Cell and Bacterial Mechanics & Motility III

3231-Pos Board B336
Thermodyanmic Control on the Torque Generation of Bacterial Flagellar Motors
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Bacterial flagellar motor is an ion-driven molecular machine that is composed of a rotor and stator units. At least 11 stator units simultaneously associate with a single rotor, and the rest units diffuse in the inner membrane. The stator units exchange rapidly with the rotor and are concerned in the torque generation. Little is understood about the detailed energy conversion mechanism. Here, we demonstrate a novel assay that controls the intermolecular interaction between rotor and stator units with high-pressure techniques [1]. The strain was enclosed in the specially designed high-pressure chamber, which could be available up to 2,000 bar. The torque-speed relationship of the motor was measured by tracking of polystyrene beads attached to flagellar filaments at pressure range of less than 800 bar. At ambient pressure (1 bar), the torque is approximately constant (at ~1500 pN nm) from stall up to a ''knee'' speed of ~150 Hz, and then falls linearly with speed, extrapolating to zero torque at ~280 Hz. As the pressure increased, both the knee and zero-load speeds decreased significantly, while the zero-speed torque was not affected. Similar relations were obtained by decreasing intracellular pH [2]. Thus, our results suggest that applied pressure decreases the rate of proton translocation in the mechanochemical energy translation, but not the actual torque generation step within the cycle by the stator-rotor interactions.
[1] Nishiyama et al., Biophys. J. 96(3) 1142-1150 (2009).
[2] Nakamura et al., J. Mol. Biol. 386(2), 332-338 (2009).

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Are Biomechanical Changes Necessary for Tumor Progression? - The Impact of Cell Mechanics on Cancer Progression
Mareike Zink, Anatol Fritsch, Tobias Kiessling, Kenechukwu D. Nnetu, Steve Pawlizak, Franziska Wetzel, Josef A. Käs.
Biophysics established a new research area which described the progression of cancer from a materials science perspective. It has been know for a long time that malignant transformation is associated with significant changes in the cellular cytoskeleton. If the cytoskeleton's alterations are necessary for malignant transformation, they have to trigger biomechanical changes that impact cellular functions. In all cancers malignant neoplasia - uncontrolled growth, invasion into the surrounding tissue and metastasis occurs. Our results indicate that all these three phenomechanisms of malignancy require changes in the active and passive biomechanics of a tumor cell. Optical stretcher experiments with tumor cell lines and primary cells clearly show that malignant transformation causes cell softening for small deformations which correlates with an increased rate of proliferation compared to normal cells. However, tumor spheroids confined in agar gel proliferate until a gel stiffness of 10^4 Pa is exceeded which results in strain-hardening of cytoskeletal filaments at larger deformations. Furthermore, cell softening of the actin cortex can increase individual cell speed for lamellipodial motion of malignantly transformed fibroblasts and breast cancer cell lines. However, all cells can migrate and the motion of epithelial cells is mainly determined by their environment, whereas fibroblasts have the capability to move freely. Thus, it is the ability of single epithelial cells to overcome the tumor barrier to metastasize. The barrier that cells feel when they try to leave their cell compartment can be lowered by reducing cell adhesion. In breast tumor samples, small numbers of cells can be found that actively contract when laser light tries to stretch them. These could play a key role in metastasis because contraction can pre-strain and thus stiffen the cells to reduce adhesion sides to adjacent cells.

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Dual Mechanical Signal Integration Reveals Non Linear Cell Behavior
Robert L. Steward Jr., Chao-Min Cheng, Philip LeDuc.
Cell signaling is a complex, dynamic process, that requires precise coordination between internal signaling cascades and pathways and external environmental cues. One such external cue is mechanical stimulation which is known to influence a variety of cell responses including motility and proliferation. While mechanical stimulation may come in the form of tension, compression, or shear via a static or cyclic mode it may also occur separately or simultaneously reflecting the diversity of mechanical signals that cells may be required to integrate into cell behavior. For example, one cell type that experiences coupled mechanical influences are endothelial cells, which undergo three mechanical stresses: parallel shear flow, perpendicular radial stretch, and circumferential wall stress. Both normal and abnormal physiological processes result from the interplay between these forces. To probe how the cell integrates multiple mechanical signals single cells were exposed to uniaxial stretching, shear fluid flow and both modes of stimulation simultaneously to examine how the cell processes multiple inputs of mechanical stimulation as a function of cellular orientation. Cells exposed to single modes of mechanical stimulation were observed to align along the direction of stress, but intriguingly when combined, the responses were out of phase where the cells aligned between the direction of applied stresses with an orientation that was neither vertical or horizontal. This signal integration was observed to be not simply an additive approach, which has been investigated for physiological processes, but rather a nonlinear combination between both modes of mechanical stimulation, which is interesting as this has implications in abnormal physiological implications. These results will help provide insight into the complexities of cell behaviour and have implications in a variety of fields including biophysics, mechanotransduction, and cell signaling.

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Substrate-Ligand Friction Controls Traction Force in Cell Adhesion
Tilo Pompe, Thomas Bischoff, Stefan Glorius, Stephanie Johne, Maria Kasimir, Martin Kaufmann, Ina Uhlmann, Manfred Bobeth, Wolfgang Pompe, Carsten Werner.
Extracellular matrices determine cell fate decisions through the regulation of intracellular force and stress. The mechanical properties of these matrices like stiffness and ligand anchorage cause a distinct signaling behavior of adherent cells in respect to adhesion receptor forces. This process is known to be tightly linked to downstream kinase activation and epigenetic events including cell proliferation and differentiation. We report now on a new mechanism controlling traction forces in cell adhesion originating from sliding friction between adhesion ligands and the supporting material.
Using polymer surfaces with a graded physicochemistry we were able to tune the non-covalent anchorage of adhesion ligands, namely fibronectin, to the materials surfaces. Traction force cytometry and in situ analysis of cell-driven ligand reorganization revealed a correlated dependence on the ligand-substrate anchorage. Ligand reorganization during the formation of fibrillar adhesions was characterized by a reaction-diffusion process with a surface mobility around 1e4 m/Ns. Based on these quantitative data we could describe the force-velocity equilibrium at the adhesion site with its extracellular and intracellular components, namely the nanoscale friction of adhesion ligands on the materials surface and the myosin motor activity at the actin stress fibers. The correlation of a linearized Tomlinson model of ligand friction on the materials surface with the characteristics of myosin motors in the actin stress fibers revealed a control mechanism of traction forces of around 1pN per receptor-ligand pair by the ligand-substrate friction.
These findings elucidate a novel mechanism in force regulation at adhesion receptors, which is proposed to be highly relevant for cell behavior on natural and artificial scaffolds in vivo and in vitro, as many adhesion ligands are found non-covalently anchored to scaffold surfaces.

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Engineering the Mechanobiological Oscillations of Single Cells
Tianzhi Luo, Douglas N. Robinson.
Recently, we found that cytoskeletal proteins, such as myosin-II and cortexillin-I, cooperatively accumulate to highly deformed regions in Dictyostelium cells and the accumulation extent increases with increasing forces. Accompanying the protein accumulation, the cellular deformation decreases even as the external forces are kept constant. We argue that the accumulation of cytoskeletal proteins sense the mechanical stimuli and accumulate locally to enhance the local mechanical resistance of the cell, i.e. the cortical stiffness, which leads to diminishing cellular deformation. We suggest that the underlying mechanism of the cooperative accumulation could be that the binding of myosin to actin enhances cortexillin binding to actin filaments. Especially, myosin, undoubtedly a force sensor, binds to actin filaments in a force-dependent manner. On the other hand, cortexillin maintains the mechanical integrity of the whole actin network and anchors it to the cell membrane. Additionally, we found that cells are able to repeat the "increased deformation-protein accumulation-decreased deformation" cycle while the external force does not change. We propose two mechanisms for the restarting of the cycles. The first is that the myosin accumulation itself leads to less force experienced by each myosin, which results in the falloff of the accumulated myosins and the subsequent cortical softening, leading to larger deformation again. The second is that blebs, which behave as increased deformation, are sometimes formed when either the cortex-membrane linkage is too weak or the local myosin associated contractility is too strong. To test these hypotheses, we use chemicals, genetic tools and their combinations to modulate the expression level, the localization and function of cytoskeletal proteins and therefore tune the cortical stiffness, the cortex-membrane linkage and the period of the cellular oscillations in a quantitative way.