4.15 Intracellular Transport: Relating Single-Molecule Properties to *In Vivo* Function

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Abbreviations

ATP adenosine triphosphate
EM electron microscopy
LD lipid droplet
MAP microtubule-associated protein

Glossary

Dynein A motor protein that moves in the opposite direction from kinesin along microtubules.
Kinesin An enzymatic protein known as a motor protein because it hydrolyzes ATP and converts the energy stored in the phosphate bond to motion.
Microtubule A stiff polymeric filament in cells used as a highway for long-distance transport; the motors kinesin and dynein 'walk' along it, with kinesin moving toward the microtubule plus end and dynein moving toward the minus end.

4.15.1 Overview

In older textbooks, there is little mention of how cells are organized and the importance of that organization. The modern view is quite different. We now know that many cellular processes depend on the existence of distinct subcellular compartments with unique properties and on the spatial organization of these compartments relative to each other, allowing controlled transport of components between them. Both the positioning of these compartments and the transport between them rely on enzymes called molecular motors that walk along cytoskeletal tracks. These motors are true nanomachines: They burn fuel (ATP) and use this chemical energy to do mechanical work, taking 8- to 36-nm steps (depending on the motor) and exerting forces up to approximately 7 pN. Typically, they are approximately 50% efficient – that is, approximately 50% of the energy released from ATP hydrolysis can be converted into useful mechanical work.

The function of these motors is fascinating, and in no small measure the tools to study these proteins at the single-molecule level have been developed motivated by the desire to understand the proteins mechanistically, at a fundamental level. There are three such classes of motors – the myosin motors that move along actin filaments, the kinesin motors that move along microtubules, and the dynein motors that also move along microtubules – and reviews describe what we know about the motors' single-molecule functions.[1,2] Thus, we know much about the way these proteins function, although there are still interesting open questions related to the mechanism.

However, single-molecule knowledge is only the start of this journey to understand cellular function and organization. We cannot easily explain how transport and organization processes in cells work using only the information gained from single-molecule *in vitro* studies on purified proteins. The challenge before us, then, is to understand how to make this transition. This chapter discusses our current understanding of this process, highlighting progress to date, and the challenges involved in using single-molecule function to understand *in vivo* transport.

4.15.2 An Introduction to the Families of Molecular Motors

Many families of molecular motors accomplish various roles in cells. This chapter focuses on cargo transport motors – that is, on motors used to move cargos from one subcellular location to another. Such cargos can include 'traditional' membrane-bound organelles, such as axonal vesicles or mitochondria, but can also include LDs or protein particles such as signaling complexes or viruses, mRNA particles, and 'large' cargos such as chromosomes or entire nuclei. Long-distance transport occurs along microtubules, which are polar filaments with a 'plus' end and a 'minus' end. The microtubules are usually arranged with their minus end close to the nucleus and their plus end away from the nucleus. For many cells with the nucleus in the cell center, this corresponds to minus end at the cell center and plus end at the periphery. However, other organizations of microtubules are frequently found in polarized cells (Figure 1). There are two classes of motors (Figure 2) that move along microtubules: the kinesin family of motors that predominantly moves toward the microtubules' plus ends and the dynein family of motors that moves toward the microtubules' minus ends. Given typical microtubule organization, one might imagine that transport of components away from the nucleus would be mediated by kinesin-powered transport, and transport toward the nucleus would be mediated by dynein. This is predominantly correct, although it is not nearly as straightforward as would initially be expected.

One interesting aspect of the kinesin and dynein motors is that they appear to have taken a slightly different evolutionary strategy. There are many kinesin family member proteins, each likely optimized for moving a subset of intracellular cargos.[3] Almost all kinesin motors move toward the microtubule plus end, although there are a few slow mitotic kinesin motors that move toward the minus end. In contrast, there are only a few cytoplasmic dynein motors (all moving toward the microtubule minus end), and these few motors each move many different kinds of cargos. Accumulating evidence suggests that for dynein, much of the 'tailoring' of the motor for specific tasks is accomplished by mixing and matching different dynein complex subunits and accessory proteins.[4] Although such a hypothesis is appealing, it is still far from proven, and certainly more experimental data on the effects of protein cofactors will be required to evaluate the extent of difference in 'tunability' between dynein and kinesin.

Microtubules are long, typically with a rather well-defined organization. In contrast, actin filaments – used by the myosin family of molecular motors – are shorter and have much more variation in their organization. As such, it is clear that actin/myosin-based transport in typical mammalian cells cannot be responsible for the majority of long-distance transport, and

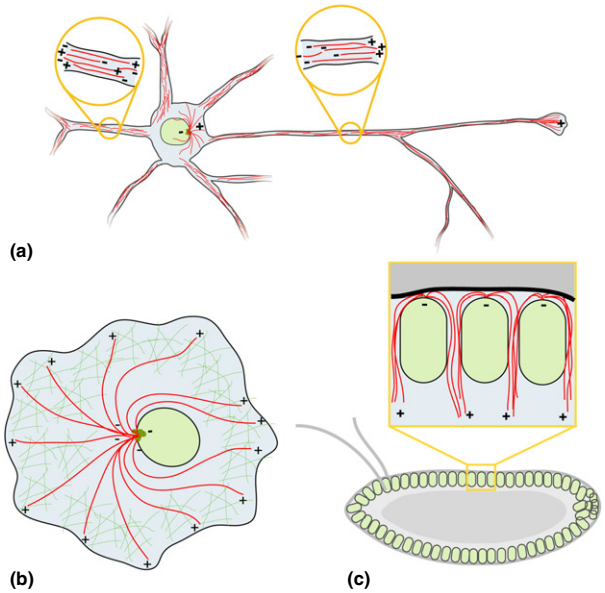

Figure 1 Different cell types have different microtubule architectures. Long-distance transport of organelles and other cargos occurs along microtubules (shown in red), which are filaments with directionality, typically indicated by referring to the 'plus' end (+) and the 'minus' end (−). The microtubules are usually arranged with their minus ends close to the nucleus and their plus ends away from the nucleus. In many cell types, the nucleus is in the cell center Fig. 2 in the International *Journal of Biochemistry & Cell biology 40*(4), 2008, pages 619–637 (b), but other organizations are possible, as in the case of the *Drosophila* embryo schematized in panel c. Given the high aspect ratio of neuronal cell processes (a), motor-based transport of cargos is especially vital. Microtubules in the axons are typically arranged with their minus ends all pointing toward the cell body and their plus ends away from it (right insert in panel a). Dendrites, on the other hand, have shorter microtubules that have a mixed organization Fig. 1 *from phys. Biol* 1 (2004) RI-RII, Hithes and yon: a review of bi-directional microtubule-based transport, steven P. Gross (left insert in panel a).

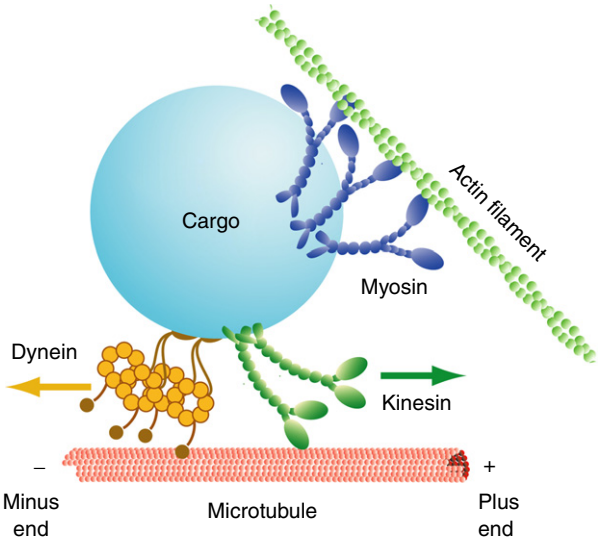

Figure 2 Molecular motors attach to both cargos and filaments. Cargos are hauled inside cells by molecular motors along cytosolic filaments. Two families of microtubule motors, kinesins and cytoplasmic dynein, are responsible for most long-distance transport. Kinesins and dyneins predominantly move toward the plus ends and minus ends of microtubules, respectively. Members of the myosin families of motors move cargos along actin filaments and translocate toward either the barbed ends or the pointed ends of the filaments, depending on the myosin isoform.

any description of transport involving them must carefully consider the actin filament organization as well as properties of the motors. However, there is significant evidence that in some cases actin-based transport is used as a local road system for transport to places that microtubules do not reach.[5–7] Also, there is surprising work demonstrating long-distance actin/myosin-based transport in special cellular projections where actin is more organized.[8]

Because actin filaments are also polar/directed, one might expect there to be myosin family motors that move in each direction along them. This is indeed correct, with almost all myosins, including the myosin V and myosin X family motors, moving toward the barbed (+) end, and the myosin VI families moving toward the pointed (−) end of the filaments.

4.15.3 Single-Molecule Properties of the Molecular Motors

Biophysical approaches have been successful at determining how single molecular motors function.[1,2] Motors are characterized by their processivity (how far a motor typically walks along its filament before detaching), their stalling force, and their velocity. Because applied load (opposing motion) alters both processivity and velocity, there are measured force–velocity and force–processivity curves. At saturating ATP, kinesin has an unloaded velocity of approximately 800 nm/s[9,10] and an unloaded processivity of approximately 1 μm. Its force–processivity and force–velocity curves are experimentally well-defined,[10,11] and it stalls at between 4 and 7 pN (depending on the source of the motor and the experimental conditions). Myosin V has also been well characterized, with a stalling force of 2 or 3 pN,[12] a mean velocity of 350 nm/s,[13,14] and experimentally measured force–velocity[15] and force–processivity curves.

Numerous experimental efforts have also investigated dynein, but here there is less consensus. A number of groups have reported that single molecules of cytoplasmic dynein from higher organisms have an *in vitro* stall force of 1.1–1.7 pN,[16,17] but it is clear that cytoplasmic dynein from yeast (which moves much more slowly) has a stall force of ~6 pN.[18] Thus, dynein's overall architecture does not intrinsically limit its force production. There is a single-molecule report of measuring a 6 pN stall force for mammalian dynein,[19] although in this study the measurements may reflect kinesin contamination because the purification did not include a kinesin removal step, and the back-stepping commonly observed in dynein single-molecule experiments was lacking. Nonetheless, it appears likely that a range of forces may be possible for dynein, although this is not yet well understood.

4.15.4 From Single Motor Function to *In Vivo* Transport: The Issues

A long-term goal of a biophysical approach is to be able to understand transport in cells starting from single-molecule properties of the molecular motors (Figure 3). As we attempt this, certain immediate challenges become apparent.

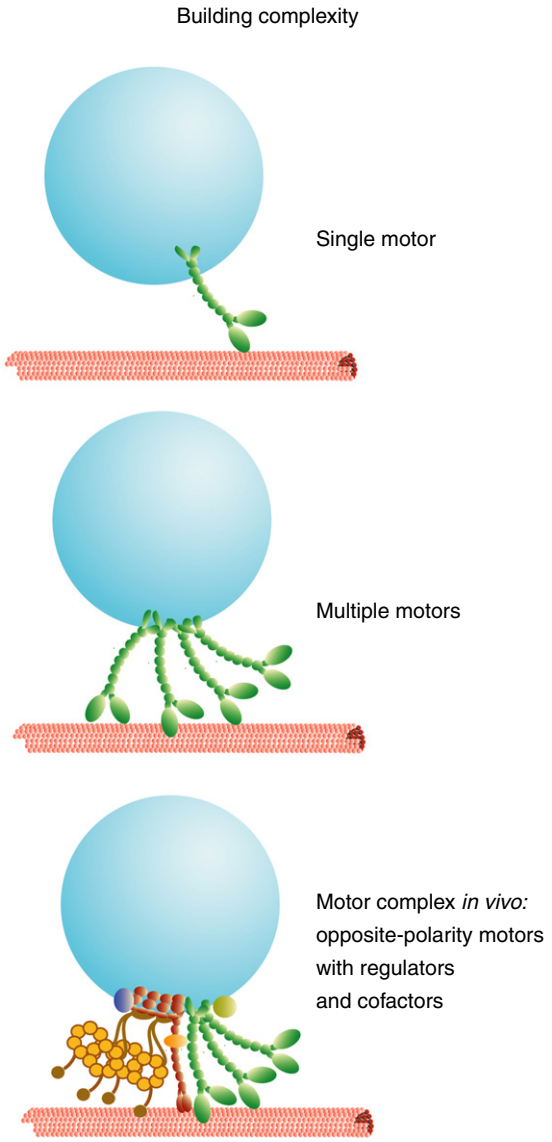

Figure 3 Different levels of complexity. Understanding the properties and the function of individual motors is an important first step in comprehending their complex behavior *in vivo*. These single-motor properties (top) lead to emergent complexity when ensembles of the motors function together (middle). Experimental and theoretical advances in understanding how multiple motors work together as reviewed in this chapter are starting to pave the way toward understanding *in vivo* function. Much more work remains, however, to obtain the whole picture of how motors behave in living cells, in which their function is modulated not only by the presence of motors of opposite polarity but also by regulatory proteins and cofactors (bottom).

First, in cells, cargos are frequently transported by more than one motor, in some cases by multiple motors of the same kind and in others by multiple sets of different classes of motors. From the *in vivo* side, we therefore need to understand how many motors move a specific type of cargo. From the *in vitro* and modeling side, we need to understand how motors of the same and different types function together in groups that are henceforth called 'ensembles'.

Second, in cells, additional accessory proteins are present that may bind either the motors – and alter the motors function – or the tracks that the motors move along, thus altering the transport. From the *in vivo* side, any description of transport of a specific cargo must identify which of those accessory factors are present and important, and from the *in vitro* side studies must be done to understand mechanistically exactly what the accessory proteins do and then must include such effects in modeling.

Third, from the *in vitro*/modeling side, we need to know which single-motor properties contribute most to ensemble performance because these are likely the properties that regulation will target to change the way cargos are moved. Once these properties are identified, of course, we need to understand the extent to which the biology takes advantage of the sensitivity and does indeed tune these specific single-molecule properties.

Fourth, cargos frequently have both microtubule and actin motors attached. In some cases, the two sets of motors may function in a coordinated manner, whereas in other cases they may function in an antagonistic manner. The reasons for this, and how this is controlled, needs to be investigated both generically and in a cargo-specific manner.

In the following sections, these issues are discussed in more depth, focusing on specific cases in which some of these issues have been investigated.

4.15.4.1 How Many Motors Move Cargos *In Vivo*?

As we start to link *in vitro* single-motor function with *in vivo* transport, a first key question to be addressed is the number of motors moving the cargo *in vivo*. It is important to differentiate between the total number of motors present on the cargo and the actual number of engaged motors or motors accessible to move the cargo together. Biochemical, immunogold EM, or fluorescence measurements can provide an estimate of the motors present on a cargo, but that merely puts an 'upper limit' on the number of motors actually engaged.[20] In principle, both cofactors that regulate motor activity, and the organization of motors on the cargo, could contribute to the actual number of active/engaged motors being significantly less than the total number of motors present. Using optical traps, however, it is possible to directly probe the actual engaged motors, which has so far been done on a few systems.

In vivo stalling force measurements were first made by Ashkin et al., who examined the motion of some small vesicular cargo (likely mitochondria) in *Reticulomyxa*.[21] That study found that a typical force of between 5 and 10 pN was required to stall the cargos, regardless of whether they were going in the plus-end or minus-end direction. By measuring the number of crosslinks between the cargo and the microtubule (visualized by EM), and assuming each crosslink represented an engaged motor, Ashkin et al. deduced that a few motors typically moved the cargo, and that the force generated by each motor was $\sim$2.6 pN.

The next two studies were on LD motion in *Drosophila* embryos. Like the Ashkin study, the LD studies found that the mean stalling forces were the same in each direction. The first study estimated a stalling force per motor of $\sim$1.1 pN based on escape-force measurements of populations of droplets.[22] However, the second study used a more sophisticated system to directly measure stall forces of individual droplets and arrived at a value of $\sim$2.6 pN per motor – in good agreement with the earlier Ashkin study. In addition, by measuring decreased stalling forces in a mutant background with decreased numbers of wild-type kinesin motors, the second lipid droplet study showed conclusively that *in vivo* cargos can indeed be moved by more than one motor. In that study, a histogram of stall forces with peaks at $\sim$2.6 and $\sim$5.2 pN was observed, consistent with *in vitro* studies that have found that for low numbers of motors, stalling forces are additive. That is, relative to a cargo moved by a single motor, it takes approximately twice as much force to stall a cargo moved by two motors. These studies on lipid droplet transport gave an estimate of the maximum number of motors hauling them of approximately five motors, with most droplets being moved by less than five. The action of multiple motors to move a cargo as well as the additive nature of the forces of motors was later found to similarly hold *in vivo* for motors hauling lipid droplets in lung cancer cells.[23] The force of the single motor ($\sim$3 pN) inferred from the stall force histograms measured by Sims and Xie was consistent with that for lipid droplets in the *Drosophila* embryos given the reported uncertainties.[23]

For several other systems, the number of molecular motors hauling cargos was estimated indirectly. Although inconclusive due to the fact that counting motors was achieved either by extrapolating from *in vitro* measurements or by counting cargo-bound motors rather than active motors, these works all suggest that multiple motors move the different cargos. Stall forces as large as $\sim$60 pN were measured for beads attached to FMG-1 transmembrane protein patches in *Chlamydomonas* flagella.[24] Based on stall forces of individual motors as previously reported *in vitro*, this force was interpreted as resulting from 10 motors teaming up to move the protein patch. Although strongly suggesting transport by multiple motors, more work on this system will likely unravel the identity of the motors and the details of their interaction. It is possible that multiple motors moving membrane-bound cargos behave differently than those hauling cytoplasmic cargos.[25]

The action of multiple motors hauling endosomes in *Dictyostellium* cells was also indirectly inferred.[17] Endosomes were purified and their motion reconstituted *in vitro*. The maximal force ($\sim$5.5 pN) generated by motors hauling them was measured and compared to the stall force of individual motors moving plastic beads *in vitro*. This was used to assert that five dynein motors were moving these purified cargos and utilized as indirect evidence for multiple dyneins moving the endosomes *in vivo*.

Counting the number of active motors via force measurements was not accessible for other cytoplasmic cargos, but use of other means suggested the action of multiple motors. mRNA localization in *Drosophila* embryos was shown to require dynein-mediated transport along microtubules.[26,27] Bullock et al.[28] reported that the localization efficiency of mRNA particles correlated with the amount of dynein that they assembled as quantified by pull-down experiments. With different mRNA transcripts localizing differently in the embryos, tuning the number of motors was suggested as a mechanism to achieve this variability.

Multiple actin-based motors were also found to team up *in vivo*. In one study, total internal reflection fluorescence imaging was used to quantify fluorescently tagged myosin Vc motors trafficking secretory granules in MCF-7 cells – a cell line derived from human mammary gland adenocarcinoma.[8] From the integrated intensity measured per puncta, Jacobs et al.[8] estimated that at least 36 myosin motors were present on the granules. Although the presence of multiple myosins on different cargos is known via EM,[20] this work goes a step further in showing that these motors function together to haul the granules because the individual myosin Vc had been reported to be nonprocessive. This is consistent with past work suggesting that by changing the number of engaged myosin V motors, their switching properties at actin–actin intersections may be changed.[29]

In summary, stall force measurements on cargos *in vivo* have provided direct evidence for the simultaneous engagement of multiple motors in hauling the cargos. Moreover, evidence suggests that this is likely the norm for many classes of cargos in a variety of organisms. Best estimates for vesicular cargos moving along microtubules are consistent with the general hypothesis that they are typically moved by less than six motors, although there are likely exceptions such as the IFT particles mentioned previously, and transport of nuclei. The number of myosin motors moving a cargo is less well-established. Overall, it is crucial to understand how multiple motors function together and how their collective behavior diverges from that of the individual motor. Consequently, the following question arises: Do organisms exploit these possible differences in transport to regulate transport by regulating the number of motors? Our limited understanding of such regulation is discussed later. First, however, *in vitro*, theoretical, and *in silico* work that has boosted our understanding of how motors work together is discussed.

4.15.4.2 How Do Multiple Motors Function Together *In Vitro*?

4.15.4.2.1 Motion along a single filament

A single kinesin-1 or dynein motor has a run length on the order of 800 nm; that is, on average, such a motor remains bound to a microtubule through approximately 100 enzymatic cycles, taking approximately one hundred 8-nm steps before detaching. However, the distances that must be traveled in cells are frequently much longer, with cargos in axons often needing to travel tens of micrometers or even millimeters. This is beyond what might be expected from single motors, if indeed they function the same way in the cell as they do in purified systems (at least single motors appear to have similar *in vivo* run lengths, as detailed later). Something else is thus required, and at least part of what contributes to increased ability for long-distance transport is likely the use of multiple motors – a hypothesis supported by a variety of *in vivo* studies reviewed previously. Thus, one challenge is to understand exactly how groups of motors function together.

This problem has been addressed both experimentally and theoretically. From an experimental perspective, the original studies investigating how multiple kinesin[30] or multiple dynein motors[31] function together each concluded that more motors move cargos significantly farther, and that at least in the low-motor regime that was investigated, motor stalling forces were approximately additive. As might be expected from processive motors that remain attached to microtubules through their complete enzymatic cycle, when moving a cargo in an unloaded state, more motors did not move a cargo more rapidly, with the multiple motor ensemble traveling at the same average velocity as a single motor.[9,30,31] Note that in some circumstances, particularly when tightly coupled, multiple motors can interfere with each other, the ensemble can move more slowly than the single motors.

Although the stalling force and velocity measurements and interpretation were relatively straightforward, the geometry of the experiments made determination of the relationship between run lengths and motor number somewhat challenging. In the optical trap bead assays, motors were attached to the cargo in random locations to achieve a state in which, on average, approximately two motors were engaged. Hence, there were a number of additional motors present on the cargo. Although these other motors on average could not reach the microtubule when the two motors were engaged and moving the cargo (i.e., when the two attached motors held the cargo in a particular orientation relative to the microtubule), the additional motors in principle 'came into play' when one of the two motors detached. At that point, the cargo was free to rock or pivot about the one motor that was still attached; thus, in addition to the motor that had previously been active, these other motors were also in principle able to reach the microtubule. Then, the effective on-rate for a second motor attaching was increased because any of these motors could play the role of the second motor. For instance, if there were five such motors that had been inactive due to their position on the cargo, the effective on-rate of attachment of the second motor would have been approximately six times that for a single second motor (each of the five had a chance of binding the microtubule, as did the original motor that had detached from the microtubule and could rebind). Because of this potentially very high on-rate, the effective travel of a cargo that typically had approximately two motors active/engaged, as measured in the experiments on dynein[31] or kinesin-1,[30] was higher than what might be expected for a cargo that had only two motors attached to it at a single point.

These experimental challenges highlight the power of a theoretical approach, in which everything can be controlled. Importantly, in contrast to the multiple-motor experiments, for the experimental single-molecule case, the geometry is well-defined, allowing the precise measurement of the single-molecule properties such as the force–processivity curve and force–velocity curve. These measured properties can then be used as the building blocks of theoretical descriptions.

How multiple motors work together was investigated in a mean field model for the large motor-number limit by Julicher and Prost.[32] They predicted phase transitions that lead to periods of directed motion. Evidence for tens of the nonprocessive myosin Vc hauling vesicles suggests behavior similar to that predicted by these models.[8] However, implications of these early works are not likely to apply to cases in which a few processive motors (approximately five) haul a cargo. A more recent theoretical description of how multiple kinesin motors function together suggested mean cargo travel

increased dramatically with increasing numbers of motors.[33] For instance, if kinesin has a travel distance of 1 μm, the theory suggested that two motors should move a cargo 3 μm on average and that three motors should move a cargo 10 μm on average (however, see the later discussion on the importance of cytosolic drag). This rapid increase in mean cargo transport with increasing motor number suggests that, in principle, cargos may not need that many motors because typical cellular microtubules are between 10 and 50 μm in length. Although the theory as presented was quite elegant, there are a number of assumptions that seem likely to limit its practical applicability.

First, it is a mean field theory and assumes that motors share load equally; that is, it assumes that if a force F opposes the motion of a particular cargo, and there are N total motors, with n ($n \leq N$) attached motors, then each motor experiences a force of F/n as it attempts to step forward. In the large motor limit (N larger than six or seven motors), this assumption may be reasonable, but it is unlikely to be true in a smaller N limit – that is, the exact situation relevant for many *in vivo* cargos.[34] Depending on the exact properties of the motors, the possibility that the assumption is wrong may turn out to be important. For instance, in the two-motor case, for motors that are relatively insensitive to detachment under load, it predicts a qualitatively wrong force–velocity curve.[34] Second, the Klumpp model assumes that the single-motor force–velocity curve is linear; this is not true for kinesin, and it may be important, depending on which properties are of interest.

Third, and quite important, the motors are assumed to be relatively prone to detachment when a load larger than their stall force is applied; in particular, the theory assumes an exponentially increasing probability of detachment as a function of force. Experimentally, this is unlikely to be true. The details of detachment kinetics under load can significantly alter ensemble function,[35] so one must carefully match theoretical assumptions to the particular experimental system under consideration.

A somewhat different theoretical approach was taken by Kunwar et al.,[34] who used a Monte Carlo simulation approach to avoid the mean field assumption of equal load sharing. In addition to allowing for unequal load sharing, the study by Kunwar et al. implemented more likely assumptions about detachment under load and also a more realistic force–velocity curve. Compared to the original Klumpp et al. model, this new theoretical approach led to a prediction of better performance of multiple motors under load, which appears to be borne out experimentally. Thus, although the various simplifications present in the Klumpp et al. model likely do not matter significantly for predictions of how far cargos hauled by multiple motors will move when not opposed by significant load, they likely do matter in the more challenging case of motion under high load and also in the small N limit. It must be stressed that the Klumpp et al. theory is not incorrect from a formal standpoint: Although these differences are important from a practical standpoint, from a theoretical standpoint most of the assumptions (although not equal load sharing) are quite easy to alter.

One interesting issue raised in the Kunwar et al. theory is the importance of motor–motor coupling. In these basic theories, the 'coupling' is assumed to be the simplest possible and as such occurs through the cargo: When one motor moves, it exerts force on the cargo, causing the cargo to move, and this motion then changes the forces felt by the other motors attached to the cargo. It is clear that the details of how the motors are attached to the cargo are important for how much the motion of one of them is sensed by and influences the others. For instance, if the motors are all attached to the cargo by 'floppy' linkages – that is, by linkages whose spring constants are such that when the motor advances by an 8-nm step, the force applied by the newly stretched linkage is not substantially changed – then when one motor advances by 8 nm, its linkage stretches, and the force it exerts on the cargo does not change very much. In this case, a motor does not 'know' much about what the other motors are doing, and the mean field model is quite accurate – they all share load approximately equally. Now imagine the converse: The motors are attached to the cargo via a 'stiff ' linkage – that is, a linkage such that when the motor steps and advances, the linkage does not stretch very much, causing the cargo to advance by close to 8 nm as well. In this case, the other motors 'know' what the first motor is doing. In particular, if the first motor happens to advance ahead of the other motors, that motor can, in principle, support additional load, relieving load from the motors in the rear (which are then free to advance, essentially in an unloaded state). Thus, in a stiff-linkage case, the load can in principle be very different between different motors, and interesting motor–motor interactions can emerge.

This tight-coupling case is quite intriguing but somewhat difficult to achieve experimentally. *In vivo*, membranes are floppy, so if motors are attached to the membrane, but not to each other directly, they are going to be in the weak-linkage state. However, some experimental data *in vivo* are consistent with theoretical predictions from a moderately tight-coupling model, suggesting that perhaps multiple kinesin motors may be directly linked to each other.[36] The exact organization and coupling of motors *in vivo* remains an interesting and mostly open question. *In vitro*, allowing motors to attach nonspecifically to a rigid polystyrene sphere appears to lead to relatively tight coupling, as judged by relatively good ensemble performance under high load.[34] However, it does not lead to coordinated stepping:[37,38] Individual motors step independently from their neighbors, leading to fractional steps in cargo motion both at very low ATP levels under no load[38] and at more physiological saturating ATP levels under some load.[37]

After the Kunwar study, there appeared a more elaborate theoretical treatment of multiple interacting kinesins,[39] which included a treatment of the motor compliance as distinct from the cargo–motor attachment linkage stiffness. One important innovation in this study was the development of specific measures of motor–motor interaction and coordination. Although this study had a slightly different focus from that of the Kunwar et al. study, the general conclusion was similar: Motors work relatively well together, and in the cell they are likely to be loosely coupled. However, under high loads, they start to interact more, and this interaction can lead to improved ensemble function.

With work, it is possible to achieve even tighter coupling between motors, such that the motors are so tightly coupled that they start to interfere with each other. To achieve this, the

motors need to be attached to very stiff substrates, and the motors' own compliance needs to be decreased: The long 'tail' of the motors provides enough compliance to avoid a majority of the motor–motor inhibition.[40] Such inhibition occurs because these increased constraints make it so that the motors cannot separate from each other. Suppose one tries to take multiple steps while another is momentarily paused. In this case, the stepping motor runs into problems because the paused motors provide opposition to its forward motion. However, if there is sufficient compliance, then when one gets ahead, the linkages simply stretch and do not significantly impede its forward progress. This motor–motor inhibition when tightly coupled and tail-less was initially investigated over a range of motor concentrations by the Surrey group[40] and in the two-motor case by the Diehl group.[41] Such studies are interesting because they shed light on the conditions required for motors to interfere with each other, but they suggest that such interference is unlikely to be generally relevant *in vivo*, where motors are full-length. However, biology often tends to take advantage of many options, so future studies *in vivo* may discover that under some conditions motor–motor coupling is increased at least temporarily by additional unknown cofactors.

In summary, in the simplest case in which motors are attached to cargos, the ensemble velocity is the same as the single-motor velocity, but stalling forces are higher (increasing almost linearly with motor number) and mean travel of the cargo increases much more rapidly than linearly. Thus, if one simply puts multiple motors of the same type together, they work quite well without requiring additional factors to 'coordinate' activity. However, the coupling between the motors is important, and if one achieves extremely tight coupling, the motors can interfere with each other. Whether such interference is ever relevant for *in vivo* function remains to be determined.

4.15.4.2.2 Motion involving two filaments

In the crowded environment of the cell, intersections between different microtubules and between microtubules and actin filaments are common and can be important for the proper overall distribution or delivery of cargoes as manifest, for example, in the case of pigment granule transport.[5,7,29] At such intersections, the effect of multiple motors of opposite polarity is likely to play an important role in determining the fate of a cargo (Figure 4). Two studies have examined this. In the first study, by Vershinin et al.,[30] it was found that artificial cargos moved on average by small numbers (approximately one) of kinesin motors were able to frequently switch at microtubule–microtubule intersections and avoided getting stuck. However, when cargos were moved by significantly more kinesins (approximately two or three), they tended to get stuck at intersections. The second study, by Ross et al.,[42] concluded that dynein and kinesin behave differently when

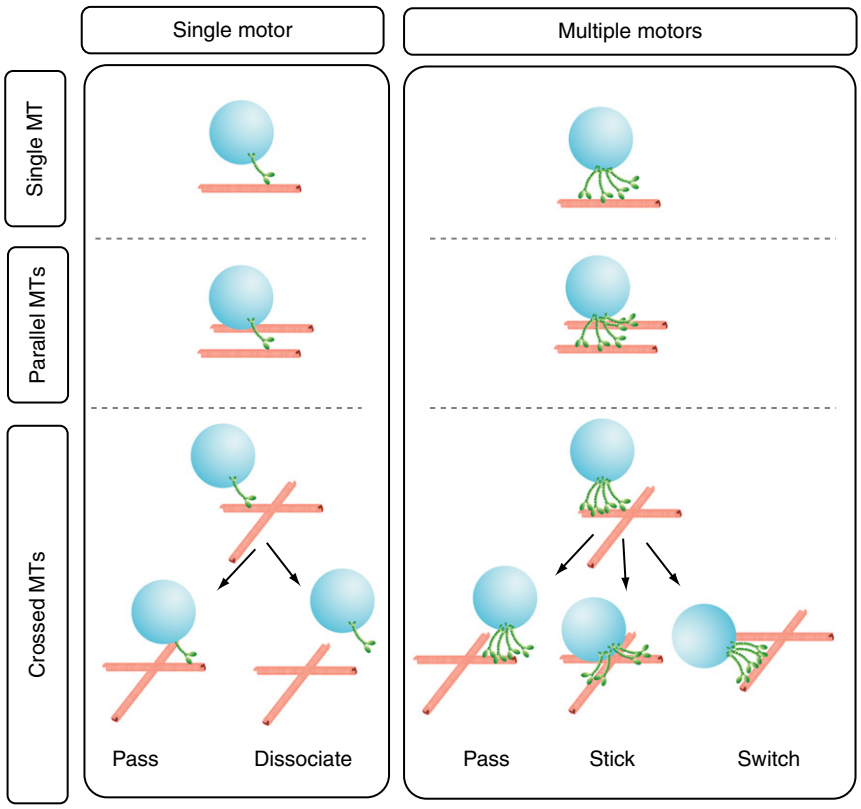

Figure 4 Cargos *in vivo* may encounter a variety of different filament organizations. *In vivo*, numerous filament organizations are possible, and part of what will determine the 'net' transport of many cargos of a specific type is how they react to these different organizations. These studies are in their infancy, but work already indicates that the number of active motors on a cargo may influence how they react to filament crossings (bottom). Intuitively, it also seems likely that a cargo with multiple motors on it may be able to move simultaneously along parallel filaments (middle), although this has not yet been investigated experimentally.

encountering intersecting microtubules. Although multiple kinesin-hauled cargos typically either pass the intersection or switch to the intersecting microtubule, they report that multiple dynein-hauled cargos pause. At reduced motor numbers, kinesin behaves similarly, whereas dynein displays a varied response by passing, switching, pausing, or reversing. Although the findings of the two studies with regard to kinesin appear at odds, the observed differences can likely be accounted for by hypothesizing that the 'high' motor studies were done at different concentrations of kinesin per bead. Indeed, although the study by Ross et al. hypothesized they were in the multiple-motor limit, no optical trap data were used to directly measure the forces, so the actual mean number of engaged motors could not be determined. It seems likely that the 'high' motor limit in the study by Ross et al. was the 'moderate' limit in the study by Vershinin et al.; under this hypothesis, both studies are consistent. Overall, there may well be differences in the function of opposite-polarity motors at intersections with regard to the concentration of motors that is required to stall the cargo at the intersection, and this in principle could be exploited by the cell to tune the delivery or the distribution of the cargos. Other work has begun to examine filament–filament switching between the actin and microtubule transport systems.[43] Although much remains to be done in this regard, it is becoming clear that the different classes of motors may influence each other in interesting ways when both filaments are present.[44]

4.15.4.3 Is Regulation of the Number of Engaged Motors Used to Regulate Transport *In Vivo*?

4.15.4.3.1 Possible regulation via cargo attachment

Because changes in the number of engaged motors can have a significant effect on overall cargo motion, a natural question concerns the extent to which *in vivo* regulation controls transport by tuning the number of motors that are attached to the cargo. There is not a unique answer to this question: It appears that there may be a great deal of variation, depending on the system. First, for bidirectionally moving cargos, one level of regulation targets overall cargo motion (via kinases),[45] and some of this regulation somehow affects motor activity rather than cargo attachment,[5,7,45] so it is clear that motor activity (rather than attachment) can be directly targeted in melanophores, with regard to regulation of pigment granule motion. Similarly, optical trap studies measuring the number of engaged motors moving lipid droplets (discussed previously) show that whereas the number of engaged motors can change slightly, coincident with regulation of motion, the number of motors attached to the cargo does not appear to vary significantly, and opposite motor forces remain balanced, regardless of net direction of transport. Therefore, at least for many bidirectionally moving cargos, there does not appear to be significant evidence that regulation involves changing the number of motors attached to the cargo. However, work shows that in neurons, phosphorylation of the Huntington protein can play a role both in the interaction of kinesin with the dynactin complex and in the recruitment of kinesin to the cargo.[46] It is clear that this phosphorylation plays an important role in regulating net direction of transport of these cargos, but further work must be undertaken to determine whether the key regulatory event is altering the kinesin–dynactin interaction (with a secondary effect on kinesin cargo attachment) or whether the altered kinesin localization is the critical event accounting for the changed transport.

For more unidirectionally moving cargos, there is considerably more evidence that regulation of the number of motors on the cargo may be quite important. First, increased kinesin function is required for synaptic plasticity, and particularly induction of long-term facilitation in *Aplysia*, and this requires increased kinesin heavy chain production,[47] consistent with increased recruitment of kinesin to cargos. Second, a number of studies suggest that kinesin may detach from cargos when they arrive at certain locations to facilitate cargo delivery.[48–50]

Further work must be performed to understand exactly how important is control of motor recruitment to the cargo, relative to control of motor activity. Almost certainly, both are important.

4.15.4.3.2 Possible regulation via filament modification

A second way in which the number of engaged motors can be altered is to control the ability of motors to bind to the filament (either microtubule or actin filament). This has long been understood to be important for myosin regulation, particularly in muscle, but the importance of regulating filament availability for vesicular transport was unclear. However, work suggests that it may be important. The general idea is that by altering filament properties, in principle, transport can be spatially controlled. This could be important to promote filament–filament switching or to alter the outcome of conflicts, for example, between microtubule and actin-based transport.[30] Because some microtubule binding proteins, such as tau, can decrease kinesin's on-rates,[30,51] and different tau isoforms have different effects,[30] by controlling which isoform is localized where, the number of motors that are engaged might be controlled locally.[30] Furthermore, because tau has a significant effect on kinesin but not dynein, the presence of tau could be used to tune the relative contributions of kinesin versus dynein on the same cargo.[30,51,52] Other work suggests that a second family of microtubule-association proteins (MAPs), ensconsin, helps recruit kinesin to microtubules, so specific MAPs can either increase or decrease the number of engaged motors.[53]

In addition to filament binding proteins, the filaments themselves can be subject to post-translational regulation, and these modifications can alter filament–motor interactions. For instance, microtubules can be both glutamylated and acetylated. Studies have shown that microtubule glutamylation increases axonemal dynein function,[54] and older work showed that kinesin has a 2.8-fold higher affinity for glutamylated microtubules relative to tyrosinated microtubules.[55] Other work has shown that *in vitro*, microtubule acetylation can alter the strength of microtubule–motor interactions[56] and that modulation of such acetylation can be used to regulate transport.[57] The microtubule-associated histone deacetylase 6 regulates epidermal growth factor receptor, endocytic trafficking, and degradation.[58] Thus, regulation of filament properties can also be used to tune transport in interesting ways, in part likely by altering filament–motor interactions.

4.15.4.4 Do Motors *In Vivo* Function the Same Way as They Do *In Vitro*?

The discussion of filament-level alteration of motor function highlights one of the challenges of trying to use *in vitro* single-molecule studies to understand transport *in vivo*: There is not a 'single' *in vivo* environment but, rather, numerous different environments. It is not surprising, then, that quantification of motor transport parameters *in vivo* reveals similarities and differences to their *in vitro* function, both at the single motor level and for motor ensembles.

Because many endogenous cargos *in vivo* are hauled by multiple motors of the same kind and of opposite polarity and/or cytoskeletal filament affinity, direct comparison with *in vitro* properties of individual motors is not straightforward. Multiple works have thus resorted to introducing exogenous fluorescently labeled motors into cells to visualize them. Cai et al.[59] used COS mammalian cells transfected with a plasmid-encoding citrine-labeled kinesin. The kinesin was truncated so that the tail could neither autoinhibit the motor domain nor bind cargos or microtubules. The velocity and processivity ($\sim$0.8 μm/s and 1.2 μm, respectively) of the single kinesins they imaged were in agreement with *in vitro* values measured for the same motor constructs purified from the transfected cells and for recombinant kinesin-1. This agreement confirmed previous reports that used individual kinesins labeled with quantum dots incorporated in endocytosed vesicles[60] or labeled via a direct biotin–avidin link.[61] Given that the quantum dots tracked by Nan et al.[60] were in vesicles, it is possible that multiple motors were hauling them, making it difficult to isolate the *in vivo* properties of the individual motors without a means to probe for motor number. The same authors later used optical trapping to measure the forces exerted in hauling a different endogenous cargo, and they used these data to estimate motor numbers.[23] On the other hand, Courty et al.[61] used cargo-free kinesin that was labeled with quantum dots via an avidin–biotin bond to ensure that they were probing individual motors. The labeled kinesin was endocytosed by the cells and released into the cytosol via osmotic rupture of the vesicles. Although the reported velocity and processivity were similar to values for individual motors *in vitro*, it was observed that individual motors were able to diffuse in close proximity to the microtubule and then reattach and move along for an average of $\sim$6 μm – much longer than the 1-μm processivity of kinesin. Work on perfused quantum dot-labeled myosin V in HeLa cells showed similar agreement between its processivity and velocity with values *in vitro*.[62]

These studies confirm that the crowding of the cytosol and the modification of filaments in cells do not affect the way individual motors translocate or their kinetic rates – on condition that they are able to bind the filaments. Thus, *in vitro* properties such as the processivity, velocity, and step size for individual motors can form a basis for understanding complexity arising from the association of ensembles of motors and can be used in models to predict how ensembles will function.

Although it was important to confirm in these cases that the individual motors functioned *in vivo* as they do *in vitro*, this result should not be overinterpreted: They were not on endogenous cargos, and thus possible regulators on such cargos that could alter the motors' function were not present. The velocities and run lengths of endogenous cargos have been quantified in many systems and for many cargos (lipid droplets,[22] mitochondria,[63] pigment granules,[5,7] synaptic vesicles,[64] etc.). Velocities and run lengths are reminiscent of those of individual motors, but the number of motors moving these cargos was rarely measured.

However, a few works have studied how motors haul endogenous cargos in systems in which the number of motors moving them was measurable, enabling meaningful comparison with work *in vitro*. The well-characterized system of lipid droplet transport in *Drosophila* embryos has given insight into how multiple motors function *in vivo*.[22] Force measurements have shown that multiple motors (five or less, but most often more than) haul the lipid droplets.[36] Nevertheless, the run lengths of cargos in both the plus- and minus-end directions ($\sim$0.5 μm) are close to processivities of single motors measured *in vitro* and are considerably less than the multiple micrometers measured for two motors moving a plastic bead.[30] Thus, Although multiple motors can move a cargo, there appears to be regulation that shortens runs. This was further confirmed in the lipid droplet system using mutants having a smaller number of motors resulting in most lipid droplets being hauled by one motor. In these mutants, the run lengths were found to be similar to their values measured in the wild-type embryos and not shorter (as would be expected if motor number alone were regulating run length).

Indeed, it has been shown in other systems that the presence of kinesins on a cargo does not guarantee its transport because kinesin function can be regulated. Axonal transport of mitochondria, for example, was shown to be regulated by the mitochondrial protein miro, which binds the kinesin-1 motor domain in a Ca^{2+}-dependent manner. Kinesin was still present on the mitochondria, but its ability to bind microtubules was impaired and the mitochondria were halted.[65] Thus, the extent to which additional cofactors alter motor function on cargos remains to be investigated.

The velocity of the motors, on the other hand, does not change with increasing motor number *in vitro* (in the absence of significant load) and, similarly, barely changes with increased motor number *in vivo*, as shown by Shubeita et al.[36] for the lipid droplets. Theoretical models of multiple motors (described previously) predict a slight decrease in the average velocity in going from one motor to two motors, when moving under slight load, due to the uncoordinated stepping of the motors, coupled with preferential detachment of the forward motor.[34] A slight but statistically significant decrease in velocity with increased motor number is indeed observed for the droplets *in vivo*. It is conceivable that in systems in which the drag experienced by motors is significant, more motors would haul the cargos faster, as suggested in previous works,[66–69] with the maximal velocity always being the unloaded single-motor velocity. However, a direct correlation between motor number and cargo velocity is yet to be measured *in vivo*.

Two studies measured the stall forces of individual motors *in vivo* using lipid droplet transport in *Drosophila* embryos[36] and in mammalian cells.[23] The stall forces of individual motors were measured as the smallest force peak in a stall

force histogram. For lipid droplets in *Drosophila* embryos, Shubeita et al.[36] found that the opposite-polarity motors hauling them – kinesin and cytoplasmic dynein – exert equal forces. The force measured for the individual kinesins was ~2.6 pN. This value was confirmed to be that of individual motors by using mutants expressing less kinesin to decrease overall copy number. The stall force histogram in these mutants showed an enhanced first peak at the expense of higher forces. A value of 3 pN was later measured for kinesin in the mammalian system by Sims and Zie,[23] in agreement with the reported uncertainty and similarly consistent with the early measurement by Ashkin et al. of ~2.6 pN in a third system.[21] This value is less than the reported values measured for single kinesin-1 *in vitro* (4–7 pN). The difference likely reflects the distinct behavior of the motor as part of a transport complex compared to its behavior in isolated form. Other works that measured stall forces of cargos *in vivo* did not report distributions of stall forces and used the known *in vitro* stall force of individual motors to infer the number of motors, as described previously.[17,24]

Differences between the opposite-polarity microtubule motors have been observed *in vitro*. For example, whereas kinesin has been shown to reproducibly take 8-nm steps, Mallik et al.[16] reported that cytoplasmic dynein takes steps in multiples of 8 nm in a load-dependent manner. Yeast cytoplasmic dynein was later shown to also take variable step sizes (4–24 nm) *in vitro*.[18] Measurements *in vivo* with high temporal resolution show that the distribution of step sizes is indeed broad for dynein but narrow and centered around 8 nm for kinesin.[23,60,70] Kural et al. reported similar distributions of step sizes centered around 8 nm for both kinesin and dynein hauling pigment granules in melanophores.[71] They reported the step size of myosin V hauling the granules along actin to be ~36 nm, consistent with values measured *in vitro*[72] and for quantum dot-labeled single myosin V in HeLa cells.[62] Compared to kinesin, dynein was also observed to have a larger tendency to move side-to-side while stepping along microtubules *in vitro*.[73–75] An enhanced lateral motion was similarly observed for minus-end moving lipid droplets in *Drosophila* embryos,[22] which were later shown to bemoved by dynein.[76]

4.15.5 Summary

Advances have started to bridge the gap between what single-molecule techniques are able to probe *in vitro* and *in vivo* and our understanding of overall transport *in vivo*. This has enabled relation of motor properties as observed in artificial systems to their function in living cells, revealing similarities and differences at many levels. These single-molecule approaches, when combined with the established cell biology and genetic techniques, will likely continue to reveal ingenious ways in which the cell exploits the properties of the single motors. However, the complexity of the cell will almost certainly reveal new ways in which the properties of the individual motors are tuned or otherwise altered by association to motor regulators to achieve the complex behavior observed when similar and dissimilar ensembles of motors function together to haul a cargo.

References

[1] Gennerich, A.; Vale, R. D. Walking the walk: How kinesin and dynein coordinate their steps. *Curr. Opin. Cell Biol.* **2009**, *21*, 59–67.
[2] Hirokawa, N.; Nitta, R.; Okada, Y. The mechanisms of kinesin motor motility: Lessons from the monomeric motor KIF1A. *Nature Rev. Mol. Cell Biol.* **2009**, *10*, 877–884.
[3] Vale, R. D. The molecular motor toolbox for intracellular transport. *Cell* **2003**, *112*, 467–480.
[4] Mallik, R.; Gross, S. P. Molecular motors: Strategies to get along. *Curr. Biol.* **2004**, *14*, R971–R982.
[5] Gross, S. P.; Tuma, M. C.; Deacon, S. W.; Serpinskaya, A. S.; Reilein, A. R.; Gelfand, V. I. Interactions and regulation of molecular motors in *Xenopus* melanophores. *J. Cell Biol.* **2002**, *156*, 855–865.
[6] Reilein, A. R.; Tint, I. S.; Peunova, N. I.; Enikolopov, G. N.; Gelfand, V. I. Regulation of organelle movement in melanophores by protein kinase A (PKA), protein kinase C (PKC), and protein phosphatase 2A (PP2A). *J. Cell Biol.* **1998**, *142*, 803–813.
[7] Rodionov, V.; Yi, J.; Kashina, A.; Oladipo, A.; Gross, S. P. Switching between microtubule- and actin-based transport systems in melanophores is controlled by cAMP levels. *Curr. Biol.* **2003**, *13*, 1837–1847.
[8] Jacobs, D. T.; Weigert, R.; Grode, K. D.; Donaldson, J. G.; Cheney, R. E. Myosin Vc is a molecular motor that functions in secretory granule trafficking. *Mol. Biol. Cell* **2009**, *20*, 4471–4488.
[9] Coy, D. L.; Wagenbach, M.; Howard, J. Kinesin takes one 8-nm step for each ATP that it hydrolyzes. *J. Biol. Chem.* **1999**, *274*, 3667–3671.
[10] Visscher, K.; Schnitzer, M. J.; Block, S. M. Single kinesin molecules studied with a molecular force clamp. *Nature* **1999**, *400*, 184–189.
[11] Schnitzer, M. J.; Visscher, K.; Block, S. M. Force production by single kinesin motors. *Nature Cell Biol.* **2000**, *2*, 718–723.
[12] Cappello, G.; Pierobon, P.; Symonds, C.; Busoni, L.; Gebhardt, J. C.; Rief, M.; Prost, J. Myosin V stepping mechanism. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 15328–15333.
[13] Cheney, R. E.; Oshea, M. K.; Heuser, J. E.; Coelho, M. V.; Wolenski, J. S.; Espreafico, E. M.; Forscher, P.; Larson, R. E.; Mooseker, M. S. Brain myosin-V is a 2-headed unconventional myosin with motor activity. *Cell* **1993**, *75*, 13–23.
[14] Mehta, A. D.; Rock, R. S.; Rief, M.; Spudich, J. A.; Mooseker, M. S.; Cheney, R. E. Myosin-V is a processive actin-based motor. *Nature* **1999**, *400*, 590–593.
[15] Clemen, A. E.; Vilfan, M.; Jaud, J.; Zhang, J.; Barmann, M.; Rief, M. Force-dependent stepping kinetics of myosin-V. *Biophys. J.* **2005**, *88*, 4402–4410.
[16] Mallik, R.; Carter, B. C.; Lex, S. A.; King, S. J.; Gross, S. P. Cytoplasmic dynein functions as a gear in response to load. *Nature* **2004**, *427*, 649–652.
[17] Soppina, V.; Rai, A. K.; Ramaiya, A. J.; Barak, P.; Mallik, R. Tug-of-war between dissimilar teams of microtubule motors regulates transport and fission of endosomes. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 19381–19386.
[18] Gennerich, A.; Carter, A. P.; Reck-Peterson, S. L.; Vale, R. D. Force-induced bidirectional stepping of cytoplasmic dynein. *Cell* **2007**, *131*, 952–965.
[19] Toba, S.; Watanabe, T. M.; Yamaguchi-Okimoto, L.; Toyoshima, Y. Y.; Higuchi, H. Overlapping hand-over-hand mechanism of single molecular motility of cytoplasmic dynein. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 5741–5745.
[20] Gross, S. P.; Vershinin, M.; Shubeita, G. T. Cargo transport: Two motors are sometimes better than one. *Curr. Biol.* **2007**, *17*, R478–R486.
[21] Ashkin, A.; Schutze, K.; Dziedzic, J. M.; Euteneuer, U.; Schliwa, M. Force generation of organelle transport measured *in vivo* by an infrared-laser trap. *Nature* **1990**, *348*, 346–348.
[22] Welte, M. A.; Gross, S. P.; Postner, M.; Block, S. M.; Wieschaus, E. F. Developmental regulation of vesicle transport in *Drosophila* embryos: Forces and kinetics. *Cell* **1998**, *92*, 547–557.
[23] Sims, P. A.; Xie, X. S. Probing dynein and kinesin stepping with mechanical manipulation in a living cell. *ChemPhysChem* **2009**, *10*, 1511–1516.
[24] Laib, J. A.; Marin, J. A.; Bloodgood, R. A.; Guilford, W. H. The reciprocal coordination and mechanics of molecular motors in living cells. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 3190–3195.
[25] Mallik, R.; Gross, S. P. Intracellular transport: How do motors work together? *Curr. Biol.* **2009**, *19*, R416–R418.
[26] Hughes, J. R.; Bullock, S. L.; Ish-Horowicz, D. Inscuteable mRNA localization is dynein-dependent and regulates apicobasal polarity and spindle length in *Drosophila* neuroblasts. *Curr. Biol.* **2004**, *14*, 1950–1956.
[27] Wilkie, G. S.; Davis, I. *Drosophila wingless* and pair-rule transcripts localize apically by dynein-mediated transport of RNA particles. *Cell* **2001**, *105*, 209–219.

[28] Bullock, S. L.; Nicol, A.; Gross, S. P.; Zicha, D. Guidance of bidirectional motor complexes by mRNA cargoes through control of dynein number and activity. *Curr. Biol.* **2006**, *16*, 1447–1452.

[29] Snider, J.; Lin, F.; Zahedi, N.; Rodionov, V.; Yu, C. C.; Gross, S. P. Intracellular actin-based transport: How far you go depends on how often you switch. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 13204–13209.

[30] Vershinin, M.; Carter, B. C.; Razafsky, D. S.; King, S. J.; Gross, S. P. Multiple-motor based transport and its regulation by Tau. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 87–92.

[31] Mallik, R.; Petrov, D.; Lex, S. A.; King, S. J.; Gross, S. P. Building complexity: An *in vitro* study of cytoplasmic dynein with *in vivo* implications. *Curr. Biol.* **2005**, *15*, 2075–2085.

[32] Julicher, F.; Prost, J. Cooperative molecular motors. *Phys. Rev. Lett.* **1995**, *75*, 2618–2621.

[33] Klumpp, S.; Lipowsky, R. Cooperative cargo transport by several molecular motors. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 17284–17289.

[34] Kunwar, A.; Vershinin, M.; Xu, J.; Gross, S. P. Stepping, strain gating, and an unexpected force–velocity curve for multiple-motor-based transport. *Curr. Biol.* **2008**, *18*, 1173–1183.

[35] McKenney, R. J.; Vershinin, M.; Kunwar, A.; Vallee, R. B.; Gross, S. P. LIS1 and NudE induce a persistent dynein force-producing state. *Cell* **2010**, *141*, 304–314.

[36] Shubeita, G. T.; Tran, S. L.; Xu, J.; Vershinin, M.; Cermelli, S.; Cotton, S. L.; Welte, M. A.; Gross, S. P. Consequences of motor copy number on the intracellular transport of kinesin-1-driven lipid droplets. *Cell* **2008**, *135*, 1098–1107.

[37] Carter, B. C.; Vershinin, M.; Gross, S. P. A comparison of step-detection methods: How well can you do? *Biophys. J.* **2008**, *94*, 306–319.

[38] Leduc, C.; Ruhnow, F.; Howard, J.; Diez, S. Detection of fractional steps in cargo movement by the collective operation of kinesin-1 motors. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 10847–10852.

[39] Hendricks, A. G.; Epureanu, B. I.; Meyhofer, E. Collective dynamics of kinesin. *Phys. Rev. E* **2009**, *79*.

[40] Bieling, P.; Telley, I. A.; Piehler, J.; Surrey, T. Processive kinesins require loose mechanical coupling for efficient collective motility. *EMBO Rep.* **2008**, *9*, 1121–1127.

[41] Rogers, A. R.; Driver, J. W.; Constantinou, P. E.; Jamison, D. K.; Diehl, M. R. Negative interference dominates collective transport of kinesin motors in the absence of load. *Phys. Chem. Chem. Phys.* **2009**, *11*, 4882–4889.

[42] Ross, J. L.; Shuman, H.; Holzbaur, E. L. F.; Goldman, Y. E. Kinesin and dynein–dynactin at intersecting microtubules: Motor density affects dynein function. *Biophys. J.* **2008**, *94*, 3115–3125.

[43] Ali, M. Y.; Krementsova, E. B.; Kennedy, G. G.; Mahaffy, R.; Pollard, T. D.; Trybus, K. M.; Warshaw, D. M. Myosin Va maneuvers through actin intersections and diffuses along microtubules. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 4332–4336.

[44] Ali, M. Y.; Lu, H.; Bookwalter, C. S.; Warshaw, D. M.; Trybus, K. M.; Myosin, V. and kinesin act as tethers to enhance each others' processivity. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 4691–4696.

[45] Deacon, S. W.; Nascimento, A.; Serpinskaya, A. S.; Gelfand, V. I. Regulation of bidirectional melanosome transport by organelle bound MAP kinase. *Curr. Biol.* **2005**, *15*, 459–463.

[46] Colin, E.; Zala, D.; Liot, G.; Rangone, H.; Borrell-Pages, M.; Li, X. J.; Saudou, F.; Humbert, S. Huntingtin phosphorylation acts as a molecular switch for anterograde/retrograde transport in neurons. *EMBO J.* **2008**, *27*, 2124–2134.

[47] Puthanveettil, S. V.; Monje, F. J.; Miniaci, M. C.; Choi, Y. B.; Karl, K. A.; Khandros, E.; Gawinowicz, M. A.; Sheetz, M. P.; Kandel, E. R. A new component in synaptic plasticity: Upregulation of kinesin in the neurons of the gill-withdrawal reflex. *Cell* **2008**, *135*, 960–973.

[48] Guillaud, L.; Wong, R.; Hirokawa, N. Disruption of KIF17–Mint1 interaction by CaMKII-dependent phosphorylation: A molecular model of kinesin-cargo release. *Nature Cell Biol.* **2008**, *10*, 19–29.

[49] Horiuchi, D.; Collins, C. A.; Bhat, P.; Barkus, R. V.; DiAntonio, A.; Saxton, W. M. Control of a kinesin-cargo linkage mechanism by JNK pathway kinases. *Curr. Biol.* **2007**, *17*, 1313–1317.

[50] Morfini, G. A.; You, Y. M.; Pollema, S. L.; Kaminska, A.; Liu, K.; Yoshioka, K.; Bjorkblom, B.; Coffey, E. T.; Bagnato, C.; Han, D.; et al. Pathogenic huntingtin inhibits fast axonal transport by activating JNK3 and phosphorylating kinesin. *Nature Neurosci.* **2009**, *12*, 864–874.

[51] Dixit, R.; Ross, J. L.; Goldman, Y. E.; Holzbaur, E. L. F. Differential regulation of dynein and kinesin motor proteins by tau. *Science* **2008**, *319*, 1086–1089.

[52] Vershinin, M.; Xu, J.; Razafsky, D. S.; King, S. J.; Gross, S. P. Tuning microtubule-based transport through filamentous MAPs: The problem of dynein. *Traffic* **2008**, *9*, 882–892.

[53] Sung, H. H.; Telley, I. A.; Papadaki, P.; Ephrussi, A.; Surrey, T.; Rorth, P. *Drosophila* ensconsin promotes productive recruitment of kinesin-1 to microtubules. *Dev. Cell* **2008**, *15*, 866–876.

[54] Suryavanshi, S.; Edde, B.; Fox, L. A.; Guerrero, S.; Hard, R.; Hennessey, T.; Kabi, A.; Malison, D.; Pennock, D.; Sale, W. S.; et al. Tubulin glutamylation regulates ciliary motility by altering inner dynein arm activity. *Curr. Biol.* **2010**, *20*, 435–440.

[55] Liao, G. J.; Gundersen, G. G. Kinesin is a candidate for cross-bridging microtubules and intermediate filaments: Selective binding of kinesin to detyrosinated tubulin and vimentin. *J. Biol. Chem.* **1998**, *273*, 9797–9803.

[56] Reed, N. A.; Cai, D. W.; Blasius, T. L.; Jih, G. T.; Meyhofer, E.; Gaertig, J.; Verhey, K. J. Microtubule acetylation promotes kinesin-1 binding and transport. *Curr. Biol.* **2006**, *16*, 2166–2172.

[57] Hammond, J. W.; Huang, C. F.; Kaech, S.; Jacobson, C.; Banker, G.; Verhey, K. J. Posttranslational modifications of tubulin and the polarized transport of kinesin-1 in neurons. *Mol. Biol. Cell* **2010**, *21*, 572–583.

[58] Gao, Y. S.; Hubbert, C. C.; Lu, J.; Lee, Y. S.; Lee, J. Y.; Yao, T. P. Histone deacetylase 6 regulates growth factor-induced actin remodeling and endocytosis. *Mol. Cell Biol.* **2007**, *27*, 8637–8647.

[59] Cai, D.; Verhey, K. J.; Meyhofer, E. Tracking single kinesin molecules in living cells. *Biophys. J.* **2007**, *92*, 4137–4144.

[60] Nan, X. L.; Sims, P. A.; Chen, P.; Xie, X. S. Observation of individual microtubule motor steps in living cells with endocytosed quantum dots. *J. Phys. Chem. B* **2005**, *109*, 24220–24224.

[61] Courty, S.; Luccardini, C.; Bellaiche, Y.; Cappello, G.; Dahan, M. Tracking individual kinesin motors in living cells using single quantum-dot imaging. *Nano Lett.* **2006**, *6*, 1491–1495.

[62] Pierobon, P.; Achouri, S.; Courty, S.; Dunn, A. R.; Spudich, J. A.; Dahan, M.; Cappello, G. Velocity, processivity, and individual steps of single myosin V molecules in live cells. *Biophys. J.* **2009**, *96*, 4268–4275.

[63] Pilling, A. D.; Horiuchi, D.; Lively, C. M.; Saxton, W. M. Kinesin-1 and dynein are the primary motors for fast transport of mitochondria in *Drosophila* motor axons. *Mol. Biol. Cell* **2006**, *17*, 2057–2068.

[64] Wagner, O. I.; Esposito, A.; Kohler, B.; Chen, C. W.; Shen, C. P.; Wu, G. H.; Butkevich, E.; Mandalapu, S.; Wenzel, D.; Wouters, F. S.; Klopfenstein, D. R. Synaptic scaffolding protein SYD-2 clusters and activates kinesin-3 UNC-104 in *C. elegans*. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 19605–19610.

[65] Wang, X. N.; Schwarz, T. L. The mechanism of Ca^{2+}-dependent regulation of kinesin-mediated mitochondrial motility. *Cell* **2009**, *136*, 163–174.

[66] Hill, D. B.; Plaza, M. J.; Bonin, K.; Holzwarth, G. Fast vesicle transport in PC12 neurites: Velocities and forces. *Eur. Biophys. J. Biophys. Lett.* **2004**, *33*, 623–632.

[67] Kural, C.; Kim, H.; Syed, S.; Goshima, G.; Gelfand, V. I.; Selvin, P. R. Kinesin and dynein move a peroxisome *in vivo*: A tug-of-war or coordinated movement? *Science* **2005**, *308*, 1469–1472.

[68] Levi, V.; Serpinskaya, A. S.; Gratton, E.; Gelfand, V. Organelle transport along microtubules in *Xenopus* melanophores: Evidence for cooperation between multiple motors. *Biophys. J.* **2006**, *90*, 318–327.

[69] Macosko, J. C.; Newbern, J. M.; Rockford, J.; Chisena, E. N.; Brown, C. M.; Holzwarth, G. M.; Milligan, C. E. Fewer active motors per vesicle may explain slowed vesicle transport in chick motoneurons after three days *in vitro*. *Brain Res.* **2008**, *1211*, 6–12.

[70] Nan, X. L.; Sims, P. A.; Xie, X. S. Organelle tracking in a living cell with microsecond time resolution and nanometer spatial precision. *ChemPhysChem* **2008**, *9*, 707–712.

[71] Kural, C.; Serpinskaya, A. S.; Chou, Y. H.; Goldman, R. D.; Gelfand, V. I.; Selvin, P. R. Tracking melanosomes inside a cell to study molecular motors and their interaction. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 5378–5382.

[72] Rief, M.; Rock, R. S.; Mehta, A. D.; Mooseker, M. S.; Cheney, R. E.; Spudich, J. A. Myosin-V stepping kinetics: A molecular model for processivity. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 9482–9486.

[73] Gelles, J.; Schnapp, B. J.; Sheetz, M. P. Tracking kinesin-driven movements with nanometre-scale precision. *Nature* **1988**, *331*, 450–453.

[74] Reck-Peterson, S. L.; Yildiz, A.; Carter, A. P.; Gennerich, A.; Zhang, N.; Vale, R. D. Single-molecule analysis of dynein processivity and stepping behavior. *Cell* **2006**, *126*, 335–348.

[75] Wang, Z. H.; Khan, S.; Sheetz, M. P. Single cytoplasmic dynein molecule movements: Characterization and comparison with kinesin. *Biophys. J.* **1995**, *69*, 2011–2023.

[76] Gross, S. P.; Welte, M.; Block, S. M.; Wieschaus, E. F. Dynein-mediated cargo transport *in vivo*. A switch controls travel distance. *J. Cell Biol.* **2000**, *148*, 945–956.