

Molecular Motors: Strategies to Get Along Review

Roop Mallik and Steven P. Gross

The majority of active transport in the cell is driven by three classes of molecular motors: the kinesin and dynein families that move toward the plus-end and minus-end of microtubules, respectively, and the unconventional myosin motors that move along actin filaments. Each class of motor has different properties, but in the cell they often function together. In this review we summarize what is known about their single-molecule properties and the possibilities for regulation of such properties. In view of new results on cytoplasmic dynein, we attempt to rationalize how these different classes of motors might work together as part of the intracellular transport machinery. We propose that kinesin and myosin are robust and highly efficient transporters, but with somewhat limited room for regulation of function. Because cytoplasmic dynein is less efficient and robust, to achieve function comparable to the other motors it requires a number of accessory proteins as well as multiple dyneins functioning together. This necessity for additional factors, as well as dynein's inherent complexity, in principle allows for greatly increased control of function by taking the factors away either singly or in combination. Thus, dynein's contribution relative to the other motors can be dynamically tuned, allowing the motors to function together differently in a variety of situations.

Introduction

Cells are organized with different compartments — the nucleus, the Golgi complex, the endoplasmic reticulum, and so on — that act as factories. Each factory generates a unique set of products, which are then distributed to 'consumers', which could be either end-users or other factories. The distribution system is complex, and uses three sets of molecular transporters: the myosin, kinesin and dynein motors. Intracellular transport occurs along two sets of paths, both of which are similar to rail systems: the more or less randomly oriented actin filaments, used by myosin; and the (typically) radially organized microtubules used by both kinesin and dynein. Transport occurs along each of these when the appropriate motor binds to a cargo through its 'tail' and simultaneously binds to the rail through one of its 'heads' (Figure 1). The motor then moves along the rail by using repeated cycles of coordinated binding and unbinding of its two heads, powered by energy derived from hydrolysis of ATP (reviewed in [1–4]).

Microtubules are polar, and are typically organized with 'minus ends' clustered at a microtubule-organizing center situated close to the nucleus. The microtubule 'plus ends' spread outwards from the organizing center, and this leads to a radial organization (see Figure 1A) of the microtubule network in some interphase cells, such as fibroblast cells [5], pigment cells [6,7] and certain mammalian cells [8]. Microtubule organization is cell-type specific and in some cases, such as neurons [9] and epithelial cells [10], differs significantly from the radial organization shown in Figure 1A; the microtubule organization in neurons is shown schematically in Figure 1B.

Motor proteins are able to recognize the microtubule polarity, and so the organization of the rails combined with the specific motor employed determines the direction of transport. Most kinesin-family motors that have been studied move toward the plus-end of the microtubules [1,2,11], and thus kinesin-mediated transport is usually used to bring cargos toward the cell periphery. In contrast, dynein moves in the other direction — toward the microtubule minus-end [1,2,12] — and is typically used to move cargos toward the cell center (and nucleus).

Actin filaments are more randomly oriented, and can be used by unconventional myosin motors, such as myosin-V, to ferry cargos [13]. Actin filaments are significantly shorter than microtubules [6,14] and have been suggested to bridge the gap between microtubules, for example in cultured rat axons [15,16]. In this way, local transport can occur on actin filaments in regions where there are few microtubules, as at the axon terminal [16]. As with microtubules, the organization and density of actin filaments is cell-type specific. In some cases, actin filaments have an ordered structure close to the cell surface [17,18] with barbed (plus) ends pointed outwards, which could allow myosin-V — which moves toward the actin filament plus end — to transport cargos to the very edge of the cell.

In one example of how transport might work, it has been suggested that kinesin-mediated transport brings vesicles to neuronal termini, at which point the kinesins are degraded and the vesicles are subsequently transported along actin filaments by myosin-V in the actin-rich neuron terminus [13,19]. In contrast, during endocytosis, myosin-VI — which moves in the opposite direction from myosin-V, toward actin filament minus ends [20] — can be used to bring recently internalized cargos into the cell [21]. At least in some cases, however, further inside cells the actin filament network is approximately randomly oriented and has sufficient density to make it a good local transport system [17,22]. This random distribution of actin filaments can be used to spread out cargos [22], enabling the cell to achieve a more uniform distribution of cargos than would be possible by moving on microtubules alone [7,23].

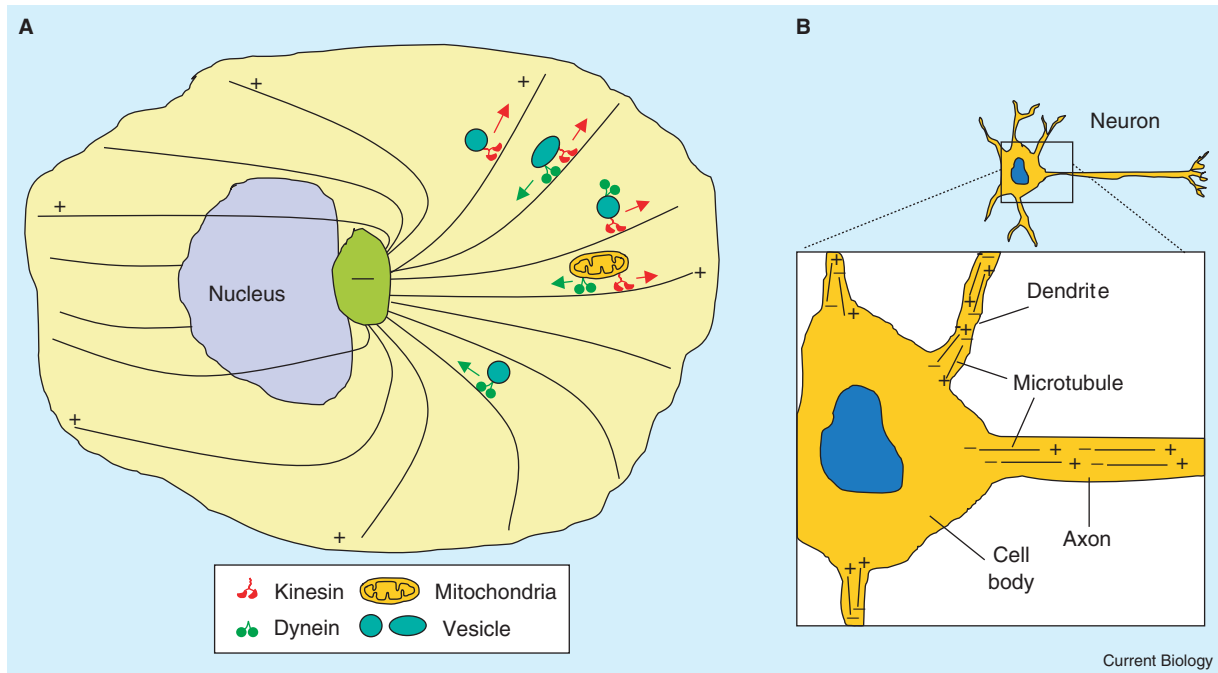


Figure 1. Organization of microtubules in a eukaryotic cell.

(A) An interphase fibroblast-type cell showing the roughly radial arrangement of microtubules (dark lines). Microtubules nucleate at the organizing center (green), with their fast-growing plus ends extending toward the cell periphery. A few different forms of cargo and associated molecular motors are also shown. (B) A neuronal cell, showing the organization and polarity of microtubules within the axon and a dendrite.

In some systems, the same cargo can move on both microtubule and actin filaments, switching between motors in the course of motion. Cargos moving this way include pigment granules [6,7], axonal vesicles [24,25], mitochondria [26] and endosomes [27,28]; for reviews, see [16,24]. A functional collaboration [29] can then exist between microtubule and actin filament networks, and there have been suggestions that motors associated with each network coordinate to achieve the requisite subcellular distribution of cargo [13,16,24]. At a global level, therefore, the intracellular transport machinery appears to regulate the relative activity of different classes of motors.

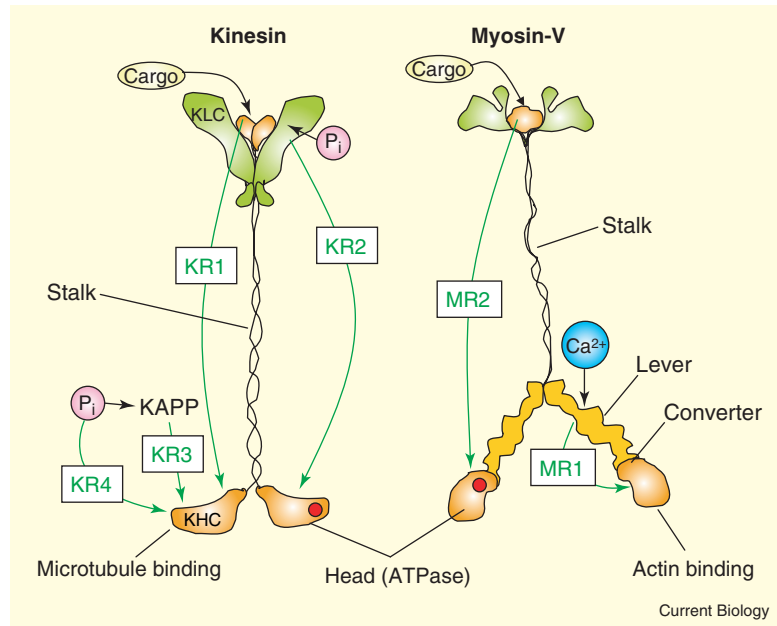
Surprisingly, motors also often appear to work together locally — intracellular transport often employs multiple motors of different classes on the same organelle. For example, multiple dyneins and kinesins attach to, and move, single lipid droplets along microtubules in bidirectional (back and forth) fashion inside the syncytial *Drosophila* embryo [30,31]. Such a strategy seems quite widespread [23,32–43] though it is not clear why an energy-inefficient mode of transport with oppositely inclined motors is necessary. So to understand intracellular transport, we have to understand both how the activity of individual motors can be controlled, and also how a certain class of motors is regulated with respect to another class.

Two complementary approaches to such research can be visualized. In a ‘top-down’ approach, within a complex and intact transport system, one could investigate how motor activity is controlled to achieve net regulated motion. Here, an *in vivo* system is

typically under investigation, such as lipid droplets in *Drosophila* [31] or pigment granules in melanophores [23]. Attempts are made to both understand particular molecular interactions, and also model the system dynamics in all its complexity [44]. Recent reviews [45,46] summarize what is known from such approaches. In contrast, in a ‘bottom-up’ approach, one can start from single-molecule properties of the motors themselves and attempt to understand what specific adaptations of each motor make it amenable to regulation by the cellular transport machinery. This review takes the latter approach. By summarizing how individual motor function can be regulated, we develop a hypothesis about how these properties allow motors to work well together.

A review now appears appropriate in view of recent results [47–53] on dynein. As our understanding of this most complex of motors evolves, we can consider other motors in a new light. We begin with a brief summary of the kinesin and myosin motors, though the interested reader should consult several excellent reviews [2–4,54–56] for further details. We then discuss dynein, emphasizing how it differs in fundamental ways from kinesin and myosin. The implications of these differences are discussed in the spirit of the aforementioned bottom-up approach. We conclude with a discussion of how these different motors might fit into the bigger picture of cellular transport. As properties of the processive organelle transporters kinesin-1, myosin-V and now cytoplasmic dynein are better understood, we present this review in the spirit of understanding how these three motors might work

Figure 2. Overview of processive molecular motors kinesin-1 and myosin-V. The motor schematics are based on figures in [2]; regulatory pathways shown are from other work, as indicated. Possibilities for kinesin-1 regulation (KR) and myosin regulation (MR) are shown by green arrows. KR1: cargo-binding dependent folding inhibits ATPase activity and microtubule binding [55,128]. KR2: Ca^{2+} -dependent binding of calmodulin to kinesin-1 light chain (KLC) inhibits ATPase activity; KLC may integrate various regulatory signals to control kinesin-1 activity [130] KR3: ATPase activity can be regulated through phosphorylation of kinesin-1-associated phosphoproteins [131]. KR4: phosphorylation of kinesin-1 heavy chain could regulate motor activity [127,129]. MR1: Ca^{2+} -binding to myosin-V induces conformational change to enhance motor activity [146–148]. MR2: reminiscent of KR1, cargo binding to myosin-V tail activates the motor, which can now undergo motion depending on Ca^{2+} levels [147].



together. It should be noted that a wide variety of molecular motors function *in vivo* [1,2], and that the functions of many of these are poorly characterized either *in vivo* or *in vitro*. Thus, the extent to which the hypotheses presented here are applicable to other instances of molecular motor based transport remains to be determined.

Myosin and Kinesin

Many molecular motors are dimers with two ‘heads’ connected together at a ‘stalk’ region and a ‘tail’ domain opposite the heads to which the cargo attaches (Figure 2). The kinesin motor family is large, so to avoid confusion we use the new nomenclature for kinesin [57]. For both kinesin and myosin family motors, the head domains bind directly to the cytoskeletal substrate, microtubule or actin filament. Kinesin-1 and myosin-V have a single ATP-binding site per head [3], and these motors function as an enzyme to hydrolyze a single ATP molecule per step during motion [58–61]. Some kinesin and myosin-family members are known *in vitro* to be able to take many consecutive steps [59,61–64] before detachment, a property known as processivity. Processive motors are specially suited to function as vesicle transporters in the cytoplasm, because if a motor detaches from the filament, the cargo is likely to diffuse away.

The fraction of its cross-bridge cycle that a motor remains attached to the rail, microtubule or actin filament, is known as the ‘duty ratio’ [56]. Processive motors, such as kinesin-1 [59,62], kinesin-2 [65] and myosin-V [63], have a high duty ratio (~1) and so rarely detach during motion. The remarkable similarity of the core motor domain of kinesin and myosin suggests that they function in a similar manner and arose from a common ancestral G protein [66,67]. This structural conservation implies functional conservation, in the

sense that similar conformational changes might occur within the motor domain in response to nucleotide hydrolysis.

The manner in which this conformational change is subsequently amplified to result in processive motion is different for the two classes of motors. For myosin, this small conformational change — on the order of angstroms — is amplified to produce a stepsize of several nanometers through the molecular equivalent of a lever, called the light-chain binding domain [3,56,68]. This lever couples the ATP-hydrolysis-induced conformational change to the actin filament through a ‘converter domain’ in the head (Figure 2). For kinesin-1, it has been suggested that structural changes in the neck-linker, a region that links the conserved motor domain to coiled-coil stalk, serves to amplify ATP-hydrolysis-induced conformational changes into mechanical motion [69–71].

There is now overwhelming evidence that both kinesin-1 [72,73] and myosin-V [74] move using a ‘hand-over-hand’ mechanism, where the occupancy state of each head — either empty or with bound ATP, ADP or ADP-Pi — determines the binding affinity of the head to a filament. An important ingredient in such models of processive motion is coordination between the two heads of the motor, such that the nucleotide state of each head, and therefore its binding affinity, can be regulated in a stereotyped fashion. This is necessary to ensure that both heads do not detach at the same time. It has been suggested that this coordination is mediated through a strain developed between the heads in a two-head-bound configuration of the processive cycle [61,70].

To fully understand the function of a molecular motor, it is important to establish the exact cycle of events — ATP hydrolysis, conformational change, filament binding, hydrolysis product release and so forth — and how these are coupled to the mechanical

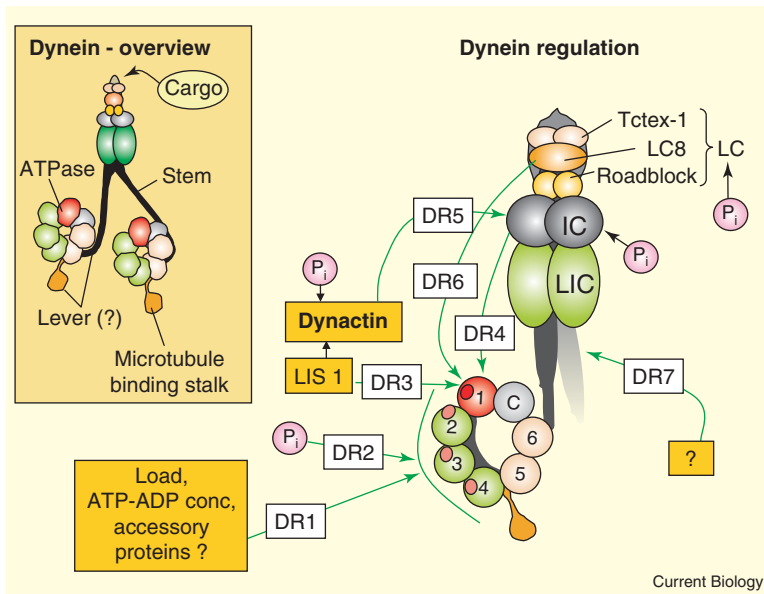


Figure 3. Potential ways in which dynein might be regulated, with an inset highlighting dynein's various structural components.

The schematics are based on figures in [2]; regulatory pathways shown are from other work, as indicated. Dynein regulation (DR) could occur at multiple levels. Different domains of the motor are labeled: IC, intermediate chain; LIC, light intermediate chain; LC, light chain. The motor head, formed by the dynein heavy chain (HC), is a ring with numbered spheres, 1 to 6, denoting AAA domains (C is not an AAA domain). Only one dynein head is shown for the sake of clarity. ATP/ADP (red sphere) is shown bound on AAA1, and putatively bound on AAA2–4 (light red sphere); P_i , phosphate. DR1: nucleotide occupancy at AAA2–4 is proposed to vary, depending on load and ATP availability [50]. Unknown accessory proteins binding to AAA2–4 might in principle control ATP/ADP binding. The linker region of the stem [49], (dark gray) curves across rear face of the ring and contacts AAA1–4; this

linker could mediate interactions with AAA1–4, possibly in a nucleotide-dependent manner [47]. DR2: cytoplasmic dynein HC can be phosphorylated to control dynein motor activity [149–151]. DR3: Lis1, a dynein regulatory protein can interact with AAA1, the site for ATP hydrolysis [132] and may regulate motor activity. DR4: IC acts as a negative regulator of dynein ATPase activity [135]; differential expression of IC [152] could explain observed differences in ATPase activity of dynein from different tissues. IC phosphorylation regulates dynactin binding [136] and could therefore influence dynein function through DR5. DR5: dynactin associates with IC [136], and allows a whole new set of possibilities for dynein regulation through secondary regulation by dynactin binding proteins (see text for details). DR6: dynein ATPase activity can be regulated by the phosphorylation of LC in a dynactin-dependent manner [137]. DR7: other proteins are also implicated in dynein regulation — for example Halo [153], Klar [30], BicD [139] — but their interactions with dynein are not fully understood.

events that generate processive motion. This is a formidable problem, and kinetic, biophysical and biochemical studies have been brought to bear on it, resulting in considerable scientific debate [3,55,56,70,75]. We shall avoid such issues here, instead focusing on how the well-characterized properties of these motors — their stall force, processivity and so on — can be rationalized in terms of their *in vivo* function.

How are single-motor properties of kinesin and myosin-V adapted to *in vivo* function? Kinesin-1 has a relatively simple structure (Figure 2). Many forms of the kinesin motor have evolved [1,2], but the best studied motors are kinesin-1 [55,58,59,62,76], kinesin-2 [65,77] and kinesin-3 [78,79]. Kinesin-1 is a two-headed homodimer (Figure 2) with a single ATP-binding site within each head. The head can also bind microtubules during motion [69,76]. In the following, we assume that the properties of the single motor determined *in vitro* are maintained when it functions *in vivo*. Such assumptions are useful, but in most cases have not yet been confirmed by careful *in vivo* experiments.

When moving *in vitro*, a single kinesin-1 motor typically takes about 100 steps with a fixed step size of 8 nm [59,62]. The motor can exert a maximum force of ~6 pN and this value is almost independent of the ATP concentration [59]. The kinesin-1 velocity is significantly reduced only at loads >3 pN [59]. Even under load, kinesin-1 rarely slips backward [80,81]. It moves along a single microtubule protofilament [64,82] hydrolyzing one ATP molecule for each 8 nm step [59]. Finally, the velocity of microtubule gliding driven by

kinesin-1 [83] or kinesin-2 [65] is independent of motor number at physiological ATP concentration and we would therefore expect that, for cargos driven by these motors *in vivo*, the characteristics of motion would not be significantly affected by the number of motors driving the motion unless there was significant drag (for example because of high cytoplasmic viscosity).

In vivo, the velocities of plus-end-directed, kinesin-driven motion can vary significantly. While this variability might reflect regulation (see below), it might equally likely derive from the complicated *in vivo* environment, where there could be local obstacles. Such obstacles could be actin filaments impeding the cargo's motion, or multiple motors (of different classes) functioning on a given cargo that could 'load' the single kinesin motors leading to lowered velocities [59,62]. Additionally, in neurites there is the suggestion that the effective viscosity is large enough that cargo velocity is dependent on the number of active motors [84].

To sum up: *in vitro*, kinesin-1 is a robust and efficient transporter — a single motor can attach to a cargo and take many successive steps before detaching from the microtubule [59,62]. *In vivo*, we expect kinesin to function similarly, though this has not yet been fully established.

Amongst the myosins, myosin-V (myosin XI in plants) is the primary motor involved in vesicle transport [2]. The *in vitro* stall force of myosin-V is ~3 pN [63], and does not depend on the ATP concentration. Myosin-V does not show any significant back-steps up to loads of ~1 pN. At a load of ~2 pN, the backstep frequency increases and intermediate steps of half the

usual step size are seen [61]. Such intermediate steps are rare, and appear to be associated with increased compliance in the motor. The frequency of such intermediate-size steps is determined by load alone, and is not a significant function of ATP concentration [61,85]. The similar structures of kinesin and myosin head domains imply some similarity between the functional properties of kinesin-1 and myosin-V. Indeed, a well-defined step size independent of load, and a constant stall force independent of ATP, support this notion.

Dynein

This third superfamily of molecular motors produces force toward the minus end of microtubules, and its function is necessary for a wide variety of processes [1,2,86]. Dyneins are found in many kinds of cells, and can be classified into two forms: axonemal and cytoplasmic dyneins. About 15 forms of axonemal dynein [87,88] have been implicated in the bending of cilia and flagella of eukaryotic cells. Axonemal dyneins are not required to be processive since they function as a large linear array of motors. We shall focus on processive cytoplasmic transport motors, and not discuss the vast and interesting literature on the non-processive muscle myosins [89–92] and axonemal dyneins [87,88,93].

Only two cytoplasmic dyneins have been discovered: cytoplasmic dynein 1b, which mainly drives slow transport within the flagellum [94,95]; and cytoplasmic dynein 1 [12], which displays an immense range of functions during mitosis [96], neuronal transport [35,97,98], maintenance of the golgi [99,100] and transport of a wide variety of intracellular cargoes, such as mRNA, endosomes, viruses and so on. *In vitro* studies have shown that cytoplasmic dynein 1 is a processive motor [50,101,102]. Henceforth, unless otherwise mentioned, 'dynein' will refer to cytoplasmic dynein 1. Like kinesin-1 and myosin-V, dynein is also a homodimer of two identical heavy chains, which make up the two motor domains (Figure 3). The head domains in dynein are massive (~520 kDa) and much more complex than those of kinesins or myosins. Because of this complexity, it has been difficult to isolate this motor in quantities and condition required for experimental studies. We shall summarize the more complicated geometry of dynein, and then discuss what is known about its function.

Dynein Structure

Sequence analysis studies of dynein show that it belongs to the AAA — ATPase associated with diverse cellular activities — class of proteins, which makes the structure of dynein fundamentally different from that of kinesin or myosin [93,103]. This difference is also obvious in form and function — with a molecular weight of about 1.2 MDa, cytoplasmic dynein is a massive multisubunit complex almost ten times bigger [104–107] than kinesin-1. In contrast to the single ATP binding in kinesin and myosin heads (Figure 2), dynein has multiple ATP binding sites (Figure 3) in each head [108–110]. Further, dynein requires the help of various accessory proteins, such as the dynactin complex, for *in vivo* function [111]. Electron microscopy studies

[105] have shown that the dynein head has seven globular domains, out of which six are AAA domains, arranged in a ring-like conformation around a central cavity (Figure 3). AAA5 and AAA6 do not have the ability to bind ATP, whereas AAA1–AAA4 can bind ATP, though with varying binding affinities [51,52,108–110,112]. AAA1 appears to be the primary site of ATP hydrolysis [112,113], though recent reports indicate AAA2–AAA4 might also have some hydrolytic activity [52,53].

Dynein is thus unique in that the different AAA domains of its ring have distinct properties, yet they have evolved from a single peptide. An unusual feature of dynein is that the dynein head makes contact with the microtubule through an unusual 13 nm long microtubule-binding stalk [107] (Figure 3). Another elongated projection, called the stem, emerges out of the ring and mediates interaction of the dynein head with other parts of the dynein complex (Figure 3). Burgess and colleagues [47–49] have proposed that a 10 nm portion of this stem, called the linker, curves around one face of the dynein ring. This geometry makes the linker a candidate mediator of multiple interactions with domains AAA1–AAA4, possibly in a nucleotide-dependent manner.

Dynein Function

To visualize the dynein power stroke, Burgess and colleagues [48,49] took images of axonemal dynein after locking the motor into its presumed pre-power and post-power stroke conformations (respectively, in the ADP-vanadate bound state and in the absence of nucleotide). They found that product release after ATP hydrolysis leads to rotation of the ring-like dynein head around the motor–stem junction. This translocates the microtubule by ~15 nm, proposed to be the mean value of the powerstroke [48]. Electron microscope reconstructions of cytoplasmic dynein [105] show a structure similar to axonemal dynein, so it is likely that the powerstroke occurs in a similar way.

Compared to kinesin and myosin, dynein has two potential lever arms: the microtubule-binding stalk and the stem. Coordinated action of both these levers, initiated through and accompanying rotation of the dynein head, appears to be the most plausible route of the powerstroke. The action of two lever arms also opens up the possibility of a longer stepsize than kinesin, as observed for cytoplasmic dynein [50]. The difficulty of implementing this mechanism is obvious from the large distance over which communication of force has to occur: ~20 nm, the distance from AAA1 to the tip of the microtubule-binding stalk via AAA2–AAA4 (Figure 3). The AAA2–AAA4 region of the ring is hypothesized to play a regulatory role in the transmission of force during dynein powerstroke, and is therefore called the 'regulatory domain' of the dynein head [47–51,108,109,112].

Recent work [51–53] has focused on understanding the properties and functions of these different AAA domains. Kon *et al.* [53] systematically studied dynein function by blocking nucleotide binding at individual AAA1–AAA4 domains, through mutations in their ATP binding P loops (P1–P4). The most severe effect on

motility of cytoplasmic dynein was observed when P1 or P3 were mutant, consistent with earlier studies on P3 mutant cytoplasmic dynein in *Drosophila* [114]. Interestingly the P1 mutant shows vanadate-mediated photocleavage at the P4 site, as if a disruption of nucleotide binding/hydrolysis at the primary P1 site induces hydrolytic activity at P4. The functional significance of this observation is unclear, though the results emphasize that domains P3 and P4 modulate the coupling between the microtubule-binding site and the primary ATPase site P1 in a non-trivial, possibly nucleotide-dependent manner. However, the effect of an applied load [50] cannot be factored into these experiments. Future single-molecule measurements on mutant dyneins, using optical trap methodology, should provide a better understanding of dynein function.

Single molecule studies of cytoplasmic dynein are, by comparison with those on kinesin-1 and myosin-V, in their infancy. We do not know whether dynein walks using a hand-over-hand mechanism. Cooperation between the two dynein heads may be important for processive motion, as a single-headed dynein is able to bind and hydrolyze ATP but cannot detach from the microtubule [115]. Recent reports [116,117] of robust microtubule gliding by single-headed cytoplasmic dynein constructs suggest that such head-to-head communication might be mediated through load from the microtubule. For kinesin-1 and myosin, such communication between the two heads is believed to arise out of internal mechanical strain generated in an intermediate configuration in which both heads are bound [61,70]. Considering the massive size of dynein heads and the flexible nature of the dynein molecule, it will be interesting to understand how the heads can communicate to achieve processive motion.

In spite of our limited knowledge of dynein function, it is already clear that dynein works in a very different manner from other motors. This difference in function arises to some extent from the dynein motor domain, which, with multiple ATP-binding sites (Figure 3), has a very different architecture. First, dynein has a lower stall force [50,118] than either kinesin-1 [59,62] or myosin-V [61,63]. Second, in contrast to these other motors, dynein mechanics is strongly altered by available ATP [50]: as one goes from micromolar to millimolar ATP concentrations, dynein's stalling force increases by a factor of three, in contrast to the 20% change seen for kinesin-1 [59]. Similarly, dynein's step size changes significantly as a function of load [50]. We do not yet know how the velocity of a single dynein motor changes with load and ATP concentration, but again it is likely more variable than kinesin-1 and myosin-V, given the reported variability in step size. Following this theme of increased variability, in contrast to the path of kinesin-1 along single protofilaments [64], dynein appears to follow a more random path along the microtubule surface, frequently switching between protofilaments [101]. Dynein also shows frequent backward motion and pauses, even when moving under no load. As a consequence, the distribution of velocities for single cytoplasmic dynein-carried beads *in vitro* is much broader than kinesin-1 [119].

The mean run lengths for dynein are less than half of that for kinesin-1 [102]. *In vivo*, the loss of accessory proteins such as dynactin [100,120,121] inhibits dynein function, and *in vitro* studies indicate that single dynein processivity is doubled by the presence of dynactin [102]. It has been suggested [119] that dynein might make more than one attachment per monomer with the microtubule: a first tight binding, which mediates the power stroke, and a second, weaker attachment to keep the head stuck to the microtubule when contact at the first site is lost. In support of this, single cytoplasmic dynein motors often show linear diffusive motion along the microtubule while moving a bead in *in vitro* assays [122]. Consistent with this hypothesis, optical trap data (our unpublished observations) for bead displacement show instances of backward sliding of the motor-bead complex over distances of ~20 nm. In this situation, the duty ratio of the strong binding site could be smaller than 0.5, but the 'effective duty ratio' of the two-headed motor approaches 1 — similar to the duty ratio value of kinesin-1 — by virtue of the weak residual binding. Thus, even though multiple consecutive steps occur, forward motility itself might not be as smooth with dynein as with kinesin-1.

Regulation of Motor Activity at the Single-Molecule Level

In spite of dynein's apparent shortcomings at the single-motor level, dynein-based transport in the cell is robust [30,123]. It is possible that this apparent discrepancy is resolved through the use of accessory proteins and multiple motors. Dynein's processivity is increased by the dynein regulatory complex dynactin [102]. Dynein-based transport of lipid droplets in *Drosophila* is robust, and occurs over distances of a few microns [30,31]. During such motion, the stall force is regulated in units of 1.1 pN — equal to the single-dynein stall force [50] — but is typically between 3.3 and 5.5 pN, strongly suggesting that the motion is driven by multiple dynein motors. The *in vivo* ramifications of moving a vesicular or bead cargo by multiple dynein motors remains to be investigated, but the smooth gliding of microtubules, presumably driven by multiple dyneins [124,125], suggests that such multiple-dynein motion will be more robust than single-motor motion. Quite likely, *in vivo* a combination of multiple motors and accessory factors increases the cargo stall forces and processivity, and suppresses back steps, though the details are as yet unclear.

At first glance, it seems odd that the predominant motor used for minus-end transport is so apparently mediocre and sensitive to external conditions, requiring significant accessory factors to function. In the world of cellular transport, is there any advantage of having a poor performer that requires external help? We hypothesize that inherent in the complexity of dynein is the opportunity for regulation at multiple levels. The simpler structures of kinesin and myosin do not allow such extensive regulation, as can be inferred from the insensitivity of key motor properties, such as step size and stall force, to external conditions, such as load and ATP concentration. To investigate this hypothesis

further, we shall discuss what is known about the regulation of motor activity of each class of motors.

Kinesin and Myosin-V: Simplicity Allows Limited Scope for Regulation

The relative insensitivity of kinesin-1 and myosin-V to external parameters *in vitro* might suggest that these motors provide limited opportunity for regulation by the cellular machinery. In contrast, dynein can be regulated at multiple levels. Whether this scenario holds true in general for *in vivo* transport — which potentially employs multiple forms of motors on a single cargo — is not yet clear, though there is some evidence that this is the case.

In several cases, it appears kinesin-1 and myosin-V are regulated predominantly by simply turning the motor ON — ‘attach to cargo’ — or OFF — ‘remove from cargo’. For example, cell-cycle regulation of myosin-V has been investigated by treating melanosomes with interphase or metaphase-arrested *Xenopus* egg extracts [126]. It was found that transport mediated by myosin-V can be downregulated by phosphorylation of the motor domain; however, this phosphorylation causes no change in ATPase activity — rather, it dissociates the motor from the vesicle.

Immunolocalization studies on rat nerve preparations [11] showed that kinesin dissociates from organelles at the nerve periphery (microtubule plus end), but remains attached strongly to organelles at the proximal (microtubule minus end). Thus, the motor gets on to organelles at the microtubule minus end, transports them to the plus end and then falls off [127]. Limited possibilities for regulation of kinesin-1 motor activity do exist, and are summarized in Figure 2.

For kinesin-1 not bound to cargo, the motor’s globular ‘tail’ domain can fold back onto the head in such a manner that the ATPase activity is blocked [55,128]. Such self-inhibition has also been suggested to occur when kinesin-1 is complexed with myosin-V through a common light-chain-binding domain [13]. Kinesin-1 motor activity can also be regulated *in vivo* through phosphorylation of both heavy and light chains [127,129,130], and also through phosphorylation of proteins interacting with kinesin [131]. From *in vitro* studies of kinesin-1 [59], there is no indication that it is possible to regulate kinesin’s stalling force, though *in vivo* stall forces have not been measured. Also, as we will see below, in comparison to dynein the role of accessory proteins in altering kinesin-1 function appears somewhat limited. The limited evidence that myosin-V motor activity can be regulated at the single motor level has been summarized in Figure 2.

Dynein and the Need for Complexity: Regulation at Multiple Levels

Three distinct modes of dynein regulation can be visualized: regulation within the motor domain itself; regulation through accessory proteins; and regulation by controlling the number of dynein motors functioning together. These possibilities are discussed in more detail in the subsections below.

Regulation within the Motor Domain

Cytoplasmic dynein is the only motor reported to take steps of variable size — 8, 16, 24 and 32 nm — with a proposed load-induced reduction in step size, analogous to a gear mechanism. According to the model of Mallik *et al.* [50], load-induced binding of nucleotide to domains AAA2–AAA4 can ‘compactify’ the dynein ring, leading to a shorter step size and increasing the force generated. In this scenario, the unique architecture of dynein motor domain allows regulation of function at multiple levels.

It is possible that a four-fold variation in step size would require contribution from elements outside the dynein head. An attractive element is the linker region [47–49], which curves across one face of the dynein ring, and appears to make contact with all the potential ATP/ADP binding sites (Figure 3). This linker could be a key structural element for force transmission from AAA1 to the microtubule binding tip, as its rigidity could be modulated through multiple nucleotide-dependent interactions with empty/occupied AAA domains. Other non-motor proteins might bind to the linker, or bind directly to the AAA domains in a manner that regulates the nucleotide binding affinity of sites AAA1–AAA4. For example, the dynein regulatory protein Lis1 binds AAA1 [132], though the functional consequences of this interaction remain to be elucidated. Thus the force production or hydrolysis rate of the motor could in principle be regulated at multiple levels. In the absence of such regulatory proteins, and if ATP is abundant, the motor would be free to change gear in a manner regulated by the load under which it functions [50].

Regulation through Accessory Proteins

If the functions of dynein and architecture of its head domain are complex, its components outside the head do not lag far behind. Each dynein molecule consists of heavy chains (HCs), intermediate chains (ICs), light-intermediate chains (LICs; two each) and several light chains (LCs), which vary in number [1,86,88]. The list of proteins [86] that interact with these units is bewildering, and gives a preview of the versatility of this motor. How does association with this long list of proteins modify dynein function?

While several proteins play the role of recruiting dynein on to the right cargo [133], there is evidence that function of the dynein motor itself can be regulated at multiple levels (Figure 3). For example, heterogeneity of the LIC subunits within dynein could lead to differential regulation of the motor [134]. While the dynein motor could be regulated indirectly, through the action of accessory proteins such as dynactin, is it possible that the motor ATPase activity itself is also regulated? Removal of the dynein IC from rat testis cytoplasmic dynein leads to a four-fold enhancement of ATPase activity [135], implying that the dynein IC functions as a negative regulator of the motor domain.

Dynactin associates with the dynein IC through a phosphorylation-dependent mechanism [136], and this association might indirectly modulate the way the enhancing effect of the dynein IC on dynein’s ATPase

activity. Indeed, phosphorylation of dynactin is reported to influence dynein's ATPase activity [137]. We do not yet know what such modulation in ATPase activity could achieve, as far as dynein function *in vivo* is concerned. It does not appear likely that dynactin modulates dynein's stall force, because *in vivo* estimates [118] of the single dynein stall force are 1.1 pN, the same as found by *in vitro* measurements [50].

Dynactin has also been suggested [102] to be a processivity factor for dynein, from the observations that dynactin can bind independently to microtubule and that dynactin increases the run-length of single dynein motors in an *in vitro* assay. Proteins associating with dynactin might in principle also regulate dynein function via dynactin. For example, Lis1 interacts with both dynein and the dynein IC, and is important for the stability of the dynein–dynactin complex [132], which in turn should determine processivity of the complex. Similar possibilities could also arise out of association of the dynein–dynactin complex with casein kinase II [138], BicD [139] and other proteins [86].

We do not know what the major role of dynactin is: to regulate dynein motor activity, or just to enhance dynein processivity by providing the motor a second attachment to the microtubule. At this stage, it is certainly clear that dynactin interacts with several other accessory proteins, and this opens up an entire second level of regulatory possibilities for the dynein motor. The fact that no such regulatory complex has been found to be essential for kinesin function *in vivo* strengthens the view that dynein is extensively regulated by comparison with the kinesins and myosins.

Multiple Dynein Motors Work Together

For non-processive motors such as muscle myosin, processivity can be increased by multiple motor molecules combining to form higher-order assemblies [140]. Sufficient motors can then always make contact with their polymer track, so that the cargo is not lost through detachment. It is possible that dynein might use a similar strategy, though on a more limited scale. Might three or four dynein motors combine to drive processive motion comparable to that of kinesin-1, where linear diffusion and backward sliding are reduced? The minus-end-directed motor kinesin-14, for example, is not processive at the single motor level, but multiple kinesin-14 motors driving motion of a bead in an optical trap show significantly enhanced processivity [141]. The single-headed motor kinesin-3 also shows processivity-enhancement through dimerization [79], suggesting at least that a similar strategy is employed elsewhere.

Indeed, several observations imply that multiple dyneins may be active simultaneously on a given intracellular cargo *in vivo*. Thus, the *in vivo* stall force for dynein-driven motion in *Drosophila* can be as large as ~6 pN, and is quantized in multiples of 1.1 pN, the single-motor stall force [118]. Electron micrographs show that multiple dyneins are present on individual cargoes, and more than one appear to bind the microtubule at the same time [142]. The number of dyneins driving motion in *Drosophila* appears to be developmentally regulated [30]. The

average run length for *in vivo* dynein-associated structures [30,118,123] is often larger than that observed for single cytoplasmic dynein driven *in vitro* motion [101,102]. Dynein is the only known molecular motor which makes contact with the microtubule through a long (~13 nm) stalk [49,107], which might be a special adaptation allowing multiple cytoplasmic dynein molecules to overcome space restrictions to attach to the same cargo during transport [143]. When moving, do dynein motors in such a 'team' coordinate? How is the organism able to regulate the number of active dyneins on a specific organelle? These will be interesting questions for the future.

As well as overcoming processivity limitations and potentially suppressing pauses and backsteps, the use of multiple dynein motors together on the same cargo in principle allows the relatively weak dynein, with a stall force of only ~1.1 pN, to compete against kinesin-1 and myosin-V. There is evidence that motor stalling forces are additive [30], so that three dynein motors together would be expected to exert ~3.3 pN, comparable to the force from a myosin-V motor. Just as accessory proteins can be removed to downregulate dynein at the single molecule level, the use of multiple dynein motors inherently allows regulation of dynein's competitive state relative to kinesin-1 and myosin-V by altering the number of active dynein motors. A possible example of such regulation is discussed below.

Putting it All Together

We thus suggest that the multiple avenues available to regulate dynein function potentially allow the cell to tune dynein's contribution relative to the other motors. Are there any known examples where this appears to be the case? During pigment granule motion in fish [6] and *Xenopus* [7] melanophores, switching from microtubule-based to actin-based transport occurs only during microtubule minus-end-directed motion, which is dynein-driven. On the basis of this, a model [6] was suggested in which myosin-V, with a stall force ~3 pN, loses in a tug-of-war with kinesin-1 or kinesin-2, with a stall force of ~6 pN, but wins in a tug-of-war against dynein. When dynein loses, granules are handed over from microtubules to actin filaments. But during pigment aggregation in these cells, however, the goal is to move pigment granules to the cell center, so in this case granules should be handed over from actin filaments to microtubules. This is indeed achieved, as during aggregation dynein motion is up-regulated and 'wins' against myosin-V activity.

It is interesting to speculate that one or more of the forms of dynein regulation discussed above are employed to achieve this change in relative dominance of the motors, but a careful experimental investigation of these possibilities has not yet been done. We are left with a possible rationalization of the hierarchy of motor strength (kinesin-1 or kinesin-2 > myosin-V > dynein) and processivity (kinesin-1 or kinesin-2 > dynein) as potentially useful for cooperative microtubule and actin filament-based motion. While such a scenario is appealing, this is still very much in the realm of hypothesis, as *in vivo* stalling

forces for kinesin-1 or kinesin-2 and myosin-V have not yet been measured (though they are likely the same as the measured *in vitro* forces, as observed for dynein). We will have to wait for *in vivo* estimates of force for kinesin and myosin, and also results from other *in vivo* systems to test this conjecture.

Microtubule-based transport is a complicated story in its own right, apart from possible interactions with actin-based transport. Multiple, oppositely directed cargos are simultaneously transported on a given microtubule. How does efficient transport arise out of such seemingly chaotic motion? If several massive dynein motors are to remain attached continuously to bidirectionally moving cargo, why do they not obstruct motion? In short, how can the cell avoid 'traffic jams' on microtubules? This is clearly desirable, as such traffic jams could have serious consequences, such as neuronal degeneration [98]. Motor function and architecture may be adapted to avoid this.

Dynein may have adaptations giving it a 'yield when necessary' strategy, including the following features. First, flexible and long stalks [49,107,143], which would impart a certain degree of maneuverability to the motor. Second, poor processivity and a tendency to back-step [101]: dynein might clear the way to give 'right-of-way' to oppositely moving cargo. Third, unlike kinesin-1, dynein can re-attach to adjacent protofilaments of the microtubule [101]; thus, dynein could move laterally away to give 'right-of-way' to oppositely directed cargo. And fourth, a second weak attachment to the microtubule, which permits linear diffusional motion [122]; short segments of such diffusive motion might extract the motor from a jam.

Kinesin-1, on the other hand, might have adaptations giving it a rather different strategy — 'it's my right of way' — including the following features. As a stronger motor, which takes few backsteps [59,62], it could bulldoze its way against dynein. Its high processivity [59] will reduce the probability of it yielding to dynein. Its track is a single protofilament [64,82], so might be able to displace dynein-driven cargo to an adjacent protofilament. This 'right-of-way' scenario appears true in at least one *in vitro* reconstitution of organelle motion, where kinesin-1-driven organelles dominate [144]. This is clearly not the whole story, however, because it is certainly possible to regulate kinesin activity even when it remains bound to the cargo the entire time (Figure 2). Such control of kinesin likely involves additional higher-order structures used in bidirectional transport [45].

Conclusion

We have attempted to identify at a qualitative level specific properties that allow single motor proteins to function in harmony with other motors. The focus has been on dynein, as recent results have enhanced our understanding of this motor and allowed us to integrate this information into the 'big picture' of intracellular transport. We hypothesize that the complexity of dynein allows it to be regulated at multiple levels, and this imparts maneuverability to the cellular transport machinery. The kinesin and myosin motors are the performance-oriented 'lean and mean' workhorses of

transport, with regulation often restricted to dissociation from the organelle or inactivation through folding. In contrast, dynein can be regulated in more subtle ways at multiple levels.

While this is potentially a useful framework for rationalizing the relative contributions of different motors, it is certainly a simplification. In some systems, there may in fact be regulation of different kinesin motors, for instance in the case of intraflagellar transport [145]. Thus, our understanding of how motors work together *in vivo* is still evolving. To some extent we now understand how many (thousands) of motors function together in muscle, and also how motors like kinesin, myosin and dynein function at the single motor level. A crucial next question lies in between these levels: we need to clarify the function of small ensembles, where a few motors, possibly of different families, work together. Is single-motor processivity relevant at all within this ensemble, or are run-lengths determined by a regulatory mechanism operative at a higher level of control? How does cellular organization on a global scale arise out of the seemingly chaotic motion of single motors? How is work done at the nanoscale manifested at a macroscopic level? We believe that these issues go beyond the field of molecular motors, and are relevant to biological structure in general.

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