Current Biology *17*, R478–R486, June 19, 2007 DOI 10.1016/j.cub.2007.04.025

Cargo Transport: Two Motors Are Sometimes Better Than One

Review

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Molecular motor proteins are crucial for the proper distribution of organelles and vesicles in cells. Much of our current understanding of how motors function stems from studies of single motors moving cargos *in vitro*. More recently, however, there has been mounting evidence that the cooperation of multiple motors in moving cargos and the regulation of motor–filament affinity could be key mechanisms that cells utilize to regulate cargo transport. Here, we review these recent advances and present a picture of how the different mechanisms of regulating the number of motors moving a cargo could facilitate cellular functions.

Introduction

Cells are exquisitely organized, with multiple compartments and active transport of a variety of cargos between different locations driven by molecular motors. In addition, motors help to regulate signaling interactions by transporting a variety of receptors [1,2] and help to create specific protein distributions directly — by transporting proteins [3] — and also indirectly — by moving mRNA particles [4–6]. Motor function is also especially important in neurons, as illustrated by many neuronal disorders linked to transport malfunction [7].

Transport is often not 'all or none': rather than moving a set of cargos as fast as possible to a definitive location, cells instead frequently establish distributions of the cargos within the cells. What's more, the distributions change significantly in response to a variety of signals [8,9]. Thus, to understand transport and its regulation we must move beyond the study of single motors to consider how combinations of filaments, motors, and regulatory proteins work together to control cargo distributions. We would like to start with the properties of individual motors and understand which properties are responsible for which aspects of essential *in vivo* transport. The next goal is to combine single-molecule function with properties of cargos (such as the number of motors on the cargo and their organization on the cargo) and properties of the filaments (such as their affinity for motors, and how that might be changed, as well as the organization of multiple filaments), to ultimately understand how the cell can achieve specific transport tasks. This review will discuss recent work investigating how multiple motors work together, the ramifications of different numbers of motors on transport in the cell, and how the number of motors might be regulated to control this transport. Although we briefly discuss cargo-based regulation, we focus more on the regulation of motor–microtubule interactions from the microtubule perspective.

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Transport by a Single Motor

Molecular motors can be grouped into three families (Figure 1): kinesins and dyneins, which move along microtubules, and the myosins, which move along actin. Most kinesin family motors transport cargos towards the plus end of microtubules, while cytoplasmic dynein moves toward the microtubule minus end. The minus-end-directed kinesins are typically slow, and so far there are very few examples [10] of such kinesins playing a role in vesicular transport. Vesicular transport also occurs in both directions along actin filaments, with myosin V moving toward the barbed end [11], and myosin VI moving toward the pointed end ([12], and see [13] for a more complete discussion of the architecture of the individual motors).

Single-molecule *in vitro* studies have extensively investigated how single motors work in the absence of other proteins. For instance, kinesin-1, cytoplasmic dynein, and myosin V are each processive, meaning that they go through repeated enzymatic cycles without releasing from their filament. Thus, in principle, a single motor is sufficient to move a cargo some distance. *In vitro*, kinesin's processivity is approximately between 800 and 1200 nm [14,15], and myosin V's processivity is between 700 and 2100 nm [16]. Because kinesin takes 8 nm steps, this means kinesin takes on average about 100 steps before detaching from the microtubule, i.e. it has about a 1% chance of detaching per step. Because myosin V takes 36 nm steps, even though its average travel distance is the same or slightly higher than that of kinesin, it has a higher probability of detaching per step, and takes between 20 and 60 steps before detaching [17,18]. *In vitro*, at saturating (~1 mM) ATP, both kinesin [19] and myosin V [20] move on the order of 600 nm/sec, although significant deviations from this typical mean value are observed in different *in vitro* experiments. Dynein has a processivity of approximately 1000 nm [21], and because it can take steps of different sizes [22] has somewhat lower processivity than kinesin. Its processivity can be increased by dynactin [21], as can the processivity of kinesin-2 [23]. Note that the processivities measured *in vitro* may not directly translate to what occurs *in vivo.* For instance, *in vitro* experiments are typically carried out in the presence of non-physiological salt conditions, which can affect the motor's processivity [14].

Molecular motors undergo a mechano-chemical cycle, that is, as they go through their enzymatic cycle, converting fuel (ATP) into product (ADP + Pi), they translocate along a filament and are able to convert the energy released from hydrolyzing the phosphate

bond into mechanical work. Conversely, the application of mechanical force (i.e. load, opposing forward motion) can slow down the enzymatic cycle. Therefore, the force–velocity curve declines from maximum velocity at zero load to zero velocity at stall force [24]. Applied load decreases processivity [14,15] in addition to velocity, increasing the probability of detachment per step. Thus, in relating single-molecule *in vitro* experiments to the way motors function to move cargos in organisms, we need to carefully consider the loads that the motors encounter *in vivo* — applied load could significantly reduce how far a single motor would be expected to transport the cargo, as well as how fast it would go.

A recent study examined the way in which single motors move *in vivo* [25]. The study used artificial cargo–motor complexes (quantum dots coated with streptavidin and coupled to biotinylated kinesin), which were internalized in live HeLa cells via induced pinocytosis. The authors concluded that the motor's processivity and velocity are approximately the same *in vivo* as *in vitro*, suggesting that the above-mentioned concern of different ionic conditions may not be that important in comparing *in vitro* and *in vivo* data. However, the data presented in this study appear to suggest a greater variation in travel velocity than that observed *in vitro* [25]. While in general the approximate agreement between *in vitro* and *in vivo* single-molecule function is encouraging, as far as validating the *in vitro* single-molecule studies, such studies must not be too broadly interpreted — the actual full-length endogenous motors on endogenous cargos may still behave quite differently. In particular, regulatory proteins might be used to alter the function of motors specifically when the motors are bound to endogenous cargos and incorporated into cargo-specific complexes.

Transport by Two Motors

A number of studies *in vitro* have investigated how multiple motors work together. When motor proteins were first studied *in vitro*, gliding assays were performed in which motors were adsorbed to glass surfaces and filaments were then observed to move around in the flow cell. In addition to demonstrating that individual motors were sufficient to move cargos (at low motor density, nodal-point pivoting was observed, consistent with a filament being moved by a single motor) [26], the gliding assays were crucial in demonstrating that the velocity of a cargo moved by multiple processive motors is the same as that of a cargo moved by a single motor, as long as the load per motor is negligible. That is, barring severe load, more processive motors do not move a cargo faster [27].

However, because the filaments could not diffuse away from the surface rapidly when released from the motors, the gliding assays could not provide significant information about cargo persistence, i.e. how far a cargo driven by more than one motor would go before detaching from the filament it was moving along. Early bead assays revealed that beads moved by multiple kinesins did in fact move further than beads moved by a single kinesin [28], but the magnitude of the effect and the number of motors required were not investigated. Recent work has revisited this

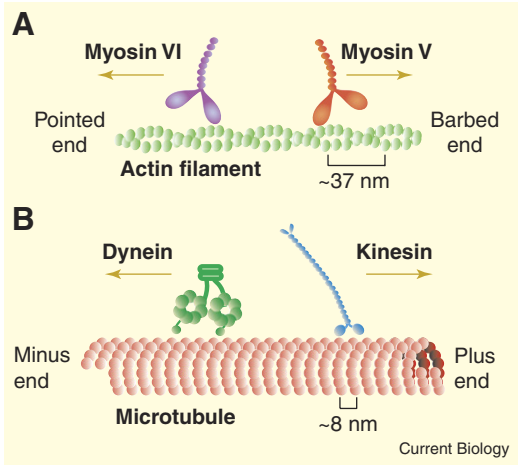

Figure 1. The families of vesicular molecular motors.

(A) Myosin motors move along actin filaments; the most well-known myosin that moves towards the barbed end is myosin V, and towards the pointed end is myosin VI. Structurally, the myosin motors share substantial similarity. (B) The two families of microtubule motors, with most of the kinesin-family motors moving towards the plus end, and cytoplasmic dynein moving towards the minus end. Structurally, kinesin and dynein are very different.

question in more detail, and suggests that approximately two engaged motors (either kinesin or dynein) are sufficient to move a cargo extremely long distances (i.e. more than 8 μm) [29,30]. Theoretical work confirms that long cargo travels are expected from two kinesin motors functioning together [31]. The magnitude of the increase in travel distance for the experiments is larger than what was predicted theoretically, but the two cannot be perfectly compared, because the geometry used in the theory assumes only one group of clustered motors, whereas in the experiments motors were randomly attached to the bead's surface. Therefore, although the cargos were most often propelled by two motors, when one of the two motors detached, more than one other motor was available to reattach, potentially resulting in a higher than expected on-rate (see supplement of [29] for a discussion of this). The extent to which this geometry issue resolves the differences between theory and experiment remains to be investigated.

Regardless of the exact magnitude of the change, it is clear that a cargo moved by approximately two motors can move more than twice the distance of a cargo moved by a single motor, and when three motors are able to be engaged simultaneously, both theory and experiment concur that the expected average travel is more than 13 μm. Thus, the increase in travel distance is a very non-linear function of the number of motors. The reason for this is that under conditions in which more than one motor is present, when one motor detaches, the second (engaged) motor holds the cargo close to the filament, so that the detached motor has the possibility to rebind the filament (Figure 2) [29]: as long as the detached motor rebinds before the other motor releases, the cargo keeps on going.

In principle, then, two motors should be sufficient to move a cargo over very long distances, with the caveat

that how far a set of motors is expected to move a cargo will be influenced by the opposing force they feel (see below). Most cells have a radius of tens of microns or less, and individual microtubules are rarely longer than 50 μm [32]. Combined with the observation that individual cargos are frequently transported along microtubules between organelles that are radially arranged, and separated by a few microns, it seems likely that in most cases a few motors should be sufficient to move a cargo where it needs to go, with minimal likelihood of the cargo falling off the track and diffusing before it arrives at its destination. For actin-based transport, in most cases even a single motor would have sufficient processivity to reach the filament end because actin filaments are typically short [29]. The fact that the travel distance of a cargo is so sensitive to the number of engaged motors (when small numbers of motors are employed) suggests that control of the number of engaged motors might be a useful way to regulate transport. In order for this general model — altering transport by tuning the average number of engaged motors between none or one and two or three — to be viable, cargos must in general be driven *in vivo* by relatively few motors. Data supporting this proposition are discussed next.

The Number of Motors Moving Cargos *in Vivo*

To be able to tune transport by altering the number of engaged motors, there must be a moderate number (somewhere between one and five or so) of motors engaged — if cargos are driven by many motors, the cargo would be expected to go so far that a single motor engaging or dis-engaging (e.g. a change from seven to eight motors) would effectively be irrelevant, unless additional regulation is present. We therefore propose that most *in vivo* cargos are indeed moved by a limited number of motors. Below, we review a variety of studies that are consistent with our hypothesis. We also review the limitations of these studies to outline the bounds of our current knowledge and to highlight the need for more research in this area.

There are two distinct questions of interest regarding the number of motors involved in moving cargos *in vivo*. The first is the number of motors engaged in moving the cargo at any particular time in any particular direction, and the second is the number of motors altogether present on the cargo.

There is currently no experimental methodology for visualizing the number of engaged motors on an *in vivo* cargo at any particular time. The best guidance to date comes from cryo-electron microscopy (cryo-EM) studies that examined the number of cross-bridges between microtubules and cargos [33,34]. A detailed count of cross-bridges for both anterograde and retrograde-moving vesicles in the squid axoplasm revealed a distribution centered around two and ranging between one and five [34]. The above studies did not establish the nature of the cross-bridges, so it is unclear whether all cargo–microtubule linkages are active functional motors. These reports therefore provide an upper bound on the number of engaged motors and their estimates agree well with our proposal that the number of engaged motors is typically (though perhaps not always) small.

It is also of interest here to review studies where the motors attached to cargos were visualized using immunogold EM [34–44]. These complement the cross-bridge studies nicely because the antibodies conjugated to the gold nanoparticles specifically recognize the molecular motors, so the above concern that some of the cross-bridges might not reflect motors is alleviated. Examining the number of nanoparticles then provides an indication of the number of motors present on cargos. For kinesin and dynein, these EM images show a distribution of motors typically ranging in number between one and seven on a wide variety of cargos such as various vesicles [35,37,38,43], and melanosomes [41,42]. Reports of the number of mitochondrion-bound motors range from few (1–2) [43] to many (100–200) [35]. These discrepancies could reflect organismal differences, but the exact explanation is unknown. Notably, a maximum of four mitochondrion–microtubule cross-bridges have been reported [33]. While most of these studies appear consistent with our limited motor number hypothesis, there are many caveats in interpreting these data. First, not all motors present may be labeled, either because the antibodies may not penetrate deeply or because some of the epitopes recognized by the antibodies may not be preserved during sample preparation. Second, the EM studies show cross-sections, so a careful estimate of the overall number of motors on the cargo would require serial cross-sections and integration of the number of motors. Third, because most of the above immunogold studies were not trying to quantify the number of motors present, the 'representative images' shown may not be representative with regard to the number of motors present. Fourth, motors such as dynein are known to fall off some cargos during purifications, so the studies (e.g. [35,37,38,43]) in which the EM was carried out on purified cargos may under-represent the motors present because some have been lost.

Given the large sizes (>500 nm) of many cargos like melanophores, lipid droplets, mitochondria, and endoplasmic reticulum (ER) vesicles, a random distribution of a small number of microtubule motors on the cargo's surface would make it unlikely that more than one motor would be able to reach the microtubule at any one time. Many immunogold EM images, however, appear to show motors that are grouped in clusters of two or more and that, in many cases, all of the motors on the cargo are localized in one cluster. While evidence for this clustering can be found for kinesin [35,40,42], dynein [41,43] and some myosins [36,39,44], a study in which both kinesin and myosin V were co-labeled on ER vesicles with gold particles of different sizes shows that kinesin is mostly clustered while myosin V is randomly distributed on the surface of the vesicles [40], suggesting that the clustering apparent in the micrographs may not be an artifact of the preparation or imaging. Further, this last study observed many myosin V motors but few kinesins. Thus, myosin V appears to serve as an internal control, indicating that the immunogold–EM technique has the sensitivity to observe many motors (if present). Because of this, the fact that all the studies observe only a few sites that are immunogold-labeled by the anti-kinesin- or anti-dynein-complexed gold beads suggests that only

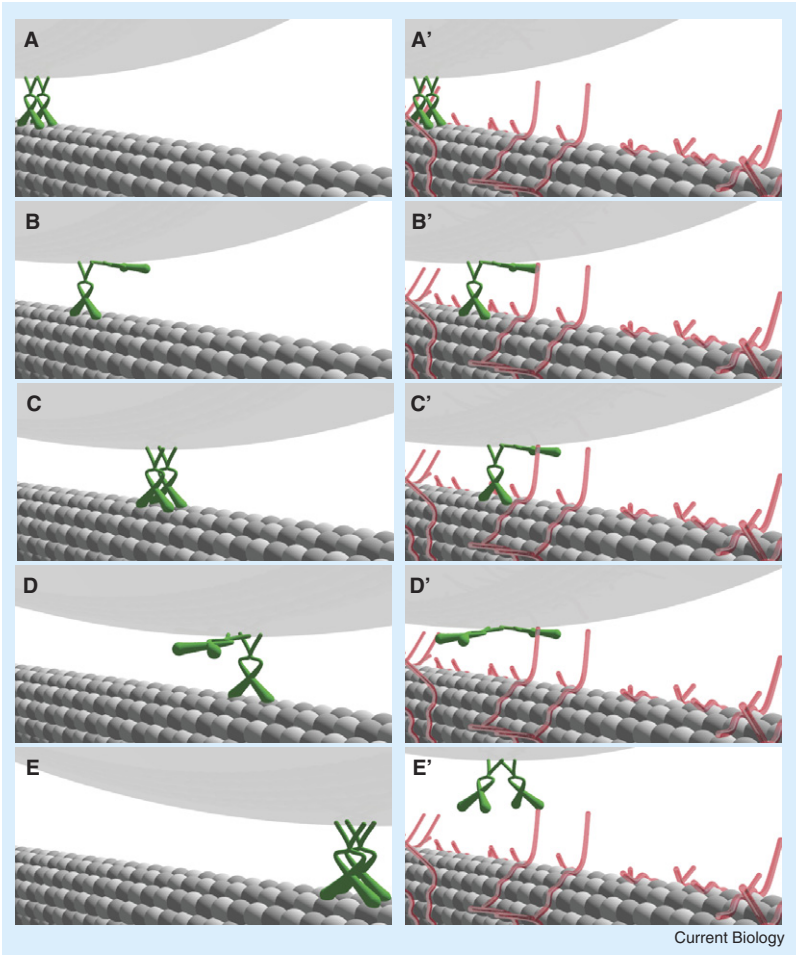

Figure 2. Two motors moving a cargo along a microtubule.

Left: the motors start out both bound to the microtubule (A), but while one of the two detaches (B), the second remains, keeping the cargo close to the motor and allowing the detached motor the opportunity to rebind (C). This type of detachment–reattachment continues (D, E), and the cargo is transported very long distances. Right: the situation changes due to factors such as tau (red filaments) that decrease the motors' on-rates. Then, although both motors start out engaged (A'), when one detaches (B'), it does not re-attach to the microtubule (C') because of its decreased on-rate. Then, the second motor detaches from the microtubule (D') before the first motor has a chance to rebind, and ultimately the cargo is no longer bound to the microtubule but instead diffuses away (E').

a few of these motors are bound to the cargo and that these motors are clustered.

Here too, however, there are caveats in interpretation. When gold clusters appear, one of the common concerns is that the antibodies to which they are bound are partially denatured or precipitated, so that the clustering represents problems with the antibodies rather than intrinsic clustering of the recognized proteins. Furthermore, it is difficult to design a careful control for this effect, because antibody denaturation can be different for different primary antibodies. Thus, in the example [40] where myosin V is observed unclustered and all around the cargo but kinesin is not, there is the formal possibility that the kinesin clustering is due to conditions resulting in denaturation and subsequent aggregation of the anti-kinesin antibody, but not denaturation of the anti-myosin V antibody. In conclusion, then, these EM labeling studies appear to be consistent with our hypothesis of few clustered cargo-bound microtubule motors, but alone are not definitive. We note, however, that the EM studies visualizing cross-bridges are not prone to these types of errors and came to effectively the same conclusion.

Assuming our interpretation is correct, these EM studies set the upper limit on the number of motors that can be engaged in moving a cargo at any one time. The number of motors actually engaged in moving lipid droplets in *Drosophila* embryos was inferred from stall force measurements [45,46]. Using an optical trap and counting the percentage of cargos escaping a trap of increasing power, combined with changes in this percentage under different conditions, the average number of motors moving cargos was inferred. The numbers ranged between three and five depending on the phase of development of the embryo [45]. These numbers agree with the number of available motors visualized in electron micrographs in other systems and are consistent with the notion of having only a few motors whose collective function can be regulated by tuning the transition between single- and multiple-motor-based transport.

Recently, three studies used peaks in the velocity distributions of moving cargos to infer the number of motors moving them [47–49]. To that end they assumed that the motors are moving against a substantial viscous drag so that changes in the speed of the cargo can be directly attributed to the loss or gain of a motor. Using that argument they went on to determine the number of motors moving a cargo of a certain velocity. Analysis of peroxisomes [47–49] reported a maximum of 11 plus-end motors and 12 minus-end motors, while analysis of pigment granules and neuronal vesicles [47–49] reported a maximum of approximately four motors.

We have recently contributed to two studies that examine the use of velocity as a readout of the number of

engaged motors. First, the basic assumptions and analysis in the three papers above [47–49] were re-examined [50] and were found to be inconsistent with known properties of molecular motors, namely, with the well-established fact that velocity decreases with increasing opposing load [15]. Furthermore, we carried out a systematic analysis of velocities of moving lipid droplets in *Drosophila* embryos and found no defined peaks in the velocity distribution [51]. Our studies therefore suggest that regularly spaced peaks in cargo velocity distributions are either specific to the system under study or are an artifact of data analysis and normalization.

While we have argued extensively that velocity is not a good indicator of the number of engaged motors, the prospect of being able to measure the instantaneous number of motors moving a cargo *in vivo* is appealing. Measuring the force that motors exert in moving an individual cargo provides a way to do just that, because the force exerted by multiple motors is approximately additive [29,30], so motility contributions from several motors can be discerned from *in vivo* force measurements. Moreover, motor attachment and detachment dynamics can potentially be obtained via force measurements at various positions as the cargo moves along. Reports on overcoming the experimental hurdles that stood in the way of such measurements were recently presented by our lab [52] and confirm the picture that lipid droplets are moved by relatively few motors.

Control of the Number of Engaged Motors

Alteration of the number of active motors could be achieved in two distinct ways. First, the cell could control the number of motors physically present on the cargo, thus affecting the number that can be engaged, i.e. it can control motor recruitment. Second, it could leave fixed the number of motors on the cargo but alter how many are typically working, i.e. it could control motor engagement. There are many ways in which motor engagement can be regulated: the motors can be turned off completely (e.g. via a post-translational modification or binding partner); or their affinity for the tracks can be tuned.

At a molecular level, there are a number of ways to alter motor recruitment. It can be altered by Rab function [53] and in principle by recruitment of motor-binding proteins to the cargo's surface [54], as well as by control of interactions between motors and docking proteins via phosphorylation. Two examples of such regulation have been cleanly described: the release of cytoplasmic dynein from membranes due to phosphorylation by Cdc2–cyclin B1 kinase (to turn off transport vesicular transport during metaphase) [55], and localized release of kinesin from vesicles due to GSK3-mediated phosphorylation of the kinesin light chain [56]. These types of model have been previously considered [53,57] and will not be discussed extensively in this review.

While control of motor recruitment is a prevalent model for regulation of transport and is clearly used to control the transport of some cargos, it is perhaps not quite as prevalent as is generally perceived. Control of motor engagement (while the motor(s) remain bound to the cargo) in the effective regulation of the number of functioning motors has received less attention and is the focus of the remainder of this review. There is evidence of alteration of cargo motion while the number of microtubule motors on the cargo remains constant. For instance, motors of a given polarity are known to remain on cargos while those cargos move in the reverse direction [46,58–61], suggesting that *in vivo* control of motor engagement plays an important role in regulation of transport. For melanophores, the amount of kinesin-II or dynein bound to the cargo is the same, regardless of whether the cargo's average motion is towards the plus-end or minus-end of microtubules (see Figure 9 in [62]). Similarly, for lipid droplets the force required to stall the droplets (reflecting the number of engaged motors) changes [45], but dynein levels appear not to alter (S.P.G and S.C., unpublished observations).

How might motor engagement be controlled? Below, we focus predominantly on alteration of on-rates and off-rates, but the motors' enzymatic activity could also be targeted by binding partners or phosphorylation — for example, Lis-1 may play this role in part for dynein [63], phosphorylation has been reported to alter kinesin's enzymatic activity [64], and recent work shows that there are binding partners [65] that alter kinesin's overall function. Similarly, in the case of lipid droplets, LSD2 alters both plus-end- and minus-end-directed droplet motion but does not affect the overall amount of dynein on the droplets [61].

The off-rate of a motor reflects the probability of its detachment from the filament. This probability is influenced by the affinity of the motor to the track as well as external environmental influences, such as load. The off-rate is well characterized by the motor's processivity. In contrast, special care needs to be taken when defining the on-rate. In this discussion the on-rate is not the rate at which a motor in solution diffuses and binds to the microtubule, nor is it a rate at which a free-floating cargo (with a motor attached) docks to a microtubule. Instead, the on-rate here is referring to the case when a cargo is already tethered to a microtubule, and the motor in question is restricted by the cargo to be in the vicinity of the microtubule. Thus defined, the on-rate reflects the rate at which the motor is able to bind to the microtubule and can be modified by proteins bound to the microtubule, by any microtubule post-translational modifications, by geometric restrictions on the motor's position, and by the properties of the medium in which the motor is diffusing. Frequently, factors that affect the off-rate affect the on-rate as well.

Factors that affect motor binding strongly may only have a weak effect on motor processivity and vice versa. One example where the on-rate and the off-rate are regulated differently is cargo transport along microtubules obstructed by the microtubule-associated protein (MAP) tau (see below).

The Effect of Load on Velocity and Processivity

In vitro, the application of load opposing motion decreases both motor velocity and processivity, although the details of the effect may be different for each motor. For kinesin, the response is non-linear. Kinesin's velocity is relatively insensitive to small

to moderate load; at approximately half the force required to stall the motor (½ max stall), velocity decreases by only about 20%. The remaining 80% decrease in velocity occurs between ½ max stall and stall. In contrast, kinesin processivity is quite sensitive to load; between no-load and ½ max stall it decreases by approximately 70%, with the remaining 30% decrease occurring between ½ max stall and stall [15]. For dynein, the situation is less clear because of differences between current *in vitro* measurements. One set of measurements [22], coupled with a theoretical model [66], suggest that dynein's function should be quite sensitive to load, while another set of measurements [67] suggests that functionally dynein's response to load (in terms of velocity) is almost the same as that of kinesin. Further experiments are needed to understand the nature of these significant differences. Based on these *in vitro* findings, one plausible mechanism contributing to the control of the number of engaged motors is to control the load felt by the motors. Below, we evaluate the potential importance of a few sources of load.

A Possible Source of Load: Viscous Drag from the Cytosol

The cytosol is a viscous medium, so that a cargo moving inside a cell will experience an opposing force — the faster it moves, the more the cytosol opposes its motion. Theoretical work [31] suggests that in some cases cytosolic load could have a significant effect on cargo motion. The viscosity of the cytosol is thus potentially very important for intracellular transport.

Unfortunately, viscosity is a local property and may change not only between cells but also within each cell. Reports of the magnitude of viscous drag vary widely [68] and a systematic understanding of these effects is lacking. The actual effect of the load from the cytosol on velocity and mean travel for an *in vivo* cargo driven by multiple motors is still under investigation. However, with respect to velocity, we believe that the effect of cytosolic load may be relatively small. We base this on two arguments: there is little evidence in support of it, and some against it. The only proposed direct support for a strong role of cytosolic drag in determining velocity is the reported peaks in velocity distributions *in vivo* [47–49] (where those peaks were attributed to transport by different numbers of motors and reflected cytosolic load). We pointed out [50] that the model employed by these papers is not quantitatively consistent with the basic property of molecular motors, such as dyneins and kinesins, i.e. that they slow down under opposing load. Second, recent experiments from our lab have observed no significant correlation between the velocity of moving cargos and the force required to stall them (S.P.G. and G.T.S., manuscript in preparation).

Other Sources of Load: Molecular Brakes?

In principle, in addition to viscous drag, one can envision other sources of load (which could be more amenable to regulation). For instance, a number of proteins have the ability to bind to microtubules independent of the motors and such proteins could be part of a complex that links the cargo to the microtubule. Depending on the nature of the binding to the microtubule, such independent cargo–microtubule linkages could be hard to slide along the microtubule, providing load [69]; regulation of mean motor transport distances could then occur by tuning the strength of the sliding linkage (e.g via phosphorylation). The extent that such a mechanism is actually used in an organism to alter travel distances remains to be determined.

The Effect of Non-Load Methods on Transport

Load is not the only way to regulate typical travel distances; the off-rate of the engaged motors can be regulated via their interaction with various proteins that either affect motor performance directly or affect cargo processivity by providing an additional link between the cargo and the microtubule. This motor regulation has been observed for actin-based motors (myosin V processivity can also be regulated by calcium [70]), for cytoplasmic dynein [21], and for kinesin [23,64]. Similarly, motor processivity can be regulated via changes to the motor-binding substrate (the microtubule or actin filaments) either directly or via substrate-binding proteins. For instance, myosin–actin binding is sensitive to the actin charge distribution [71]. Similarly, kinesin–microtubule binding is strongly ionic and is sensitive to local charges of individual tubulins (modifiable via post-translational modifications) [72–74] and the ionic strength of the solution [75,76]. Finally, MAPs such as tau and MAP2 can decrease motor processivity [29,77].

The Effect of Filament-Binding Proteins

The tau protein has many microtubule-related roles *in vivo*, including stabilizing and organizing microtubules. Another key role now coming into focus is its pronounced effect on transport — excess tau in living cells produces a transport defect phenotype (particularly strong for kinesin-based transport) [78,79], although perhaps not in all model systems [80].

Initial studies of a recombinant truncated form of kinesin suggested that the *in vitro* velocity and processivity of single kinesin motors moving along microtubules was unaffected by the presence of tau [81]. A more recent study, using full-length bovine kinesin attached to a cargo, suggests that the presence of tau does indeed decrease individual kinesin's processivity. However, the effect on single motors was far smaller than that observed for multiple-motor-based transport [29]. Additionally, *in vitro* force production of single kinesin motors was shown to be unaffected by tau's presence [29]. Taken together, these results suggest that tau does not have a dramatic effect on single kinesin function once the motor is bound to a microtubule.

In contrast, tau's effect on kinesin's on-rate is more pronounced. Tau, at concentrations close to what is found in white matter axons [82], severely inhibits kinesin binding to microtubules *in vitro* [29,81]. This produces a major change in motility of a cargo with several active kinesin motors available for transport [29]. First of all, fewer kinesins are able to initially dock with the microtubule, providing fewer tethers between the cargo and its track. Furthermore, even though each motor only feels a moderate effect of tau while bound

to the microtubule, once it dissociates from the microtubule its re-binding is strongly inhibited by tau. *In vitro* tau can thus reduce potential multiple-motor-based transport to the single-motor limit [29] and does this primarily by altering the motor's effective on-rate. We propose that this effect is not unique to tau because other MAPs such as MAP2 also alter kinesin's on-rate [77,81]. Thus, alteration of the on-rate, resulting in tuning of the number of engaged motors, is likely a general paradigm for the function of an entire class of fibrous MAPs; such a suggestion has a precedent in muscle, where myosin activity is regulated via tropomyosin [83]. This idea further raises the exciting possibility of having specific MAPs that interact particularly strongly with particular motors, allowing for specialized highways favoring certain motors over others. While this idea is still speculation, we do know that some MAPs have special localizations, for instance tau is found in axons whereas MAP2 localizes to dendrites [84].

The Effect of Post-Translational Modifications

MAPs are not the only way to modify transport from the microtubule side of the microtubule–motor interaction — post-translational modifications of tubulin can also regulate motor on-rate and off-rate. For instance, the affinity of kinesin for microtubules containing detyrosinated [73] and acetylated [72] α-tubulin is higher than for those with non-modified tubulin. For cargos with a moderate number of potentially active motors, such a change in the on-rate or off-rate could shift the average number of engaged motors from one to two, resulting in a very large change in transport. Of note also is the recent observation that excessive polyglutamylation abolishes ciliary motility due to inhibition of axonemal dynein function [85], suggesting that this modification can also regulate processive motors. Unfortunately, the details of how these and other modifications affect the on-rate and off-rate of motors is currently unclear, and hence so are the quantitative details of their effect on multiple-motor-based transport. In particular, we do not know whether the magnitude of their effect on motor on-rate or off-rate is sufficient to significantly alter the average number of engaged motors.

Ramifications of the Number of Engaged Motors

Above, we examined how two motors can move a cargo much further than a single motor and discussed the range of potential mechanisms used by a cell to tune the number of engaged motors. There are other ways that changing the number of engaged motors might be useful in the biological context of achieving particular cargo distributions, particularly with respect to events at filament–filament intersections.

Increased robustness of transport is not always synonymous with increased efficiency. For instance, *in vitro* experiments have shown that cargos driven by many motors take longer to choose which way to go at microtubule intersection or branching points and exert more strain on the microtubules while at the intersection [29]. The likely mechanism here is that active motors attached to the cargo explore all possible microtubule tracks and the final choice is made via a tug-of-war between all engaged motors. More engaged motors thus naturally implies higher forces exerted on the microtubules and a longer time until motors detach from all but one possible track. The strain and traffic stoppages associated with such contention may be undesirable in many polar cells which rely on efficient transport for their function and internal maintenance (the most notable example being neurons).

The above-mentioned MAP tau may provide an example of how neurons address traffic efficiency issues at microtubule branch points. Six isoforms of tau are found in human neurons. It is now well established that these isoforms differ in the extent of their effect on kinesin-based transport. Notably, the 3RS isoform effects a much stronger reduction in kinesin on-rate than the 4RL isoform at the same concentration on microtubules [29,81]. Curiously, a recent study examined the localization of three-repeat and four-repeat tau constructs in the IMR-32 neuroblastoma cell line [86]. The three-repeat construct was observed in puncta near the axonal branch points, while the four-repeat construct was found elsewhere in axonal processes. This is consistent with the neuron using a local decrease in engaged motors at a branch point to avoid stranded cargos with motors engaged in futile tug-of-wars. Thus, through its localization, three-repeat tau may help achieve efficient transport through axonal branch points, though a causal relationship has not been established to date.

More generally, it has been shown *in vivo* that cargos do switch between filaments and that this process can be regulated both for actin–actin switching [87] and for microtubule–actin switching [8]. This regulation is not well understood but, similar to microtubule–microtubule switching (see above), actin–actin switching probably involves both changes in the number of engaged motors [87] and alteration of the function of individual motors [88–90]. For microtubule–actin switching, the role of the number of motors is also likely to be important: the number of motors driving a cargo along a microtubule affects the cargo detachment force. *In vitro* experiments [29] recently illustrated this effect. We expect that the more force the actin-based motors are able to exert relative to the microtubule-based motors, the more likely it is that the tug-of-war will result in a switch to actin-based transport. Supporting such a hypothesis, *in vivo,* we note that tau protein is typically distally enriched in neurons [91], presumably resulting in a gradual decrease in the number of engaged kinesin motors as the cargo approaches the growth cone. Such a decrease could help favor the myosin V motors so that the cargos would switch to actin filaments for shorter-range local transport and delivery.

Conclusions

In this review we have highlighted how cargo transport in cells can be regulated via tuning motor–track attachment. The dramatic difference between the single-motor-based and multiple-motor-based transport observed *in vitro* suggests that tuning the number of engaged motors is most relevant when the number of available motors on typical cargos is low. A survey

of existing reports suggests that many types of intracellular cargos do indeed have such a low number of motors, making such tuning a potential regulatory strategy. We further highlighted ways in which motor–track attachment strength can be regulated, including post-translational modifications of motors and tracks, as well as track-associated proteins (e.g. MAPs of the tau family). Such filament–motor-directed (as opposed to the more studied motor–cargo-directed) regulatory mechanisms are likely to influence the shunting of cargos down side-branches in neurons, the transfer of cargos from microtubules to actin (or vice versa), and so on. In addition, post-translational modifications of motors and tracks and/or MAPs could regulate binding of specific motors to specific tracks, allowing them to segregate and guide cargo transport.

As we look ahead, we face two exciting challenges: first, to understand the extent to which this filament-based regulation is used and tuned, and second, to understand how different regulatory strategies are integrated within the organism to allow the exquisite control that clearly exists.

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