Platform: Cell Mechanics, Mechanosensing, and Motility

1514-Plat

Mechanosensitive ion channels are essential for the durotaxis of pancreatic stellate cells

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Malignant neoplasms, such as pancreatic ductal adenocarcinoma (PDAC), show pathologically increased rigidity, which results in a stiffness gradient between tumorous and healthy tissue. Cells are able to detect such differences in rigidity and to migrate into the direction of higher stiffness which is referred to as durotaxis. In PDAC, pancreatic stellate cells (PSCs) are mainly responsible for the production of the rigid tumor environment. Thereby they indirectly attract more PSCs, and ultimately, promote the invasiveness of cancer cells. The molecular sensors behind durotaxis are largely unknown. The aim of this work is to investigate the importance of mechanosensitive ion channels for the durotaxis of PSCs. In our experiments, we developed a two-dimensional model system of durotaxis using ultraviolet polymerized polyacrylamide gels containing a linear rigidity gradient. These gradient gels mimick the stiffness environment of PDAC which we validated via atomic force microscopy. Afterwards, we investigated the influence of mechanosensitive ion channels such as Piezo1, TRPC1 and TRPV4 on the durotaxis of primary murine PSCs by pharmacological and genetic channel modulation. Our results show that PSCs migrate towards a more rigid substrate. Both an inhibition of the Piezo1 channel and its activation lead to a suppression of durotaxis. A similar impairment of durotaxis is present by knocking out the TRPC1 channel with simultaneous TRPV4 inhibition. Overall, our results indicate that mechanosensitive ion channels, especially the Piezo1 channel, are involved in the process of durotaxis of PSCs by sensing and transducing the mechanical microenvironment. Moreover, Piezo1 relies on the concurrent functionality of TRPC1 or TRPV4 in order to mediate the stiffness-guided migration of the cells.

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Hypotonic stress triggers delayed cell volume increase via crosstalk between myosin, calcium signaling, and ion transport

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Mammalian cells can maintain a relatively homogeneous cell volume distribution, while tumorigenic cells tend to have large cell size variability. At the short timescale, cell volume is determined by water flow across the membrane, which is driven by osmotic and hydrostatic pressure gradients across the cell surface. During processes such as mitotic swelling, spreading on the substrate, and response to change in media osmolarity, cells are able to dramatically change their volume within minutes or a few hours. To understand the principles underlying short timescale cell volume regulation, we explored cell volume changes after hypotonic shock using the fluorescence exclusion method (FXm) in several cell lines. We found that the change in media osmolarity triggers a rapid cell volume increase, and then HT1080 fibrosarcoma cells and MDA-MB-231 breast cancer cells can recover their volume within 5-10 minutes. Strikingly, instead of undergoing a monotonic volume recovery, 3T3 fibroblast and MCF-10A breast epithelial cells show a delayed volume increase. After 5-10 minutes of initial volume decrease, cell volume increased again by 20% over the next 30 minutes, followed by a second phase of volume decrease. We further discovered that while HT1080 and MDA-MB-231 rely mostly on Na/K ATPase for volume recovery, 3T3 and MCF-10A volume responses to hypotonic stress are regulated by a combination of myosin activation, Calcium signaling, and ion transport. Our findings reveal that mechanosensing and associated signaling are central mediators of cell volume regulation, with important implications for cell size regulation over longer time scales of the cell cycle.

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Distinct self-organized actin patterns explain diverse parasite gliding modes

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During host infection, single-celled apicomplexan parasites like *Plasmodium* and *Toxoplasma* use a unique form of locomotion called gliding that differs fundamentally from the swim-or-crawl paradigm of eukaryotic cell motility. Gliding is powered by a thin layer of actin and a specialized myosin sandwiched between the plasma membrane and an inner membranous scaffold. How is this actomyosin network organized to generate coherent traction forces, and drive the diverse cell movements observed during gliding? Here, we used single-molecule imaging to track individual actin filaments and myosin complexes in living *Toxoplasma gondii.* Based on these data, we drew on flocking theory to develop a continuum model of actin self-organization in the unusual confines provided by parasite geometry. Deriving a parameterization-free surface formulation of our governing equations enabled finite element method simulations on detailed reconstructions of the *Toxoplasma* cell surface. In the presence of rapid actin filament depolymerization, our model predicts the emergence of rearward steady-state actin flows. By contrast, at low depolymerization rates, emergent stable actin patches recirculate up and down the cell in a ''cyclosis'' that we observed experimentally for drug-stabilized actin bundles in live parasites. These findings indicate that actin turnover governs a transition between distinct self-organized actin states, whose different properties can account for the disparate gliding modes observed experimentally: unidirectional (helical, circular, twirling) and bidirectional (patch, pendulum, rolling). More broadly, our experimental observations and theoretical model illustrate how different forms of gliding motility can emerge as an intrinsic consequence of the self-organizing properties of actin filament flow in a complex confined geometry.

1517-Plat

Dynamics of cell adhesion on fluid substrates

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Adhesion is a ubiquitous process for virtually all cells from those that form together tissues and organs of multicellular organisms to single immune cells that migrate adhering to the extracellular matrix in search of pathogenic cells. Cells adhere to substrates or other cells not only to maintain physical cohesion, but also to receive information on their microenvironment. Adhesion is thus a platform for mechano-chemical communication between a cell and a substrate or another cell.

In this work, we focus on integrin-mediated adhesion. Integrins are the main players of this mechano-chemical communication that assemble in clusters in a mechanosensitive manner. Mechanical stimuli that act on these clusters are converted into biochemical signals and transmitted inside the cell. Although cell adhesion on rigid substrates is very well studied, much about adhesion on soft fluid substrates like membranes remains unknown.

Here we study the evolution of integrin clusters in cells on fluid supported lipid bilayers (SLBs) functionalized with cell ligands of different affinities (RGD peptide, bacterial protein *Invasin*). Ligand affinity to integrins is very important in integrin activation and consequent formation of adhesion clusters. We have used advanced confocal microscopy, fluorescence calibration and custom-made image analysis routines to quantify integrin densities in adhesion clusters. We have found that integrin densities in clusters on *Invasin* are significantly higher than the ones on RGD reaching the densities reported for focal adhesions on glass. Additionally, we have observed the recruitment of the proteins that are associated with mature adhesions to integrin clusters like zyxin and VASP. Finally, we have shown cells are able to deform SLBs by pulling membrane tethers from them. These results suggest the presence of mechanotransduction in integrin clusters on fluid substrates and that ligand affinity is a critical parameter in integrin cluster growth.

1518-Plat

The adipocyte secretome inhibits T-cell activation by dampening T-cell receptor mechanotransduction

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