rotation. Here we used a polydimethylsiloxane (PDMS) microfluidic device that decreases photodamage via deoxygenation to study the initial stages of ring formation in absence of photodamage-related effects to kinesin motors. Specifically, valves on the devices were used to control the addition of biotinylated microtubules and streptavidin-coated quantum dots to the system, while effectively removing oxygen from the sample. This approaches enabled us to use relatively high excitation intensities and high frame rates to characterize the early events of microtubule ring formation. We show that both proposed mechanisms occur, and we characterize the conditions under which each mechanism predominates. In the pinning mechanism, it was observed that a single microtubule may be pinned at the head, leading the tail to loop and contact itself, forming a single ring. Once the initial ring is formed, additional bundling occurred as other microtubules encountered the ring and became attached. For the mechanical strain mechanism, microtubule oligomers were observed to undergo ring formation in the absence of detectable pinning events. Here, the presence of non-13mer microtubules induces oligomers to rotate axially, resulting in twisted nano-domains that introduce mechanical strain, coercing the bundle to move in a curved path and form a ring. Together, these data provide a critical insight as to the mechanism underlying the self-assembly of dynamic material structures based on active transport systems.

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Crystal and EM Structures Reveal a Mechanism for Long-Range Allosteric Communication in the Yeast Dynein Motor Domain

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Dynein, a member of the AAA family of ATPases, ''walks'' toward the minus end of microtubules using energy from ATP hydrolysis. Recent atomic structures of the dynein motor domain with no nucleotide (apo; yeast) or ADP (Dictyostelium) in the main hydrolytic site (AAA1) have begun to yield insights into how this fascinatingly complex machine may work. Here, we have solved the crystal (3.5 Å) of the yeast dynein motor domain with a nonhydrolyzable nucleotide (AMPPNP) bound at AAA1. By comparison to the prior apo structures of yeast dynein, the new structure reveals that AMPPNP causes a large conformational change of AAA domains in the dynein ring in which AAA2 moves closer to AAA1. The linker, the proposed mechanical element of dynein, remains largely in a similar conformation with its N-terminal docked onto AAA5, but new interactions are made with AAA2. Further EM analysis of dynein bound to several ATP analogs showed that the linker remains docked on the ring in all states, with a small conformational change observed when bound to the analog ATP-vanadate. Mutagenesis studies show that disruption of a single salt-bridge between the linker and AAA2 blocks microtubule stimulation of AAA1 ATPase activity and nucleotide-induced changes in microtubule binding affinity. Collectively, our data reveal a new nucleotide triphosphate bound state of the dynein motor domain and suggest that the linker serves not only in dynein mechanics but also plays a critical role in the long range allosteric communication between dynein's main ATPase site and the microtubule binding domain.

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Load-Sharing Mechanism of Cytoplasmic Dynein

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Cytoplasmic dynein is a homodimeric AAA^+ motor protein responsible for minus end-directed transport along microtubules. The two heads of dynein are not strictly coordinated during processive motility, unlike those of dimeric kinesins and myosins. Therefore, tail-tethered optical trapping experiments yield insufficient information about the force production of individual heads. Here, we connected a trapped bead to a dynein dimer through a rigid DNA tether and directly measured the stepping motion of a single head under load. We observed that even though the step size of a head decreases under load, the head only stalls at half the stall force of the dimer and must rely on its partner head to walk against near-stall loads. The bulk of the force is produced when a head is bound to the microtubule, and stepping direction in the unbound state can be reversed by as little as 0.5 pN of backward load. Force-velocity curves indicate that the forward stepping rate of a dynein head is strongly inhibited by external force, while the backward rate is slow and nearly force-independent. Our results provide evidence for tension gating in dynein motility and lead us to propose a load-sharing mechanism between the two heads of a dynein dimer.

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The Mechanism of Dynein's Minus End Directionality and an Engineered Plus End Directed Dynein Motor

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Cytoplasmic dynein is responsible for nearly all minus-end directed transport along microtubules (MTs). However, little is known about how dynein achieves its directionality. Using an optical trap, we find that the interaction between the dynein microtubule binding domain (MTBD) and the MT is highly asymmetric. Release towards the plus-end is slow and force independent, whereas minus-end directed force greatly accelerates MT release. Motor directionality was reversed by exchanging the MTBD with a catalytically inactive kinesin motor domain that favors release towards the plus-end. High resolution assays revealed that this construct moves processively by taking 8-32 nm steps, similar to dynein. However, it stepped backward more frequently than dynein, which may be the result of its relatively low asymmetry in MT-release. To test if interhead tension alone is sufficient for motility, we generated dynein constructs that do not undergo nucleotide-dependent MT-release by attaching the N-terminal linker and the tip of the stalk of a dynein monomer to two artificial protein handles terminating with the dynein MTBD. This construct showed processive motility, suggesting that asymmetric MT-release in combination with tension generated by the motor domains is sufficient to drive minus-end directed motility. We propose that MTBD, not the ATPase ring, determines dynein directionality by asymmetry in MT release under tension and preferential rebinding to MTs when the stalk is tilted towards the plus end.

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Single-Molecule Study of the Communication between the Two Primary Sites of Cytoplasmic Dynein

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Cytoplasmic dynein is the primary minus-end directed microtubule (MT) motor in eukaryotic cells. The motor domain of dynein consists of six AAA^+ subunits, forming a ring. Previous studies showed that AAA1 is the main site of ATP hydrolysis, responsible for force generation and MT detachment. The AAA3 site is also required for robust motility, but its role in dynein's mechanochemical cycle is unknown. In this study, we use single-molecule fluorescence to elucidate the role of AAA3 and its interactions with AAA1 during stepping. We find that AAA3 regulates MT detachment. AAA3 mutants of dynein are gated by MT release and AAA1 requires timely detachment to proceed through its own hydrolysis cycle. High-resolution fluorescence tracking indicated that substrate release from AAA1 is rapid compared to catalysis, consistent with the open conformation of AAA1 observed in crystal structures. Consistent with loose substrate binding at AAA1, the catalytic activity of AAA3 can be specifically inhibited by a nonhydrolyzable ATP analog at low concentrations. Analysis of analog-induced pause density indicates that two analogs are required to initiate pausing, and that the analogs bind in an uncoordinated manner. By analyzing the pausing behavior at different velocities and at different inhibitor concentrations, we gain insight into the nature of communication between AAA1, AAA3, and the MT-binding domain. On the basis of our results, we propose a model for the role of AAA3 in dynein's stepping mechanism.

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A Structural and Functional Analysis of the Dynein Light Intermediate Chain

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The concentrated cytoplasm of the cell must obtain spatial and temporal organization, and molecular motors are one means such organization can be achieved. Dynein is a multi-subunit microtubule motor complex that is implicated in a large range of functions including cargo transport and cell division. Many questions about dynein remain unanswered, including the mechanisms of cargo selection and attachment. The N-terminal tail subunits of dynein are crucial for cargo transport, yet it is not known how they select and attach cargo. In particular, the light intermediate chain (LIC) is the least studied tail subunit of dynein. Knockdown of the LIC leads to a number of mitotic and cargo-transport defects, and different LIC isoforms have demonstrated specificity in cargo selection. However, it is not understood how the LIC carries out these functions, and structural information about the LIC is lacking. We have solved