of CD1 nanoclusters upon disruption of the actin cytoskeleton was observed for Kv2.1 clusters

in transfected human embryonic kidney (HEK) cells [47]. In cells treated with latrunculin A, Kv2.1 clusters become more mobile and they coalesce into larger domains.

By imaging hemagglutinin and actin using two-color photo-activated localization microscopy (PALM), it was shown that hemagglutinin localize to actin-rich membrane regions. Further analysis of individual trajectories revealed that the mobility of hemagglutinin was negatively correlated to the cortical actin density [48]. A striking revelation by stochastic optical reconstruction microscopy (STORM) imaging was the fact that the axonal actin-spectrin cytoskeleton in neurons organizes in ring-shaped structures wrapping around the circumference of the axon, evenly spaced with a periodicity of 180–190 nm [14]. Recently, single-particle tracking of lipid-anchored molecules was analyzed in the AIS and the mobility was correlated to the periodic submembrane cytoskeleton [49••]. This elegant correlative microscopy was performed in several steps: first single-particle tracking was implemented by uPAINT (universal point accumulation for imaging in nanoscale topography) using labeled antibodies against specific GPI constructs in transfected neurons plated on gridded coverslips. Then the neurons were fixed and immunostained against βII-spectrin or actin. At this point STORM super-resolution images were obtained in the same cells. The single-particle tracking data were registered with the super-resolution cytoskeleton images using fiducials on the coverslip. These experiments showed that actin rings in the AIS behave as diffusion barriers to GPI-anchored molecules, thus confining molecules to equidistant stripes.

In a recent study, we employed simultaneous single-particle tracking of quantum dot-tagged ion channels and dynamic PALM super-resolution imaging of actin in live HEK cells to elucidate the interactions of these molecules with the cortical cytoskeleton [18••]. Further, the details of the cortical actin cytoskeleton structure were found with high resolution after cell fixation using STORM (Figure 2b). As expected, the actin cytoskeleton was observed to delineate dynamic compartments that transiently confined the motion of ion channels. However, the proteins exhibited scale-free dynamics with correlations that were independent of lag time. Scale-free dynamics were shown to be caused by an actin cytoskeleton with self-similar fractal topology. These observations have direct consequences on our interpretation of the membrane compartmentalization by the actin cytoskeleton. On one side, the measured averaged compartment size is not enough to characterize protein motion. For a narrow compartment size distribution, molecules would exhibit 'normal' Brownian dynamics both at times shorter than the time required to explore an individual domain (with a large diffusion coefficient) and at times longer than the characteristic time to hop between compartments (with an apparent reduced diffusion coefficient). However, a fractal cytoskeleton structure causes the protein motion to display subdiffusion behavior for very long times. On the other side, a fractal structure suggests a hierarchical membrane organization where molecules are confined over multiple time and length scales. These findings bridge small actin-delimited compartment sizes found for small residence times [5] and large compartment sizes characterized by anomalous diffusion at long times [42].

Active processes

Active cellular processes can play important roles in segregating membrane components. As discussed above, delivery and retrieval of membrane molecules, that is, exocytosis and endocytosis, at specific membrane locations can influence the formation of membrane domains [40•,50]. In addition, membrane clustering and local composition can be modulated by cytoskeleton-driven flows. The actin cortex is currently understood to form a dynamic network, composed of at least two filament subpopulations with different turnover rates, one population with free barbed ends and fast turnover dynamics and another population of longer and more static filaments associated to formins [51]. The existence of dynamic actin filaments in conjunction with myosin motor activity led to a theoretical framework for the appearance of tangential active stresses and membrane currents [52]. Such stresses and membrane currents affect local membrane composition and transiently cluster molecules into actin nodes. Experimental observations of GPI-anchored proteins have verified that the motion of membrane molecules depend on actomyosin activity [52,53]. Furthermore, actin nodes, also known as asters, have been shown to self-organize into nanoscale structures containing formins and actin crosslinkers [54]. A fundamental part of this framework is the coupling of the plasma membrane to a dynamic actin cytoskeleton via motifs that bind either actin filaments or actin-binding proteins. Molecules in the plasma membrane can then be actively driven to reaction sites via interactions either directly with actin or indirectly via actin-associated proteins.

Some of the most convincing evidence for the role of actomyosin dynamics in the organization of the membrane comes from *in vitro* measurements in reconstituted model membranes. By linking a minimal actin cortex to a model membrane, it was found that actin alters the organization of the membrane [55] and that actomyosin contraction mediates membrane compartmentalization [56•]. Of special relevance, by employing a minimal system of a supported lipid bilayer with actin-binding components, actin filaments, and myosin motors, Mayor and coworkers have provided experimental evidence for membrane clustering governed by actomyosin contractility in a fashion that strongly resembles clustering

observed in live cells [57••]. In particular, in this system, it was shown that contractile actomyosin flows advect membrane components and that this advection requires specific interactions between the membrane and F-actin. These experiments show that actomyosin contractility directly alters the segregation behavior of membrane domains.

Conclusions and outlook

The compartmentalization of the plasma membrane appears to be ubiquitous in cell biology. Interestingly, the mechanisms responsible for compartmentalization are much more complex than originally suspected. There are two different ways by which cells segregate membrane components: the formation of barriers that passively hinder free motion and the active delivery of membrane components to specific locations. These two mechanisms act jointly in order to adjust local membrane composition. The last few years have seen significant progress in deciphering the organization of the plasma membrane. Recent studies have demonstrated the feasibility of employing *Drosophila* and *C. elegans* embryos to study the effect of active actin processes on the plasma membrane compartmentalization [58,59]. However, a thorough understanding is still lacking and many questions remain unanswered. As imaging tools for live cell imaging continuous to progress toward higher spatial and temporal resolution and better theoretical biophysical models are developed, it is foreseen we will see the answer to many of these questions in the near future. Future studies may provide answers regarding the coupling of the membrane to the cytoskeleton and the roles of active cytoskeleton processes in membrane organization. Particularly, understanding the plasma membrane compartmentalization will enable a deeper understanding of intracellular and intercellular signaling pathways.

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Conflict of interest statement

Nothing declared.

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- •• of outstanding interest

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