binding of myosin II induces conformational changes in subdomain 2 and the proteolytic digestion of the D-loop disturbs the motor function of myosin II. However, although as many as 24 classes of myosin have already been found and their *in vivo* roles are completely different, the contribution of the D-loop to actin-myosin interaction has so far been studied only for myosin II.
In this study, to determine whether the D-loop contributes to the interaction with myosin V and if so, in what way it affects its motor function, we prepared actins modified in the D-loop and analyzed the effects of modifications on the motile properties of myosins II and V. We found that the D-loop modifications, namely, the proteolytic digestion with subtilisin and the M47A point mutation, significantly decreased the gliding velocity on myosin II-HMM in an *in vitro* motility assay, due to a weaker generated force. On the other hand, single molecules of myosin V "walked" with the same velocity on both the wild-type and modified actins; however, the run lengths decreased sharply, correlating with a lower affinity of myosin for actin due to the D-loop modifications.These results show that the D-loop strongly modulates the force generation by myosin II and the processivity of myosin V, presumably affecting actin-myosin interaction in the A.M.ADP.P_i state of both myosins. Our findings are important to understand the principles how an actin molecule may regulate diverse *in vivo* functions of various myosin isoforms.

2891-Pos
Spontaneous Oscillations of a Minimal Acto-Myosin System Under Elastic Loading
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Spontaneous mechanical oscillations occur in various types of biological systems where groups of motor molecules are elastically coupled to their environment. By using an optical trap to oppose the gliding motion of a single bead-tailed actin filament over a substrate densely coated with heavy meromyosin molecules, we mimicked this condition in vitro. We show that this minimal acto-myosin system can oscillate spontaneously. Our finding accords quantitatively with a general theoretical framework where oscillatory instabilities emerge generically from the collective dynamics of molecular motors under load.

2892-Pos
Kinetic Characterization of Non-Muscle Myosin IIB Single-Headed Heavy Meromyosin on Single Molecule Level with Optical Tweezers
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Non-muscle myosin IIB (NMIIB) is a cytoplasmic conventional myosin, which plays an important role in development of the brain and heart, and in directed growth cone motility by maintaining cortical tension in motile cells. It forms short bipolar filaments with ~14 myosin molecules on each side of the bare zone. NMIIB is a very slow myosin both in terms of actin-activated ATPase activity and actin translocation capability. Our previous studies showed that the NMIIB is a moderately high duty ratio (at least 20-25%) motor. The ADP release step (~0.35 s-1), of NMIIB is only ~3 times faster than the rate-limiting phosphate release (0.13 $\pm$ 0.01 s-1). Because of its slow ADP off-rate, acto-NMIIB has the highest ADP-affinity reported so far for the myosin superfamily ($<$0.15 μM). To examine the mechanics and kinetics of NMIIB at the single-molecule level we used a dual-beam optical tweezer to perform the "three-bead" assay. The surface-immobilized bead was coated with recombinantly engineered single-headed heavy meromyosin-like (NMIIB-SH-HMM) molecules. We measured the lifetimes of unitary actomyosin interactions and determined the actin-detachment kinetics with varying ATP concentrations. Results showed that at physiological ATP concentration (1 mM), the rate of detachment of acto-NMIIB-SH-HMM interactions was ~0.51 s-1, similar to the ADP release rate and steady-state ATPase rate reported from solution kinetic studies. Decreasing the ATP concentration to 1 μM did not alter this rate of detachment (~0.47 s-1). Also, our results showed that the power-stroke of NMIIB-SH-HMM was ~8 nm. We will discuss our single-molecule results from the perspective of the essential cellular functions of NMIIB in cell locomotion, tension generation and maintenance.

2893-Pos
Myosin-1 Function Extends to Microtubule-Dependent Processes
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Class 1 myosins are motor proteins found on various organelles and at defined structures at the cell periphery, where they play important roles in organelle translocation, intracellular transport, and cytoskeleton organization. Here, we investigated the cellular function of *Dictyostelium* myosin-1C, a long-tailed, monomeric motor with three tail homology domains called TH1, TH2, and TH3. We identified that in addition to its actin-dependent function in endocytotic processes during interphase, in *Dictyostelium* cells the motor co-localizes with centrosomes and spindle microtubules (MT) during mitosis. *In vitro* TIRF microscopy experiments and MT-cosedimentation assays using truncated myosin-1C tail constructs revealed a direct binding of myosin-1C tail to MTs. Only constructs containing TH1 and TH2 bound efficiently to MTs. Moreover, these two domains were sufficient to prevent MT depolymerization at low nanomolar concentrations of myosin-1C tail, while MT formation, i.e. nucleation and elongation, was unaffected. Additionally, we observed myosin-1C tail mediated cross-linking of MTs to F-actin. In cells, myosin-1C constructs lacking the motor domain did not associate with the spindle. This demonstrates that actin-dependent motor function is required for the cell cycle-dependent relocalization of myosin-1C from actin-rich structures at the cell periphery to MT-associated mitotic structures. Cells producing a hydrolysis deficient full-length myosin-1C mutant exhibited reduced growth rates with increased size of nuclei due to defects in spindle alignment and prolonged mitosis. Single kinesin molecule motility assays showed that MT-bound myosin-1C reduced the attachment rate of kinesin-1 to MTs without affecting its velocity and run-length. From this we propose that myosin-1C may regulate MT-dependent motility and MT dynamics. Our cell biological and functional characterization of a long-tailed class 1 myosin shows that myosin function is not limited to the F-actin network but extends to MT associated processes.

2894-Pos
Myo1e Binds Anionic Phospholipids with High Affinity
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Myo1e is a single-headed motor protein that has been shown to play roles in clathrin-mediated endocytosis in HeLa cells (Krendel *et al.* 2007. *FEBS Letters*. 581:644-650) and podocyte function in the kidney (Krendel *et al.* 2009. *J. Am. Soc. Nephrol.* 20:86-94). The myo1e C-terminal tail domain includes a basic region that is required for localization to clathrin-coated vesicles and is homologous to regions of other myosin-1 proteins that have been shown to bind phospholipids. However, the phospholipid binding properties of myo1e have not been examined. We used sedimentation assays and stopped-flow fluorescence to determine the membrane binding affinities and kinetics of a fluorescently labeled recombinant myo1e-tail construct. We found that the myo1e-tail binds tightly ($K_{eff}^{lipid} < 5$ μM) to large unilamellar vesicles (LUVs) containing physiological concentrations of the anionic phospholipids phosphatidylinositol (4,5)-bisphosphate (PIP_2) and phosphatidylserine (PS). Unlike myo1c, myo1e can also bind to physiological concentrations of PS in the absence of PIP_2. While myo1e has a slightly higher affinity for PIP_2 over PS, this selectivity is much less than observed with myo1c, which contains a putative pleckstrin-homology (PH) domain and shows strong specificity for phosphoinositides. Soluble inositol phosphate headgroups, such as inositol (1,4,5)-trisphosphate, can compete with PIP_2 for binding, but the apparent affinity for the soluble inositol phosphate is substantially lower than that for PIP_2. The rate of myo1e attachment to LUVs is similar to that of myo1c, but the rate of detachment from LUVs is slower than that found for myo1c. The high affinity of the myo1e-tail for phospholipids suggests that, *in vivo*, myo1e is strongly attached to membranes where it plays a role in endocytosis and other physiological processes.

2895-Pos
Control of Myosin-I Force Sensing by Alternative Splicing
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Tension sensing by myosin motors is important for numerous cellular processes, including control of force and energy utilization in contracting muscles, transport of cellular cargos, detection of auditory stimuli, and control of cell shape. Myosins have evolved different tension sensitivities tuned for these diverse cellular tasks, thus it is important to determine the mechanisms and regulation of force sensing within the myosin superfamily. In this study, we examined force sensing by the widely expressed myosin-I isoform, myo1b, which is alternatively spliced in its light chain binding domain (LCBD), yielding proteins with lever-arms of different lengths. We found that the step sizes of the myo1b proteins are not linearly related to the number of IQ motifs in the LCBD, suggesting that splicing introduces a structural feature into the LCBD that affects the lever arm size. We also found the actin-detachment kinetics of the splice isoforms to be extraordinarily tension sensitive, with the magnitude of tension sensitivity linearly related to lever arm length. Thus, in addition to regulating step-size, motility rates, and myosin activation, the LCBD is a regulator of force sensing. Finally, we found that myo1b is substantially more

tension sensitive than other myosins with similar length lever arms, suggesting that different myosins have different tension-sensitive transitions.

2896-Pos
Myosin-I Dependent Membrane-Cytoskeleton Adhesion as a Regulator of Cell Surface Morphology
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All cellular functions involving deformation of the plasma membrane (e.g. endocytosis, exocytosis, the formation of surface protrusions) are regulated by the apparent membrane tension (T_m), a composite of the surface tension (σ) and the adhesion provided by molecules linking membrane to the actin cytoskeleton (γ). Using an optical trap based tether force assay, we recently demonstrated that class I myosins, a family of membrane-binding actin-based motor proteins, control membrane tension by mediating membrane-cytoskeleton adhesion. More specifically, these studies revealed that the membrane-cytoskeleton adhesion provided by myosin-I increases apparent membrane tension as indicated by the force required to pull a single membrane tether from the cell surface. Interestingly, the physical links to the cytoskeleton provided by myosin-I also allow the cell to form multiple adjacent tethers. However, when multiple tethers are pulled, tether lifetimes appear to decrease as apparent membrane tension increases. Here we present a thermodynamic model, which accounts for the impact of apparent membrane tension on the lifetime of multiple tethers. We argue that, in the context of our experimental geometry, the global increase in apparent membrane tension that results from greater membrane-cytoskeleton adhesion, works locally to accelerate the rupture of bonds between the membrane and cytoskeleton, which would otherwise prevent individual membrane tethers from coalescing. We also elaborate on this concept to develop a separate model, which shows that tuning the level of membrane-cytoskeleton adhesion may enable cells to vary the density of surface protrusions (i.e. # of structures per unit area membrane). As such, myosin-I dependent membrane-cytoskeleton adhesion emerges as the key regulator of cell surface morphology.

2897-Pos
Kinetics of Myosin-I-Membrane Detachment Under Load
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Myo1c is a single-headed motor that links cell membranes to the underlying actin cytoskeleton. Actin binding occurs via the motor domain, while the tail domain interacts with phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) through a putative pleckstrin homology (PH) domain. In this study, we measured the strength and lifetime of the myo1c attachment to spherical supported lipid bilayers (SSL) composed of 1 μm diameter silica or polystyrene beads coated with various mole fractions of PI(4,5)P2, dioleoyl-phosphatidylcholine (DOPC), and dioleoyl-phosphatidylserine (DOPS). The SSLs trapped by a laser beam were brought into contact with immobilized spherical (2 μm) silica pedestals sparsely labeled with the myo1c tail domain. Upon repeated contact and retraction cycles, binding events and subsequent rupture forces were measured. The most probable rupture forces (< 10 pN) from membranes containing 2% PI(4,5)P2 were found to be largely independent of the loading rate (pN/s). Nevertheless, the frequency of single myo1c molecules interacting with membranes containing 2% PI(4,5)P2 - 98% DOPC was considerably higher than with membranes that contained 20 - 80% DOPS. Lifetime measurements of myo1c attachment to 2% PI(4,5)P2 - 98% DOPC membranes under constant pulling forces (1 - 3 pN) were fitted to Bell's equation and the extrapolated duration of the bond at zero force was found to be ~ 25 ms. Although phosphoinositide binding is crucial for the proper cellular targeting of myo1c, our results suggest that it is unlikely that this connection is a suitable anchor for force generation.

2898-Pos
Kinetics and Thermodynamics of Nucleotide Binding Pocket Opening/closing in Myosin V Monitored with FRET
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Kinetic and structural studies of both muscle and non-muscle myosins have revealed that the enzymatic cycle of these motors frequently contains more than one actomyosin ADP state. Interestingly, the rate of ADP release in myosin motors is thought to be the main determinant of sliding velocity in muscle, suggesting strain dependent ADP release may be a critical mechanism of mechanochemical coupling. Our previous work has demonstrated that labeling myosin V in the upper 50 kDa domain with the biarsenical dye FlAsH (MV FlAsH) can serve as an acceptor for fluorescence resonance energy transfer studies with mant labeled nucleotides. We also determined that this donor-acceptor pair likely monitors opening/closing of the nucleotide binding pocket. Currently, we utilized the FRET signal to examine the kinetics of nucleotide binding pocket opening during the process of mantADP release from acto-MV FlAsH. We obtained evidence that the nucleotide binding pocket goes from a closed to an open conformation prior to the release of ADP. We also explored the temperature dependence of the closed to open transition and nucleotide release steps. We find that at lower temperatures the closed conformation is favored while at higher temperature the open conformation is favored. The more rapid ADP release step which follows nucleotide binding pocket opening is also temperature dependent. Therefore, since both steps are temperature-dependent they likely require significant conformational changes. We also compared our FRET results to the rate of ATP-induced dissociation from actin in the presence of ADP monitored by light scatter. Understanding how strain alters either of these two steps may be critical for elucidating the structural mechanism of strain-dependent ADP release in myosins.

2899-Pos
Coupling the Actin Binding Cleft and Nucleotide Binding Pocket in Myosin V
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Previously we have demonstrated in fluorescence resonance energy transfer (FRET) studies that mant labeled nucleotides and IAEDANS actin can act as good donor probes for a FlAsH labeled acceptor site in the upper 50 kDa domain of myosin V. We examined the temperature dependence of the FRET signal between mantADP and MV WT FlAsH in the presence and absence of actin. We found that at low temperature (4-15°C) a high FRET state dominates (closed pocket) while at high temperature (30-35°C) a low FRET state dominates (open pocket). This transition is reversible suggesting a temperature-dependent conformational change. However, the mutant E442A, which is incapable of hydrolyzing ATP, remains in a high FRET state (closed pocket) with mantATP bound in the presence or absence of actin. Our results suggest a more flexible conformation of myosin in the presence of ADP compared to ATP which allows myosin to populate two actomyosin.ADP state conformations. These results are supported by the lifetime FRET analysis, and by computational FIRST/FRODA analysis of the intrinsic flexibility found in different x-ray crystal structures. We also plan to explore the temperature dependent conformational dynamics of the actin binding cleft using the IAEDANS actin (donor) and MV FlAsH (acceptor) pair in the presence of ATP, ADP, and absence of nucleotide using steady state and lifetime based FRET measurements. Our results will provide critical insights into the mechanocoupling that may occur between the nucleotide-binding pocket and actin binding cleft in myosin motors.

2900-Pos
Interaction of a Class V Myosin from Budding Yeast with its Adapter Protein
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Like their mammalian counterparts, class V myosins in *S. cerevisiae* (Myo2p and Myo4p) bind to various adapter proteins to target a particular cargo for transport. Myo4p uses the adapter proteins She3p and She2p in order to transport mRNA from the mother cell to the bud. She3p binds to the rod of Myo4p, and prevents it from dimerizing, thus forming a single-headed motor complex (Hodges et al., 2008; Bookwalter et al., 2009). Because the Myo4p/She3p complex is single-headed, the question arises as to whether enough motors can bind to a single She2p to enable continuous cargo transport. The She2p crystal structure suggested that She2p exists as a dimer (Niessing et al., 2004). In contrast, our sedimentation equilibrium measurements of She2p were consistent with formation of a tetramer in solution, in principle allowing for binding of four motor heads. We showed that Myo4p/She3p forms a complex with tetrameric She2p in the absence of mRNA, based on sedimentation velocity experiments and co-purification. Mutation of Ser 120 to Tyr converts She2p to a dimer. The ability of the motor complex to bind to this and other She2p mutants is being tested in order to map the binding interface. Total internal reflection fluorescence microscopy is being used to test whether the native She2p tetramer can bind enough single-headed motors to support continuous movement on actin. The ability of She2p mutants to support correct bud tip localization of *ASH1* mRNA in living yeast cells will also be assessed. These studies will help elucidate how a non-processive single-headed motor can act as a cargo transporter.

2901-Pos
The Mechanical Properties of a Single Myosin V Motor Domain During Gait Motion
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