In recent years, a considerable number of DNA-based molecular devices have been developed whose motion can be controlled by nucleic acid ''effector'' strands. For instance, the paradigmatic DNA ''tweezers'' system consists of two double-stranded arms connected by a flexible single-stranded ''hinge'' that can be closed or opened by the addition of so-called ''fuel'' strands. We have recently shown that this motion can also be driven with RNA rather than with DNA effectors. In order to realize an autonomously running biochemical system, we now utilized an artificial gene regulatory circuit in vitro to control the temporal behavior of the DNA tweezers. The gene circuit is a minimalistic feedback system that contains two genes from which regulatory RNA molecules are transcribed. The regulators mutually influence their production in an activatory and inhibitory manner, respectively,resulting in oscillatory network dynamics. We experimentally demonstrate how this transcriptional oscillator can be used to ''clock'' the motion of the DNA nanodevice in a variety of different ways. Furthermore, we investigate the robustness of the oscillator system with respect to increasing ''load'', i.e., tweezers concentrations.

Minisymposium 3: Tug of War: Molecular Motor Interactions

2226-MiniSymp

Opposite-Polarity Motors Activate One Another to Trigger Cargo Transport in Live Cells

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Intracellular transport, unlike in vitro, is typically bi-directional – consisting of a series of back and forth movements. Kinesin-1 and cytoplasmic dynein require each other for bi-directional transport of intracellular cargo along microtubules i.e. inhibition or depletion of kinesin-1 abolishes dynein-driven cargo transport, and vice versa. Using Drosophila S2 cells, we demonstrate that replacement of endogenous kinesin-1 or dynein with an unrelated motor of the same directionality, and targeted to peroxisomes, activates peroxisome transport in the opposite direction. However motility-deficient versions of motors, that retain the ability to bind microtubules and hydrolyze ATP, do not activate peroxisome motility. Thus any pair of opposite-polarity motors, provided they move along microtubules, can activate one another. These results demonstrate that mechanical interactions between opposite-polarity motors are necessary and sufficient for bi-directional organelle transport in live cells.

2227-MiniSymp

Motor Number Controls Cargo Switching at Actin-Microtubule Intersections *in vitro*

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Cellular activities such as endocytosis and secretion require that cargos switch between the microtubule (MT) and actin filament (AF) networks. Previous evidence suggests that switching may be regulated through a tug-of-war between MT and AF motors. To test the hypothesis that motor number can be used to direct the outcome of this tug-of-war, we reconstituted cargo switching at MT-AF intersections in a minimal system. We attached varying numbers of myosin-V and dynein-dynactin molecules to polystyrene beads and used an optical trap to position these beads near MT-AF intersections. Beads displayed a median pause time of 9 s at the intersection before exiting on a track. At least 23% of beads underwent rotation at intersections suggesting that competing motors apply a torque on their cargo. Force measurements to quantify the number of actively engaged motors show that stall force scales with the number of myosin-V motors as has previously been shown for kinesin-1 and dynein. Largely independent of whether it enters the intersection on the MT or AF, a bead with a myosin-V:dynein-dynactin force ratio of 0.5 (1 myosin-V to 4 dynein-dynactins) has a $>$85% probability of exiting on the MT. A bead with a myosin-V:dynein-dynactin force ratio of 1 (1 myosin-V to 2 dynein-dynactins) has an approximately equal probability of exiting on the MT, exiting on the AF, or remaining at the intersection. A bead with a myosin-V:dynein-dynactin force ratio of 4 (2 myosin-Vs to 1 dynein-dynactin) has a $>$95% probability of exiting on the AF. We have developed a statistical model that delineates the relationship between switch probability and motor number. Thus, cargo switching can be tuned via combinations of 1-4 myosin-V and dynein-dynactin motors through a simple force-mediated mechanism. Supported by P01 GM087253.

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Myosin Va and Myosin VI Engage in a "tug of war" on Actin Tracks While Transporting Cargoes *in vitro*

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Myosin Va (myoVa) and myosin VI (myoVI) are processive molecular motors that transport cargo in opposite directions on actin tracks. Since myoVa and myoVI may colocalize to the same cargo *in vivo*, these motors may undergo a tug of war. Therefore, we sought to characterize the stepping dynamics of single myoVa and myoVI motors *in vitro* as they mechanically interact when linked together by a Qdot cargo. Expressed myoVa-HMM with an N-terminal biotin tag were labeled with streptavidin-Qdots (565nm) while expressed dimerized myoVI-HMM were Qdot(655nm)-labeled on an exchanged calmodulin. The effective tug of war on actin filament tracks (25mM KCl, 2mM ATP, 22°C) was observed in TIRF with 6nm resolution, allowing individual steps to be detected. MyoVa won ~80% of the time and regardless of which motor won, its stepping rate was reduced ~50% below its unloaded value due to the resistive load of the opposing motor. Interestingly, as the winning motor stepped forwards (myoVa, 73nm; myoVI, 56nm) the opposing motor stepped backwards (myoVa, 68nm; myoVI, 65nm) at the same rate, although myoVI appeared to be dragged at times. Why does myoVa dominate when its stall force is similar to myoVI? Given the probability that both myoVa and myoVI take occasional backsteps and experience a 2-3-fold reduction in stepping rate when winning, we estimate based on optical trapping data (Altman et al., 2004; Kad et al., 2008) that myoVa exerts a 50% greater resistive load compared to myoVI, providing a potential advantage to myoVa. Differences in the length of the myoVa and myoVI constructs could lead to each motor experiencing different vectorial force components, the potential that this may influence the outcome of the tug of war is being investigated.

2229-MiniSymp

Collective Behavior of Antagonistically Acting Kinesin-1 Motors

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Active cellular transport along microtubules is driven by the concerted operation of molecular motors. This often leads to complex dynamic behaviors such as stop-and-go or bidirectional movements. An important situation arises when motors act antagonistically in a tug-of-war scenario. In order to mimic the action of antagonistic motors, we performed gliding motility assays of antiparallel microtubule doublets driven by kinesin-1. In this configuration the lengths of the individual microtubules of the doublet determined the numbers of motors available to act against each other. At high motor density, we found two possible modes of movement: slow movements, where the doublets were almost stalled, and fast movements, where the doublet velocity was close to the velocity of single microtubules. Moreover, we observed a range of microtubule length differences where both modes coexisted. We developed a theoretical description that quantitatively describes the experimental data. In order to account for the two modes of movement, as well as for the possibility of their coexistence, it was necessary to take into account (i) the finite stiffness of the linkers by which the motors are connected to the substrate, (ii) the load-dependence of the detachment rate of single motors, and (iii) a non-linear force-velocity relationship of single motors. Our results show that mechanical interactions between motors can generate coexisting transport regimes with distinct velocities.

2230-MiniSymp

Iinteractions between Motor Proteins can Explain Collective Transport of Kinesins

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The collective function of multiple motor proteins is central to a variety of transport processes in cells. Yet, how key transport parameters depend on motor number and inter-motor interactions remains unclear. Recent experiments[1] have allowed the dynamic properties of two coupled kinesin-1 molecules to be examined using 'single-molecule' biophysical techniques. These studies have revealed that negative motor cooperativity plays a significant role in collective kinesin dynamics. Current theoretical models that neglect intermolecular interactions cannot capture this behavior. We propose a new theoretical approach, based on discrete-state stochastic models, which allows us to describe complex aspects of coupled kinesin dynamics. By treating intermotor interactions explicitly, these models can be used to reconcile important differences between predictions based on non-cooperative (additive) behaviors, and observations of negative kinesin cooperativity.