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1956-Plat

Feeling the Pressure: Dynamics of Viral DNA Packaging are Controlled by the Confined Genome within the Capsid

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Double-stranded DNA viruses, including tailed bacteriophages and mammalian herpesviruses, package their genomes into pre-formed protein capsids. The packaging process is driven by a molecular complex known as the packaging motor. This motor is a ring-shaped oligomeric ATPase that utilizes the chemical energy from ATP binding and hydrolysis to perform the mechanical work. We have recently presented a detailed mechanochemical characterization for the bacteriophage phi29 motor, a homo-pentamer that translocates DNA in cycles composed of alternating dwells and bursts (1). We now aim to investigate the behavior of the motor at different stages of packaging, as the motor needs to overcome increasing amounts of internal pressure generated by the compressed dsDNA. We find that the effect of the internal pressure on the motor dynamics is more complex than simply exerting an opposing force to packaging. Through a detailed analysis using ultra-high-resolution DNA translocation data, we find that the internal pressure affects multiple kinetic transitions in the mechanochemical cycle, including events in both the dwell phase and the burst phase. This analysis allows us to make an accurate estimation of the internal force as a function of DNA filling, which is important for the understanding of the DNA organization inside the capsid and the ejection energetics. Remarkably, the motor changes its step size and displays a new class of long-lived pauses towards the completion of packaging. Finally, we determine the structural elements in the packaging complex that are responsible for the signal transduction from the capsid to the motor.

(1) G. Chistol*, S. Liu*, C. L. Hetherington, J. R. Moffitt, S. Grimes, P. J. Jardine, C. Bustamante, ''High Degree of Coordination and Division of Labor Among Subunits in a Homomeric Ring ATPase'', Cell, in press (2012).

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Friction and Wear of Porcine Articular Joint and Effects of Selective Digestions

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In this work, we explored the possible existence of a wear mechanism developing under mild conditions using surface force apparatus (SFA). By selectively digesting different components (type II collagen, Hyaluronic acid (HA), glycosaminoglycans (GAGs)) of the cartilage, we also establish correlation between the structural properties of cartilage surface and the tribological properties.
We observed stick-slip friction in articular cartilage for the first time under mild conditions. To visualize load and speed regimes of stick-slip friction occurrence we introduced 'Dynamic (friction) phase diagram'. Prolonged exposure of the cartilage surfaces to stick-slip sliding results in an increase of surface roughness similar to HA and GAGs digested cartilage suggesting that stick-slip motion is able to induce severe morphological changes of cartilage superficial zone. We also found that digestion of the different components of cartilage alters the morphology of the superficial zone in very different ways. HA and GAGs digestions increased surface roughness while collagen digestion decreased the surface roughness compared to normal cartilage. Friction forces increased up to 2, 5 and 10 times after HA, collagen and GAGs digestion, respectively, indicating no direct correlation between surface roughness and friction forces.

Platform: Microtubular Motors

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The Structural Basis of Force Generation by the Mitotic Motor Kinesin-5

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Kinesin-5s are essential for forming the bipolar spindle during mitosis in most eukaryotes. The kinesin-5 motor domain contains conserved nucleotide and microtubule binding sites and mechanical elements to generate force. However, biochemical and biophysical studies have suggested that the mechanochemistry of the kinesin-5 motor is very different from other kinesins. using cryo-electron microscopy and image reconstruction, we have calculated subnanometer resolution structures of microtubule-bound human kinesin-5 before and after nucleotide binding. Our structures reveal that, despite its mechanistic differences with conventional kinesin, kinesin-5 has the same coupled, nucleotide-dependent conformational changes as seen in conventional kinesins, including a ratchet-like docking of the neck linker, and simultaneous, parallel docking of the amino-terminal cover strand. These observations are supported by kinetic experiments that indicate a cooperative rearrangement of the kinesin-5-specific neck linker with the amino-terminal cover strand during the motor's ATPase cycle. In contrast to conventional kinesin however, our structures reveal a dramatic reorientation of Loop L5 - the binding site for allosteric inhibitors of kinesin-5-following ATP binding. This reorientation suggests that L5 is directly involved in controlling nucleotide binding by acting as an intra-molecular competitive inhibitor. Our structures indicate that allosteric inhibitors of human kinesin-5 bind to a motor conformation that occurs in the course of normal function. However, due to evolutionarily defined sequence variations in L5, this conformation is not adopted by invertebrate kinesin-5s, explaining their resistance to drug inhibition. Our data reveal the structural basis for kinesin-5 force generation that has evolved in the physiological context of the mitotic spindle.

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Kinesin-1 Motility Inhibited by Microtubule Defects

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Microtubules (MTs) provide the basic highway system for the cell, with molecular motors providing the cargo transport. Molecular motors traverse the MT network to deliver vital nutrients throughout the cell. It has been proposed that in vivo MTs, which are free of defects, provide the best tracks for motor transport, yet no study has investigated the effect of microtubule defects on transport. Moreover, in vivo defects may result when mechanisms that maintain MTs break down. Therefore, studying motor motility in the presence of defects is an important area of interest. Unlike in vivo MTs, microtubules polymerized in vitro can have many dislocation defects in their structure, such as seam or point defects. Here, we systematically study the effects of microtubule lattice defects on the transport abilities of kinesin motor proteins. We have studied the effect a step edge defect has on kinesin-1 motility, using single molecule fluorescence imaging. We created the step edge defects by end-to-end annealing Taxol-stabilized MTs with 12-13 protofilaments to GMPCPP MTs with 14 protofilaments. We find that fewer than 20% of kinesin-1 motors traverse a defect location while the rest dissociate from the MT lattice just before the defect. We speculate that kinesin-1 motility past the defect is inhibited because the step edge results in either a change in radius, or a shift in the protofilament lattice. Consequently, kinesin-1 is unable to overcome the increased step distance. This suggests that defects do play an important role in motility.

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Effects of Neck Linker Length on Kinesin-1 Force Generation and Motility

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Kinesin-1 is a dimeric, processive microtubule motor. The ATP hydrolysis cycles of its twin motor domains are tightly coupled through efficient gating mechanisms that coordinate hand-over-hand stepping. A key structural element for this coordination is the neck linker (NL), a 14 amino-acid (AA) segment that connects each catalytic head to the stalk. We have previously shown that extending the kinesin NL can lead to backstepping and diminished processivity (Clancy *et al.*, 2011, *Nat. Struct. Mol. Biol.*, **18**:1020-7; Shastry and Hancock, 2010, *Curr. Biol.***20**:939-43). Here, we used optical trapping techniques to study how force-dependent kinesin motility is affected by incrementally increasing the NL length in wild-type (WT) and cysteine-light (CL) versions of Kinesin-1 protein from both *Drosophila* and human. For *Drosophila* constructs, adding one AA into the NL reduced the velocity and run length for all forces, but further extensions of the NL exerted no appreciable, additional effect. Assisting loads (loads applied in the microtubule plus-end direction) did not affect the velocity of the WT construct, whereas slower mutant velocities were increased, approaching those of the WT. Under hindering loads, increased force dependencies were correlated with longer NLs, but more pronounced effects, including processive backstepping, were associated with CL mutations, such as those found in a previously characterized human CL construct. Our results illustrate how the NL in Kinesin-1 is optimized under external loads for force production, velocity, and run length.

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Serine 176 Phosphorylation Attenuates Kinesin's Stall Force and Biases Bidirectional Transport

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