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Membrane tension homeostasis of epithelial cells through surface area regulation in response to osmotic stress

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ABSTRACT

Osmotic stress poses one of the most fundamental challenges to living cells. Particularly, the largely inextensible plasma membrane of eukaryotic cells easily ruptures under in-plane tension calling for sophisticated strategies to readily respond to osmotic stress. We describe how epithelial cells react and adapt mechanically to the exposure to hypotonic and hypertonic solutions in the context of a confluent monolayer. Site-specific indentation experiments in conjunction with tether pulling on individual cells have been carried out with an atomic force microscope to reveal spatio-temporal changes in membrane tension and surface area. We found that cells compensate for an increase in lateral tension due to hypoosmotic stress by sacrificing excess of membrane area stored in protrusions and invaginations such as microvilli and caveolae. At mild hypotonic conditions lateral tension increases partly compensated by surface are regulation, i.e. the cell sacrifices some of its membrane reservoirs. A loss of membrane–actin contacts occurs upon exposure to stronger hypotonic solutions giving rise to a drop in lateral tension. Tension release recovers on longer time scales by an increasing endocytosis, which efficiently removes excess membrane from the apical side to restore the initial pre-stress. Hypertonic solutions lead to shrinkage of cells and collapse of the apical membrane onto the cortex. Exposure to distilled water leads to stiffening of cells due to removal of excess surface area and tension increase due to elevated osmotic pressure across the plasma membrane.

1. Introduction

The plasma membrane of cells is a highly dynamic and strongly regulated two-dimensional liquid crystal. Many cellular processes like endo- and exocytosis [1,2], cell migration [3], cell spreading [4,5] and mitosis [6] are regulated by an intrinsic feature of the plasma membrane, the membrane tension. The plasma membrane tension encompasses the in-plane tension of the lipid bilayer, and the membrane–cytoskeleton adhesion, which is actively controlled by the contractile actomyosin cortex [7]. The intricate interplay between the plasma membrane and its cortex enables cells to withstand mechanical challenges posed by the environment. Considering that membranes are thin and fragile structures sophisticated feedback circuits are necessary based on tension homeostasis to maintain an intact shell. Osmotic stress is a physiologically relevant mechanical stimulus since animal cells have to sustain substantial fluctuations in the osmolarity of external fluids, which produces considerable pressure differences between the cytosol and the environment. Osmotic pressure forces the cell to quickly adapt in order to avoid damages of the largely inextensible plasma membrane. When cells are subjected to a hypoosmotic solution they increase their volume due to influx of water and subsequently they extend their projected surface area. Groulx et al. reported that animal cells can increase their surface area by a factor of 3 and 10 times their volume depending on the cell type [8]. As membranes cannot bear large strains (3–4%), they require regulatory processes to maintain the overall plasma membrane tension below lysis tension; more precisely, tension driven surface area regulation is necessary to accommodate changes in tension. Concretely, high tension is buffered by an excess of membrane area, while a decrease in membrane area is triggered if the tension lowers [9]. To provide sufficient membrane area, cells store excess membrane in reservoirs like microvilli and caveolae, which by virtue of unfolding can buffer membrane tension. Kozera and coworkers showed that caveolae might act as membrane reservoirs to compensate for an increase in membrane tension induced by swelling [10]. So far, a comprehensive picture of membrane homeostasis is lacking since it is difficult to measure surface area and tension simultaneously to confirm the regulative relationship between the two parameters. In this study, we use a unique combination of site-specific indentation followed by tether pulling to acquire tension and excess area of the apical side of epithelial cells spatio-temporally resolved. Polar epithelial cells as those from the intestine or kidney are suitable candidates to study the impact of environmental physicochemical stimuli on changes in membrane tension since they frequently face changes in the chemical potential. Here, we

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used Madin Darby canine kidney cells (MDCK II) grown to confluence and challenged by different osmotic solutions to investigate the mechanical response of the plasma membrane with respect to tension-buffering membrane reservoirs. Therefore, indentation experiments, analyzed with an extended liquid droplet model for adherent cells, were carried out with an atomic force microscope (AFM) to simultaneously assess local changes in membrane tension and at the same time and location to monitor the excess membrane area as a function of osmotic pressure. The latter task is achieved by measuring the apparent area modulus of the plasma membrane mirroring the amount of stored excess area. In conjunction with membrane tether pulling experiments, as an independent mechanical approach, we were able to show how epithelial cells adjust their surface area in response to tension changes. Furthermore, we monitored morphological changes due to cell swelling and shrinking showing distinct alteration in tension buffering membrane reservoirs such as microvilli. We found that MDCK II cells sacrifice their microvilli to generate excess membrane readily consumed to accommodate rising tension. Lysis of the plasma membrane is prevented by loosening membrane–actin links and sacrificing membrane reservoirs. However, exposure to distilled water exhausts all existing reservoir and consequently leads to lysis. Long-term monitoring of tension and surface area reveals that membrane tension largely recovers at the expense of membrane area taken-up via an increased endocytosis rate.

2. Materials and methods

2.1. Cell culture

MDCK II cells, obtained from the Health Protection Agency, Salisbury, UK, were maintained in minimal essential medium (MEM) with Earle's salts and 2.2 g/L $NaHCO_3$ (Biochrom, Berlin, Germany) supplemented with 4 mM L-glutamine, penicillin–streptomycin and 10% FCS at 37 °C in a 7.5% CO_2 humidified incubator. Confluent cells were released with trypsin/EDTA (0.5%/0.2%) (Biochrom, Berlin, Germany) and subcultured weekly.

2.2. Cell labeling

Cells were grown in ibidi™ petri dishes (ibidi, Martinsried, Germany) to confluency and treated with mild (200 mOsm), strong (80 mOsm) hypotonic and hypertonic (700 mOsm, 400 mM glucose) solutions for 0, 0.5, 2 and 6 h. Cells were fixed with 4% paraformadehyde PFA for 20 min. F-actin labeling was carried out with Alexa Fluor 546 phalloidin or 488 (Invitrogen, Darmstadt, Germany, diluted as recommended by the manufacturer, 5 μL methanolic stock solution with 200 μL PBS for each coverslip). For ezrin labeling, cells were incubated with primary monoclonal antibody (4 μg/mL diluted in PBS) for 1 h at room temperature and subsequently labeled with secondary Alexa Fluor 488-conjugated goat-anti-mouse IgG1 antibody (BD Biosciences, Heidelberg, Germany) for 45 min (4 μg/mL in PBS).

2.3. Atomic force microscopy for imaging

AFM imaging was performed with a Nanowizard® II AFM (JPK Instruments AG, Berlin) mounted on an Olympus IX 81 inverted light microscope. Although AFM delivers images of unprecedented resolution under physiological conditions, fixation of the plasma membrane for high resolution imaging is inevitable due to membrane flexibility and lateral mobility. Therefore, cells were fixated using 4% glutaraldehyde in PBS for 20 min. Silicon nitride cantilevers (MLCT, Bruker AFM Probes, Camarillo, USA) with a nominal spring constant of 0.01 N m^{-1} were employed. Cells were imaged in PBS at room temperature with a scan rate of 0.2 Hz and processed with JPK Image Processing® software.

2.4. Atomic force microscopy for elasticity measurements

Force curves were taken continuously on a Nanowizard™ II AFM (JPK Instruments AG, Berlin, Germany) while scanning laterally across the sample referred to as force mapping [11,12]. MDCK II cells seeded on Ibidi™ petri dishes (ibidi, Martinsried, Germany) and grown to confluency were mounted in a PetriDishHeater® (JPK Instruments AG, Berlin, Germany) set to 37 °C with HEPES buffered culture medium. Force curves were processed by applying an extended liquid droplet model first described by Sen et al. [13]. The contact angle of the spherical cap measured by AFM imaging for an untreated cell was found to be $\phi_0 = 20°$. For the radius of the cap we determined a value of 10 μm. Force curves are recorded in constant force (setpoint 1 nN). Further parameters are listed in Tables 1 and 2.

2.5. Atomic force microscopy for probing tether forces

Prior to tether pulling, cantilevers (MLCT, Bruker AFM Probes, Camerillo, USA) were plasma cleaned for 30 s (Argon) and incubated with 2.5 mg/mL concanavalin A (Sigma Aldrich, Germany) in PBS for 1.5 h to establish a strong contact to the apical surface. Both approach and retrace velocities were set to 2 μm/s. Tether forces were determined from force distance retrace curves (Fig. 4) and analyzed using Eq. (2).

3. Results

3.1. Mechanical theory—the liquid droplet model

The liquid droplet model successfully describes the viscoelastic behavior of neutrophils and leucocytes in micropipette aspiration experiments [14]. These cells behave like liquid drops when suspended and deform continuously inside a micropipette. Essentially, the model envisions the cellular interior as a homogeneous viscous liquid surrounded by a shell comprising the actomyosin cortex and the plasma membrane both exhibiting a static tension. Contributions from bending are neglected [15]. The model was successfully modified to describe the indentation of an AFM probe into an immobilized cell by Rosenbluth et al. [16] and Sen et al. for single adherent cells [13]. The model relies on the assumption that curvature and volume remain conserved throughout indentation. Two sources of restoring force occur, pre-stress or lateral tension in the membrane–cortex and the finite area compressibility upon in-plane stretching of the shell. The response to indentation originates almost exclusively from lateral stretching of the membrane and work against the existing pre-stress generated by the underlying cell cortex and the boundary, i.e. the cell–cell contacts. Here, we adopt this model to quantify the indentation of a conical indenter into a confluent cell represented by a spherical cap. The spherical cap represents the apical part of the cell in the context of a confluent cell layer. The model essentially assumes a lateral tension T composed of a lateral tension or pre-stress T_0 governing the response to indentation at low penetration depth and non-linear in-plane stretching of the shell characterized by a 2-D Hookean term [13]:

$$T = T_0 + \tilde{K}_A \frac{\Delta A}{A_0} \tag{1a}$$

Table 1
Parameters for modeling indentation of a spherical cap with a conical indenter describing confluent MDCK II cells subjected to hypotonic stress.

	Isotonic 300 mOsm	Hypotonic 200 mOm	Hypotonic 80 mOsm	Hypertonic 700 mOsm
ϕ_0 [°]	20	23	30	17
R_0 [μm]	35	30	26	28