1114-MiniSymp
The Dam1 Ring Binds Microtubules Strongly Enough To Be A Processive As Well As Energy-efficient Coupler For Chromosome Motion
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Accurate chromosome segregation during mitotic division of budding yeast depends on the multiprotein kinetochore complex, Dam1 (aka DASH). Purified Dam1 heterodecamers encircle microtubules (MTs) to form rings that can function as ''couplers'', molecular devices that transduce energy from MT disassembly into the motion of a cargo. Here we show that MT depolymerization develops the 6-fold larger force against a Dam1 ring than the force exerted on a coupler that binds only one side of a MT. Wild type rings slow depolymerization 4-fold, but rings that include a mutant Dam1p with truncated C-terminus slow depolymerization less, consistent with the idea that this tail is part of a strong bond between rings and MTs. A molecular-mechanical model for Dam1-MT interaction predicts that binding between this flexible tail and the MT wall should cause a Dam1 ring to wobble as is moves with a shortening MT end, and Fourier analysis of moving, ring-attached beads corroborates this prediction. Comparisons of the forces generated against wild type and mutant complexes confirm the importance of tight Dam1-MT association for processive cargo movement under load. Since processivity in chromosome motion is an essential property, particularly for organisms in which there is only one MT per kinetochore, the Dam1 ring seems well adapted for mitotic motions in budding yeast.

1115-MiniSymp
The ATPase Cycle of the Mitotic Motor CENP-E
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Several members of the kinesin superfamily are highly processive molecular motors, capable of taking multiple steps on their track without dissociating. For the processive transport motor kinesin I, processivity results from features of this motor's enzymology, which enhance its ability to stay attached to the microtubule through multiple ATPase cycles. These include a high duty ratio, rapid rate of ATP binding, rapid rate of forward stepping, and strain-dependent gating of nucleotide binding. We had recently shown that CENP-E, a mitotic kinesin that localizes chromosomes to the midzone during metaphase, is highly processive, with run lengths similar to those for kinesin I. In this study, we have examined the CENP-E ATPase cycle to see if its enzymology explains its processive behavior. We find that some features of the CENP-E enzymatic cycle, including rapid ATP binding, multiple enzymatic turnovers per diffusive encounter, and strain-dependent gating of nucleotide binding, are indeed very similar to those for kinesin I. However, the rate of neck linker docking for CENP-E is nearly 20-fold slower, and this would predict run lengths 50-fold shorter than what we have observed. This implies that, like the mitotic motor Eg5, CENP-E processivity is shaped not only by its enzymology, but also by other, non-enzymatic features of this molecular motor as well.

1116-MiniSymp
Dimeric Centromere Protein E (CENP-E) Promotes Microtubule-elongation At The Plus-ends Of Microtubules
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Centromere protein CENP-E is a dimeric kinesin (Kinesin-7 family) with critical roles in mitosis including establishment of microtubule (MT)-chromosome linkage, processive movement of monooriented chromosomes on MTs for proper alignment at metaphase, and as a tension sensor at mitotic checkpoint to signal onset to anaphase. Fluorescence microscopy studies were performed to test the hypothesis that CENP-E promotes MT-elongation at the MT plus-ends. CENP-E constructs were engineered, expressed, and purified which yielded dimeric and monomeric motor proteins. The results show that dimeric CENP-E promotes plus-end directed MT gliding at 11 $\pm$ 0.005 nm/sec (n=173 MTs). Real-time microscopy assays were performed to image CENP-E promoted elongation of GMPcPP-stabilized polarity marked FITC MTs. The results revealed that out of the 270 polarity marked MTs examined, 164 MTs (60%) exhibited CENP-E promoted MT plus-end extension by GTP-tubulin (1.48 $\pm$ 0.37 μm/30 min; n=200 MTs) in the presence of MgATP. In contrast, dimeric Kinesin-1, dimeric Eg5, and CENP-E in the presence of AMPPNP did not show this pronounced MT elongation. These results suggest that CENP-E as part of its function for chromosome kinetochore attachment to MTs plays a direct role in kinetochore MT extension during congression. Supported by NIH GM54141 and NIH Career Development Award K02-AR47841 to SPG.

Platform V: Molecular Mechanics & Force Spectroscopy

1117-Plat
Small-Molecule Binding to DNA under Tension and Twist Studied by Magnetic Tweezers
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DNA-binding small molecules are present in the cellular environment and are ubiquitously used in biochemistry and biotechnology. Here, we use single molecule magnetic tweezers experiments to study the effect of small-molecule ligands on DNA mechanical properties.
Using the magnetic tweezers ability to control both the applied stretching force and torque, we have systematically characterized the mechanical properties of DNA in the presence of Ethidium Bromide (EtBr), a well-known intercalator, and Netropsin, an anti-microbial drug and known minor groove binder. In addition, we have characterized the interactions of Topotecan, a clinically used anti-tumor drug, with bare DNA.
Our results show a lengthening of DNA upon EtBr intercalation to an extension of ~1.5 times the initial contour length and a decrease in DNA twist by 29 +- 3 degrees per intercalation event, in agreement with previous estimates from bulk experiments. Further effects of intercalation are a stabilization of the double strand under high forces and negative torques and a decrease in both the bending and twisting persistence lengths. In contrast, minor groove binding by Netropsin does not change the contour or persistence length significantly, but increases the twist per base of the DNA.
For Topotecan, both intercalative and minor-groove binding have been postulated. Our magnetic tweezers results indicate that drug binding at $>$ 50 micromolar concentrations has consequences qualitatively similar to the changes observed for EtBr, in support of a intercalative binding mode for Topotecan.
These results point to important consequences of intercalative ligand binding for DNA torsional behavior, which we characterized at the single-molecule level using magnetic tweezers. Ultimately, such insights can help elucidate the effects of small molecule drugs in the cellular environment and their interference with DNA transcription and replication.

1118-Plat
Method to Measure the Activation Energy of the Receptor-Ligand Binding by Single-Molecule Force Spectroscopy
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Single molecule force spectroscopy (SMFS) is routinely used in biophysical research to measure the kinetic parameters of dissociation of biomolecules by affecting the dissociation kinetics with external force. We have noticed that probability to form bond between tethered ligand and immobilized receptor measured by atomic force microscopy (AFM)-based SMFS depends on time the AFM probe spends near the surface. This dependence has been attributed to the tether-constrained kinetics of forming the molecular bond between the ligand and the receptor. We have developed a kinetic model that incorporates the polymeric tether dynamics and permits measurement of the activation energy of association in bimolecular reaction. To test this model we have performed SMFS measurements using biotin tethered to the AFM probe by PEG linkers with molecular weights of 5 and 3.4 kDa and streptavidin immobilized on a flat substrate. Measurements support the developed model and yield activation energy of the binding of 9.5 $\pm$ 0.5 kT. The developed approach can be used as a common SMFS tool in characterizing the activation energy of binding in other molecular systems.