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Stress Fiber Organization and Dynamics in Cells Adhered to Substrates of Varying Stiffness

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Abstract

Cellular morphology, locomotion and division are closely related to the stiffness of the substrates onto which the cells are cultured. The stiffness of cultured cells typically increases with the stiffness of the substrate. This increase correlates with increased contractility and the development of a meshwork of parallel and cross-linked stress fibers along the contacting surface. Many questions remain regarding the mechanisms that underlie cell-level cytoskeletal remodeling during mechanosensing. To better quantify the morphology and dynamics of the actomyosin cytoskeleton as a function of substrate stiffness, we used confocal microscopy to image cultured HeLa cells that stably express myosin regulatory light chain tagged with GFP (MRLC-GFP). We cultured cells on poly-acrylamide substrates coated with collagen I, with stiffness ranging from 0.4 kPa to 60 kPa. We used image segmentation methods to measure stress fiber and cortical myosin distributions in static 3D images. Time-lapse recordings show a connected stress fiber meshwork that evolves through new stress fiber formation on the cell periphery, contractile activity, and stress fiber merging and splitting over times of order hours. Quantitative analysis is suggestive of kinetic models that explore how different cortical myosin remodeling kinetics may contribute to different cell shape and rigidity depending on substrate stiffness.

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Arp2/3 Exhibits Higher Affinity for Young than for Mature Actin Filaments

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Actin undergoes a conformational transition during polymerization, known as maturation. Caldesmon (CaD) or its C-terminal fragment, H32K, when present before the transition, inhibits this process and arrests the actin filament at the pre-transitional ''young'' state, which is otherwise transient (Huang et al., J Biol Chem 285, 71-79, 2010). However, CaD in cells usually binds to stable actin filaments, except when it is phosphorylated and translocated to the cell leading edge where actin is being actively assembled (Kordowska et al., Exp Cell Res 312, 95-110, 2006); this may be the only occasion that CaD could interfere with actin maturation in vivo. We hypothesized that the young actin filaments stabilized by phosphorylated CaD are rendered more amenable to remodeling. Electron microscopic images indeed show that these young filaments have a rough and fuzzy morphology (Collins et al., BioArchitecture 1, 127-133, 2011). The question then arises: Could such an irregular appearance in fact provide more available docking sites for other actin-binding proteins, and thereby facilitate actin dynamics? To test this idea, we have performed binding experiments between Arp2/3 and actin filaments formed under the condition favoring either the young or the mature state. Actin filaments polymerized in the presence and absence of the CaD fragment H32K were incubated with the Arp2/3 complex and centrifuged; the amount of actin-bound Arp2/3 in the pellet was then analyzed by immuno-reactivity toward anti-Arp2 after serial dilutions. We found that the level of Arp2/3 cosedimented with actin filaments polymerized in the presence of H32K was higher than that bound to CaD-free F-actin, indicating stronger binding of Arp2/3 to the young actin filaments than to the mature filaments. These results appear to support our hypothesis. Supported by a grant from NIH.

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Breaking the Dynamic Instability of the Actomyosin Cortex Triggered by a Single Mechanical Cue Drives Cell Polarity in Non-Adherent Cells

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The ability of cells to establish and maintain polarized states is essential for numerous developmental and physiological processes. A key role in animal cell polarization is played by myosinII which generate contractile forces that result in producing a tensile stress in the actin network. Polarity establishment requires a symmetry-breaking event, resulting in an axis along which the cell will organize itself. While the polarization can occur spontaneously it could be triggered by a mechanical cue implying the presence of a mechanical threshold beyond which the cortex ruptures.

We propose to study the role of asymmetric and weak mechanical cue on the first steps of 3T3 fibroblast polarity establishment. We setup a dual-objective system that combines quantitative micromanipulation using optical tweezers and three-dimensional fluorescence imaging. 3T3 cells were maintained in suspension using glass coverslips coated with a hydrophobic co-polymer.

In absence of mechanical and chemical perturbations, cells exhibit an oscillatory behavior driven by the dynamical instability of the actomyosin cortex. Application of a single fibronectin-coated trapped bead is sufficient to induce the growth of an irreversible protrusion in the direction of the cue. In addition, the MTOC reorients toward the newly cue-defined polarity axis. Inhibition of myosinII contractile activity prevents any deformations and MTOC reorientation, suggesting thus far that an ''unimpaired'' actomyosin contractile activity is necessary for driving cell polarity. Finally, we report a directed cortical flow of actomyosin at the opposite side of the polarizing cue which suggest the idea of a latent migration polarity axis. Depletion of actomyosin filament as well as constant flow away of the polarizing cue zone supposes that the actomyosin cortex is a self-renewing contractile engine rather than a pre-tensioned network releasing once stored tension.

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Tension in the Cytokinetic Contractile Ring Depends on the Mobility of Actin and Myosin-II Anchors

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Cytokinesis, the physical process of cell division, is accomplished by constriction of an actomyosin contractile ring anchored to the plasma membrane. In fission yeast, the contractile ring assembles via coalescence of ~65 membrane-anchored precursor nodes containing myosin-II and actin filaments whose barbed ends are capped by formin Cdc12p. Once assembled, constricting rings have irregular, random organizations and the available evidence suggests that actin barbed ends and myosin-II remain anchored. However, whether the anchors are mobile in the membrane and how mobility of ring components could affect ring organization and tension are unknown. We developed a stochastic computer simulation of the fission yeast contractile ring with a minimal set of assumptions based on established biochemical mechanisms. Simulated rings have random organization and actin and formin undergo continuous turnover as observed experimentally. In simulations with myosin-II and actin filament barbed ends immobilized by strong anchoring, rings generated ~38 pN of tension due to stalled myosin-II motors pulling on immobilized actin filaments. Increasing actin or myosin-II anchor mobility decreased ring tension because myosin-II exerts reduced forces at higher velocities and because relative sliding of actin and myosin-II dissipates the ring organization and tension, similar to the polarity sorting phenomenon. Thus, ring tension is promoted by rapid actin turnover that refreshes the ring organization over the ~30 s turnover time. We also calculated correlation functions characterizing myosin-II and formin motions that could be used to quantify component mobility in experimentally observed rings. Interestingly, while rings with high actin mobility were unable to produce tension, rings with unanchored myosin-II generated ~10 pN of tension. Thus, our results suggest that strong anchoring of actin filaments is required for ring tension, but myosin-II anchoring is dispensable.

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Model of Condensation of an Actomyosin Network into a Contractile Ring for Fission Yeast Cell Division: The Effect of Actin Cross-Linkers Alpha-Actinin and Fimbrin

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Quantitative studies of the assembly of the contractile ring in the fission yeast S. pombe provide insights of how actin, myosin motors, and cross-linkers self-organize into contractile structures within cells. The fission yeast contractile-ring assembles through the condensation of a broad band of membrane-bound nodes that form in the middle of the cell and contain myosin-II and formin. In the Search, Capture, Pull, and Release (SCPR) model (Vavylonis et al., 2008), formins nucleate actin filaments from nodes and establish transient actomyosin connections among them. Myosin pulls and condenses the band of nodes into a ring. However, the role of actin cross-linkers