determine the high resolution crystal structure of the human spastin protein both alone and in complex with a domain of tubulin. To this end, we have expressed and purified a construct of human spastin and a C-terminal tubulin construct, to which spastin binds. We have also crystallized spastin and collected a 3.3 angstrom data set and are now attempting to crystallize the spastin-tubulin complex. Visualization of such a complex will provide details as to how spastin binds to and severs microtubules, and will also help to explain how point mutations in the spastin gene lead to altered protein activity and a disease phenotype.

1880-Pos
Characterization of Tau Interactions with Lipid Vesicles
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A number of neurodegenerative diseases, including Alzheimer's disease, are associated with the deposition of aggregated tau protein in the form of neurofibrillary tangles (NFTs). Although a causal relationship between NFTs and disease has not been conclusively resolved, it is hypothesized that either the aggregates themselves or the process of aggregation is pathological. Tau is an intrinsically unstructured protein expressed in neurons, primarily functioning to stabilize and catalyze microtubule assembly. Studies indicate that tau binding of lipid vesicles may serve as a mimic of microtubule binding. We measured the affinity of the various constructs of tau encompassing the microtubule binding region to synthetic lipid vesicles using fluorescence correlation spectroscopy (FCS). Importantly, we observe that the binding behavior is dramatically affected by solution pH. At pH 7.4, the protein binds stably and we are able to extract a partition coefficient for both wild-type and the disease-associated point mutant, P301L. However, at low pH, binding to lipid bilayers triggers rapid aggregation of the tau fragments, which we were able to confirm as amyloid using Thioflavin T binding and electron microscopy.

1881-Pos
Tubulin is an Amphiphilic Protein Whose Interaction With Membranes is Regulated by its Charged Carboxy-Terminal Tails
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Tubulin is an acidic heterodimeric protein whose negative charges are significantly (~40% of total negative charge) located on the 10-15 residue long, glutamic acid-rich, unstructured, carboxy-terminal tails (CTT) found on both α- and β-subunits (though not on the monomeric γ-tubulin). Unsurprisingly, tubulin is a quite water-soluble protein. However, it has been consistently reported to be a component of highly purified membranes, including plasma membranes, intracellular membranes such as Golgi and mitochondrial outer membranes, and vesicle membranes as in clathrin-coated endocytic vesicles. In these preparations, tubulin is non-microtubular and is tightly associated with the membrane, often requiring detergent for solubilization. Tubulin has also been shown to associate tightly with liposomes make of purified lipid only, including neutral lipid. The exact mechanism of tubulin-membrane association has not been defined, nor has it been shown that there is only one mechanism. Tubulin could dock with lipid-embedded proteins, for example. We have shown that tubulin binds to VDAC in the mitochondrial outer membrane, with functional consequences for mitochondrial function, and this binding requires and is mediated by the CTT. Thus the CTT can enhance membrane binding of tubulin. This cannot be the mechanism for liposome interaction, since there is no protein present other than tubulin. We show by charge-shift electrophoresis and non-ionic detergent extraction that (a) tubulin behaves as an amphiphilic protein, and (b) the CTT can regulate interaction with amphipathic molecules.

Cell & Bacterial Mechanics & Motility II

1882-Pos
Mechanics of the Cell Nucleus as a Function of Lamin Expression in Granulocyte Differentiation
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The ability of cells to deform through narrow spaces is critical for processes ranging from immune function to metastasis. Neutrophils are the most abundant white blood cell, which are required to transit through spaces less than 1/5 of the cell's diameter. As the nucleus is typically the stiffest organelle in the cell, the lobulated shape of the neutrophil nucleus is thought to facilitate its transit. However, neither the mechanical properties of the nuclei, nor the mechanism underlying the transition from ovoid to lobulated nuclear shape, are fully understood. We used HL60 cells as a differentiable model system to study nuclear shape transitions and the effects on cell mechanics. To elucidate the effects of the nuclear envelope protein, lamin A, we genetically modified the cells to generate subpopulations of cells with well-defined lamin A levels. Quantitative image analysis revealed that increased lamin A expression inhibits the nuclear shape transition to the typical lobulated morphology. To determine the effect on whole-cell mechanics, we measured cell deformability by flowing cells through channels of a microfluidic device, monitoring transit time and nuclear deformation. In addition, we performed functional assays to test if the impaired transition in nuclear morphology is also associated with defects in phagocytotic function, and thus reflect overall impairment of differentiation due to increased lamin levels. These results help to elucidate the molecular mechanism of granulocyte differentiation, and may have possible implications for understanding reduced immune function in aging, where lamin A has been reported to accumulate at the nuclear envelope.

1883-Pos
Model for Microtubule-Actin Interactions in Growth Cone Motility
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A growth cone is a motile structure on the tips of axons that guides axon extension to synaptic targets during nervous system development. In order to translate chemotactic signals into a mechanical response, the microtubule and actin filaments in the growth cone self-organize into a motile lamellipodial structure. A meshwork of actin filaments in the lamellipodium are continually transported inward by myosin-driven forces, at a speed that matches actin polymerization at the leading edge. This creates a stationary actin treadmill when actin adhesion to the substrate is low, and allows leading edge protrusion when actin adhesion increases in response to guidance cues. A population of highly dynamic microtubules that explore the P domain in stochastic bursts of growth and shrinking have also been shown to play an essential role in growth cone steering. Cooperation between these two filament systems is known to be essential for directed motility. We present initial results from a theoretical model of the growth cone cytoskeleton in the lamellipodium, testing the hypothesis that dynamic microtubules and actin work cooperatively to guide growth cone motility. We simulate dynamically unstable microtubules that transiently attach to actin retrograde flow, actin-myosin-adhesion force balance, and test several scenarios for feedback between microtubules and actin. Our theoretical work is guided by direct visualization of actin and microtubule dynamics during growth cone advance with fluorescent speckle microscopy.

1884-Pos
Examining Mechanical Properties of Vertebrate Meiotic Spindles
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Accurate chromosome segregation during cell division relies on the self-organization of a dynamic multi-component apparatus, the microtubule-based bipolar spindle. Through genetic, biochemical, and cell biological approaches, the complete 'parts list' of the components, including microtubule-based motor proteins, which are responsible for the assembly and maintenance of the meiotic spindle are now available. However, how spindle self-organization is controlled through integration of forces generated by multiple biomolecular processes remains mysterious. Here we report a calibrated microneedle-based system that allows application of sub-nanoNewton forces at specific sites within the metaphase spindle assembled in Xenopus egg extracts. Our set-up allows direct force measurements and can be combined with multi-mode high resolution microscopy along with chemical perturbations of specific spindle components. Using this system we applied sinusoidal strain to the metaphase spindle, keeping the range $<5\%$ to minimize nonlinearities of the response. The stress response was measured over the frequency ranged between 0.01 Hz and 2 Hz. Based on the resultant stress-strain relationship we determined the frequency-dependent mechanical properties of the spindle. The spindle response showed a typical characteristic time-scale of ~50 s, at which the mechanical property changed from solid-like to liquid-like. Additional experiments examining stress relaxation, which may reflect the dynamics of structural reorganization, revealed a similar time-scale. This transition disappeared when the spindles were treated with AMPPNP, a slow-hydrolyzing ATP analog, suggesting that the characteristic time-scale is determined by an ATP-dependent processes within the spindle. The contribution of key mitotic motors such as dynein and kinesin is being examined by pharmacological or immunological perturbations. Together, these analyses will allow us discuss models for how forces in the self-organizing meiotic spindle are integrated.