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Intracellular Transport: Kinesins Working Together

While most *in vitro* experiments with motor proteins focus on the behavior of individual motors, in cells most cargo are transported by multiple motors and even multiple classes of motor. How these motors cooperate and compete in transporting cargo is not clear. Recent experimental and theoretical work suggests that motors attached to a given cargo interact in both expected and unexpected ways.

William O. Hancock

Microtubule-based transport of intracellular cargo, such as vesicles and organelles, is carried out by kinesin and dynein motor proteins. Experiments in cells have helped to define which motors move which cargos, while single-molecule investigations have defined many of the performance characteristics and underlying mechanisms of individual motors. What is less clear is how multiple motors attached to a cargo interact mechanically to achieve long-distance cargo transport that can withstand significant viscous and elastic loads.

One characteristic of transport motors is their processivity — their ability to walk multiple steps along their filament track without detaching. Clearly, having multiple motors attached to a given cargo will increase the cargo's transport distance because, when one motor detaches, other motors will maintain association with the track. How the forces of multiple motors sum is somewhat less clear — are forces shared equally by all motors such that the maximal load a cargo can move against is simply a multiple of the single-motor stall force, or is the relationship more complex? And intuition really starts to be taxed in predicting the force–velocity relationship of a cargo transported by multiple motors. Under load, do a fraction of the motors become particularly taxed and slow down the group, or do cooperative phenomena minimize load-induced slowing?

These questions are important for understanding the workings of motors in cells. For instance, in bidirectional transport, as seen for melanosomes, intraflagellar transport and axonal transport [1,2], how many motors need to be turned on or off to trigger directional switching? And what sorts of regulation and cooperative interactions underlie the complex oscillations of chromosomes seen during metaphase? While understanding the characteristics of the individual motors involved in these processes is important, there is clearly another level of complexity that needs to be considered when developing realistic physical models of these processes.

Current efforts to attack these questions rely on a paired approach of *in vitro* experiments using cargos functionalized with many motor proteins and theoretical models that extrapolate from single-motor to multi-motor behavior. In this issue of *Current Biology*, Kunwar and colleagues [3] describe a mechanochemical model consisting of two, three, and four kinesin motors attached to a rigid cargo. The model builds upon previously developed models of single motors [4,5] and, importantly, includes a compliant linker domain that connects the motor domains to the cargo. Individual motors are allowed to independently step along the microtubule and the position of the cargo is tracked. Loads are imposed on individual motors both from random variations in the stepping rates that cause the motor–cargo linkages to stretch, and from external loads imposed on the cargo (as in optical-trapping experiments).

An important innovation in the Kunwar model [3] is the approach to load sharing. In an earlier model of multi-motor transport, Klumpp and Lipowsky [6] assumed that the load was shared equally by every attached motor. A more realistic picture is that,

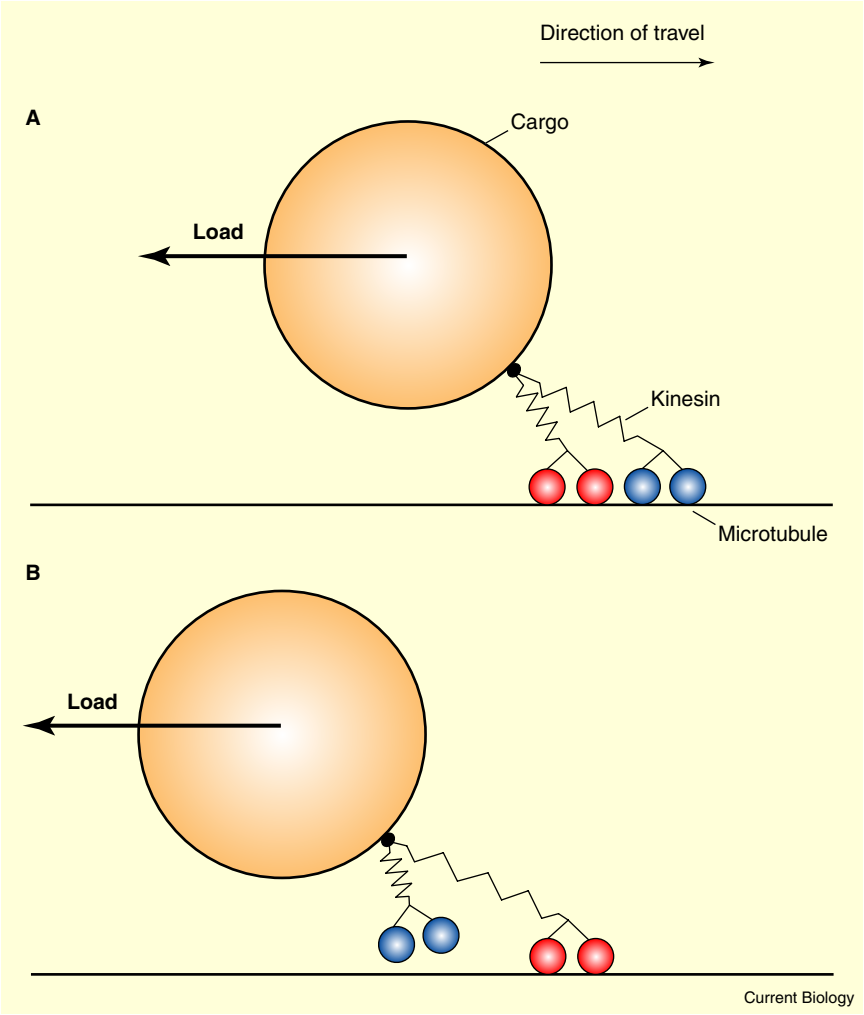

Figure 1. Mechanical model of two kinesin motors transporting a cargo against an external load, adapted from Kunwar *et al.* [3].

Due to the stochastic nature of stepping, one motor will step ahead of the other. (A) Because the motors are attached to the same point on the cargo, the compliant motor–cargo linkages will be unequally stretched and the lead motor (blue) will support the majority of the load. For stiff motor–cargo linkages, this effect will tend to cluster and synchronize the motors because the front, loaded head will step more slowly than the rear (unloaded) head (red). (B) The second effect of this unequal load sharing is that the front head (blue) will preferentially detach from the microtubule. Detachment leads to a backward displacement of the cargo both because of the rearward attachment point of the remaining (red) motor and because the compliant linker on the remaining motor is supporting the entire load.

against an imposed load, different motors (usually the leading motor) will shoulder more of the work, and the degree of coupling between the motors will depend on how stiffly they are connected to one another. This idea is reminiscent of current models for how the two heads of processive motors like conventional kinesin and myosin V coordinate – intermolecular strain tension between the two motor units through their mutual dimerization domain modulates the mechanochemistry of each head [7,8].

By including motor–motor interactions in the simulation, it is possible to test how changing the stiffness of the motor–cargo linkage affects cargo transport. The general idea is that with very compliant linkages the motors don't 'feel' one another and their additive effects are minimal, while stiff motor-cargo linkages lead to tighter mechanical coupling and better 'synching' of the motors. Increasing the external load that a cargo is moving against also causes the motors to synchronize – because motors slow with increasing load, when one motor pushes ahead and shoulders more of the load, it tends to slow down, allowing the lagging motor to catch up and share more of the load.

Another interesting prediction from the model is that a cargo moving against a load will periodically slip backwards due to detachment of the lead motor from the microtubule (Figure 1). With multiple motors on a cargo, the lead motor will shoulder more load and tend to preferentially detach from the microtubule. This has two consequences: first, since the attachment points of the other motor(s) are to the rear, the cargo will be pulled back; second, because the load is now pulling against fewer motors, the remaining motor–cargo linkages are shouldering more load and hence they stretch, pulling the cargo back even further. This effect is considerable with fewer motors (i.e. going from two to one) and with more compliant linkages, and it leads to the prediction that motors with compliant linkages will have considerably shorter run lengths at high loads.

Importantly, the Kunwar model [3] is shown to be consistent with experimentally determined stall forces and step sizes measured using an optical trap and beads functionalized with multiple motors. However, there is considerable potential for developing the model further. The Hookean spring approximation for the motor–cargo linkage is a reasonable starting point, but, because of its importance in determining motor coupling, further simulations using different compliant elements like freely jointed chains are warranted. A second aspect of the model that deserves further investigation is defining the point in the kinesin hydrolysis cycle where force acts. With stiff linkages, when one motor takes a step it bears a considerable fraction of the load. Kunwar *et al.* [3] chose to use the pre-step load as the determinant of the stepping kinetics rather post-step load or the mean load during the step, which might be more appropriate.

To experimentally characterize the motor–cargo linkage stiffness and define its role in motor–motor coupling, a novel and potentially fruitful approach is to use protein engineering to create peptide scaffolds that assemble motor proteins into defined geometries. Diehl and colleagues [9] recently attached kinesin heads to an artificial polypeptide backbone such that the

motors were placed in defined positions along the scaffold. Further, the flexibility of the scaffold could be externally modulated to alter coupling between the different motors. This approach enables considerably more precision in defining the mechanics of these multi-motor systems and experiments using coupled dimeric motors are underway.

While irreversibly binding motors to glass beads is an optimal approach for *in vitro* mechanical experiments and may be a good model for attachment to protein complexes in cells, this arrangement differs quite a bit from the attachment of kinesins to intracellular cargo, such as vesicles, Golgi, and mitochondria. When attached to a membrane, kinesins are free to move laterally in the fluid bilayer, which on one hand reduces the potential mechanical coupling between motors but on the other hand allows dynamic clustering of motors, leading to significant forces. This geometry has been successfully recapitulated *in vitro* by attaching kinesins to giant unilamellar vesicles and characterizing the extraction of membrane nanotubes by the attached motors [10,11]. Importantly, as is predicted for the rigid attachment to cargo, motors with different degrees of processivity show considerably different cooperative dynamics in this system.

Interesting and non-intuitive phenomena are observed when groups of motor proteins are attached to beads, protein scaffolds, and membranes. The power of these *in vitro* systems is the ability to vary experimental parameters and use quantitative models to predict and interpret experimental findings. Ongoing experiments and modeling should lead to important insights regarding transport by groups of motors in cells.

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Candida Biofilms: Is Adhesion Sexy?

The development of *Candida albicans* biofilms requires two types of adhesion molecule — the Als proteins and Hwp1. Mutational analyses have recently revealed that these molecules play complementary roles, and their characteristics suggest that they may have evolved from primitive mating agglutinins.

David R. Soll

In the past decade, bacteriologists, and more recently mycologists, have begun to realize that the microbes they study frequently infect hosts, not as free-living, planktonic organisms, but as multicellular biofilms that form on tissues, prosthetics and catheters [1–3]. Biofilms protect a pathogen from host defenses and antibiotics, and provide it with a degree of spatial stability and autonomy in controlling its own microenvironment. A biofilm utilizes sophisticated intercellular communication systems (such as quorum sensing in bacteria [4]), involves the formation of an extracellular polymeric matrix, depends on adhesion both to substrates and between cells, and can be composed of multiple cell types. Many of these characteristics are shared with tissues of higher eukaryotes, an analogy that evokes the hypothesis that biofilms formed by microbes may represent the first steps in the evolution of multicellularity in higher eukaryotes.

Candida albicans, the most pervasive fungal pathogen that colonizes humans, also forms biofilms on tissues, prosthetics, and catheters [2,3]. Although usually associated exclusively with host colonization, the formation of a biofilm by *C. albicans* may have preceded host colonization in the evolution of the organism, perhaps as a mechanism to protect cell propagation in a hostile environment, such as in soil or on a rock at the edge of a pond. In the formation of a *C. albicans* biofilm (Figure 1), cells first adhere to the substratum. This results in the formation of a confluent basal layer of cells that divide and produce compartmentalized hyphae, long tubular projections that intertwine in the upper region of the biofilm (Figure 1). Cells in the developing biofilm release a stable extracellular matrix of polymeric substances. Adhesion must play a major role throughout the development of a *C. albicans* biofilm: firstly, it must secure cells to the substratum and may bind them to one another in the formation of a basal layer, the first step in biofilm formation(s); and secondly, it may bind hyphae to each other, thus stabilizing the maturing biofilm. In both bacteria and fungi, our understanding of the adhesive forces involved in biofilm formation is rudimentary. Elucidating such adhesive mechanisms would be extremely useful in developing new