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forces do not. These results indicate that the structure of actin networks is permanently altered by different external forces, giving rise to very different mechanical properties. Our AFM-TIRF measurements provide new insight into the role of force in the assembly and mechanical properties of dendritic actin networks.

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Toxoplasma Gondii Targets the Host Actin Cytoskeleton during Invasion, GO Figure

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T. gondii belongs to the phylum apicomplexa, which also includes Plasmodium, the agent of malaria. T. gondii quickly establishes infection as a fast-replicating form, the tachyzoite, which invades numerous cell types throughout the body. The parasite can cause encephalitis and neurologic diseases. Unlike viruses and intracellular bacteria, Toxoplasma actively penetrates the host cell without using typical host-uptake pathways that involve coated pits or actin based engulfment. During invasion, the extracellular parasite triggers the assembly of a unique zoite-cell junction and in less than 20 sec progressively propels its ~ 2um long body through the junction into the growing vacuole. How the growing PV and its parasite passes through the barrier of the host cell cortical actin remains unknown.

Here we provide evidence that Toxofilin, an actin-binding protein secreted by the invading parasites into the host cell cytoplasm at the onset of invasion, targets the host cortical actin cytoskeleton to facilitate vacuole folding and tachytozoites invasion. Indeed, Toxoplasma tachyzoites lacking toxofilin exhibit delayed invasion kinetics. Correlative light and electron tomography allowed tracking toxofilin in action within the host cell and showed that integrity of the host cortical actin cytoskeleton was disrupted at the site of parasite entry, suggesting that toxofilin induces actin depolymerization in close proximity to the zoite apex at the onset of invasion. Quantitative fluorescent speckle microscopy indicates that toxofilin facilitate tachyzoite invasion by upregulating host cortical actin filament turnover, consistent with toxofilin monomer sequestration and barbed end filament-capping capabilities in vitro. The ability of toxofilin to expedite invasion by regulating the turnover of cortical actin filaments in the host cell is likely to confer a selective advantage to the parasite.

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Probing the Interaction between the Auto-Inhibitory Filamin a Immunoglobulin Repeats Pair 20 and 21 under Tension

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The Immunoglobulin Repeats 16-23 of Filamin A (FLNa) interact with numerous binding partners. Recent studies suggest that inter-domain interactions of these repeats under tension is crucial for the mechanosensing function of FLNa: the accessibility by FLNa binding partners is inhibited when the domain pairs are in closed conformation or permitted when they are in opened conformation. For example, it was proposed that in a closed stacked conformation, the A-strand of IgFLNa 20 interacts with the CD interface of IgFLNa 21, blocking its accessibility by other molecules such as b7 integrin tail and GP1Ba peptide. Prompted by these results, we examined the inter-domain interaction between repeats 20 and 21 (IgFLNa 20-21) using a magnetic tweezers setup. We show that the stacking between the two repeats can be disrupted at ~15 pN at a loading rate range 0.1 - 2.1 pN/second and it can reform at decreased force values. Our results support the previously proposed force-dependent regulation of the accessibility of cryptic binding sites in the domain pair 20 and 21 of FLNa, which may have broad biological implications in the mechanisms of other mechanosensing proteins.

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Myosin Assembly Regulation by 14-3-3

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The regulation of non-muscle myosin II is key to a number of processes, including cell migration, tissue morphogenesis, and cytokinesis. We have been using the social amoeba *Dictyostelium discoideum* as a model organism to study how myosin II is regulated in cytokinesis. One major pathway that we have discovered is the racE–14-3-3 pathway, which maintains the uniformity of myosin II at the cortex and increases myosin II turnover, as shown by FRAP analysis and *in vitro* studies. We have demonstrated that 14-3-3 associates directly with the myosin II assembly domain to inhibit bipolar thick filament (BTF) assembly. This effect is observed in wild-type as well as in constitutively assembled or unassembled mutant forms of myosin II, and the exact 14-3-3 binding site is being mapped by mutational analysis. Furthermore, a bioinformatics analysis has predicted a similar 14-3-3 binding site in the mammalian non-muscle myosin IIA and IIB assembly domains. We are investigating if the 14-3-3–myosin II interactions that we have observed in *Dictyostelium* are also present in the mammalian system. We are thus pursuing a possible conserved mechanism for phosphorylation-independent regulation of myosin assembly by 14-3-3 proteins.

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Dynamics of Non-Muscle Myosin II Organization into Stress Fibers and Contractile Networks

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The cellular morphology of adhered cells critically depends on the formation of a contractile meshwork of parallel and cross-linked stress fibers along the contacting surface. The motor activity and mini-filament assembly of non-muscle myosin II is an important component of cell-level cytoskeletal remodeling during mechanosensing. To monitor the dynamics of non-muscle myosin II, we used confocal microscopy to image cultured HeLa cells that stably express myosin regulatory light chain tagged with GFP (MRLC-GFP). MRLC-GFP was monitored in time-lapse movies at steady state and during the response of cells to varying concentrations of blebbistatin which disrupts actomyosin stress fibers. Using image correlation spectroscopy analysis, we quantified the kinetics of disassembly and reassembly of actomyosin networks and compared them to studies by other groups. This analysis suggested that the following processes contribute to the assembly of cortical actomyosin and stress fibers: random myosin mini-filament assembly and disassembly along the cortex; myosin mini-filament aligning and contraction; stabilization of cortical myosin upon increasing contractile tension. We developed simple numerical simulations that include those processes. The results of simulations of cells at steady state and in response to blebbistatin capture some of the main features observed in the experiments. This study provides a framework to help interpret how different cortical myosin remodeling kinetics may contribute to different cell shape and rigidity depending on substrate stiffness.

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Myopodin has a Nucleolar Localization in Several Mammalian Cell Lines

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Synaptopodin 2 is a natively unfolded actin binding protein that stimulates actin polymerization in a Ca^{2+}-calmodulin dependent manner, has multiple binding partners and is rich in smooth muscle dense bodies. Various synaptopodin 2 isoforms have cytoplasmic and nuclear localizations in non-muscle cells and the distribution changes in certain cancers. We prepared an antibody against the C-terminus that recognizes isoform B and myopodin, the truncated form. Immunological staining showed a nuclear location in several cell lines. In further studies with the colon cancer cell line, HT-29 and African green monkey kidney cells, CV1, we observed intense staining in irregular areas within the nucleus. Staining was also observed at the nuclear periphery of HT-29 cells. The strong synaptopodin 2 staining pattern was 90% correlated with markers of nucleoli including a commercial antibody against nucleolin and a counter stain with DAPI. Furthermore, when Actinomycin D was used to inhibit transcription the nuclear stain disappeared. When HT-29 cells were forced to differentiate with sodium butyrate, the synaptopodin 2 antibody revealed a re-localization of the protein into the cytoplasm. RT-PCR and Western blot analysis showed that HT-29 cells lacked Synaptopodin2B, but contained the smaller isoform, myopodin. These studies were performed using cytoplasmic and nuclear fractions. Further analysis showed that the nuclear fraction contained a smaller cross-reactive protein with an apparent mass of 55-60 kDa. Both myopodin and the 60 kDa protein were immunoprecipitated along with proteins tentatively identified as actin and α-actinin. These results show that myopodin and a smaller synaptopodin-like protein are present in nucleoli along with actin and other actin binding proteins.