domains or mechanisms. Kinesin-14s share a domain structure in the stalk that consists of an N-terminal region followed by a region of coiled coil. They can vary drastically in size and share minimal sequence similarities. Features of a second microtubule-binding domain are unknown as well as regulatory elements. Most recently this lab has determined that Kinesin-14/γ-tubulin interactions at the γ-TuSC MTOC can regulate bipolarity. Other genetic and biochemical analysis indicates that Kinesin-14 interactions with other MTOC proteins also occur. We are using deletion analysis, site-directed mutagenesis and chimeric Kinesin-14 proteins with human HSET and *Drosophila* Ncd to relate structural and sequence domains in Kinesin-14s to their functional importance. Twenty-five Kinesin-14 constructs have been generated and are helping to define the minimal Kinesin-14 and to separate domains for bipolar spindle assembly versus later mitotic roles. Length requirements of the coiled-coil domain are also being examined as a critical component in models that emphasize microtubule cross-linking by Kinesin-14s. This work provides insights into Kinesin-14 functional needs and unique tools for assessing Kinesin-14 interactions with tubulin and non-tubulin proteins in conserved molecular mechanisms.

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Reconstitution Of Microtubule-driven Movement and Force Production by the Ndc80 Kinetochore Complex

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The kinetochore-microtubule junction is a central but poorly understood part of the molecular machinery that organizes and separates chromosomes during mitosis. Kinetochores maintain load-bearing attachments between chromosomes and microtubule tips, even as the tips assemble and disassemble under their grip. This end-on coupling is conserved from yeast to humans, suggesting it may be mediated by a common molecular mechanism but the existence of a conserved coupler remains uncertain. Using techniques for manipulating and tracking individual molecules, we discovered that end-on coupling can be reconstituted with the Ndc80 complex, a widely conserved component of the outer kinetochore. Beads decorated with pure Ndc80 complex bind individual microtubules through the N-terminal globular domains of Ndc80p and Nuf2p. Like kinetochores, these Ndc80-based couplers are carried by disassembling tips and they remain tip-bound even under tensile loads up to 2.5 pN. Microtubule-driven movement and force production persist at low surface densities where the number of Ndc80 complexes interacting with the filament is close to the number found at kinetochores in vivo. At the single molecule level, fluorescent-tagged Ndc80 complexes diffuse randomly along microtubules except at disassembling tips where their motion becomes biased in the direction of filament shortening. Our results indicate that Ndc80-based coupling likely depends upon a 'fluctuating ensemble' mechanism similar to that proposed by Hill on purely theoretical grounds more than two decades ago*. We also demonstrate that both the yeast and human Ndc80 complexes support microtubule-driven bead movement and force production. This conservation of function, together with the established importance of the Ndc80 complex for kinetochore-microtubule coupling across eukaryotes, strongly suggests that a fluctuating ensemble of Ndc80 complexes represents a core attachment module within the kinetochore.

* Hill, T. L. (1985) Proc Natl Acad Sci USA 82:4404-4408.

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"Cortical" Dynein Can Capture And Pull On Dynamic Microtubule Ends In Vitro

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The interplay between dynamic microtubules (MTs) and cytoplasmic dynein plays an important role in many different cellular processes. Dynein at the cortex positions the mitotic spindle through interaction with astral MTs. Within the spindle MTs interact with dynein at the kinetochore, contributing to poleward movement of the chromosomes. Despite the apparent importance of MT-dynein interactions in these and other cellular processes, very little is known about how dynein mechanically interacts with dynamic MT ends. We have set-up different *in vitro* assays that mimic the MT-cortex/kinetochore interaction. Dynamic MTs grow against a micro fabricated barrier, coated with purified dynein. An optical tweezers based technique, previously developed in our lab, allows us to monitor MT dynamics and force generation while MTs grow and shrink against the dynein-coated barrier. We show that dynein, artificially anchored to a barrier, dramatically alters MT dynamics (dynein in solution does not alter MT dynamics). It captures MT ends, inhibits growth and induces catastrophes. Subsequent shrinking of the MT is slow compared to the free walking velocity of the motor and the free shrinking velocity of the MT. In addition, the MT-dynein connection at the barrier can generate pulling forces up to 5 $\pm$ 1 pN. Our results show that the MT-dynein connection, if it is anchored to a barrier, forms a simple force-generating unit that does not require other factors to behave in a way that is similar to what is observed in living cells.

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Structure and Evolution of Tubulin C-terminal Tails

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Both α- and β-tubulin have a highly charged, acidic, unstructured, C-terminal ''tail'' peptide (CTT) that extends from the outer surface of the microtubule (MT).These peptides, ~10 residues for α and ~20 for β, do not appear in crystal structures or in cryo-EM reconstructions but are critical to the interaction of other proteins with tubulin in dimer or MT form. The CTT are the locus of isotype-defining sequence differences as well as the site of most posttranslational modifications (PTM) of tubulin. Molecular modeling shows that the CTT are flexible and mobile compared to the body of the protein, explaining why they don't diffract. MT are covered with a sparse negatvely charged brush composed of these peptides: their length is comparable to their grafting distance. CTT mediate interactions with many proteins, including the dimer with the mitochondrial outer membrane protein VDAC. Modeling shows that the tails fit into the channel, but not with branch-producing PTMs (polyglutamylation or polyglycylation). Thus PTMs could alter (prevent) tubulin from acting as a regulator of VDAC, and hence mitochondrial, function. Evolutionarily the tails are highly conserved, but not in exact sequence. Rather the residue length and charge of each CTT are closely maintained. This is found throughout the eukaryotic world, except in organisms that lack mitochondria, in which case the tail can be much shorter and/or less charged. Bacterial tubulin (αβ-tubuin, not FtsZ), arriving from an unknown eukaryote by horizontal gene transfer, shows great divergence in tail length (from total loss to much increased length) and charge (including some with many positive charges). We conclude that the interactions of the tail peptides with other proteins, including VDAC on mitochondria, provide a major selective constraint on this critical part of the tubulin molecule.

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Modeling of Motor Mediated Microtubule Bending

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Microtubules are often viewed as mechanically rigid compressive struts that help maintain cell shape and aid in the transport of cellular cargo by serving as tracks for the molecular motors. However, individual microtubules in living cells are often highly bent, and even though the different force mechanisms that contribute to microtubule deformations are known, it is not clear how all these mechanisms act together in a given cell type. Recent experiments on LLC-PK1 epithelial cells strongly suggest that F-actin dynamics and polymerization of microtubules play a minor role, and that the dominant mechanism is anterograde transport via molecular motors. Surprisingly, quantitative analysis of these deformations using curvature distributions exhibit striking similarities to in vitro gliding assays. Motivated by these experiments, both in vivo and in vitro, we have modeled the deformation of microtubules under the influence of molecular motor forces by coarse-grained simulations. In the simulations, microtubules are modeled as semi-flexible polymers with rigid bond constraints embedded in a solvent. Molecular motors exert forces on the microtubules, and walk along microtubule tracks according to their known force-velocity relations, bind and unbind stochastically. Simulation results support our experimental findings and further elucidate on the interplay between molecular motors and passive cross-linkers. Our results suggest that molecular motors are not necessarily just cargo carriers, but can play a dynamic role in the deformation and positioning the microtubule array.