Cholesterol domains regulate the actin cytoskeleton at the leading edge of moving cells

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The plasma membrane must be rigid to form a barrier between the cell interior and the extracellular milieu, while remaining sufficiently flexible to enable rapid changes in cell shape. The concept of the plasma membrane as a mosaic of microdomains can help to understand this combination of properties. A recent report shows that the polarization of cholesterol-enriched domains changes the viscoelastic properties of the membrane at the front of moving cells, permitting efficient formation of the actin network required to push the leading-edge membrane forward.

To move, a cell must acquire and maintain morphological and functional asymmetry – a process termed polarization [1]. Polarity refers to the ability of a migrating cell to change its morphology in response to chemoattractants and to maintain a stable asymmetric shape with two poles: the leading edge, which protrudes at the cell front, and the rear edge (termed uropod in leukocytes), which retracts.

The basic molecular mechanisms involved in the polarization of moving cells resemble those of other cell types. Segregation of membrane domains to spatially restricted cell locations seems to be a conserved component of the polarization program from yeast to mammals [2]. In constitutively polarized cells, such as neurons or epithelial cells, the plasma membrane forms localized microdomains with specific compositional and structural properties. The apical membrane of epithelial cells and the axon in neurons are rich in cholesterol and sphingomyelin compared with the basolateral or somatodendritic membrane [3]. Domain segregation plays an important role in the establishment and maintenance of polarity, and in the function of constitutively polarized cells. Specific membrane domains also segregate during cell migration, which is important for achieving the persistent polarity crucial for cell movement [2].

Although growing evidence suggests the existence of microdomains, it is difficult to obtain a satisfactory description of their properties, such as size, composition and dynamics. It is also evident that there are many different types of membrane domains in cells, which makes understanding the functional consequences of domain segregation more difficult. Vasanji et al. [4] have reported that the polarization of cholesterol-enriched domains might be a mechanism to deliver ‘membranes’ with specific physical properties to defined locations in migrating endothelial cells (EC). They showed a front-to-back gradient of plasma membrane microviscosity (PMM) due to cholesterol accumulation at the cell leading edge. This increase in PMM could permit the formation of a productive actin network that effectively pushes the leading-edge membrane forward. Strikingly, angiogenic growth factors increase viscosity at the leading lamella but decrease actin polymerization rates. We will discuss these findings in the context of membrane domain polarization during cell migration.

The plasma membrane is a mosaic of microdomains

Numerous studies have provided evidence that the plasma membrane comprises distinct domains of submicron size [5]. The study of microdomain properties is, nonetheless, a complex problem. Cell membranes can contain hundreds of chemically distinct lipids that might be modified metabolically in a dynamic manner. Proteins anchored to the bilayer that might associate only transiently with microdomains can modify local lipid organization.

Most domain characterization derives from examination of artificial membranes generated using the major lipids found in cells. There are three major lipid classes in eukaryotic cells (Figure 1). Glycerophospholipids are diacylglycerol-based and typically carry acyl chains of 16–18 carbon atoms, one of which is unsaturated. Consequently, these lipids have low melting temperatures (Tm). Sphingolipids are ceramide-based and usually carry saturated acyl chains of 16–26 carbons, thus having a high Tm. Glycosphingolipids belong to this lipid class, in which phosphate is replaced by sugars of varying complexity. Sterols, of which cholesterol is the major form in mammals, are based on a hydrophobic four-ring structure. Cholesterol represents ~30–50% of membrane lipid. Simple bilayers composed of either high- or low-Tm phospholipids can form two extreme phases: the quasi-solid gel phase and the liquid-disordered (l_d) fluid phase, which is sometimes termed ‘liquid crystalline’ (Figure 1). A sharp transition between gel and liquid phases occurs at a characteristic Tm. Lipid bilayers in the l_d phase are highly fluid – their lipids have high rotational and lateral mobility. In the solid gel phase, lipids are densely packed and, thus, have essentially no mobility in the bilayer plane. When high- and low-Tm lipids are mixed with cholesterol, the resulting model membranes show coexistence of multiple phases [6], one of which is liquid ordered (l_o) and cholesterol-dependent [7] (Figure 1). This phase separation seen in three-component models is largely replicated in membranes that are formed by lipids isolated...
from renal brush-border cells [8], suggesting that the cell membrane can form distinct domains.

Despite extensive work on phase separation, there is still an over-simplified view of cell membranes as a mosaic of cholesterol-enriched \( l_0 \) domains coexisting with cholesterol-poor fluid \( l_\infty \) domains. Two major \( l_\infty \) domains have been proposed in cells: caveolae, which are small, flask-shaped invaginations associated with cholesterol-binding caveolin proteins [9], and lipid rafts, which are small, cholesterol-rich domains that are morphologically distinct from caveolae and contain a large fraction of order-prefering lipids [3]. ‘Lipid rafts’ is a broad term covering many types of membrane domains [10–12] that are still poorly defined.

Some of the best data supporting the presence of microdomains derive from analysis of the lateral motion of single lipid or protein molecules in the living cell membrane [13,14]. These studies conclude that raft lipids and proteins show less lateral diffusion than their non-raft counterparts. Because protein diffusion in a lipid bilayer is inversely proportional to membrane viscosity, one interpretation is that lipid rafts have higher PMM than non-raft domains, which is probably a consequence of their cholesterol enrichment [3]. Indeed, differences in diffusion rate between raft and non-raft markers are lost when cholesterol is sequestered from the membrane. Although these data might not be unequivocal proof of the existence of lipid rafts [15], they clearly indicate extensive lateral heterogeneity in eukaryotic membranes that might be dependent on local cholesterol concentration.

Membrane domains polarize in moving cells

Asymmetric microdomain distribution has been described in several types of moving cell [2], although reports on where these domains localize are contradictory. Ganglioside-monosialic acid (GM1)-enriched domains accumulate at the leading edge of MCF-7 carcinoma cells [16], the hematopoietic progenitor KG1a [17] and fibroblasts exposed to electrical fields that induce directional movement [18]. Conversely, exclusive uropod GM1 accumulation was found in T lymphocytes [19] and neutrophils [20]. Two raft lipids segregate to each cell pole in leukocytes – GM3 to the leading edge and GM1 to the uropod [10]. Caveolin-1 polarizes to the cell front in transmigrating EC, but to the rear when cells migrate in a 2D system [21]. These differences in microdomain location could be attributed to differences either in cell types analyzed or in modes of cell migration.

The study by Vasanji et al. [4] using EC provides new proof of plasma membrane polarization during cell migration. Using fluorescence recovery after photobleaching and fluorescence anisotropy (Box 1) of cells labeled with a lipid probe with preference for \( l_\infty \) phases, they found that the migrating EC leading-edge membrane is more viscous than that at the cell rear. This pattern is more evident when EC are stimulated with angiogenic factors. The front-to-back PMM gradient is independent of the actin cytoskeleton but is sensitive to cholesterol depletion.

Box 1. Principles of fluorescence polarization anisotropy

Fluorescence polarization anisotropy is an extremely powerful technique used to analyze molecular rotational and internal motions. When a pulse of linearly polarized light of the appropriate wavelength passes through a liquid solution of a fluorophore, it preferentially excites those fluorophore molecules whose absorption or transition vectors are aligned parallel to the polarization plane of the light. Molecules with perpendicular vectors are not excited. This leads to a biased population of excited molecules that tend to relax to a randomly oriented ensemble because of Brownian rotational diffusion. Simultaneously, the initial excited population decays to the electronic ground state by fluorescence and other processes. The polarization plane of the fluorescence photon is determined by the actual orientation of the molecule at the moment of emission. Therefore, it is well defined shortly after the onset of excitation but becomes increasingly random with time. The anisotropy is, thus, related to the extent of fluorophore rotation during its fluorescence lifetime, and this randomization changes as a function of time. Fluorescence anisotropy kinetics cannot be recorded directly, although they can be extracted from the decay of polarized emission components.
Because cholesterol levels are important for PMM, the authors analyzed the cellular distribution of a fluorescent cholesterol derivative. This cholesterol probe was more concentrated at the leading edge than at the trailing edge of migratory cells, and differences between the two poles increased in EC stimulated with angiogenic growth factors. A distinct lipid probe with a preference for \( l_1 \) membrane phase was distributed homogeneously in migrating cells. Vasanji *et al.* had previously reported that cholesterol-enriched caveolae are concentrated at the cell rear [21], although, intriguingly, the cholesterol probe used in this study was not enriched at the trailing edge.

Because lipid rafts and caveolae are cholesterol-rich, a plausible hypothesis is that cholesterol accumulation at the cell front is due to polarization of raft domains. Vasanji *et al.* observed that GM1 rafts concentrate at the cell rear, whereas GM3 rafts do not seem to polarize in EC. They concluded that the cholesterol concentrated at the EC leading edge is not sequestered in lipid rafts. Because caveolae are concentrated at the cell rear [21], they are unlikely to be responsible for the cholesterol increase at the leading edge. Therefore, more studies are needed to determine the mechanism that transports cholesterol to the cell front.

**Membrane domain properties influence actin-based deformation**

One of the most prominent conclusions from Vasanji’s report [4] was that the viscoelastic properties of lipid bilayers influence actin-filament assembly in migrating cells. Dynamic assembly of a branched network of filamentous actin (F-actin) at the leading edge is an absolute requirement for cell polarity and migration [2]. Actin nucleation and branching activities of the Arp2/3 complex depend on its interaction with the Wiskott–Aldrich syndrome protein (WASP, known as Scar in *Dictyostelium discoideum*), which is regulated in turn by Rho family GTPases and phosphatidylinositol (4,5)-bisphosphate \([\text{PtdIns}(4,5)P_2]\). Thus, specific membrane properties might control the multiple interactions between proteins and lipids that are needed to form the F-actin network (Figure 2). \([\text{PtdIns}(4,5)P_2]\) generation depends on cholesterol levels at the plasma membrane (for review, see Ref. [2]). A recent report showed that Rac-1, a major regulator of actin polymerization at the leading edge of migrating cells, binds preferentially to cholesterol-enriched \( l_1 \) membranes (i.e. lipid rafts) [22], although another report found Rac-1 in \( l_4 \) membranes [23]. Thus, evidence suggests that some key elements involved in actin-filament formation at the leading edge can show binding selectivity for cholesterol-enriched domains.

However, Vasanji *et al.* showed that levels of actin-mediated deformation of large unilamellar vesicles (LUV; a \( \sim 100\)-nm bilayer vesicle system) containing physiological levels of cholesterol were greater than those in cholesterol-free vesicles. Because the only protein in this system is actin, the results suggest that intrinsic properties of cholesterol-enriched domains affect actin-induced membrane deformation. This is an apparent paradox because it is well established that cholesterol addition increases the rigidity of phospholipid bilayers and should, thus, diminish their elasticity [24]. Indeed, supraphysiological levels of cholesterol inhibit LUV deformation [4]. Lipid rafts have also been implicated in the formation of the phagocytic cup [25], reinforcing the idea that adequate cholesterol levels might be required for optimal actin-induced membrane deformation.

The results of Vasanji *et al.* [4] might also provide a framework for understanding the biphasic effect of cholesterol on cell migration [26] and could explain the very narrow protrusions observed in some cholesterol-depleted cells stimulated with angiogenic growth factors [16]. Their model implies that cholesterol does not affect actin polymerization machinery, but stabilizes the F-actin network by increasing membrane stiffness at the leading edge. This interpretation supports the unexpected

![Figure 2](image-url)
observation that actin polymerization is reduced in rapidly moving cells compared with slowly moving cells [4]. Nonetheless, the mechanisms underlying the stabilization of the F-actin framework remain unsolved.

Concluding remarks
Recent research has focused on chemotactant-elicited signaling pathways that drive cell migration; the plasma membrane – the cell structure that must channel this information – has been largely overlooked. It is now evident that lipid components of the plasma membrane are redistributed dynamically during the morphological and functional polarization of moving cells. Plasma membrane polarization offers platforms on which signaling is organized [27], in addition to domains with viscoelastic properties that permit actin-mediated deformation at membranes in cell migration has returned to center stage. Membrane systems and powerful real-time imaging techniques provide a multidisciplinary approach that involves studies in model systems for understanding the precise picture of lateral lipid organization in the plasma membrane of living cells requires redistribution. Understanding the precise picture of lateral lipid organization in the plasma membrane of living cells requires redistribution. Understanding the precise picture of lateral lipid organization in the plasma membrane of living cells requires redistribution. Understanding the precise picture of lateral lipid organization in the plasma membrane of living cells requires redistribution. Understanding the precise picture of lateral lipid organization in the plasma membrane of living cells requires redistribution. Understanding the precise picture of lateral lipid organization in the plasma membrane of living cells requires redistribution. Understanding the precise picture of lateral lipid organization in the plasma membrane of living cells requires redistribution. Understanding the precise picture of lateral lipid organization in the plasma membrane of living cells requires redistribution. Understanding the precise picture of lateral lipid organization in the plasma membrane of living cells requires redistribution. Understanding the precise picture of lateral lipid organization in the plasma membrane of living cells requires redistribution. Understanding the precise picture of lateral lipid organization in the plasma membrane of living cells requires redistribution. Understanding the precise picture of lateral lipid organization in the plasma membrane of living cells requires redistribution. Understanding the precise picture of lateral lipid organization in the plasma membrane of living cells requires redistribution. Understanding the precise picture of lateral lipid organization in the plasma membrane of living cells requires redistribution. Understanding the precise picture of lateral lipid organization in the plasma membrane of living cells requires redistribution. Understanding the precise picture of lateral lipid organization in the plasma membrane of living cells requires redistribution. Understanding the precise picture of lateral lipid organization in the plasma membrane of living cells requires redistribution. Understanding the precise picture of lateral lipid organization in the plasma membrane of living cells requires redistribution. Understanding the precise picture of lateral lipid organization in the plasma membrane of living cells requires redistribution. Understanding the precise picture of lateral lipid organization in the plasma membrane of living cells requires redistribution.