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[MgATP] from 1 mM to 0.02-0.05 mM did indeed reduce the L_P^P from 10-12 µm to 6-7 µm for both phalloidin-stabilized and phalloidin free actin filaments. Additionally, we found a negative correlation between the L_P^S and the [HMM]/actin ratio. However, this [HMM] dependent reduction observed in L_P^S was too small to account for the reduction in L_P^P seen with reduced [MgATP] in the IVMA. Monte-Carlo simulations and theoretical analysis revealed that the large reduction in L_P^P is consistent with the idea that every head attachment adds an extra angular displacement.

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1500-Pos Board B451

Phosphomimic S3D Cofilin Binds Actin Filaments but does not Sever them

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Cofilin-mediated remodeling of the actin cytoskeleton is critical for many cellular processes. Cofilin changes the filament structure and renders them more compliant in bending and twisting. Filaments partially decorated with cofilin sever more readily than bare or saturated ones, supporting a model where severing occurs preferentially at mechanical and topological boundaries between bare and cofilin-decorated segments. Phosphorylation of cofilin at serine 3 by LIM kinase is a well-established mechanism for regulating cofilin activity. Substitution of serine with aspartate at position three (S3D) is widely used for investigating the mechanism of cofilin phospho-regulation in cells and in biochemical studies with purified protein components. The S3D substitution or phosphorylation weakens cofilin binding to actin filaments, and it is thought that subsequent reduction in cofilin occupancy inhibits filament severing activity. Here, we show that S3D cofilin binds actin filaments with a lower affinity than wild-type (WT) cofilin, but with higher cooperativity. Because of its higher cooperativity, S3D cofilin will form larger clusters along filaments than unmodified cofilin and thus have fewer boundaries between bare and decorated segments where severing occurs. S3D decoration weakly affects filament bending and twisting dynamics, in contrast to WT cofilin. Weak actin filament severing is observed for S3D across a range of occupancies. Reduced boundary density and inability to alter filament mechanics make S3D severing deficient.

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Palladin's Ig4 Mutation: Exploring the Link with Pancreatic Cancer

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Palladin is a recently discovered protein that is expressed in human cells and plays a key role in cytoskeletal dynamics by directly binding and bundling filaments of actin. These processes provide an important function in cell motility and are made possible by the Ig3 and Ig4 domains of palladin. Since cancer survival is often dependent upon migration of cancerous cells to other parts of the body, palladin has been implicated as playing a critical role in cancer metastasis. In addition, a mutation from a conserved tryptophan to cysteine in palladin's Ig4 domain has recently been linked to a form of pancreatic cancer. This mutation is called ''PaTu2,'' and understanding how it affects palladin is the focus of this research project. We began by isolating the wild type and mutant Ig4 and Ig3-4 domains of palladin. Obtaining the PaTu2 mutant protein required a different approach due to the protein remaining insoluble within inclusion bodies in E. coli bacteria after cell lysis. This was amended by incorporating a maltose-binding protein tag to increase solubility so that the protein could undergo affinity purification. Next, we determined whether the mutation affected palladin's ability to bind and bundle actin filaments by conducting cosedimentation assays, with initial results showing no significant difference when compared to the wild-type. Future directions of the project include using nuclear magnetic resonance and circular dichroism spectroscopy to see if the mutation affects the structure and stability of palladin. Such analysis will provide the necessary data that lead towards a greater understanding the cause of metastatic pancreatic cancer.

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Dynamic Actomyosin Network Morphology in 3D Model of Cytokinetic Ring Assembly

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At the onset of cytokinesis in fission yeast (a model organism for studying mitosis), the locations of actin filament nucleation by formins shift from the cell tips to the cell middle. These actin filaments are captured by myosin motors bound to medial cortical nodes and bundled by crosslinking proteins, in order to form a ring. The myosin motors exert forces on the actin filaments, resulting in net pulling of nodes and filaments towards the cell equator, while cross-linking interactions help align actin filaments into a single bundle. We used these mechanisms in a 3D computational model of ring assembly, with semiflexible actin filaments growing from formins at the cell tips and at the medial cell cortex, capturing and pulling of filaments by nodes, and cross-linking among filaments through attractive interactions. The model was used to predict profiles of actin density at the cortex, morphologies of condensing node-filament networks, including the transition from actin cables to ring in different conditions of myosin activity and strength of crosslinking. Results show that cross-linking interactions can lead to confinement of actin filaments that grow from medial locations of the cell cortex. We show that the ring formation region in parameter space lies close to regions leading to clumps, meshworks or double rings, and stars/cables. Since boundaries between regions are not sharp, transient structures that resemble clumps, stars and meshworks can appear in the process of ring assembly. The presence of transient nonmedial cables linking the medial region to the cell tips can further modify these boundaries. These results are consistent with prior experiments with mutations in actin filament turnover regulators, myosin motor activity and changes in the concentration of cross linkers that alter the morphology of the condensing network.

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Human Tumor-Associated Fibroblasts can Sense the Topography of their Environment

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Cells can sense and adapt to mechanical properties of their environment. The local geometry of the extracellular matrix can control the motility, shape, and cytoskeletal structure of cells. In particular, surface topography has been shown to modulate cell morphology, migration, and proliferation. However, the underlying mechanisms by which topography is sensed by cells remain unclear. Here we investigate the effect of surface topography on the morphology and cytoskeletal dynamics of human pancreatic tumor associated fibroblast cells (TAFs). TAFs have been shown to promote the progression of pancreatic tumors, metastasis, and resistance to therapy. Mechanisms by which these cells stimulate invasiveness and metastasis of cancer cells are not well understood. We have previously shown that these cells can mechanically sense the stiffness of their environment. In this study, we used an arrangement of parallel ridges (nanopatterns), with variable spacing between the ridges, to investigate the response of pancreatic TAFs to the topography of their environment. We found that TAFs align along the direction of the ridges and modulate their shape depending on the spacing between the ridges. Fluorescence imaging of cells labeled with EGFP-palladin (an actin crosslinking protein) further revealed that actin stress fibers are organized along the nanopatterns and that the degree of this alignment is dependent on the spacing between the ridges. In order to obtain a deeper understanding of the underlying mechanisms, we tracked the movement of palladin structures in cells as this enables the measurement of cytoskeletal dynamics as a function of surface topography. Our results provide insight into the mechanisms of how cells sense and respond to substrate topography.