REVIEW ARTICLE

Hither and yon: a review of bi-directional microtubule-based transport

Steven P Gross

Department of Developmental and Cell Biology, 2222 Nat. Sci. I, University of California Irvine, Irvine, CA 926976, USA

E-mail: sgross@uci.edu

Abstract
Active transport is critical for cellular organization and function, and impaired transport has been linked to diseases such as neuronal degeneration. Much long distance transport in cells uses opposite polarity molecular motors of the kinesin and dynein families to move cargos along microtubules. It is increasingly clear that many cargos are moved by both sets of motors, and frequently reverse course. This review compares this bi-directional transport to the more well studied uni-directional transport. It discusses some bi-directionally moving cargos, and critically evaluates three different physical models for how such transport might occur. It then considers the evidence for the number of active motors per cargo, and how the net or average direction of transport might be controlled. The likelihood of a complex linking the activities of kinesin and dynein is also discussed. The paper concludes by reviewing elements of apparent universality between different bi-directionally moving cargos and by briefly considering possible reasons for the existence of bi-directional transport.

1. Microtubules and associated motors

Looking at the long, thin, processes of neurons, it is easy to suspect that cells are not simply floppy bags where numerous chemical reactions take place. Indeed, cells are highly organized, and this order is in large part achieved due to the efforts of a class of proteins called molecular motors. These enzymes function as the 'trucks' inside the cell, dragging different 'cargos', such as vesicles, to different sub-cellular locations. In fact, inside each cell is an extensive 'road network' composed of two classes of 'roads': the microtubule network that is used for long-distance transport, and the actin filament network that functions as local roads. The individual filaments in these networks are long, directed polymers, essentially one-way roads. Microtubules are typically arranged radially, with their plus-ends directed outward, at the cell periphery, and their minus-ends close to the nucleus at the cell centre (figure 1). Thus, if a cargo is at the cell's periphery (e.g., a recently endocytosed vesicle) and needs to be moved to the cell centre, this can be accomplished by moving along the microtubules towards their minus-ends, whereas a cargo could be transported to the cell's periphery by moving towards the microtubule plus-ends. Actin filaments organization is more varied. Close to the cell's edge they predominantly point outward, but inside the cell (i.e. removed from the plasma membrane) they can be randomly oriented [1].

There are three classes of molecular motors which use these filament networks: the myosin motors that move along actin filaments; the kinesin motors that move along microtubules, predominantly towards the microtubule plus-ends; and the dynein motors that move towards the microtubule minus-ends. Thus, long-distance transport is predominantly the province of kinesin and dynein. There has been an extensive study of kinesin at the single-molecule level (reviewed for example in [2]). Dynein is less well understood,
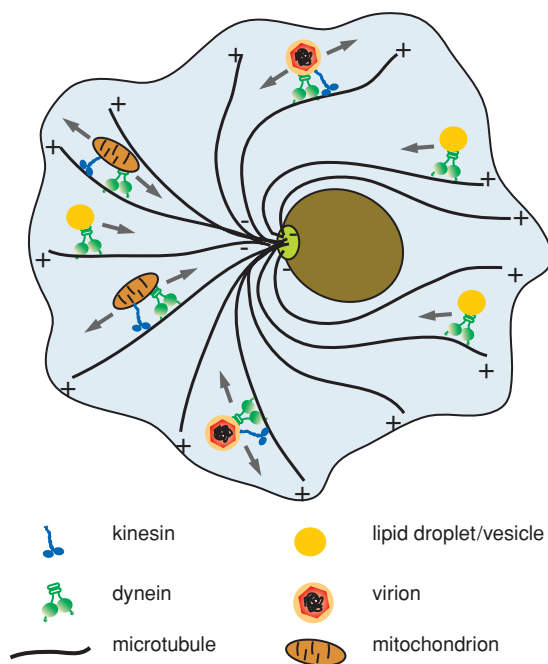
Figure 1. A diagram of a cell, showing the radial organization of the microtubule cytoskeleton, and a few bi-directionally moving cargos.

but single-molecule measurements are beginning to clarify its function [3]. In this review we will summarize what is known about how kinesin and dynein move cargos inside of cells, within the context of bi-directional transport.

2. Uni-directional versus bi-directional motion

Since the kinesin and dynein motors are uni-directional, the most obvious way that the cellular transport system could work would be to employ kinesins to move cargos towards the cell periphery, and dyneins to bring cargos back. This is in fact the dominant model for how cellular transport occurs, with the suggestion that cargos move uni-directionally just like the motors [4]. In this model, cargo transport is then regulated by controlling whether an active motor is bound to the cargo, so motor docking proteins play an essential role in regulating cargo transport. Motivated by such a hypothesis, a great deal of work has been done searching for such docking proteins (reviewed in [5] and [6]). A second line of regulation would then be controlling the activity of motors once they were cargo-bound, and work has also been done looking for an alteration of motor activity by processes such as alteration of binding of subunits [7] or phoshorylation [8]. These studies have significantly increased our understanding of how transport is regulated, especially in the case of uni-directional transport.

While the above uni-directional model makes sense, it is often not correct. Many cargos instead move bi-directionally, reversing course every few seconds. There are many examples of such motion. Chromosomes move back and forth. Mitochondria, endosomes, mRNA particles, virus particles and many vesicles of different types, all exhibit such motion. Below we will briefly discuss some of these cargos

in more detail. Since so much transport is bi-directional, we need to understand it.

For such bi-directionally moving cargos, regulation of transport could occur at two different levels. First, whether the cargo moves at all could be controlled. In this regard, the studies mentioned above, clarifying motor–cargo docking and regulation may also be directly relevant for understanding bi-directional transport. As discussed below, a complex of proteins most likely controls bi-directional transport, and thus regulation of the formation/recruitment of such a complex to the cargo could be an important part of the overall regulation of bi-directional transport. Second, once the cargo is moving and can instantaneously move in either direction, one could regulate how the net—or average—direction of transport is controlled.

We cannot fully answer either of these questions, but there has been enough work done to enable us to develop partial answers. In this review I will briefly highlight a variety of bi-directional cargos, and then combine information from a number of systems to reach a unified picture of where the field is. The review ends with a discussion of possible reasons why bi-directional transport might be employed, and open questions for future study.

3. Selected examples of bi-directionally moving cargos

Given the wide variety of bi-directionally moving cargos, this brief overview will certainly miss important cargos. A concurrent review on bi-directional transport in *Current Biology* by M Welte discusses such cargos more fully. Many 'uni-directionally' moving cargos may, in fact, be moving in a bi-directional manner, but with short enough reversals that the bi-directional character of the motion has been missed. Obviously, to detect a cargo's reversal, one needs to be sampling its position with a sufficiently high temporal resolution—if an image is captured once a second, but reversals last 0.5 s, they will almost all be missed. Recent advances in camera sensitivity, coupled with improved fluorophores, has led to a dramatic increase in the possible frame rate for fluorescent sequences of images: 20–30 frames s^{-1} is now possible, in contrast to the 1 frame s^{-1} rate common just a few years ago. This is most likely one reason that so many cargos are now realized to be moving bi-directionally.

For those cargos that have only short reversals, one might be inclined to ignore the reversals, as they do not appear to contribute significantly to the overall average motion of the cargo. However, this is almost certainly a mistake, because the fact that they do reverse suggests that both classes of motors are present on the cargo either at the same time or in rapid succession (see below). Thus, the cargo is capable of moving in either direction. In such cases, one direction of motion is actively being suppressed; however, this situation could change due to mutations or the regulatory environment that the cargo finds itself in. This is the case for fish melanophores: during net minus-end transport, the plus-end component of transport is strongly suppressed, so plus-end runs are relatively
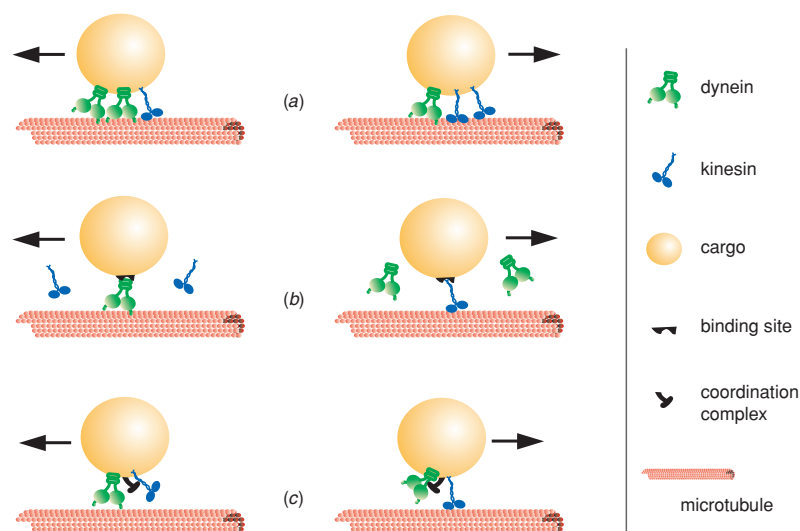
Figure 2. Possible models for how bi-directional motion occurs. In the 'tug-of-war' model (*a*), both classes of motors are simultaneously engaged. The cargo moves in the direction of whichever set of motors exerts more force. In the 'exclusionary presence' model (*b*), only one set of motors can be on the cargo at any given time. In the 'coordination' model (*c*), both sets of motors are on the cargo simultaneously, but the activity of the motors is coordinated so that they do not interfere with each other's activity.

infrequent, and quite short (∼200 nm). In this case, run lengths in each direction in this system are controlled by cyclic-AMP (cAMP)/Protein kinase A (PKA) activity, and different cAMP levels lead to different relations between the lengths of plus-end and minus-end runs [9].

Which cargos, then, move bi-directionally? Mitochondria are typically observed to move in both directions along microtubules, and appear to move to cellular locations where ATP production is necessary [10]. Pigment granules in fish and frogs [11] and also mammals [12] are observed to move bi-directionally along microtubules as well, though they can also independently move along actin filaments [12, 13]. The bi-directional motion of lipid droplets in *Drosophila* embryos has been extensively studied [14], and similar droplets in mammalian tissue culture cells also move this way [15]. Similarly, vesicles in neurons [16, 17], endosomes [15, 18] and secretory vesicles [19] all move bi-directionally along microtubules. Further, less traditional cargos such as mRNA particles [20, 21] and intermediate filaments [22] move along microtubules, though intermediate filaments are also likely to be transported along actin filaments. Finally, certain invading pathogens that co-opt the host microtubule transport system are observed to be moving bi-directionally, instantaneously reversing course. Such pathogens include Herpes virus particles [23], adenovirus particles [24] and HIV particles [25]. This list is by no means exhaustive. Thus, since many cargos move this way, it is of interest to determine how similar the mechanisms are for such motion, and how such motion can be regulated.

4. Scenarios for how cargos could move bi-directionally

Given that organelles move bi-directionally, and that *in vitro* studies indicate that the molecular motors kinesin and dynein are uni-directional, one can imagine a number of possibilities for how the activity of individual uni-directional motors could result in a bi-directionally moving cargo. We will elaborate on three of the simplest models, and summarize the experimental evidence in relation to each. The three models are 'tug of war', 'straight coordination' and 'exclusionary presence'. Figure 2 is a cartoon describing each of the three models.

4.1. The tug-of-war model

The most obvious possibility is that bi-directional transport is simply uni-directional transport 'gone bad'. In this scenario, the cargo finds itself in the unfortunate position of having both a fully functional plus-end transport system and a fully functional minus-end system, each intent on going its own way. The cargo is caught in the middle of the tug of war between the two, and moves back and forth as a result. It moves in the direction of whichever transport system is instantaneously providing more force, thus temporarily winning the tug of war. Reversals in direction of travel occur due to stochastic variation, e.g in the number of active motors in a given direction (and hence the force they produce). In such a model, the direction of net (average) transport would be controlled by determining which transport system had a higher probability of 'winning' the tug of war. Thus, for instance, net plus-end transport could be achieved by increasing the average number of active plus-end motors on the cargo, or alternatively by decreasing the number of active minus-end motors.

This scenario leads to a number of experimentally testable predictions. Most importantly, it suggests that changes in transport should be reflected in both directions; putting the plus-end motors in a better competitive position simultaneously means that the minus-end motors are in a worse competitive position. Thus, when plus-end motion is favoured, plus-end run lengths (and maybe velocities) ought to increase,

and concurrently the reverse should be observed for minus-end run lengths and velocities. Additionally, if plus-end motion is favoured, the force exerted by the plus-end motors should be larger than the force exerted by the minus-end motors, since after all the minus-end motors are being overwhelmed by the plus-end motors. From a biochemical point of view, one might expect that the number of plus-end versus minus-end motors might change; during net plus-end motion one might expect either a relative increase in the number of plus-end motors, or a decrease in minus-end motors. While this might be expected, it would not be absolutely necessary, as the total number of cargo-bound motors could remain constant while the number of active motors could shift. Finally, we would expect that if one were to impair or inactivate some of one class of motor, the transport in the other direction would improve—decreased plus-end motor activity, for example, would be expected to result in improved minus-end transport. This would be reflected in increased velocity (we know that when motors are functioning under load, they slow down [26], so decreasing the number of active plus-end motors would decrease the load the minus-end motors were under and they would speed up). Decreasing the number of active plus-end motors would also be expected to result in increased minus-end stalling forces (in the normal case, stalling a minus-end moving cargo would be easier because the active plus-end motors would be opposing its motion too, but in the absence of the plus-end motors, the optical trap would need to exert all the force by itself) and run lengths (the minus-end runs would no longer be cut short by the intrusion of plus-end motors).

All of these predictions have been tested in either the lipid-droplet model system or the melanophore model system, or both. We will review them here:

1. *Changes in transport should be reflected in both directions.* Since in this model the motion reflects the outcome of a continuous competition, the direction of transport can be regulated by altering the relative competitive position of one set of motors with respect to the other. This should alter both directions of transport. Experimentally, this was tested in three model systems: mitochondria [10], pigment granules in frog [13], Herpes virus transport (Smith *et al*, manuscript submitted) and lipid droplet transport [27] in *Drosophila* embryos. In each case, it appears possible to alter one direction while leaving the other alone, in contrast to the prediction of the tug-of-war model.

2. *Stalling forces should be unbalanced.* In a tug of war, one side typically wins by exerting more force than the opposition. Thus, one can measure whether this is true for bi-directionally moving cargos. For the sake of clarity, suppose that both plus-end and minus-end motors have the same unit stall force (which appears true in *Drosophila*; see below). From a probability perspective, if we have favoured plus-end transport by increasing the number of plus-end motors, it should be relatively common for plus-end transport to be driven by an excess of plus-end motors relative to the minus-end motors. So, on average we might expect for example an unequal competition where five plus-end motors overwhelm two minus-end motors,

yielding a net difference of three motors. In contrast, when the cargo moves minus-end, it will be due only to a slight excess of minus-end motors, for example two minus-end motors overwhelming a single plus-end motor. Thus, when we make stalling force measurements of the cargo moving in a given direction, in this model we would expect that the forces powering the favoured direction should be larger. So far, force measurements have been made only in the lipid-droplet system [27]. In contrast to the prediction of the tug of war model, it was observed that regardless of the direction of average transport, stalling forces in each direction were balanced.

3. *Impairment of transport in one direction ought to improve instantaneous transport in the other.* This is the same idea as (1), but with respect to mutant analysis rather than naturally occurring changes. It has been attempted in both melanophores and lipid droplets. In the melanophore case, a headless kinesin-II molecule was used to impair plus-end motor activity, and it was observed that minus-end velocities did not increase [13]. This suggested that in the wild type, the kinesin-II was not loading down the minus-end motors. In the lipid-droplet system, minus-end motor activity was impaired using mutations in either dynein or dynactin, and no improvement in instantaneous plus-end motion (velocity or stalling force or run lengths) was detected [14]. Thus, the conclusion from both systems is that impairment of transport in one direction does not improve the instantaneous properties of transport in the other. Of course, the *average* (as opposed to instantaneous) properties of transport could change—if the impairment of transport in one direction is significant enough, it will result in transport *on average* being in the other direction. For example, in the case of impaired plus-end transport, in the fraction of the time the cargo would be expected to move plus-end it either does not do so, or does so slowly. Then, for the fraction of time it would be expected to move minus-end, it does so normally, i.e. not faster or with more force than in the wild type.

Given that the predictions of the tug-of-war model appear incorrect in these model systems, it seems likely that this model is not an accurate description of bi-directionally moving cargos. We therefore turn to the exclusionary-presence model.

4.2. The exclusionary-presence model

This model has a simple hypothesis: while both types of motors can bind a cargo, they cannot both do it at the same time. Thus, there are frequent binding and unbinding events, where a motor attaches to a cargo and moves it; the cargo reverses when the motor unbinds, and an opposite polarity motor binds.

This model also leads to specific experimentally testable predictions. First, both motors should not be found on the cargo at the same time. Second, transport in each direction should be entirely independent: alterations in plus-end motor activity should have no effect on motion in the minus-end direction, because the plus-end motors are not on the cargo when it is moving minus-end. Finally, if one were to

biochemically purify the cargos, the mean amount of each class of motor bound should change depending on whether the mean transport was plus-end or minus-end: if the cargos are on average moving plus-end, and spend 70% of their time moving in that direction, then 70% of the purified cargos should have the plus-end motor(s) bound, whereas the other 30% should have the minus-end motors bound. By definition, these percentages would change when the cargos were on average moving in the opposite direction. These predictions have also been tested in a number of systems:

1. *Both motors should not be found on the cargo at the same time*. The key idea of this model is that the opposite-polarity motors do not interfere with each other, because they are not both on the cargo at the same time. This can be checked directly, using immunofluorescence to detect whether a specific motor is bound to a specific cargo. In a number of cases, it has been shown that both kinesin and dynein are found on the same cargo at the same time, see e.g. [28]. Additionally, in a system where all the dynein intermediate chain (necessary for dynein-based transport) was fluorescently labelled, fluorescence coming from dynein bound to cargos was observed to move bi-directionally, indicating that dynein bound to the cargo regardless of the direction of the cargo's motion [29]. Finally, it has been possible to re-constitute bi-directional motion of pigment granules [30] as well as endosomes [18] *in vitro*. In these systems, the purified cargos moved bi-directionally, and individual cargos could switch their direction of travel. However, in these *in vitro* systems there was no source of extra motors in the buffer, so both sets of motors must have been bound to the cargo all the time to allow such reversals to occur. In conclusion, most studies suggest that both sets of motors are bound to the cargo simultaneously, and none so far have suggested that only one set is bound at a time.

2. *Transport in each direction should be entirely independent*. Obviously, since the motors are not present at the same time on the cargo, they should not be able to interfere with each other. Thus, instantaneous travel in each direction (velocity and stalling force) should be independent. However, this insistence on independence might not be true for all parameters of motion. One way that reversals could come about is that a motor for one direction (A) replaces the motor for the opposite direction (B). If the affinity for docking of motor A goes up, this might well both increase run lengths in the A direction and decrease run lengths in the B direction. Thus, because there is only one set of motors bound at a time, the model predicts independence *during motion* but not necessarily at points of reversals, and thus not necessarily for run lengths and run times. This has been examined in the lipid-droplet case, where it was found that specific mutations in either dynein or dynactin could *impair* plus-end motion (stalling forces) [14]. Thus, at least in one system, the two directions are not entirely independent. Indirect data from other systems seem to support this (see (4) in section 4.3).

3. *The mean amount of each class of motor bound to the cargo should change as a function of net travel*. In this model, the net transport is determined by how much time one set of motors remains bound to the cargo versus the other. Thus, net plus-end transport is controlled by having the plus-end motors spend more time on the cargos than minus-end motors. This leads to a simple biochemical prediction: if one purifies cargos that are on average moving in the minus-end direction, they should have more minus-end motors on them than identical cargos that were purified when they were on average moving in the plus-end direction. This turns out to be false for both mitochondria [31] and for the melanophore system [13], where the amount of plus-end and minus-end motors bound to the cargo is the same, regardless of whether the cargos were on average moving plus-end or minus-end. These findings again contradict the expectations from the exclusionary-presence model. They are also in contradiction to the version of the tug-of-war model that suggests that the cell regulates the relative number of each class of motor bound to the cargo, in order to control who wins the tug of war.

In conclusion, observations from a number of systems suggest that the exclusionary presence model is unlikely to be correct. By the process of elimination, this leads to a model in which both classes of motors are on the cargo, but somehow avoid interfering with each other, i.e. some sort of coordination model.

4.3. The coordination model

This model posits that bi-directional transport is fundamentally different from uni-directional transport, and that opposite polarity motors function in such a way that they do not interfere with each other. Thus, when plus-end motors are active, minus-end motors are somehow turned off, and vice versa. In principle, such coordination could happen due to some sort of strain sensitivity, where when the motors are under a certain amount of load (e.g. from the opposite motors), they disengage. Alternatively, there could be some sort of complex that coordinates their activities. Thus, plus-end and minus-end motors would be on the cargo simultaneously, but would not get in each other's way because the motor complex would coordinate their activities: when the plus-end motors are engaged and active, the complex turns off the minus-end motors, and vice versa. In such a scenario, part of the complex would be a switch that controls which motors are engaged. Then, a reversal in direction of travel would result from a switch in the complex from, for example, the plus-end motors active state to the minus-end motors active state, and net or average transport would be controlled by the relative amount of time the complex spends in each of these two states. Such a model was previously discussed [38], and the biochemical data supporting the possibility of such a complex are summarized below.

Regardless of the exact nature of how coordination is achieved, the coordination hypothesis also makes specific predictions that can be tested experimentally. It suggests that

both sets of motors should be on the cargo simultaneously. Further, it suggests that as net transport is controlled by the activity of the switch, it might be possible to alter one direction while leaving the other alone. If coordination is being achieved due to the activity of a complex, it also suggests that a variety of non-motor proteins should exist that form part of this complex, and somehow enable the motors to be coordinated. In this model, there is no prescription about the relationship between plus-end and minus-end stalling forces, since it hypothesizes that the two sets of motors do not usually 'see' each other. However, if coordination sometimes fails, one might imagine that the opposite stalling forces should be balanced. In this way, whenever the failure occurs, the motors are more or less evenly matched, so they engage in a futile tug of war, and the cargo does not go anywhere. Then, when coordination is reestablished, the cargo starts moving again.

These predictions have also been tested experimentally, in part in studying mitochondria, in part in the melanophore model system and in part in the lipid-droplet system. Here, we summarize:

1. *Both sets of motors should be on the cargo simultaneously.* That both polarity motors are on the same cargo at the same time has been confirmed in endosomes, pigment granules and other systems (see (1) in section 4.2).

2. *It should be possible to alter one direction while leaving the other alone.* This is indeed the case. For mitochondria [10], herpes virus particles (Smith *et al*, manuscript submitted) and liquid droplets [27], plus-end runs can be increased or decreased, while leaving minus-end runs unchanged. The converse is true for frog melanophores [13].

3. *Opposite stalling forces should be balanced.* This has only been examined in the lipid-droplet case, but was confirmed there [27].

4. *There should be non-motor proteins that enable the motors to be coordinated.* This has been suggested in the lipid-droplet case (dynactin [14] and klar [27]), and also in the melanophore system (dynactin [32, 33]).

5. *Impairment of transport in one direction does not improve transport in the other, and in some cases (loss of coordination between motors in each direction) could actually impair it.* As was discussed in (3) of section 4.1, impairment of transport in one direction does not improve transport in the other. This was observed in both pigment granule transport and lipid droplet transport. Note that if coordination exists, one might expect that specific mutations could impair it, causing the motors to lose coordination and start interfering with each other. Such an effect was observed in two independent studies. The first, examining the effect of mutations in dynein or dynactin on fast axonal transport [34] was consistent with the idea of coordination between opposite motors, but it did not quantify properties of transport of individual cargos. Instead, it looked at more global phenotypes to show how transport was impaired. Thus, the data in this first study might be explained by rather indirect effects on clogging of axons, where failed transport in one direction led to traffic jams that then indirectly blocked

transport in the other direction. The second study, in lipid droplets [14], showed that altered minus-end motor activity impaired plus-end transport on the same cargo. It further showed that the minus-end motor was not in a locked up state (minus-end stalling forces were normal). This led to the conclusion that dynactin plays a role in achieving coordination of the opposite polarity motors, i.e. that minus-end motor activity could interfere with plus-end motor activity, but usually did not, in part due to the activity of dynactin. The fact that the minus-end motor activity could, under some circumstances, interfere with plus-end motion is additional evidence against the exclusionary-presence model discussed above. Additional studies have also observed that treatments designed to impair minus-end motion also impair plus-end motion [16], and the reverse has been seen as well [35].

Together, these findings from a number of systems suggest that opposite polarity motors are coordinated, so that they usually do not interfere with each other's function: when dynein is active, kinesin is turned off, and vice versa. The mechanism of such coordination is unknown, though there are some hints as to how it might be achieved (see below).

5. Number of active motors

As discussed in section 4, indications to date suggest that opposite polarity motors are in fact coordinated, so that they do not interfere with each other's function. The eventual goal is to understand how such transport occurs, and how the activity of one set of motors is turned off while the other set turned on. To develop molecular-level models of the process, we need to know how many motors are likely involved; are we talking about coordinating a single kinesin with a single dynein, or could multiple kinesin and dynein motors be involved?

Obviously, there could be many motors on a single cargo, but we are only interested in how many are engaged (and active) at any instant. Thus, we need to consider the distribution of motors on a cargo, as well as the total number, since not all the motors could reach the microtubule at the same time. One can address this question using two complementary approaches: EM techniques can image the distribution of the motors, and also the number of cargo-microtubule crosslinks (presumed to be motors), while stalling force measurements can be used to infer the number of active motors. Thankfully, both approaches lead to approximately the same conclusion: there are multiple active motors per cargo, typically somewhere between 2 and 5. We will review both approaches in the next few paragraphs.

There have been a number of studies that use EM techniques to either investigate the number and distribution of motors or to quantify the number of motors likely to be active by measuring apparent motors attached to both the microtubule and the cargo. They all tend to come to similar conclusions, i.e. that a few motors are likely to be active at any time. For instance, combining immunogold labelling of dynein with EM images, work by Habermann *et al* shows that in murine macrophages the number of dyneins on a cargo in

close proximity to each other (and hence likely to be able to bind the microtubule simultaneously) varies depending on which type of cargo is considered, but is typically from 1 to ∼7 (see EMs in [36]). Work of Ashkin *et al* [37] looked at cross-bridges and found a mean of 2.4 ± 1, with a maximum of 4. Other EM studies tend to support these conclusions, i.e. that motion is likely driven by more than one motor, but fewer than seven motors.

Stalling forces can also be used to estimate the number of active motors. This has been done predominantly in the lipid-droplet system, where stalling forces change in quantized units of 1.1 pN. From this observation, and the fact that the mean stalling force varied between 3.3 and 5.5 pN depending on the developmental phase, it was proposed that cargos on average were moved by somewhere between three and five motors, depending on developmental phase [27]. Additionally, as the minus-end motor was cytoplasmic dynein [38], it was proposed that cytoplasmic dynein *in vivo* had a stalling force of 1.1 pN. This turns out to agree with subsequent *in vitro* experiments on cytoplasmic dynein [3], where cytoplasmic dynein was indeed found to have a maximal stalling force of 1.1 pN. Thus, because the droplet stalling forces are higher than the force exerted by a single dynein, it is now well established that bi-directionally moving cargos can indeed be moved by more than one motor, and sometimes between 2 and 6, depending on the type of cargo. In the lipid-droplet system, the stalling forces in the wild type are always balanced. Interestingly, however, it appears that the average number of motors changes: during net plus-end motion there are on average five motors engaged in each direction, and during net minus-end motion there are on average four motors engaged [27]. The reason for the mean number of motors changing is still unclear.

6. Regulation of net direction

The key question, how the net direction of transport is controlled, is still predominantly unresolved. However, certain features of the control are known. First, it appears that most control centers on regulation of only one direction of transport. For example, when controlling plus-end run lengths, for net plus-end transport the system has long plus-end runs and moderate minus-end runs, and for net minus-end transport, the system has moderate minus-end runs and shorter plus-end runs. By runs, we mean periods of uninterrupted motion in a given direction (see e.g. [27]). Control of net transport through alteration of plus-end run length is found in mitochondria [10], herpes virus particles (Smith *et al*, manuscript submitted) and lipid droplets [27]; frog melanophores [13] alter minus-end runs. It is partially true in fish melanophores as well [9] (see below).

How the system alters the length of runs is still unknown. In many of the systems at least one element of the signaling/control pathway has been identified, but how this control is passed on to the motors themselves is still unclear. For the fish melanophore system, it is clear that net minus- or plus-end transport is controlled through the cAMP/PKA pathway [9]. There, high cAMP levels lead to active PKA,

and long plus-end runs and short minus-end runs. Conversely, low cAMP/PKA activity results in long minus-end runs and short plus-end runs. This regulation of both travel directions is in apparent contradiction to most of the other systems, where minus-end run lengths are not significantly altered. However, closer inspection can rationalize this discrepancy, because the plus-end run lengths are sensitive to the exact amount of PKA activity; by tuning cAMP levels over a reasonably narrow range, one can significantly tune plus-end run lengths. Over this same range, the minus-end run lengths are not altered. It is only by going to very low PKA levels that the minus-end motion is increased, and it seems to be a binary on/off transition in contrast to the fine tuning available for the plus-end motion. Thus, in this system it appears that the plus-end motion can be tuned, whereas the minus-end motion is regulated by simply turning it on or off. In principle, the other systems could function the same way, but have an increased basal level of minus-end motion, and keep the PKA signal, or its equivalent, in a narrow range. PKA also controls transport in frog melanophores, though studies have suggested a contribution of PKC as well [39]. Obviously, PKA could have many downstream targets, and these have not yet been determined. However, recently it has become clear that there is an intriguing link between PKA and Rabs (small GTPases) and molecular motors, which could in principle underlie some of the PKA-mediated transport (see section 7).

For mitochondria, what is known is similar to the pigment granules. In this case, phosphatidylinositols have been shown to be an important element in the signaling cascade, but nothing is known about the downstream targets [31, 40].

Almost nothing is known about the regulation of net transport in the case of the herpes viruses, though this is a topic of obvious interest. Similar to the melanophores, part of the regulation of adeno virus particle transport appears to include the PKA pathway [41].

For the lipid droplets, a bit more is known, but the picture is still murky at best. A recently identified small very basic protein, halo, acts as a directionality factor. When halo levels are high, net transport is plus-end, and when halo levels are low, net transport is minus-end [42]. It is not known which proteins halo interacts with in order to control the direction of transport. However, in the lipid-droplet system, another protein called klar has been shown to be required for the ability to control net transport [27]. Klar is important for controlling nuclear migration as well as lipid droplet motion [43]. As it is a large protein and has been shown to localize to nuclei [44], it also likely that it localizes to lipid droplets. It is hypothesized to be a structural protein that makes possible coordination between opposite polarity motors [27], though how it does so is still unclear. While halo-based control of transport at first glance appears different from PKA-based control of transport, because we know so few of the intervening steps, this is not necessarily the case. For instance, in the melanophore systems, PKA activity could control the recruitment of a halo-like protein to the granule's surface.

7. Physical mechanisms for achieving coordination: a motor complex?

In section 4, we summarized the evidence that the opposite polarity motors are coordinated in the pigment granule and lipid-droplet systems. Understanding such coordination is important, because once the motors are coordinated, they can be controlled by a switch that can flip from a plus-end active state to a minus-end active state. Then, it is possible to control the direction of net transport by targeting how often the switch flips states, rather than directly targeting the activity of the individual motors. How then are the motors coordinated? As should have become clear by now in this review, the answer to this question too will ultimately be 'we don't know'. However, it is likely that we will be able to answer this question in the future because a number of molecular interactions likely to play a role in the process have been identified. Taken together, these interactions suggest that there is likely a bi-directional motor complex, though many of its components are unclear.

Biophysical studies in the lipid-droplet system suggested that the dynactin complex was playing an important role in achieving this coordination [14], but did not investigate the molecular interactions responsible for this function. Recent biochemical work in melanophores [32] showed that dynactin can bind either dynein or kinesin-II (the plus-end motor for the pigment granules), but not both simultaneously. Because it is mutually exclusive, this molecular interaction could be the basis of regulated activation or inactivation of the motors: when dynactin binds dynein, it activates it, and when it binds kinesin-II it simultaneously activates kinesin-II and inactivates dynein. Such a possibility has been described in a mini review [33], though at this point it is simply a model for how such coordination could occur.

Such a model is clearly incomplete. First, it talks about how it might be possible to coordinate a single dynein with a single kinesin-II molecule. However, as discussed above, it is likely that multiple motors of each class work together. How this higher-order coordination is achieved is still entirely open. Second, other proteins are clearly involved in the coordination, such as klar (see above). The model does not include these other proteins. Finally, recent work suggests that the opposite polarity motors interact directly [28], as well as through dynactin. These interactions are probably at the heart of the proposed complex, but many additional proteins are also likely there. First, there are the Rabs. There are different Rabs on different cargos, and in some cases it is clear that altering Rab activity alters the bi-directional transport of the cargo. This results in loss of regulation of net motion [45] or redistribution of the cargo [46, 47]. As a number of Rabs have been shown to interact with dynein [48–51] or kinesin-family members [52, 53], it is likely that these are playing a central role in the complex. One appealing speculation is that it is the specific Rab that is part of the complex that allows the complex to be controlled independently of other bi-directional complexes on different classes of cargos. Further, the involvement of Rabs allow us to rationalize at least some of the effects of PKA: some studies indicate a direct interaction between Rabs and/or Rab regulators and PKA or PKA anchor proteins [47, 54]. The notion of a large complex with Rabs, PKA and/or PKA anchor proteins, dynactin and the molecular motors kinesin and dynein is further supported by the finding that at least in one case, Rab 6 binds the dynein dynactin binding protein Bicaudal D [55]. Bicaudal D has been shown to play a role in regulating a number of cargo motions, including bi-directionally moving golgi [56] and lipid droplets (our observations, manuscript in preparation). What is more, looking at the Curagen database of protein interactions in *Drosophila*, one can find a link between BicD and kinesin (through an unnamed protein, CG13474). Again, one is led to the suggestion of a multi-protein subunit complex, with many possible links between plus-end and minus-end motors. Which of these possible interactions are used, when, and how they are regulated to achieve coordination of opposite motors is still very much an open issue.

8. Universality—compare different systems

Now that we have discussed a number of bi-directional transport systems, we are in a good position to assess which features of the motion are conserved between them—and likely essential. So far, four features emerge.

(1) In all cases where the minus-end motor has been clearly identified, it is dynein. There are over ten such studies.

(2) Regulation appears to alter net transport by targeting one direction, and leaving the other direction alone. This is true in four systems.

(3) The distribution of run lengths in each direction is typically described by the sum of two decaying exponentials instead of one. This has now been reported for lipid droplet motion [38] and frog [13] and fish [9] pigment granules, and is also true for herpes virus transport (manuscript in preparation). *In vitro*, the distribution of runs of beads moved by single motors shows a single decaying exponential distribution, reflecting the fact that the motor has a constant probability of detaching from the microtubule (and hence ending the run) at each step. The fact that *in vivo* the distribution of run lengths in a single direction is described by the sum of two decaying exponentials suggests that there are two distinct ways for a run to end, each with a constant probability of occurring per step. It does not appear that this is due to two different types of motors moving the cargo in each direction, but rather to a single type of motor functioning in two ways (see [38] for more discussion). Because the short runs in each direction are slow, and we know that when under load motors slow down, one appealing suggestion is that the short-slow class of motion reflects a tug of war, where coordination has failed between opposite motors, and the long-fast state results when coordination is functioning correctly. This has been discussed in more detail in [38], but is as yet simply a hypothesis.

(4) It seems likely that transport in these situations involves multiple (2–5) motors in each direction. This has been established in the Drosophila lipid-droplet case, but is also strongly supported by the study of moving mitochondria

in Reticulomyxa [37]. The EM studies discussed above suggest it is likely the case elsewhere too.

9. Reasons for bi-directional transport

Naively, bi-directional transport seems inefficient. If one wants to move a cargo to a particular location, why constantly back up? One can formulate a number of reasons why bi-directional transport might be desirable, but most such suggestions are currently unproven. Nonetheless, here they are:

(1) With a bi-directionally moving cargo, the cargo is ready to go in either direction at any time. Thus, it is easy to rapidly change the distribution of the cargos; there is no need to recruit motors that may or may not be instantly available.

(2) The goal of the transport may not be to move the cargo to a specific place, but instead to achieve a specific distribution of cargos in the cell, e.g. to spread them out in a certain way. For instance, one could imagine that mitochondria (the cell's power plants, which move to where the demand for ATP is high) need to spread out, and the motors simply increase their rate of spreading.

(3) It could be that bi-directional transport enables stuck cargos to back up, thus getting them out of potential traffic jams. For instance, moving down an axon, a cargo might encounter a blocked microtubule (e.g. with too many microtubule-associated proteins bound to it, preventing the motor from continuing). If the cargo reverses course and backs up, it could, in principle, switch to a different microtubule that crossed the path of the first. Then, on a subsequent trip back down the axon, it might be on the new microtubule, and thus avoid the blockage. In the lipid droplet system and pigment granule system, we occasionally observe cargos switching from one microtubule to another (Gross, unpublished observations).

(4) Evolutionary efficiency. It may be useful to be able to re-use most of the different components of the transport system. If one has a bi-directional transport complex that has a switch that can be controlled, one can control transport by regulating the switch's activity rather than targeting the motors *per se*. If the switch is itself controlled by the activity of a specific 'conductor' molecule, then the only thing that needs to be changed in order to be able to transport a new cargo to a new location is to use a slightly different conductor. Everything else could be essentially the same. As there are different Rab proteins on different cargos, and they clearly play important roles in 'directing' traffic, the Rabs are candidates for such conductors.

(5) Finally, there is one place where bi-directional transport clearly excels: when the cell is trying to interface bi-directional microtubule-based transport with actin-based transport. Because many cargos can move along either microtubules or actin filaments, this may be a relatively general issue. In such cases it would be advantageous for the cell to be able to control the handoff from microtubule-based transport to actin filament-based transport. At least in the pigment granule system, the handoff occurs only when the cargo is moving towards the microtubule minus-end [13]. Thus, by controlling the amount of time the cargo spends moving plus-end versus minus-end, one can in principle control the handoff from the microtubule to actin filament cytoskeleton. This turns out to be used in at least one case [9].

In conclusion, there could be many reasons for bi-directional transport. Whether any of the above suggestions turn out to be important for rationalizing bi-directional transport is still very much an open question.

10. Open questions

In this review, I have tried to give an overview of what is currently known about bi-directional transport and how it is regulated. There are obviously a number of significant open questions. First, though some of the players have been identified, the general question of how the direction of transport is regulated is an open question. Associated with this is the issue of specificity: how can the cell differentially control different cargos in the same cytoplasmic background—endosomes and mitochondria must move to different locations, and can do so in the same cell? Thus, in addition to global signalling, there must also be some sort of local signalling to specifically control different cargos. Proteins like halo could play a part of this specificity, as in Drosophila there are actually a family of halo-like proteins [42], each of which has a different expression pattern and could in principle each be controlling a different bi-directionally moving cargo. Similarly, 'conductor' molecules such as Rabs (see section 9, part 4) might play a role in achieving cargo-specific transport.

At a more mechanistic level, there are also interesting open questions. First, the significance of the number of active motors on the cargo is unclear—why should it matter whether three or four motors are functioning? Second, how does the proposed switch work—how is one class of motors turned off when the other is turned on?

Because bi-directional transport is complex, it is likely that there will be quite a few surprises before all these questions are answered. However, as these questions and search for answers require careful fusion of biochemistry, genetics, biophysics and modelling, one of the exciting outgrowths of such studies will almost certainly be an interesting model for how to do science unfettered by traditional discipline boundaries.

Glossary

Microtubule. A directed polymer filament. Microtubules are found in all eukaryotic cells, and serve as roads along which molecular motors move. Directionality associated with a microtubule is usually indicated by referring to its plus-end and minus-end.

Kinesin. A motor protein that uses the hydrolysis of ATP to power its motion along microtubules. Most kinesin family members move towards the microtubule plus-end.

Dynein. A motor protein that uses the hydrolysis of ATP to power its motion along microtubules. Dynein family members move towards the microtubule minus-end.

Bi-directional transport. The property of many cargos moving along microtubules to frequently (every few second or less) reverse course, switching from instantaneous plus-end motion to minus-end motion, or vice versa.

References

[1] Lewis A K and Bridgman P C 1992 Nerve growth cone lamellipodia contain two populations of actin filaments that differ in organization and polarity *J. Cell Biol.* **119** 1219–43

[2] Ishijima A and Yanagida T 2001 Single molecule nanobioscience *Trends Biochem. Sci.* **26** 438–44

[3] Mallik R, Carter B C, Lex S A, King S J and Gross S P 2004 Cytoplasmic dynein functions as a gear in response to load *Nature* **427** 649–52

[4] Alberts B *et al* 2002 *Molecular Biology of the Cell* (New York: Garland)

[5] Karcher R L, Deacon S W and Gelfand V I 2002 Motor-cargo interactions: the key to transport specificity *Trends Cell Biol.* **12** 21–7

[6] Hammer J A 3rd and Wu X S 2002 Rabs grab motors: defining the connections between Rab GTPases and motor proteins *Curr. Opin. Cell Biol.* **14** 69–75

[7] Kini A M and Collins C A 2001 Modulation of cytoplasmic dynein ATPase activity by the accessory subunits *Cell Motil. Cytoskel.* **48** 52–60

[8] De Vos K *et al* 1998 The 55-kDa tumor necrosis factor receptor induces clustering of mitochondria through its membrane-proximal region *J. Biol. Chem.* **273** 9673–80

[9] Rodionov V, Yi J, Kashina A, Oladipo A and Gross S P 2003 Switching between microtubule- and actin-based transport systems in melanophores is controlled by cAMP levels *Curr. Biol.* **13** 1837–47

[10] Morris R L and Hollenbeck P J 1993 The regulation of bidirectional mitochondrial transport is coordinated with axonal outgrowth *J. Cell Sci.* **104** 917–27

[11] Nascimento A A, Roland J T and Gelfand V I 2003 Pigment cells: a model for the study of organelle transport *Annu. Rev. Cell Dev. Biol.* **19** 469–91

[12] Wu X and Hammer J A 3rd 2000 Making sense of melanosome dynamics in mouse melanocytes *Pigment Cell Res.* **13** 241–7

[13] Gross S P *et al* 2002 Interactions and regulation of molecular motors in Xenopus melanophores *J. Cell Biol.* **156** 855–65

[14] Gross S P, Welte M A, Block S M and Wieschaus E F 2002 Coordination of opposite-polarity microtubule motors *J. Cell Biol.* **156** 715–24

[15] Valetti C *et al* 1999 Role of dynactin in endocytic traffic: effects of dynamitin overexpression and colocalization with CLIP-170 *Mol. Biol. Cell* **10** 4107–20

[16] Waterman-Storer C M *et al* 1997 The interaction between cytoplasmic dynein and dynactin is required for fast axonal transport *Proc. Natl Acad. Sci. USA* **94** 12180–5

[17] Leopold P L, Snyder R, Bloom G S and Brady S T 1990 Nucleotide specificity for the bidirectional transport of membrane-bounded organelles in isolated axoplasm *Cell Motil. Cytoskel.* **15** 210–9

[18] Murray J W, Bananis E and Wolkoff A W 2000 Reconstitution of ATP-dependent movement of endocytic vesicles along microtubules in vitro: an oscillatory bidirectional process *Mol. Biol. Cell* **11** 419–33

[19] Wacker I *et al* 1997 Microtubule-dependent transport of secretory vesicles visualized in real time with a GFP-tagged secretory protein *J. Cell Sci.* **110** 1453–63

[20] Bullock S L, Zicha D and Ish-Horowicz D 2003 The Drosophila hairy RNA localization signal modulates the kinetics of cytoplasmic mRNA transport *Embo J.* **22** 2484–94

[21] Lopez de Heredia M and Jansen R P 2004 mRNA localization and the cytoskeleton *Curr. Opin. Cell Biol.* **16** 80–5

[22] Helfand B T, Loomis P, Yoon M and Goldman R D 2003 Rapid transport of neural intermediate filament protein *J. Cell Sci.* **116** 2345–59

[23] Smith G A, Gross S P and Enquist L W 2001 Herpesviruses use bi-directional fast-axonal transport to spread in sensory neurons *Proc. Natl Acad. Sci. USA* **98** 3466–70

[24] Suomalainen M *et al* 1999 Microtubule-dependent plus- and minus end-directed motilities are competing processes for nuclear targeting of adenovirus *J. Cell Biol.* **144** 657–72

[25] McDonald D *et al* 2002 Visualization of the intracellular behavior of HIV in living cells *J. Cell Biol.* **159** 441–52

[26] Visscher K, Schnitzer M J and Block S M 1999 Single kinesin molecules studied with a molecular force clamp *Nature* **400** 184–9

[27] Welte M A, Gross S P, Postner M, Block S M and Wieschaus E F 1998 Developmental regulation of vesicle transport in Drosophila embryos: forces and kinetics *Cell* **92** 547–57

[28] Ligon L A, Tokito M, Finklestein J M, Grossman F E and Holzbaur E L A 2004 Direct interaction between cytoplasmic dynein and kinesin I may coordinate motor activity *J. Biol. Chem.* **279** 19201–8

[29] Ma S and Chisholm R L 2002 Cytoplasmic dynein-associated structures move bidirectionally *in vivo J. Cell Sci.* **115** 1453–60

[30] Rogers S L, Tint I S, Fanapour P C and Gelfand V I 1997 Regulated bidirectional motility of melanophore pigment granules along microtubules *in vitro Proc. Natl Acad. Sci. USA* **94** 3720–5

[31] De Vos K J, Sable J, Miller K E and Sheetz M P 2003 Expression of phosphatidylinositol (4,5) bisphosphate-specific pleckstrin homology domains alters direction but not the level of axonal transport of mitochondria *Mol. Biol. Cell* **14** 3636–49

[32] Deacon S W *et al* 2003 Dynactin is required for bidirectional organelle transport *J. Cell Biol.* **160** 297–301

[33] Gross S P 2003 Dynactin: coordinating motors with opposite inclinations *Curr. Biol.* **13** R320–2

[34] Martin M *et al* 1999 Cytoplasmic dynein, the dynactin complex, and kinesin are interdependent and essential for fast axonal transport *Mol. Biol. Cell* **10** 3717–28

[35] Brady S T, Pfister K K and Bloom G S 1990 A monoclonal antibody against kinesin inhibits both anterograde and retrograde fast axonal transport in squid axoplasm *Proc. Natl Acad. Sci. USA* **87** 1061–5

[36] Habermann A, Schroer T A, Griffiths G and Burkhardt J K 2001 Immunolocalization of cytoplasmic dynein and dynactin subunits in cultured macrophages: enrichment on early endocytic organelles *J. Cell Sci.* **114** 229–40

[37] Ashkin A, Schutze K, Dziedzic J M, Euteneuer U and Schliwa M 1990 Force generation of organelle transport measured *in vivo* by an infrared laser trap *Nature* **348** 346–8

[38] Gross S P, Welte M A, Block S M and Wieschaus E F 2000 Dynein-mediated cargo transport *in vivo*. A switch controls travel distance *J. Cell Biol.* **148** 945–56

[39] Nilsson H M, Svensson S P and Sundqvist T 2001 L-NAME-induced dispersion of melanosomes in melanophores activates PKC, MEK and ERK1 *Pigment Cell Res.* **14** 450–5

[40] Chada S R and Hollenbeck P J 2003 Mitochondrial movement and positioning in axons: the role of growth factor signaling *J. Exp. Biol.* **206** 1985–92

[41] Suomalainen M, Nakano M Y, Boucke K, Keller S and Greber U F 2001 Adenovirus-activated PKA and p38/MAPK pathways boost microtubule-mediated nuclear targeting of virus *Embo J.* **20** 1310–9

[42] Gross S P, Guo Y, Martinez J E and Welte M A 2003 A determinant for directionality of organelle transport in Drosophila embryos *Curr. Biol.* **13** 1660–8

[43] Fischer-Vize J A and Mosley K L 1994 Marbles mutants: uncoupling cell determination and nuclear migration in the developing Drosophila eye *Development* **120** 2609–18

[44] Patterson K *et al* 2004 The functions of Klarsicht and nuclear lamin in developmentally regulated nuclear migrations of photoreceptor cells in the Drosophila eye *Mol. Biol. Cell* **15** 600–10

[45] Lebrand C *et al* 2002 Late endosome motility depends on lipids via the small GTPase Rab7 *Embo J.* **21** 1289–300

[46] Martinez O *et al* 1997 GTP-bound forms of rab6 induce the redistribution of Golgi proteins into the endoplasmic reticulum *Proc. Natl Acad. Sci. USA* **94** 1828–33

[47] Alto N M, Soderling J and Scott J D 2002 Rab32 is an A-kinase anchoring protein and participates in mitochondrial dynamics *J. Cell Biol.* **158** 659–68

[48] Jordens I, Marsman M, Kuijl C, Janssen L and Neefjes J 2003 IL-24 The small GTPase Rab7 and its effector protein RILP regulate lysosomal transport *Pigment Cell Res.* **16** 583

[49] Jordens I *et al* 2001 The Rab7 effector protein RILP controls lysosomal transport by inducing the recruitment of dynein-dynactin motors *Curr. Biol.* **11** 1680–5

[50] Short B, Preisinger C, Schaletzky J, Kopajtich R and Barr F A 2002 The Rab6 GTPase regulates recruitment of the dynactin complex to Golgi membranes *Curr. Biol.* **12** 1792–5

[51] Bielli A *et al* 2001 The small GTPase Rab4A interacts with the central region of cytoplasmic dynein light intermediate chain-1 *Biochem. Biophys. Res. Commun.* **281** 1141–53

[52] Echard A *et al* 1998 Interaction of a Golgi-associated kinesin-like protein with Rab6 *Science* **279** 580–5

[53] Nielsen E, Severin F, Backer J M, Hyman A A and Zerial M 1999 Rab5 regulates motility of early endosomes on microtubules *Nat. Cell Biol.* **1** 376–82

[54] Lonart G and Sudhof T C 2001 Characterization of rabphilin phosphorylation using phospho-specific antibodies *Neuropharmacology* **41** 643–9

[55] Matanis T *et al* 2002 Bicaudal-D regulates COPI-independent Golgi-ER transport by recruiting the dynein-dynactin motor complex *Nat. Cell Biol.* **4** 986–92

[56] Hoogenraad C C *et al* 2001 Mammalian Golgi-associated Bicaudal-D2 functions in the dynein-dynactin pathway by interacting with these complexes *Embo J.* **20** 4041–54

Further reading

Motors and neuronal disease. Cargo-carrying motor vehicles on the neuronal highway: transport pathways and neurodegenerative disease, a review written by Gunawardena S and Goldstein LS 2004 *J. Neurobiol.* **58** 258–71.

Properties of motors. Molecular motors, a review written by Schliwa M and Woehlke G 2003 *Nature* **422** 759–65.