Cell Mechanics & Motility II

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Actomyosin Contractility and Actin Polymerization Explain Dynamic Behavior of Dendritic Filopodia

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Dendritic filopodia are actin-filled highly dynamic subcellular structures that sprout densely on neuronal dendrites during early brain development. A fraction of filopodia undergo a transition to dendritic spines that later mature into synapses - a process crucial for memory formation and learning, and deficient in neurodevelopmental and neurodegenerative diseases. The dynamics of dendritic filopodia is also different from that of conventional filopodia: the former exhibit sustained length fluctuations and cease their dynamic behavior after transition into spines. While actin retrograde flow in dendritic filopodia was measured previously, there has been no detailed theoretical description of how the flow is maintained and how length oscillations are sustained in dendritic filopodia. We apply mathematical modeling and experimental techniques to dissect and analyze the components of actin-based motility in dendritic filopodia, and suggest its role in the subsequent transition into the spine shape. The simulations demonstrate that the movement of actin network influenced by myosin contractility and viscous shear stresses lead to the myosin build up at the base of the filopodium and its consequent retraction. When myosin is inactive, the filopodium grows with the rate of actin polymerization. However, when myosin is active, the processes of polymerization at the tip, and cytoskeletal contraction due to myosin, and resistance to the flow out of the filopod at the base, conspire to produce an actin flow gradient along the filopodial axis. We have measured average rates of growth and retraction of filopodia in cultured hippocampal neurons using a custom tip-tracking algorithm. The simulated length fluctuations and actin retrograde flow compare well with the experimental data. We estimate the resistive force at the base of filopodia necessary to maintain the pattern of growth and shrinking and suggest new experiments to test the proposed mechanism.

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Spatiotemporal Domains in Active Actin Gels

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Using a standard actin filament gliding assay, rhodamine-phalloidin labeled actin filaments were visualized moving over a myosin-II coated surface by fluorescence microscopy and filament tracks were analyzed by computer. When micromolar amounts of non-fluorescent phalloidin-stabilised actin were added, filament motion became aligned into a common direction (Butt et al., 2010, *J Biol Chem***285**:4964-4974). Experiments conducted at low filament densities show that, on average, there is a small angular deflection that tends to align the paths of individual colliding filaments. Path alignment is independent of relative track directionality and increases with collision incident angle. Filament elastic deformation during collision events indicates the collision bending energy is ~20k_BT. As increasing amounts of bulk actin are added, individual filament paths become straighter and start to move in a common direction. Spatial correlation analysis shows that increased alignment is due to incremental recruitment of filament tracks into one orientation rather than fusion of seed domains to larger ones. Experiments performed over a wide range of actin filament concentrations and HMM surface densities, show that ordering of filament motion peaks over a range of conditions and decreases at extreme values. This finding differs from theoretical prediction of a critical phase transition (Kraikivski et al., 2006, *Phys Rev. Lett.***96**:258103). Loss of order at high bulk actin concentration might be explained if there is increased filament exchange between bulk, disordered, actin and surface bound material. Loss of order at high myosin surface densities might arise from increased probability of motor attachment that would reduce ability of actin to change its path following a collision. Although our results are broadly compatible with generation of long-range order from mechanics of individual actin filament interactions; other phenomena become important at high protein concentrations.

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Nanoscale Cell Membrane Fluctuations Measured by Scanning Ion Conductance Microscopy

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Nanoscale membrane fluctuations in epithelial cells connected to neighboring cells were quantified by scanning ion conductance microscopy (SICM), which is intrinsically a non-contact technique and widely used for imaging soft cell surfaces at high resolution. In this study, we first proposed a model for the estimation of apical cell membrane fluctuations from the ion current curves, i.e., the ion current vs. the tip-surface distance. Mapping the ion current curves with a commercial SICM apparatus (XE-Bio, Park Systems, Korea) revealed that, in untreated Madin-Darby canine kidney (MDCK) epithelial cells in a confluent condition, the fluctuation amplitude of apical membranes increased towards the cell center. Moreover, it was found that the spatial dependence disappeared when actin filaments were disrupted, which caused a significant enhancement of cell membrane fluctuations. The measurements of the complex shear modulus of cells by atomic force microscopy (AFM) suggested that the mechanical properties of the cells are not directly correlated with cell membrane fluctuations. The results indicate that membrane fluctuations are highly constrained at the cell-cell interface, in the vertical direction to the apical cell surface and by the underlying actin filaments.

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Adhesive Interactions between the Load Surface and the Actin Filament Tips Control the Mechanical Response of Branched Actin Networks

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The force-velocity relationships of branched actin network have been investigated intensively, which yields conflicting results both in theory and in experiments. We previously established an integrated model that unifies different aspects of the dynamics of branched actin network, including the growth, the branching, and the capping events. The stochastic simulations of our model showed that upon the resistance from the load, the branched actin network is capable of reinforcing itself, which underscores the basis of both the concave and the convex force-velocity relationships. In the current work, we extend our model by incorporating the adhesive interactions between the actin filament tips and the load surface. Our simulation results reveal that such adhesive interactions critically impact the force-velocity relationships and the geometry of branched actin network.

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Stressed Stress Fibers

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Cultivated animal cells often display prominent actin bundles, also known as stress fibers, throughout their cytoskeleton. These bundles are thought to provide mechanical tension and might take part in cellular mechanosensing. To test the functions of these bundles we cultivated endothelial cells on soft silicone elastomer films. Strain was applied to cells by either globally stretching the whole elastomer film [1] or by locally deforming it with a microtool [2]. In both methods actin bundles are stretched. Resulting molecular rearrangements were studied by immuncytochemistry and by live cell imaging of cells transfected with zyxin, alpha-actinin or VASP fused to fluorescent proteins. We observed strain-dependent formation of zyxin-rich striations within actin bundles similar to [3]. Colocalization of zyxin, alpha-actinin and vinculin on actin bundles was quantified by digital image processing and the time course of dissociation was determined. From these data we conclude that zyxin and VASP act in close concert while alpha-actinin plays a different role.

[1] Faust et al PLoSOne 6 (2011) e28963
[2] Kirchenbüchler et al J. Phys. Cond. Mat. 22 (2010) 194109
[3] Hoffman et al. Mol. Biol. Cell 23 (2012) 1846-1859

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Modeling Mechanotransduction Signaling through Actin Filament Network Deformation Linked to Biochemical Response

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It is becoming more and more evident that the mechanical forces previously taken for granted actually play a pivotal role in influencing biological phenomenon from cancer metastases to vasculogenesis. Recent literature provides strong evidence for a causative link between the mechanical stretching of the cytoskeleton and the release of mechanotransductive signaling molecules. Understanding the links between mechanical input, the corresponding morphological changes in the actin cytoskeleton, and the resulting biochemical response is not well understood yet is a significant challenge in the field of mechanotransduction. Here, we present a model that integrates actin filament network remodeling under stretch with a novel biophysical model of molecular release to further elucidate the interplay between actin network morphology and resultant biochemical signaling.