In neurons, microtubule motor driven transport is crucial for communication between processes and the cell body. Disruptions in transport are associated with a variety of neurodegenerative diseases. Previous studies implicate phosphorylation of serine 176 in kinesin-1 in the impaired axonal transport associated with Huntington's disease. In isolated squid axoplasm, introduction of pathogenic huntingtin protein activates the kinase, JNK3, which specifically phosphorylates kinesin at S176 (Morfini, Nature Neuroscience, 2009). The mechanism by which S176 modification leads to impaired transport is not very well understood. It is not known whether phosphorylation of kinesin alone is sufficient to cause impaired cargo transport. To investigate the effect of residue 176 on kinesin transport, we use optical trapping and single-molecule fluorescence imaging to study purified kinesin. We employ two constructs, S176A and S176D, truncated at residue 888 to remove the autoinhibition domain, resulting in constitutively active motors. There is no significant difference in the processivity, ATPase, or microtubule binding activity between the phosphomimetic S176D construct and the non-phosphorylatable S176A construct. However, we find that S176D has an attenuated stall force (5pN) compared to S176A (7 pN). Furthermore, polystyrene bead cargos coated with dynein and S176D are transported preferentially in the minus direction in comparison with cargos coated with equivalent concentrations of dynein and S176A. We also perform phosphorylation assays using JNK3 on both 888-truncated and full-length wild-type kinesin in which radiolabeling is used to quantify the percentage of protein phosphorylated in the assay. The pattern of stall force attenuation and directional bias observed for the S176D mutant is also observed in JNK3 phosphorylated samples in which 60-80% of the wild type protein has been phosphorylated. These results show that modification of serine 176 alone is sufficient to alter the behavior of kinesin.

**1962-Plat**

**Measuring Collective Transport by Defined Numbers of Processive and Nonprocessive Kinesin Motors**

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Molecular motors usually work together in teams within the cell. However, studying coordination among these nanomachines has posed a challenge. Here, we used experimentally defined assemblies in vitro to investigate how the functions of molecular motors depend on the motor number and arrangement. The assembly forms a linear array of motor proteins with defined number and spacing on a DNA scaffold. using this method, we linked together multiple molecules of either two different types of kinesin motors, kinesin-1 or Ncd (kinesin-14), which shows a processive and nonprocessive movement, respectively. Regardless of the motor type, their processivity scaled exponentially with the motor number at low load. While single Ncd motors showed short, diffusive movement along microtubules, the assemblies composed of two Ncd molecules moved processively for more than 1 μm. Force measurement revealed that small groups of Ncd can generate an additive force that is much larger than can single motors, while multiple kinesin-1 can rarely share an external load. Numerical simulations suggest that the coordination among Ncd motors strongly depends on the fast microtubule binding kinetics of individual motors. Moreover, we found that Ncd can exert a larger drag force when pulled backward despite its small active force. These features would make Ncd suited for one of its cellular functions to dynamically crosslink microtubules while antagonizing the opposing force. Thus, our experimental system may provide a platform to study the collective behavior of motor proteins from the bottom up.

**1963-Plat**

**A Force Dependent Gating Mechanism Inhibits ATP Dependent Release of Dynein from Microtubules**

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Cytoplasmic dynein is a processive minus end directed microtubule motor involved in a wide range of cellular functions. By studying how dynein monomers respond to load in an optical trapping assay, we discovered a force dependent gating mechanism that prevents ATP driven release of dynein from the microtubule. Our results show that tension on dynein's linker domain causes the microtubule release rate of the motor to be insensitive to ATP, while dynein motors with an unloaded linker release at a much faster rate at high ATP concentrations. We found that dynein monomers preferentially release when force is applied towards the minus end of the microtubule rather than the plus end. This strong asymmetry is observed even in the absence of dynein's ATPase ring, implying it is an intrinsic property of the stalk and microtubule binding domain. To test what role these phenomena play in dynein motility, we studied ATPase mutants with altered translocation velocities and measured their force dependent release kinetics. On the basis of these results we developed a minimal model of dynein motility and show how intramolecular tension, powerstroke and force dependent release are main determinants of dynein's motility properties and directionality.

**1964-Plat**

**Collective Function of Kinesin and Dynein in the Bidirectional Transport of Intracellular Cargoes**

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Teams of several kinesin and dynein motors are bound to intracellular cargoes and drive their bidirectional transport along the microtubule cytoskeleton. To probe the collective dynamics of an endogenous complement of motors, we examined the forces on bidirectional cargoes in living cells. We previously developed methods to calibrate optical traps in living cells, taking into account the local viscoelastic environment of each cargo. using these techniques, we examined forces on latex beads taken up into mammalian macrophages, which are encased in native phagosomes that are transported by kinesin-1, kinesin-2, and dynein motors. Forces exerted by kinesin-1 and kinesin-2 are indicative of 1-3 motors which often detach at forces below the unitary stall force of ~5 pN. In contrast, multiple peaks in the dynein force histogram at 1-2 pN intervals suggest collective transport by many motors (up to 13). During high-force ($|F| > 10$ pN) events driven by multiple motors, the cargoes often advanced in 8-nm steps, suggesting that multiple kinesin and dynein motors step in a correlated manner at high loads. We compared forces in living cells to those produced by isolated latex bead-containing phagosomes along taxol-stabilized microtubules. The force histograms display similar peaks, indicating that individual motors produce similar forces in vitro and in living cells. However, forces up to ~ +/-20 pN were observed in living cells compared to maximum forces of ~ +/-12 pN in vitro, suggesting that for the same type of cargo, more motors are engaged in the cell. The dense and viscoelastic cellular environment may promote motor binding by constraining diffusion, or by allowing motors on a single cargo to interact with multiple microtubules. Supported by NIH grants GM087253 to YEG and ELFH and GM089077 to AGH.

**1965-Plat**

**Synthetic Biology Approaches to Dissecting Multiple Motor Functions in Living Cells**

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Dissecting how the coordination of cytoskeletal motor proteins, (kinesin, dynein and myosin) has become increasingly important to characterizing various transport processes in eukaryotes. Most subcellular commodities (ribonucleoprotein, particles, organelles, vesicles etc.) are driven by ''teams'' of motor proteins that are composed of multiple copies of the same motors or even mixtures of different motors that move in opposite directions, with different velocities, or along different types of filament tracks. Consequently, numerous intracellular transport processes and regulatory mechanisms depend fundamentally on how these collections of similar and dissimilar motors either cooperate or compete with one another. Understanding these behaviors are important to elucidating various disease pathologies stemming from motor and other transport-related protein mutations since their impact on cargo transport will ultimately depend on the extent to which they perturb the composite dynamics of motor systems that also contain wild type motors. Our work has focused on developing genetically engineered cells that can be used to characterize endogenous cargo transport and trafficking response to variation in motor number, type and ratio and cargo size. While integrating multiple genetic control elements, these cells also exploit drug- and light-dependent protein dimerization switches to allow competitions between different types of wild-type and mutant motors to be adaptively regulated. We are also developing micro-fabrication techniques to pattern these cells into defined shapes as a way to control the organization of their cytoskeleton and facilitate direct comparisons of transport responses within the cell population. Given these unique handles, these cells provide a powerful platform to examine fundamental mechanisms of intracellular transport regulation and assess the impact of disease-related motor mutations.