The role of the membrane confinement in the surface area regulation of cells

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We propose a new in vitro system to study the mechanics of surface area regulation in cells, which takes into account the spatial confinement of the cell membrane. By coupling a lipid bilayer to the strain-controlled deformation of an elastic sheet, we show that upon straining the supported lipid bilayer expands its surface area by absorbing adherent lipid vesicles and upon compression decreases its area by expelling lipid tubes out of its plane. The processes are reversible and closely resemble in vivo observations on shrinking cells. Our results suggest that the mechanics of the area regulation in cells is controlled primarily by the membrane tension and the effects of the membrane confinement.

Cells communicate with the environment through their membranes, and consequently the membrane surface is a critical parameter for cell function. The membrane defines the cell shape and volume, which vary throughout the life cycle of the cell, e.g., during cell migration, cell division or physiological volume changes in neuronal and plant cells. Moreover, some specialized tissues, i.e., in the urinary bladder or the lung are subject to cycles of mechanical stretching and compression. Because the cell membrane is inelastic, cells use specific mechanisms to preserve their membrane integrity during area variations. These mechanisms have been discussed in several reviews in references 3, 5 and 8. For example, cells that are prone to changes in their area maintain an intrinsic membrane reservoir in the form of lipid vesicles. Where there is a demand for area expansion, vesicles are added to the cell membrane (exocytosis) and are retrieved upon compression (endocytosis). The complex morphological transformations of the cell membrane during exo- and endocytosis inevitably require specific protein machinery, but only recently have the material properties of the lipid matrix been recognized as significant. Experiments with giant vesicles have shown that many of the membrane transformations can be explained by simple energy minimization principles; for example, the invaginations in membranes of heterogeneous composition can be driven by their spontaneous curvature, membrane fission arises from lipid phase separation, and increased lipid tension facilitates membrane fusion.

A common feature of the cells in multicellular organisms is that their membranes are in a confined state, i.e., adhered to other membranes or an extracellular matrix. Internally, the membrane is pinned to the cytoskeleton. Membrane adhesion is achieved by non-specific physical forces and specific molecular bonds. For example, the adhesion of pure lipid membranes to a substrate or other membranes is a competition between steric repulsion and attraction due to van der Waals and electrostatic forces. In addition, cell membranes adhere specifically through various transmembrane and surface proteins, forming desmosomal contacts, tight junctions, receptor-ligand bonds, etc. The adhesion forces influence the bending and stretching dynamics of the membrane and may induce local variations in the membrane tension; adhesive interactions also restrict the available

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volume of the interstitial space. As a result, (i) the mechanics for surface area regulation in spatially confined cells is expected to differ significantly from that in freely suspended cells and (ii) it must be a coordinated process in order to preserve the cell contacts. Nevertheless, the effects of the confinement on the membrane dynamics remain presently unexplored.

In our recent study, we used supported lipid bilayers, coupled to an elastic substrate to address in vitro the effects of the membrane adhesion on the area regulation. By modulating the strain of the substrate, we were able to observe the dynamical response of the strongly adhered bilayer to biaxial expansion and compression. We showed that a lipid membrane subject to lateral stretching expands its area, without compromising its integrity, by incorporating vesicles that are initially adhered to it. The extent to which the membrane can expand depends on the number of adhered vesicles, suggesting their role as a reservoir of lipids. The vesicle absorption is a self-regulated mechanism through the formation of a fusion pore (inset).

Instead, we observed an alternative fusion pathway, which had been suggested previously by molecular simulations. Pores were formed in the unadhered portion of the vesicle membrane, through which the vesicle content was expelled (iii). The empty vesicle then collapsed (iv) and got absorbed into the expanding supported bilayer (v). This fusion mechanism offers a pathway for area expansion without transporting volume in and out of the cell, which may play an important role during the expansion of cells, such as those confined in tissues.

In our experiments with supported lipid bilayers under lateral compression, we show that the confined membrane expels a multitude of lipid tubes to reduce its area in the plane. The tubes nucleate as a result of the destabilization of the bilayer above a certain compression threshold, as has been theoretically suggested and elongate throughout the compression (Fig. 2). The lipid tubes are energetically more favorable than spherical buds because the confined membrane tries to minimize the portion of the unadhered membrane and due to insufficient volume to fill the membrane invaginations (i.e., there is only a small interstitial volume and slow water permeability through the membrane). At the cessation of the compression, if left undisturbed the lipid tubes remain stable for a few hours, whereas they retract rapidly and get absorbed by the bilayer if the latter is subjected to stretching (Fig. 2). Under a new cycle of expansion and compression, tubes form and get absorbed reversibly and at roughly the same locations as in the previous cycle.

Our in vitro findings closely reproduce observations on shrinking neurons, T-tubules in skeletal muscles, renal cells, plant cells, etc. Because of their reversibility under cycles of area expansion and compression, the membrane tubes are energetically less demanding than the well-known mechanisms of vesicle endo- and exocytosis. In addition, the local reduction of surface area during compression has been proposed as a mechanism for cells to preserve their adhesion contacts during area variations.

In summary, we have demonstrated that the membrane confinement plays an important role in the surface area regulation of adhered cells. Our experimental setup can be used to study different aspects of membrane adhesion, i.e., inter-membrane distance, covalent pinning, etc., as well as effects of strain rate, membrane composition and osmotic pressure on the morphology of confined membranes.

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