Cell Mechanics & Motility III

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The Molecular Landscape of Mechanosensing by the Cell Cortex
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Mechanical forces direct a host of cellular and tissue processes. While much emphasis is placed on cell adhesion complexes as force sensors, these forces must be transmitted through the cortical cytoskeleton. However, how the cortex senses and transmits forces and how cytoskeletal proteins interact in response to these forces are poorly understood. Here, by combining molecular and mechanical experimental perturbations with theoretical multi-scale modeling, we decipher cortical mechanosensing from molecular to cellular scales. Forces are shared between myosin II and different actin crosslinkers with myosin having potentiating or inhibitory effects on certain crosslinkers. Furthermore, different types of cell deformations elicit distinct responses: myosin and α-actinin respond to dilation while filamin mainly reacts to shear. The kinetics of each protein’s accumulation may be explained by its molecular mechanisms. Finally, protein accumulation, along with the cell’s viscoelastic state, can also explain the monotonic or oscillatory contraction against mechanical load in different mutant strains.

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Intracellular Microarchitecture in the Presence of Myosin-Generated Forces in Living Cells
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The mechanics of cells are governed by a network of cytoskeletal filaments and motor molecules forming a dynamic mechanical entity. A recent experimental study by Mizuno showed local shear modulus of a synthesized cytoskeletal network could increase in the presence of myosin-generated internal stresses. It was speculated whether similar behaviors could also take place in cells. To examine this, we used an experimental method similar to Mizuno’s that combined active and passive microarchitecture to measure myosin-generated fluctuations and the local intracellular stiffness. Our data obtained from measurements in 15 HeLa cells showed a linear relationship between the magnitude of the fluctuating forces and the time-averaged shear modulus. Moreover, when myosin activities were inhibited by ML7, we saw a dramatic decrease in the intracellular forces, but surprisingly no significant changes were observed in the time-averaged intracellular stiffness by ML7. While we did not see a direct correlation between the mean intracellular shear modulus and the motor-generated fluctuating force, we did observe an increase in the fluctuation of the shear modulus with increasing force.

While it is not clear why results from the intracellular study were so different from the actin construct, some differences between the two systems may be attributed to the difference. First, the intracellular fluctuating forces were found to be weaker than that in the actin construct, thus perhaps not strong enough to modulate the cell stiffness. Secondly, the steady-state internal tension, which could dictate mechanical properties of a network, may be different between the two systems. Unfortunately, the experimental method we used could only measure fluctuating forces but not the steady-state tension in the system. Further studies that measure both fluctuating and steady-state forces at the same time may be required to address this problem.

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The Role of Myosin-II in Cell Spreading on Soft Hyaluronan-Fibronectin Substrates
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Studies using polyacrylamide (PAA) substrates show that cells respond to the resistance of the substrate by modifying spread area, focal adhesions (FA) and cytoskeletal morphology. Cells on a soft substrate (E<300 Pa) generate low traction forces, which do not support cell growth, FA formation and maturation or the assembly of actin stress fibers. We show that soft hyaluronan-fibronectin (HA-Fn) substrates support cell growth and survival similarly to rigid substrates, suggesting that the link between increased tension at the cell-substrate interface and cell growth could be altered by the substrate composition.

To address this question we measured epithelial and endothelial cell growth and morphology on soft HA-Fn substrates in the presence of agents that affect the myosin II-dependent contractile mechanism, such as the ROCK inhibitor Y-27632, and using myosin II knockdown cells.

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Photo-Regulation of Cell Division using Photochromic Inhibitors for Kinesin Eg5
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Previously it has been demonstrated that some kinesins known as an ATP driven motor protein are directly involved in regulation of the cell division cycle. Eg5 is one of the kinesin and its biochemical properties and structure were well studied. It is suggested that that Eg5 is activated in M phase of cell cycle and performs stabilizing and positioning of spindle. It is also known that Eg5 is overexpressed in tumor cell and induce significant cell division. Monastrol and STLC, which are potent inhibitor specific for kinesin Eg5, shut off mitotic division and result in apoptosis. Therefore, these inhibitors are attracting as anti-cancer drug. Azobenzene and spiroypran, a widely studied photochromic compound, can be reversibly isomerized between the cis and trans forms by ultra-violet (UV) and visible (VIS) light irradiation, respectively. We have recently demonstrated the Monastrol and STLC analogues composed of photochromic molecules inhibit ATPase activity of Eg5 reversibly upon UV and VIS light irradiation in vitro. Therefore, it is strongly expected that the photochromic inhibitors are applicable to reversible photo-regulation of cell mitosis. In this study, we have examined the effects of the photochromic inhibitors for mammalian cells. Effects of the photochromic inhibitors were evaluated by survival number of mammalian cells, HeLa cells or A172 cells. Survival numbers of cells were quantitatively analyzed by MTS reagent and cell divisions were observed by phase-contrast microscope. spindle behavior of the cells cultured in the presence of photochromic inhibitors were also examined using immunofluorescent staining. These experimental results suggested that the effects of photochromic inhibitors for the cell division were controlled by UV and VIS light irradiations reversibly.

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Testing the Tug-of-War Model of Bidirectional Transport In Vivo
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Molecular motor proteins are responsible for force generation in myriad cellular processes. Much of our understanding of how motors function has benefited from force measurements and manipulation at the single molecule level in vitro. We report on novel optical trapping methodology capable of precise in vivo stall-force measurements of individual endogenous cargoes hauled by molecular motors in their native environment. We present unprecedented stall force histograms of motor-driven lipid droplets in Drosophila embryos. Force measurements show that equal numbers of kinesin-1 and cytoplasmic dynein haul each cargo in opposite directions. Critically, by measuring cargo dynamics in the optical trap, we find that there is memory: it is more likely for a cargo to reorient in the same direction -rather than reverse direction- after the motors transporting it detach from the microtubule due to the trap force. This suggests that only motors of one polarity are active on the cargo at any instant in time and is not consistent with the tug-of-war models of bidirectional transport where both polarity motors can bind the microtubules at all times. We further use the optical trap to measure, in vivo, the detachment rates from microtubules of kinesin-1 and dynein-driven lipid droplets. Unlike what is commonly assumed, we find that dynein’s but not kinesin’s detachment time in vivo increases with opposing load. This suggests that dynein’s interaction with microtubules behaves like a catch bond and further argues in favor of a regulatory model for bidirectional transport.

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