in metazoans. In contrast, yeast cytoplasmic dynein is involved in a single, nonessential function, nuclear positioning. Interestingly, whereas mammalian isoforms exhibit a stall force of 1-2 piconewton (pN), *S. cerevisiae* dynein stalls at 5-7 pN. In addition, in the absence of load, mammalian dyneins move faster than yeast dynein (800-1,100 nm/s *vs.* 100 nm/sec respectively), and, under opposing force, maintain attachment to microtubules much less tenaciously (milliseconds to seconds *vs.* tens of seconds, respectively). The basis for these functional differences is unknown. However, the major structural difference between mammalian and yeast dyneins is an ~30 kDa C-terminal extension (CT) present in higher eukaryotic dyneins, but missing in yeast. To test whether the CT accounts for the differences in function, we produced recombinant rat dynein motor domains (MD) with (WT-MD) and without (**Δ**CT-MD) the CT region, using a baculovirus expression system. Amino-terminal glutathione S-transferase (GST) tags induced formation of a dimeric, "two-headed" motor. We found that, like yeast dynein, the **Δ**CT-MD ATPase lacks the signature vanadate inhibition characteristic of higher eukaryotic dyneins, and exhibited a strikingly higher Km(ATP). To characterize motor function, we performed single-molecule optical trapping studies. Single WT-MD stalls at ~1 pN and detaches from microtubules after brief stalls. In sharp contrast, but similar to yeast dynein, **Δ**CT-MD stalls at 6 ± 1 pN (mean ± SD), with stall durations up to tens of seconds. These results identify the CT as an important new regulatory element for controlling cytoplasmic dynein mechanochemistry, perhaps gating ATP access. The CT thus appears to represent the structural basis for differences in mechanochemical function between yeast and higher eukaryotic dyneins.

2243-Plat
Regulatory Proteins Enable the Kinesin Kip2 to Overpower Cytoplasmic Dynein
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Cytoplasmic dynein and kinesin are opposite-polarity, microtubule-based motors that create movement and spatial organization within eukaryotic cells. As a minus-end-directed motor that typically moves cargo toward the cell interior, dynein faces a directionality problem: how is dynein initially targeted to the cell periphery? Previous studies have shown that in *S. cerevisiae*, this problem is solved in part by the action of Kip2, a plus-end-directed motor that is posited to co-transport dynein and another protein called Bik1 toward the microtubule plus-end. Here, we investigate the interplay between dynein and Kip2 by coupling them to a three-dimensional DNA origami scaffold, or "chassis", and using fluorescence microscopy to visualize the emergent motile behavior of these assemblies. In the absence of regulators, dynein-chassis-Kip2 structures move predominantly in the minus-end (dynein) direction. However, the frequency of plus-end-directed movements is markedly enhanced by the addition of Bik1 (a homolog of the cytoplasmic linker protein Clip170) and Bim1 (a member of the EB [end-binding] protein family). Moreover, Bik1, Bim1 and Kip2 co-elute as a ternary complex by size-exclusion chromatography. Thus, the addition of two regulatory binding partners can enable Kip2 to overcome dynein's intrinsic minus-end-directed motility and transport dynein toward the microtubule plus-end.

Symposium: Structural Dynamics of Molecular Machines

2244-Symp
Posttranslational Modifications as Modulators of Mechanical Protein Folding
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Understanding the molecular basis of muscle elasticity is an important challenge in medicine. For example, mutations in the giant elastic protein titin are implicated in the etiology of dilated cardiomyopathies. We use single molecule force-clamp spectroscopy to study the folding and unfolding dynamics of single polyproteins composed of tandem repeats of titin Ig domains. Protein elasticity is due to the diffusional extension and collapse of a polyprotein along its free energy landscape. Using single molecule force-clamp traces we calculate the free energy of a polyprotein along its stretching coordinate under different mechanical force regimes. At high stretching forces the free energy is dominated by changes in entropy as the molecule unfolds and extends. When the force is quenched, enthalpic interactions become important as the polyprotein slowly progresses from collapsed states to molten globules and then to the mechanically stable native state. We also show that while the polyprotein is in the extended state, cysteine residues that are normally buried become exposed to the solution where they can be post-translationally modified. We have found that S-glutathionylation of such cryptic cysteines greatly decreases the mechanical stability of an Ig domain as well as its ability to fold, favoring more extensible states in titin. Thus, the free energy landscape of a protein extending and folding under force is strongly modulated by the redox state of a cell. We propose that posttranslational modification of cryptic cysteine residues is a major regulatory pathway of tissue elasticity. Accurate predictions of elastic phenotypes in titin will be possible after we fully understand how cellular chemistry modulates the physics of a polypeptide extending under force.

2245-Symp
A Zoo of Slow Dynamics
Yasmine Meroz.
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Significantly slow dynamics, namely subdiffusion, is ubiquitous - especially in the biological context. The fingerprint of subdiffusive dynamics is that the mean squared displacement grows sublinearly in time - but there are very different physical mechanisms that can lead to this same behavior! Discerning between the relevant underlying physical realities is crucial to the understanding of the system at hand. A toolbox is presented with the aim of clarifying the differences between the different mechanisms, and presenting possible complications. We focus on a test for discerning between ergodic models, presenting an interesting application which sheds light on the structure of the energy landscape of proteins.

2246-Symp
Direct Measurements of Transcription Factor Binding and Dissociation at Individual Chromosomal Operators
Johan Elf.
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I will discuss some of our recent progress in studying transcription factor kinetics at the level of individual molecules in E. coli. I will in particular describe an assay for measuring the rate of dissociation for a LacI repressor from an individual chromosomal operator site. When combined with the corresponding association rate measurement, the assay allows us to test the commonly used assumption that TF kinetics can be considered to be at equilibrium and that the gene expression is proportional to the time the operator is free .

2247-Symp
ClpX, a Stochastic Protein Unfolding and Translocation Machine
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During protein degradation by ClpXP, AAA+ ClpX hexamers grip protein substrates using GYVG pore loops and harness the energy of ATP binding and hydrolysis to the drive conformational changes necessary for the mechanical denaturation and translocation of the substrate into the ClpP chamber. We have determined how the number and configuration of wild-type GYVG pore loops affect substrate unfolding and translocation. Single-molecule experiments reveal that ClpXP can perform mechanical work under load, show fast and highly cooperative unfolding of individual protein domains, give a translocation step size of 5-8 residues, and support a model in which each individual power-stroke has a relatively low probability of resulting in substrate denaturation. In multiple crystal structures of hexameric rings, some ClpX subunits adopt nucleotide-loadable conformations, others adopt unloadable conformations, and each class of subunits exhibits variability. Using mutagenesis of individual subunits in covalent hexamers, crosslinking, and fluorescence methods to assay the conformations and nucleotide-binding properties of individual subunits, we find that dynamic interconversion between unloadable and loadable conformations is necessary for ClpX to perform mechanical work. ATP binding to different classes of subunits initially drives staged allosteric changes that set the conformation of the ring and allow hydrolysis and linked mechanical steps. Subunit switching between loadable and unloadable conformations subsequently resets the configuration of the nucleotide-loaded ring and is required for mechanical work, possibly as a fail-safe method to circumvent stalling.