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Spatiotemporal Analysis of Traction Work Produced by Migrating Amoeboid Cells

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Amoeboid cell motility is a complicated process requiring the regulated activity and localization of many molecules and resulting in the cyclic repetition of a relatively small repertoire of shape changes. These changes are driven by the traction work produced by the cell, which can be estimated by measuring the forces and displacements exerted by the cells on their substrate during migration. We have developed and applied a novel implementation of Principal Component Analysis to identify and sort out the most important shape changes in terms of traction work produced by chemotaxing *Dictyostelium* cells. For this purpose, we acquired time-lapse recordings of cell shape and traction forces of *Dictyostelium* cells migrating on deformable substrates. Using wild-type cells as reference, we investigated the effect of altering myosin II activity by studying myosin II null cells and essential light chain null cells. Our results indicate that the spatio-temporal variation of the traction work produced by *Dictyostelium* cells can be described with a reduced number of modes. In fact, only four modes are needed to account for 65% of the traction work exerted by all cells lines studied. Furthermore, the first mode alone accounts for more than 40% of the traction work. Spatially, this mode consists of the attachment of the cell predominantly at two areas at front and back, contracting towards the center of the cell. The time evolution of this mode is approximately periodic and coincides with the time evolution of cell length. Each one of the remaining modes accounts for less that 10% of the traction work. Their temporal and spatial organization is less clear, suggesting that the cell performs a traction work cycle composed of a repetitive sequence of steps over which random fluctuations are imposed.

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Dynamic Force Generation within the Immune Synapse

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Increasing evidence suggests mechanical forces modulate T cell function. In this report, we investigate forces applied by mouse CD4+ T cells onto an underlying substrate as a model of the interface between T cells and antigen presenting cells. Traction force microscopy was carried out using microfabricated arrays of elastomer (polydimethylsiloxane, Sylgard 184, PDMS) pillars; cells induce deflections of the pillars which can be measured and used to estimate force applied to each structure. We chose a pillar geometry of 1 micrometer diameter and 5-9 micrometer height. These pillars were coated with a 1:1 mix of activating antibodies to CD3 and CD28, which ligate and activate the TCR complex and costimulatory CD28 signal. Traction force microscopy carried out on mouse naïve CD4+ T cells 1 hour after seeding revealed that naïve cells exert forces onto these pillar arrays with magnitude on the order of 50 pN per structure. Moreover, force application by a given cell is periodic, with a cycle on the order of minutes. To investigate the physiological implications of these forces, we measured IL-2 secretion by T cells seeded onto planar PDMS substrates of varying rigidity, which was controlled by varying the ratio of base : curing agent, yielding bulk moduli of 2 MPa to 25 kPa. Substrates were coated with activating antibodies to CD3 and CD28 prior to cell seeding, and the per area concentration of antibodies varied less than 10% across the different moduli. Activation of naïve T cells, measured as IL-2 secretion over six hours, was 50% greater on the stiffest vs. softest elastomers, and each condition was statistically different from all others (Kruskal-Wallis methods, alpha = 0.05). These results demonstrate a functional impact of mechanical forces on T cell activation, and reveal new dynamics of the immune synapse.

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Opposing Activities of Hydrostatic Pressure and Actomyosin Contraction Drive Mitotic Cell Rounding

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During mitosis adherent tissue culture cells undergo a dramatic shape change, from essentially flat to nearly spherical. The forces and mechanisms that drive this shape change remain unexplained. Here we investigate the nature of mechanical forces associated with mitotic cell rounding. We show that as cells round up they generate substantial outward directed forces, at times exceeding 100 nN in our assay. The force depended on both an intact actomyosin cortex and transmembrane ion gradients. A functional actomyosin cortex was required to establish a rounding force and disrupting it during mitosis triggered an increase in cell volume. Addition of pore forming toxins to disrupt cation gradients decreased the rounding force and allowed actomyosin dependent cell shrinkage. We postulate that mitotic cell rounding is governed by regulating both an outwardly directed hydrostatic pressure and an inwardly directed cortical tension. Together osmolaric pressure and actomyosin cortex dependent surface tension control cell volume and shape.

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Strain Distribution and Relaxation in the Stretched Axons of Sensory Neurons

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The mechanical environment of a neuron strongly influences its function. In response to an externally applied tensile load, a number of morphological responses have been demonstrated in the axons of cultured neurons. We have developed methods to understand cellular mechanisms governing these responses. Rat sensory neurons were seeded onto a flexible silicone substrate and were imaged during substrate stretch. This configuration resulted in uniform tensile loading along the length of the neuron. Stationary mitochondria, believed to be docked to the axonal cytoskeleton, were used as fiduciary markers for elements of the cytoskeleton. Their positions were determined before and after an applied substrate strain (percent change in length) of 10%, and used to calculate the resulting ‘‘instantaneous’’ strain of regions along the axon. There was dramatic heterogeneity in the measured strain along the length of the stretched axons. This variability was particularly evident in regions of the axon less than 35 microns long. Measured strain in regions longer than this was less variable and was closer to the expected 10% strain. These results suggest a length scale over which local structural elements may be altered to modulate the biomechanical response of the axon. Following the initial stretch, the substrate was held at 10% strain and the axons imaged for 20 minutes during ‘‘relaxation.’’ Compared to unstretched axons, mitochondrial pairs in stretched axons showed little coordinated movement with each other at all length scales. Additionally, mitochondria in stretched axons showed larger displacements during the initial phase of relaxation, but after 18 minutes, the displacements were much smaller than those seen in unstretched axons. Collectively, this work presents the axon as a dynamic and heterogeneous structure, which interacts mechanically with the extracellular environment in more complex ways than previously thought.

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On the Relationship of Tissue Surface Tension to Microscopic Parameters

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It has long been established that biological tissues are viscoelastic materials, and that embryonic tissues in particular have an ability to flow over large distances on long time scales. The mechanical properties of these tissues likely play an important role in cell movements and pattern formation during embryogenesis, cancer and regeneration, and they can be measured using rheology techniques such as tissue surface tensiometry (TST).

Over the past 40 years, two theories have been advanced to explain the microscopic origins of tissue surface tension: the differential adhesion hypothesis (DAH) and the differential interfacial tension hypothesis (DITH). While the DAH contributes surface tension to adhesive energies, the DITH contributes it purely to cortex tensions. The DAH has been able to successfully explain a vast range of data on embryonic and cancer cell aggregates and tissues over the last decades, however, recent studies on single cells appear to support the DITH.

Here we show that a simple mechanical model which accounts for cell-cell adhesion, cortical tension, and fluid incompressibility can explain the two types of experimental data within a single framework. We find that tissue surface tension is a careful balance between adhesive and tensile forces, which are interdependent and cannot be altered independently; the relative strength of adhesive and tensile forces determines the measured macroscopic surface tension. In addition, the model predicts that cells on the surface of an aggregate alter their morphology as the surface tension changes, and we will present experimental data from embryonic tissues that are consistent with these predictions.