

Bidirectional cargo transport: moving beyond tug of war

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Abstract | Vesicles, organelles and other intracellular cargo are transported by kinesin and dynein motors, which move in opposite directions along microtubules. This bidirectional cargo movement is frequently described as a ‘tug of war’ between oppositely directed molecular motors attached to the same cargo. However, although many experimental and modelling studies support the tug-of-war paradigm, numerous knockout and inhibition studies in various systems have found that inhibiting one motor leads to diminished motility in both directions, which is a ‘paradox of co-dependence’ that challenges the paradigm. In an effort to resolve this paradox, three classes of bidirectional transport models — microtubule tethering, mechanical activation and steric disinhibition — are proposed, and a general mathematical modelling framework for bidirectional cargo transport is put forward to guide future experiments.

Long distance transport in neurons is carried out by the molecular motor proteins kinesin and cytoplasmic dynein that bind to cargo (vesicles, organelles, and protein and mRNA complexes) and transport them along axonal and dendritic microtubules^{1,2}. Almost all cargos are simultaneously bound to both plus-end-directed kinesin motors (responsible for anterograde transport towards the cell periphery) and minus end-directed cytoplasmic dynein motors (responsible for retrograde transport back to the cell body)^{3–9}. Whereas individual motors move unidirectionally along microtubules, the directional transport of intracellular cargo is generally achieved through back-and-forth movements with an overall net directionality towards the proper destination. It has been hypothesized that the selective advantage of this seemingly inefficient transport behaviour is to facilitate the manoeuvring of molecular motors around ‘roadblocks’, such as organelles or microtubule-associated proteins, or to provide a type of ‘proofreading’ mechanism whereby the correct cargo destination is reached by a directional bias that is reinforced over many passes rather than by an ‘all-or-nothing’ decision that is determined by the initial directionality^{10–12}. The importance of understanding the molecular mechanisms underlying bidirectional transport is highlighted by its potential role in neurodegenerative diseases. Alzheimer’s disease, for example, is characterized by tangles of the microtubule-associated protein tau¹³ that inhibit axonal transport, and amyotrophic lateral sclerosis, Huntington’s disease and Parkinson’s disease are all thought to involve deterioration in anterograde and/or retrograde axonal transport^{14–18}.

In vitro reconstitution of microtubule-based transport, structural studies and biochemical investigations have uncovered many of the molecular details of motor protein function. However, there remains a considerable gap between the insights that have been gained into motor protein behaviour at the single-molecule level and a comprehensive understanding of cargo transport in living cells. In contrast to the relatively simple model of a single molecular motor moving along an individual microtubule, cargo transport *in vivo* involves multiple motors of both directionalities transporting cargos of diverse geometries through a ‘crowded’ cytoplasm along microtubules that harbour diverse post-translational modifications and that are decorated by microtubule-associated proteins. Hence, a key challenge in the field is to bridge the knowledge gap between single-motor function *in vitro* and intracellular transport *in vivo*.

Bidirectional cargo movement is frequently described as a ‘tug of war’ between oppositely directed molecular motors attached to the same cargo. However, although the tug-of-war model is consistent with many experimental observations, numerous knockout and inhibition studies in various systems have found that inhibiting one motor leads to diminished motility in both directions, which is in direct conflict with this paradigm. In this Review, I first provide an overview of bidirectional cargo transport and a description of experimental and modelling studies that support the tug-of-war model. This is followed by a critical analysis of the model and a discussion of the experiments that conflict with it. Finally, in an effort to resolve this ‘paradox of co-dependence’,

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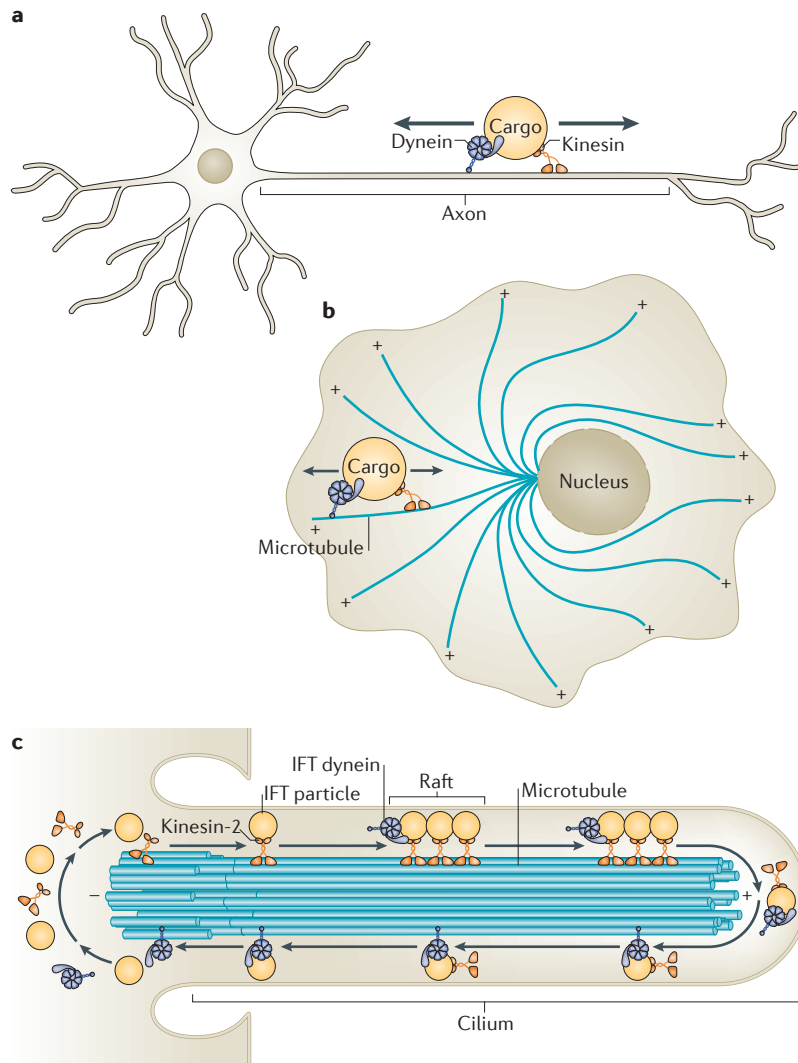


Figure 1 | Three classes of bidirectional transport in cells. **a** | Neurons contain numerous membrane-bound vesicles that are transported bidirectionally by kinesin and dynein motors in both axons and dendrites. **b** | In many diverse cell types, larger organelles, such as mitochondria, melanosomes, peroxisomes and lipid droplets, are transported bidirectionally along microtubules. **c** | Intraflagellar transport (IFT) involves the bidirectional transport of proteins (IFT particles) along axonemal microtubules in cilia and flagella by kinesin-2 family motors (KIF3 heterotrimer complex) and dynein-2 (IFT dynein) that are attached to the cargo.

Amyloid- β precursor protein (APP). A protein that is proteolyzed to generate amyloid β (A β) protein, which forms plaques in Alzheimer's disease.

Prion protein
The protein responsible for transmissible spongiform encephalopathy (including scrapie and mad cow disease).

Myosin V
A processive myosin motor involved in cargo transport along actin filaments.

I propose three classes of bidirectional transport mechanisms — microtubule tethering, mechanical activation and steric disinhibition — and a general modelling framework to guide future experiments.

Diversity in bidirectional transport

Cargos with a range of geometries and intracellular functions move bidirectionally along microtubules, and important model systems have emerged to study transport diversity. For the purpose of this discussion, cargos that are transported bidirectionally are separated into three general groups: neuronal vesicles, larger organelles (with sizes ranging from a few hundred nanometres to a few micrometres) and cargo that are transported by intraflagellar-transport (IFT) (FIG. 1). These model

systems have been reviewed in detail¹¹, and several informative reviews have been written on the general topic of bidirectional transport^{10,19–24}.

Neurons have long been used as a model system for studying bidirectional transport, and the prevalence of human neurodegenerative diseases makes a complete understanding of bidirectional transport in neurons clinically important^{1,14}. Axons and dendrites contain a diverse array of bidirectionally transported vesicles that generally range in size from a few tens of nanometres to a few hundred nanometres. These include secretory vesicles^{25–27}, lysosomal vesicles^{7,28}, autophagosomes²⁹, and vesicles containing amyloid- β precursor protein (APP)^{30–32} and the mammalian prion protein⁸.

Larger organelles, such as mitochondria^{20,22}, melanosomes^{3,4,33–39}, peroxisomes^{12,40} and lipid droplets²³, constitute a second class of cargo that are transported bidirectionally and have emerged as model systems for understanding the underlying molecular mechanisms of bidirectional transport. Mitochondria move bidirectionally in axons and are transported by kinesin-1 family members and cytoplasmic dynein motors^{20,22,41,42}. Melanosomes, which are transported by kinesin-2 family members (KIF3 heterotrimer complex) and dynein (together with myosin V), constitute another well-studied system that has revealed important molecular details^{3,4,33–39}. Moreover, the bidirectional transport of lipid droplets in *Drosophila melanogaster* embryos has proven to be a very useful model system, especially because the droplets can be manipulated using optical tweezers and the molecular mechanisms can be dissected using the tools of fly genetics^{23,43–49}.

A third category of bidirectional transport is IFT, in which protein complexes in cilia and flagella are transported along axonemal microtubules by kinesin-2 family members (KIF3 heterotrimer complex) and dynein 2 (IFT dynein)^{50–60}. One hallmark of IFT is that, instead of the directional switching and pausing observed in most bidirectional transport, the cargos move unidirectionally without pause until reaching their cellular destination and then after some time turn around, and make a similar return journey^{50,59–61}. This behaviour implies a different regulatory mechanism, although, like most of these bidirectional transport examples, the molecular details of directional switching are not entirely clear. However, this Review focuses on vesicle and organelle transport, and directional switching in IFT is not discussed here.

As mentioned above, these diverse cargos range in size from a few tens of nanometres to nearly ten micrometres. For small cargos, steric constraints will set an upper limit for the number of motors that can simultaneously attach, whereas for large cargos, the forces required to move through the viscous cytoplasm may set a lower limit for the number of motors required. Furthermore, different cargos are transported by different kinesins (primarily members of the kinesin-1, kinesin-2 and kinesin-3 families), and these different motors move at different speeds, have different degrees of processivity and respond differently to external loads: kinesin-1 motors can walk against sustained loads,

whereas kinesin-2 and kinesin-3 motors tend to detach under load^{62–68}. Cytoplasmic dynein (hereafter referred to simply as dynein) contains two heavy chains and numerous intermediate and light chains. Its complex structure enables numerous avenues of regulation, and several dynein regulatory molecules, such as Klarsicht (Klar), Halo, Lissencephaly-1 (Lis1) and NudE, have been identified^{11,19,31,44,47,49,69–81}. Due to this diversity, it is important to be cautious when trying to unify these phenomena into a common model to describe bidirectional transport. However, despite their different intracellular functions and the involvement of different molecular players, most of these cargos display qualitatively similar transport behaviours, specifically stochastic directional switching interspersed by pauses. Thus, although future progress will inevitably lead to the division of the mechanisms that underlie bidirectional transport into distinct subclasses, currently, it is still instructive to approach these questions in terms of finding common mechanisms, which has generally been the approach taken in the field.

The Tug of war model

The tug-of-war model for bidirectional transport (also known as the mechanical competition model) describes the mechanical competition between antagonistic kinesin and dynein motors bound to the same cargo. Although the term was used previously by Salmon and Reider to describe opposing poleward forces on sister kinetochores during mitosis⁸², and the concept of directional switching due to the forces of opposing kinesin and dynein motors was explored by Vale *et al.*⁸³, the use of the phrase tug of war to describe bidirectional transport along microtubules was originally coined by Welte, Gross *et al.* in 1998 to describe lipid droplet transport in *D. melanogaster* embryos⁴⁸. Over the ensuing 15 years, numerous experimental and theoretical studies have used the tug-of-war framework to analyse the intracellular transport of neuronal vesicles, mitochondria, lysosomes and melanosomes in cells, as well as precisely engineered multimotor complexes *in vitro*^{4,7,46,84–90}.

Experimental support for the model. The use of tug-of-war models for interpreting experimental data was greatly facilitated by the development of a mean field computational model put forward by Müller, Lipowsky and colleagues^{84,88,91}. Using experimentally derived parameters for motor velocity, run length, stall force and detachment rate, the Müller tug-of-war model was able to qualitatively, and in some cases quantitatively, recapitulate the back-and-forth vesicle trajectories observed *in vivo*. An excellent demonstration of the usefulness of this model was its application to kinesin- and dynein-driven vesicle transport in neurons⁷. In this work, the authors estimated the number of kinesin-1, kinesin-2 and dynein motors on purified vesicles that moved bidirectionally on isolated microtubules *in vitro*. When the specific motor parameters for kinesin-1, kinesin-2 and cytoplasmic dynein motors were incorporated into the model together with estimated motor numbers per vesicle, the simulated cargo trajectories included distinct

runs in either direction that were punctuated by pauses, thereby matching the experimental results (FIG. 2). Furthermore, inhibition experiments using antibodies raised against dynein could be recapitulated with this model by reducing the number of dyneins per vesicle.

Support for the tug-of-war model also comes from a study investigating the transport of endosomes in *Dictyostelium discoideum* cells and on isolated microtubules *in vitro*⁹⁰. Directional switching of endosomes was observed to involve a phase of slower velocity during which vesicles elongated, which is consistent with being pulled by opposing motors (similar deformations have been observed for mitochondria)⁹². Optical trapping was used to quantify parameters for the kinesin-3 family member Unc-104 (known as KIF1A in humans) and *D. discoideum* cytoplasmic dynein; the results were incorporated into a computational model (similar to the Müller mean field model)⁸⁴ that was able to recapitulate the observed directional switching behaviour. These simulations suggest that each kinesin is balanced by 4–8 dynein motors in this system. A related study using optical tweezers⁹³ found that, compared with beads bound only by kinesin, both intracellular cargo and artificial beads associated with dynein and kinesin had lower stall forces, which suggests that motors attached to the same cargo mechanically compete as predicted by the tug-of-war model.

Hence, it is clear that in some systems bidirectional switching can be qualitatively and quantitatively accounted for by the tug-of-war model. In addition, based on the fact that motors generally remain attached to their cargo during directional switching^{3–9}, it seems unavoidable that mechanical competition between opposing kinesins and dyneins will occur during bidirectional transport. This raises two questions. First, is mechanical competition, as delineated by the tug-of-war model, the dominant mechanism underlying bidirectional transport, or are these experimental tug-of-war examples isolated exceptions? Second, is the tug-of-war model, as quantitatively described by current computational models, in fact an accurate description of the underlying molecular mechanisms? Although the simulations are able to recapitulate the experiments, it is important to remember that demonstrating the agreement of a 14-parameter computational model with experimental results does not prove the model to be correct. Hence, because the Müller mean field model is so successful in reproducing experimental data, it is worth examining the underlying model assumptions before moving forwards.

Critiquing the model. The first critique of the Müller mean field model is how the force-dependence of motor detachment was taken into account. As transport direction is determined by the number of attached plus-end-directed and minus-end-directed motors, as well as by their mechanical strengths, the kinetics of detachment under load is the most important factor defining model behaviour. Consistent with the standard theory^{94,95}, the force-dependent off rate is defined as $k_{\text{off}}(F) = k_{\text{off}}(0)e^{F/F_{\text{detach}}}$ (in which $k_{\text{off}}(0)$ is the motor

Optical tweezers

An experimental technique that uses light to trap and manipulate transparent objects, which enables measurements of forces and displacements.

Processivity

The ability of a motor to walk many steps along its filament before detaching.

Sister kinetochores

Regions of duplicated chromosomes that interact with microtubules and regulate chromosome movement during mitosis.

Mean field computational model

A model in which all of the molecular motors of a particular type are assumed to share the mechanical load equally and step in unison.

Run length

The distance a molecular motor moves before detaching from the filament and diffusing away.

Stall force

The force against which a motor cannot walk any further.

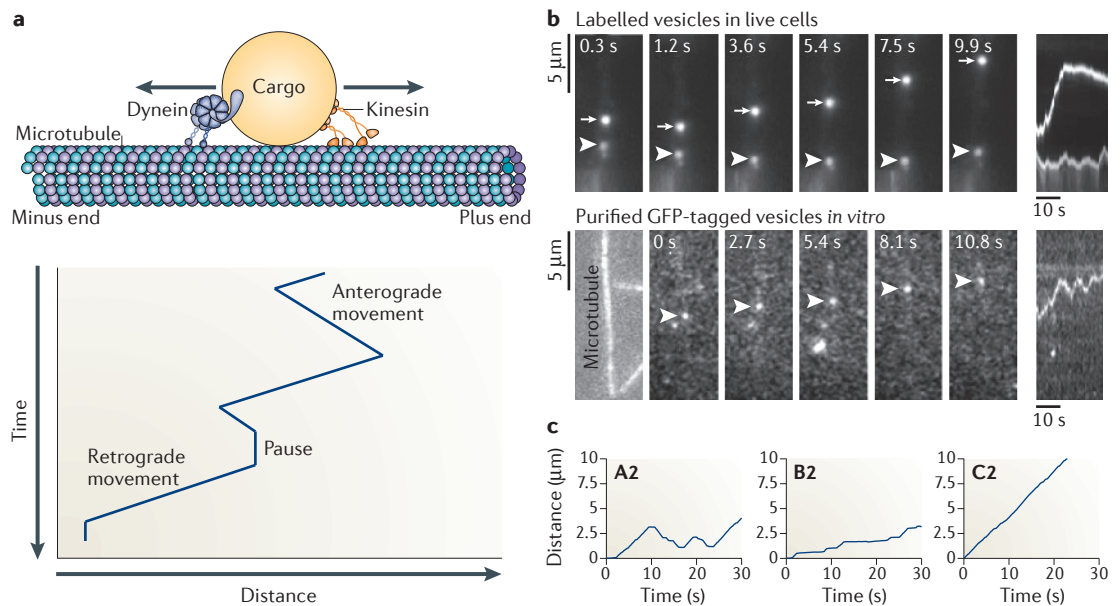


Figure 2 | The tug-of-war model. **a** | Schematic of bidirectional transport showing cargo with bound dynein motors that move to the minus ends of microtubules (retrograde transport) and bound kinesin motors that move to the plus ends of microtubules (anterograde transport). Cargo movement is bidirectional and interrupted by periodic pauses. The bottom plot is a sketch of a typical kymograph. Diagonal lines denote fast movement and vertical lines denote no movement. **b** | Experimental observations of bidirectional transport. In this experiment by Hendricks *et al.*⁷, bidirectional transport of neuronal vesicles was visualized both by live-cell imaging in neurons and by *in vitro* reconstitution on immobilized microtubules. (Top) Montage of six images taken over 10 seconds and the resulting kymograph (far right), showing the bidirectional transport of LysoTracker-labelled vesicles in neurons. Whereas the upper vesicle takes long runs in both directions, the lower vesicle shows only small fluctuations. (Bottom) Similar results are seen for purified vesicles containing dynactin–GFP moving along immobilized microtubules *in vitro*. Image of rhodamine-labelled microtubules is shown on the left, followed by a montage of five images taken over 11 seconds and the resulting kymograph. **c** | Results from simulations of a theoretical tug-of-war model. Simulations by Müller *et al.*⁸⁴ show that model-generated bidirectional transport dynamics can recapitulate experimental data and that the specific transport characteristics strongly depend on the choice of parameters and numbers of motors used. In these simulations, six kinesin and six dynein motors were modelled, using motor parameters estimated from lipid droplet experiments. Simulation A2 used the default parameters and shows robust runs in both directions, which are analogous to those observed experimentally. In simulation B2, the dynein stall force was reduced by a factor of 2.4 and the dynein dissociation rate constant was reduced by 12%, which results in net plus-end movement with occasional stalls. In simulation C2, the dynein stall force was also reduced by a factor of 2.4, but the dynein dissociation rate constant was increased by a factor of 2, which results in smooth plus-end movement (dominated by kinesins). Part **b**: this article was published in *Curr. Biol.*, 20, Hendricks, A. G. *et al.*, Motor coordination via a tug-of-war mechanism drives bidirectional vesicle transport, 697–702, Copyright Elsevier (2010). Part **2c** from Müller, M. J., Klumpp, S. & Lipowsky, R. Tug-of-war as a cooperative mechanism for bidirectional cargo transport by molecular motors. *Proc. Natl Acad. Sci. USA* 105, 4609–4614 (2008). Copyright (2008) National Academy of Sciences, U.S.A.

detachment rate at zero load and F_{detach} is the key scaling parameter that defines the sensitivity of the motor–microtubule bond to the load). A simple problem arises from the fact that the motor detachment rate from the microtubule is equal to the motor velocity divided by the distance a motor moves before detaching ($k_{\text{off}} = \text{velocity/run length}$). The first issue is that hindering loads (for example, exerted by oppositely directed motors attached to the same cargo) are known to reduce both the kinesin motor velocity and the run length^{62,68,96}, thus the ratio of kinesin motor velocity to run length is expected to be a complex function of load. The second issue is that directional switching involves motors transitioning from forwards walking (when the force is less than the stall force) to backwards walking (when the force exceeds the stall force) with a transition point at the stall force, at which the velocity is zero. Optical

tweezer experiments using kinesin-1 family members confirm that motor behaviour differs substantially under substall and superstall forces^{68,96}, and experiments of dynein point to even more complex ‘catch-bond’ behaviour at high loads⁹⁷. Thus, because detachment kinetics are such an important determinant of model behaviour, the load dependence of motor dissociation must be modelled as a more complex function than simply as an exponential dependence (as has been explored by others^{90,96}), and it is vital that the governing parameters are tightly constrained by experiments.

A second critique of the Müller mean field model is the assumption that load is shared equally between all motors of a given type (kinesin or dynein), which is known as the mean field approximation. This approximation would be valid if every motor would ‘step’ in a synchronized manner and there was no motor–cargo

Kymograph

A graphical presentation of spatial position over time that uses data from a stack of images or movie. The intensity profile along a line is recorded for every image in the stack, and the lines are assembled to create an image in which the distance is displayed on one axis and time on the other.

compliance. However, the domains of motor proteins connecting the microtubule-binding site to the cargo-binding site acts as a nonlinear elastic tether, and motors step stochastically, sometimes waiting short or long periods of time between steps⁹⁸. Thus, as a motor diverges from the 'pack' due to fluctuations in stepping rates, it will stretch and shoulder a greater proportion of the load, which will decrease its velocity and increase its detachment rate. One result of this phenomenon is that two motors attached to a single cargo do not carry out transport nearly as well as one might expect, an issue that has been explored extensively through both experiments and computational modelling^{86,87,89,96,99–104}.

One model system in which experimental results are not well described by the tug-of-war model is the bidirectional transport of lipid droplets in *D. melanogaster* embryos. Quantitative characterization of the transport kinetics, switching rates, and *in vivo* and *in vitro* forces that govern lipid droplet transport indicates instead a 'coordination model' between kinesin and dynein to account for bidirectional switching. Several potential regulatory proteins have been put forward as potential 'coordinators' of motor activity, including Klar, Halo, Lis1, NudE and Dynactin^{10,11,19,44–49,75}, but the precise molecular mechanism underlying the coordination of kinesin and dynein motors in this system remains elusive. To investigate more comprehensively whether lipid droplet transport can be quantitatively described using a tug-of-war framework, the Müller mean field model was expanded into a stochastic model that included motor–cargo tethers, stochastic motor stepping and experimentally determined force-dependent off rates for kinesin and dynein⁹⁶. Despite this considerable expansion of the model, which addresses essentially all critiques of the Müller mean field model described above, the experimental data could still not be fit into a tug-of-war framework. This finding rules out the tug-of-war model for describing bidirectional transport in the lipid droplet system.

A final detail of bidirectional transport that warrants further experimental work is the definition of the pause state. A consistent feature of many bidirectional transport measurements in cells is that the cargo spends a considerable amount of time in a paused or static state^{8,31,46,105,106}. These pauses could be due to three underlying mechanisms (FIG. 3). The first possibility is that the motors are pulling with equal and opposite forces, such that all motors are stalled (a 'draw'). Microtubule binding by a cargo-bound protein other than kinesin or dynein could achieve a similar non-motile stall state. The second possibility is that all motors are detached and the cargo is actually diffusing freely but at a sufficiently slow rate as to seem static. The third possibility is that motors are engaged but moving only very slowly and/or are rapidly switching directions, which results in a velocity close to zero (the 'kicking and screaming' mechanism)⁸¹. The Müller mean field model makes very specific and falsifiable predictions about this pause state. Specifically, it postulates that apparent pauses are due to slow backwards motions resulting from the very slow superstall velocities of attached kinesin and dynein motors (see the kicking and screaming model in FIG. 3).

Experimental characterization of cargo kinetics during pauses is hindered by limits in spatial resolution (for example, due to inherent fluctuations in cells and potential artefacts from using centroid tracking on deformable cargo) and temporal resolution (for example, due to finite frame rates and inherent difficulties in calculating instantaneous velocities). However, these fluctuations provide information-rich constraints for testing specific transport models, and experimental efforts to increase the spatial and temporal resolution of these fluctuation measurements are of high importance for the field.

The paradox of co-dependence

A consistent feature of all existing tug-of-war models is that, because the two motor types are in competition, inhibiting one motor should lead to increased motility in the opposite direction. As detailed above, this is what has been observed in many studies^{7,77,90,93,107,108}. However, despite the intuitive nature and widespread acceptance of the tug-of-war model, several investigations over many years have found precisely the opposite result — inhibiting one motor diminishes transport in both directions. These puzzling observations, which I refer to as the paradox of co-dependence, call into question the validity of the tug-of-war-modelling framework in explaining *in vivo* bidirectional transport and point to a need for developing new quantitative models of bidirectional transport. Before describing novel mechanisms that could explain the paradox of co-dependence, this section reviews some of the key studies that demonstrate it.

Over 30 years ago, even before the discovery of kinesin, it was observed in *Aplysia* spp. that axonal injection of an inhibitor of the retrograde transport motor dynein surprisingly inhibited anterograde vesicle transport¹⁰⁹. Later studies in extruded squid axoplasm showed that antibody-mediated inhibition of either kinesin or the p150^{Glued} subunit of the dynein adaptor protein dynactin led to a strong reduction in bidirectional vesicle movement on isolated microtubules^{110,111}. A series of studies in transgenic *D. melanogaster* larvae extended these findings. Mutations in the gene encoding dynein resulted in the same phenotype as a kinesin knock-out — axonal swellings filled with immobilized cargo rather than cargo accumulation either at the cell body or in the periphery¹¹². Mutating *unc-104*, the kinesin-3 family motor responsible for the anterograde transport of synaptic vesicles in flies, resulted in vesicles moving with slower velocities and shorter run lengths in both the anterograde and retrograde directions compared with wild type²⁷. Importantly, the dynamics of both mitochondria and neurosecretory vesicles, which are transported by motors other than Unc-104, were unaffected in this study, confirming that this phenomenon was not simply a result of global effects on the microtubule cytoskeleton.

Similar results were observed for fluorescently labelled cellular prion protein (PrP^c) vesicles in mouse hippocampal neurons⁸. Knocking out the gene encoding kinesin light chain 1 (KLC1) or short hairpin RNA-mediated knockdown of the KLC2-encoding gene (both of which are involved in linking kinesin-1 family protein to PrP^c vesicles) diminished vesicle run lengths

Substall

Forces that are less than the stall force of a molecular motor. They enable the forward 'stepping' of motors.

Superstall

Forces that are greater than the stall force of a molecular motor. They cause motors to either remain stationary, walk backwards or detach.

Centroid tracking

A method used to determine the position of an object in a microscope image and to follow its change in position in successive images. The centroid is also known as the centre of mass.

Aplysia spp.

A genus of sea slugs that is used as a model system in neurobiology.

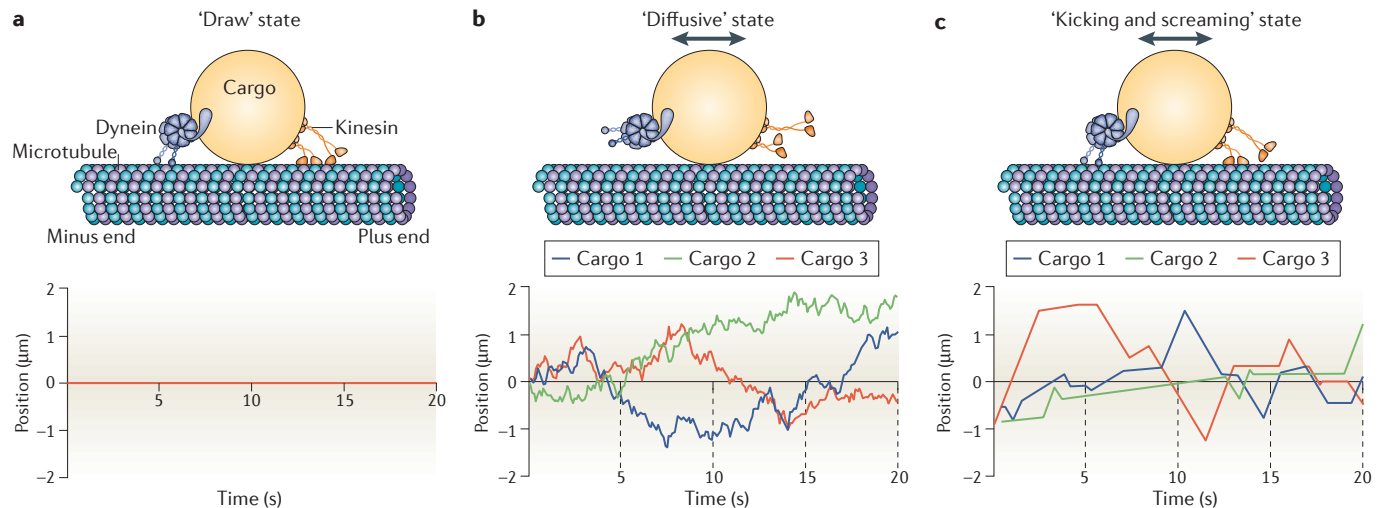


Figure 3 | Different mechanisms for the pause state. **a** | In the 'draw' state, both motors are engaged and stalled. The resulting static position of the cargo in the pause state is represented as a line at position zero in the corresponding plot. **b** | In the 'diffusive' state, both motors are detached and cargo is diffusing. The corresponding plot shows one-dimensional diffusion of three simulated cargos, each having a diffusion constant of $0.05 \mu\text{m}^2$ per second. As illustrated, diffusion can result in substantial excursions of the cargo in both directions. **c** | In the 'kicking and screaming' state⁸¹, both motors are engaged and moving slowly owing to their inherently slow reverse walking speeds. The plot shows results from a stochastic simulation of the tug of war between one kinesin-1 family member and seven dyneins, using the modelling framework and kinesin-1 and dynein parameters from REF. 84. Three 20 second periods of minimal displacement are shown, highlighting that although the displacements are generated by a different mechanism, they can seem qualitatively similar to the excursions seen in the diffusive state.

and increased the pause frequency in the retrograde direction, the opposite of what was expected (FIG. 4a). Similarly, the knockdown of dynein heavy chain 1 in this study resulted in shorter runs and more frequent pauses in both the retrograde and anterograde directions. As the knockdown of a kinesin-1 family motor was previously shown to alter microtubule dynamics¹¹³, it is important to confirm that these results are not due to off-target effects. To this end, it was shown that the knockdown of the gene encoding KIF5B (a kinesin-1 family member), which increased pausing of PrP^c vesicles, did not alter synaptophysin vesicle transport, which indicates that the results cannot be explained by a global repression of microtubule-based transport.

In mouse dorsal root ganglia, knockdown of the gene encoding the p150 subunit of the dynein adaptor protein dynactin decreased the number of anterograde transport events, thereby countering expectations, and increased the number of vesicles remaining in paused states¹⁰⁶ (FIG. 4b). In a different study, deletion of the dynactin subunit Actin-related protein 1 (Arp1) in *D. melanogaster* neurons led to decreased vesicle transport velocities in both anterograde and retrograde directions, and to a substantial increase in the number of paused vesicles¹¹⁴.

To investigate the role of motor forces in bidirectional transport, fluorescently labelled peroxisomes were tracked in *D. melanogaster* S2 cells with different genetic backgrounds¹². Depletion of either Kinesin heavy chain (kinesin-1 is also known as KHC) or Dynein heavy chain 1 (Dhc1) arrested cargo transport (as opposed to causing mislocalization to the microtubule plus or minus ends, which would be predicted by the tug-of-war model).

The next question was whether this transport arrest was due to the inhibition of the specific motor species or the specific motor activity. In cells lacking kinesin-1, bidirectional motility could be rescued by the kinesin-3 family member Unc-104 but not by a non-motile kinesin-1. Similarly, in dynein-knockdown cells, bidirectional transport could be rescued by the minus-end-directed kinesin-14 family member Non-claret disjunctional (Ncd). Hence, in this system a specific motor activity and not the particular motor species is key to maintaining bidirectional transport.

The finding that inhibiting one motor also inhibits the activity of the oppositely directed motor suggests some sort of co-dependence — the activity of one is required for the activity of the other. I call this paradox 'co-dependence among antagonistic motors'. In addition to the above studies, this apparent co-dependence has also been observed for the bidirectional transport of lipid droplets⁴⁶, melanophores³³, mitochondria^{22,42,112,115}, neurofilaments¹¹⁶ and ribonucleotide granules¹¹⁷. As the dominant paradigm in the field, the tug-of-war model, cannot explain this large body of results, there is a need for new models of bidirectional transport beyond existing frameworks that can account for the large body of disparate data.

Mechanisms to explain co-dependence

In an effort to resolve the paradox of co-dependence between kinesin and dynein, three hypothetical mechanisms of bidirectional transport are proposed below (FIG. 5). The approach advocated here follows Platt's 'strong inference' method to scientific inquiry^{118,119}:

propose multiple hypotheses to describe a phenomenon, devise and carry out crucial experiments to falsify one or more of the hypotheses and repeat as necessary. Owing to the complexity of bidirectional transport, progress forwards requires a tight integration of modelling and experiments. To provide more rigour to the conceptual mechanisms presented in FIG. 5, a modelling framework for developing these (and other) hypothetical mechanisms into quantitative models is presented in FIG. 6. The general mathematical framework can be thought of as a collection of kinesin and dynein motors that can exist in different states (such as stepping, diffusing, stalled and inhibited). Transition rates between these states depend on motor properties and the current state of the system (such as the number of motors attached, cargo velocity and the forces being generated by motors). In this way, the model structure is not unlike the Müller tug-of-war model, but the key differences are the definitions of the on and off states and how parameters that control the transition rates are specified.

The microtubule tethering mechanism. The first mechanism that may account for the paradox is the microtubule-tethering mechanism, in which the key characteristic is that instead of simply attaching and detaching motors switch between states of directed transport and a weak binding or diffusive transport. Molecular motors are generally characterized *in vitro* by their run length and the distance they move along the microtubule before detaching¹²⁰. However, there are many documented cases in which motors diffuse in one dimension along microtubules. This one-dimensional diffusion is often compared to DNA-binding proteins, which are known to bind electrostatically to the negatively charged DNA and diffuse until finding the proper sequence, at which time they switch to a strongly bound state^{121–123}. The kinesin-3 family member KIF1A was the first kinesin to clearly demonstrate this one-dimensional diffusional movement. It was shown that an engineered monomeric KIF1A moved processively along microtubules in a low ionic strength buffer by a

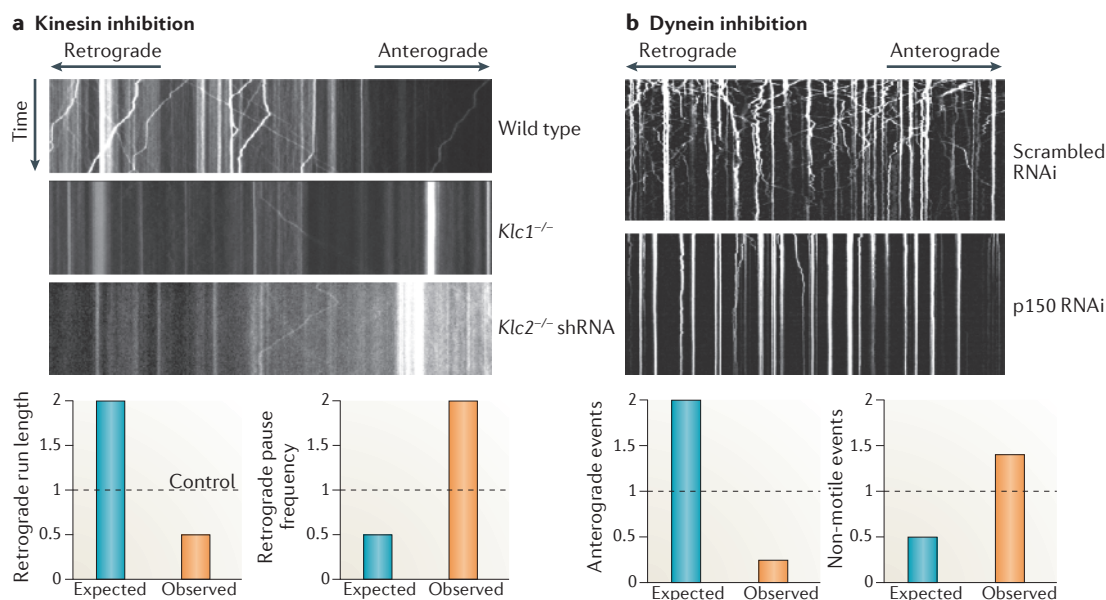


Figure 4 | Examples of antagonistic motor co-dependence. **a** | The anterograde motor kinesin was inhibited in mouse neurons by knocking out the gene encoding kinesin light chain 1 (Klc1^{-/-}) or by short hairpin RNA (shRNA)-mediated knock down of the gene encoding KLC2, both of which are involved in linking kinesin-1 to cellular prion protein (PrP^c) vesicles. Vesicles were labelled by YFP-PrP^c and their transport dynamics were analysed by kymographs. The wild-type kymograph (top) shows left and right diagonal tracks, indicative of retrograde and anterograde cargo transport, respectively, as well as some vertical lines indicative of stationary vesicles. By contrast, the kinesin inhibition kymographs (middle and bottom) show very little transport in either the retrograde or anterograde directions, and many stationary vesicles. Schematic plots of the data demonstrate that although kinesin inhibition was expected to result in longer retrograde run lengths and fewer pauses during retrograde movement, the opposite was observed. Data are all plotted relative to control values. Note that although the observed results in the schematic approximate the published data, the expected results are only qualitative estimates for comparison. **b** | In mouse neurons, dynein was inhibited by knocking down the p150 subunit of the dynein adaptor protein dynactin, which links dynein to vesicles. Vesicles were labelled by RFP-tagged lysosome-associated membrane protein 1 and their transport was analysed by kymographs. In the control kymograph at the top (scrambled RNAi), numerous bidirectional transport events (diagonal lines) were observed, whereas following dynein inhibition (p150 RNAi) very little transport in either direction was observed, and almost all vesicles were stationary (vertical lines). Schematic plots of the data show that although dynein inhibition is expected to result in more anterograde transport events and fewer non-motile events, the opposite was observed. Part **a**: this article was published in *Cell*, 144, Encalada, S. E., Szpankowski, L., Xia, C. H. & Goldstein, L. S., Stable kinesin and dynein assemblies drive the axonal transport of mammalian prion protein vesicles, 551–565, Copyright Elsevier (2011). Part **b**: this article was published in *Neuron*, 74, Moughamian, A. J. & Holzbaur, E. L., Dynactin is required for transport initiation from the distal axon, 331–343, Copyright Elsevier (2012).

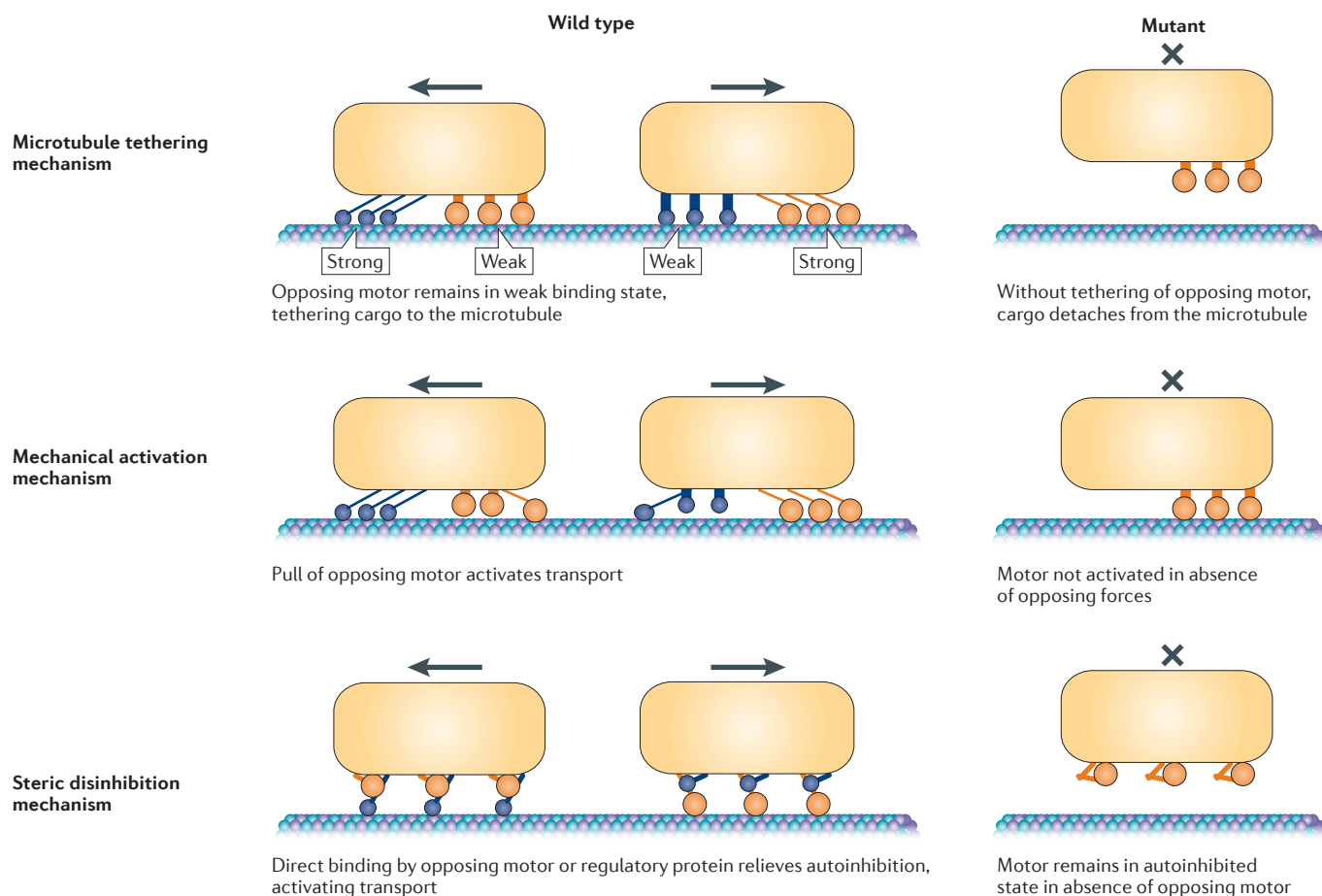


Figure 5 | Three hypothetical mechanisms for resolving the paradox of co-dependence. In the microtubule tethering mechanism, motors are proposed to transition between a strong binding state when the motor is walking and a weak binding state in which the motor is inactive but remains tethered to the microtubule. Diminished cargo transport in mutants results from a lack of tethering. In the mechanical activation mechanism, motors are posited to be in an inactive state until an opposing motor pulls on the cargo and activates them. The absence of one class of motor in a mutant diminishes cargo transport because the opposing motor is not mechanically activated. In the steric disinhibition mechanism, motors are proposed to remain in an inhibited state even when bound to their cargo. Direct binding by opposing motors or other regulatory proteins relieves inhibition, resulting in transport. The diminished cargo transport in mutants results from motors remaining in their inhibited state.

combination of diffusive and directed transport^{64,124,125}. The non-covalent forces keeping the motor associated with the microtubule were mapped to the positively charged K-loop of the motor and the negatively charged carboxy-terminal tail of tubulin. Although it was subsequently shown that *in vivo* kinesin-3 family motors dimerize through cargo binding or other mechanisms^{63,126,127}, KIF1A represents an important model of motor transport in which continuous transport is achieved by the motor switching between a motile state and a diffusive state, while continuing to remain bound to the microtubule.

Subsequent to the work on KIF1A, others have shown that the mitotic motor Eg5 (a kinesin-5 family member) switches between diffusive and motile states¹²⁸ and that at low ionic strengths mouse KIF5B alternates between stepping and diffusing along the microtubule¹²⁹. Furthermore, dynein links to cargo through the adaptor protein dynactin that can bind to and diffuse along

microtubules¹³⁰. Hence, there is clear support from *in vitro* studies that molecular motors (or motor-adaptor complexes) can diffuse along microtubules. However, it is unclear what triggers the switch between the diffusive and the directed transport states. Furthermore, the implications of extrapolating these diffusive properties of isolated motors to the motor–cargo complex have not been explored in detail.

One prediction from the microtubule-tethering mechanism is that, in the paused state, one or both sets of motors remain attached to the microtubule in a weakly bound state. This situation contrasts with the active stall (in which both sets of motors are attached and their forces are balanced) inherent in tug-of-war models. In this context, the reason why inhibiting one motor diminishes cargo transport by the opposing motor is that the tethering of the cargo to the microtubule is reduced, and the cargo then tends to dissociate from the microtubule (FIG. 5).

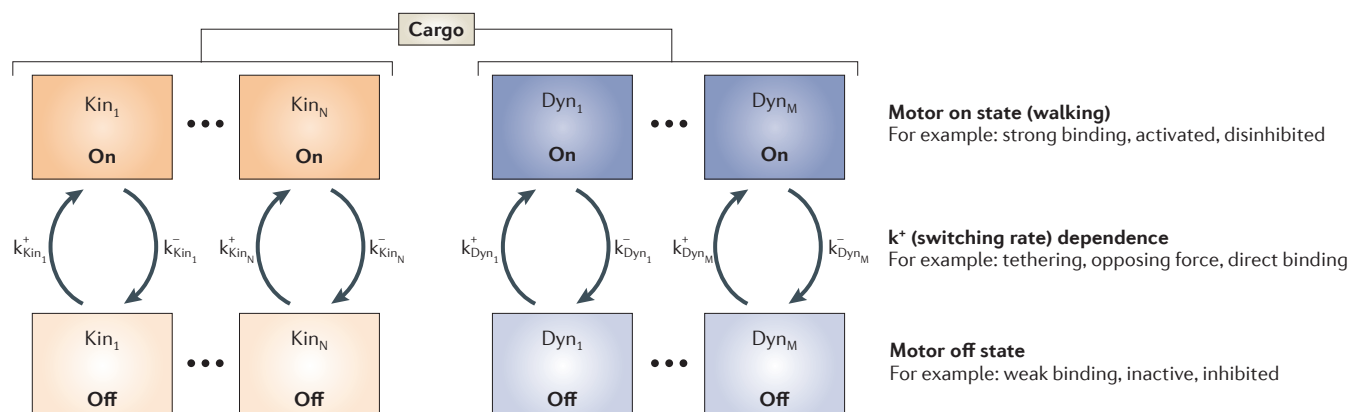


Figure 6 | Mathematical modelling framework for bidirectional transport models. Hypothetical mechanisms can be explored and quantitatively tested using mathematical models of cargo transport by kinesin and dynein motors. On a given cargo, several kinesin motors (Kin_1 to Kin_N) and dynein motors (Dyn_1 to Dyn_M) can be bound. Motor states are defined generically as on and off, and different hypothetical mechanisms (for example, microtubule tethering, mechanical activation, and steric disinhibition) involve different on and off states. For each motor, the rate constant k^+ defines the rate by which the motor switches from the off state to the on state, and the rate constant k^- defines the rate by which the motor switches from the on state to the off state. For different hypothetical mechanisms, these switching rates will depend on different variables in the system (such as the force acting on that motor, the cargo position or the activity of the opposing motors). Subscripts on the rate constants (for example, $k^+_{Kin_1}$) indicate that each motor has its own switching rates that vary over time and depend on other system variables. In this modelling framework, the switching rate magnitudes and parameter dependencies are the principal determinants of overall model behaviour. These models readily lend themselves to standard Monte Carlo (Gillespie Stochastic Simulation Algorithm)¹⁶⁴ approaches.

In the modelling framework displayed in FIG. 6, the simplest formulation is that the on state is stepping and the off state is diffusing, and the transition rates depend on the number of motors in the stepping and diffusive states. A slightly more complex model would include a second off state (distinct from the diffusive state), in which the motor is completely inactivated or detached.

The mechanical activation mechanism. The mechanical activation hypothesis postulates that motors are, at low load forces, predominantly in an inhibited state due to tight binding to the microtubule, failure to properly engage with the microtubule or another reason. The forces generated by opposing motors bound to the same cargo pull the motor out of this inhibited state, freeing it for active motion. According to this paradigm, the reason that inhibiting one motor diminishes transport in both directions is that in the absence of opposing forces the remaining motors are generally in an inactive state (FIG. 5).

Despite the fact that beads with a uniform population of attached motors move robustly *in vitro*, there is experimental support for mechanical activation as a mechanism to control motor activity. First, optical trap experiments, in which beads are dragged back and forth along immobilized microtubules, have shown that the dissociation of kinesin motors from microtubules depends not only on the magnitude of the external load but also on its direction — the dissociation being faster when the load is pulling in the assisting (plus end) direction than in the hindering (minus end) direction^{68,131–133}. Furthermore, a recent optical trapping study that analysed both phagosomes in intact cells and

artificial beads *in vitro* found that the duration that dynein motors stalled increased with increasing stall forces, which is reminiscent of a catch bond⁹⁷. A similar study using rat brain dynein bound to polystyrene beads also found that at superstall forces, the binding duration of dynein increased with increasing load⁹⁶. Granted, these studies demonstrated increased attachment and not necessarily activation due to opposing motor forces; so, evidence is still lacking for the precise mechanism underlying activation. Notably, the principal result from the aforementioned study of fluorescent peroxisomes in *D. melanogaster* S2 cells¹² was that, following the knockdown of the genes encoding KHC or dynein, bidirectional transport could be rescued by any motor as long as it was functional and pulled in the appropriate direction. Although it does not prove the mechanical activation mechanism, this result is in line with this proposed model. In terms of the modelling framework depicted in FIG. 6, the on and off states in the mechanical activation mechanism would be walking and inactive (that is, stuck, weak binding or fully detached), respectively, and transitions between the states would be regulated primarily through the forces generated by the competing motors. Another possibility is that the mechanical activation results from other mechanisms than motor effects per se. For example, when opposing sets of motors simultaneously pull on a cargo, there will be a force of attraction towards the microtubule surface, which will bring motors and microtubules in closer proximity and increase on rates following motor dissociation. This force will be diminished when one set of motors is inhibited.

Catch bond

The dissociation rate of this type of bond decreases (the bond strength increases) with increased pulling forces.

The steric disinhibition mechanism. Kinesin motors exist in autoinhibited conformations in which the tail folds back to shut down the motor activity^{127,134–141}. Activation is thought to result from binding to cargo or binding by regulatory proteins^{142,143}. Although there is no clear autoinhibitory mechanism for dynein, there are regulatory proteins that control its activation^{79,80}. Moreover, it has been shown that the nucleotide state of their secondary AAA domains can also alter dynein processivity and microtubule affinity¹⁴⁴. Hence, it is reasonable to hypothesize that the motor activity of kinesin and dynein motors can be altered even while the motors remain bound to their cargo. The steric disinhibition mechanism posits that motors remain in their autoinhibited state even after cargo binding and that transport results from the activation of cargo-bound motors. This activation could result from direct binding of one motor to the other or through a regulatory protein that relieves this autoinhibition through direct binding, phosphorylation or some other mechanism. Thus, the reduced motility following the inhibition of one motor results from the fact that the opposing motor is no longer activated (FIG. 5).

In support of this model, there is evidence that kinesin-1 family members directly bind to dynein⁶ and that both the kinesin-2 accessory protein KAP3 and dynein bind directly to dynactin³³. Recent work has shown that the adaptor protein JNK-interacting protein 1 (JIP1) directly binds to and relieves the autoinhibition of KHC, and that it also binds to dynactin, leading to the speculation that JIP1 differentially regulates the two motors, perhaps acting as the master control switch³¹. Perhaps the strongest support for this mechanism involves the mitochondrial Rho GTPase Mitochondrial Rho (Miro) and the adaptor protein Milton (Milt; the homologue of mammalian TRAK proteins). They link kinesin-1 motors and dynein to mitochondria in a Ca^{2+} -dependent manner, which results in the positioning of mitochondria at sites of increased Ca^{2+} in neurons. In *D. melanogaster*, both the dynein–dynactin complex and KHC bind directly to Milt, and in the presence of Ca^{2+} Miro binds to the KHC heads, thereby inhibiting the motor activity and resulting in the arrest of transport^{115,145}. Thus, low Ca^{2+} levels relieve the inhibition of KHC. Although transport arrest mediated by similarly high Ca^{2+} concentrations is observed in mammalian neurons, experiments suggest that mammalian KIF5 binds directly to Miro1 at low Ca^{2+} levels but dissociates at high Ca^{2+} concentrations, which suggests that the same results are achieved through a different mechanism¹⁴⁶.

In terms of the modelling framework shown in FIG. 6, the on and off states of the motor would be disinhibited and inhibited, respectively, and transitions between these states would be regulated by binding of oppositely directed motors or adaptor proteins, or possibly by phosphorylation.

Other considerations

To obtain a comprehensive picture of bidirectional microtubule-based transport in cells, it is also important to take into account possible roles of the actin cytoskeleton and myosin motors. For example, it has been shown

that in addition to kinesin and dynein, myosin V motors are also attached to melanosomes and that the dispersion and aggregation of melanosomes involve both actin filaments and microtubules^{4,36,147,148}. In particular, the cortical actin network seems to be important for anchoring cargo at the periphery, as depolymerization of actin leads to melanosome aggregation. This spatial segregation between the roles of actin and microtubules, with actin-based mechanisms dominating at the periphery, is also thought to have a role in endocytosis and the subsequent trafficking of endosomes²¹. However, in other cellular systems, depolymerizing the actin cytoskeleton has minor effects on microtubule-based cargo transport^{97,149}. Finally, myosin V interacts with microtubules and increases kinesin processivity *in vitro*, which suggests another potential crosstalk between actin- and microtubule-based transport^{150–152}. Hence, in the context of the three hypothetical mechanisms discussed above, actin and myosin probably have minimal roles in bidirectional cargo transport in neurons, but they may have an important role in organelle dynamics in other systems, particularly in transport and docking at the cell cortex.

Although the connection of actin filaments and microtubules to antagonistic disinhibition is not entirely clear, there are other regulatory molecules that have been shown to alter the activities of kinesin or dynein or both, and thereby alter bidirectional transport. For instance, the protein huntingtin, which is mutated in Huntington's disease, facilitates bidirectional transport, most likely by acting as a scaffold for binding motors and/or motor binding partners^{108,153–155}. JUN N-terminal kinases (JNKs) and JIPs are important regulators of bidirectional transport in neurons — JIPs act as scaffolding proteins that link motors to cargo, and JNK phosphorylation leads to some combination of reduced cargo binding, reduced activity and diminished mechanical performance of kinesin-1 motors^{16,31,156–159}. JNKs are also thought to increase microtubule dynamics and promote microtubule stability, which provides another avenue for altering transport^{160–163}.

Another issue in uncovering mechanisms controlling bidirectional transport is the question of timescales. Direct measurements of transport, such as visualizing vesicle deformations and transient pauses, provide potentially a very different readout than the behaviour of cargo following hours or days of inhibition. For example, the injection of antibodies raised against dynein into non-neuronal cells resulted in rapid anterograde vesicle movement in the short term, followed by diminished overall transport at later times⁷⁷.

The way forward

The value of using models to uncover biological mechanisms is that they provide testable hypotheses for designing experiments and a framework for interpreting data. In developing new models of bidirectional transport, incorporating the core concept of the tug-of-war model — motors of opposite directionality mechanically compete during bidirectional transport — seems

Mean-squared displacement

A parameter commonly used to measure diffusion. It is calculated by squaring the displacement of an object from its original position. Its rate of change with time is proportional to the diffusion constant.

Table 1 | Mechanisms by which different models account for experimentally observed cargo behaviour

Model	Experimentally observed cargo behaviour		
	End of run	Pause state	Rescue from pause
Tug of war	Stall due to stochastic motor detachment or attachment of opposing motor	Both motors stalled in 'a draw' (with potential cargo deformation)	One motor 'wins', and the other motor detaches or backsteps (with potential recoil of cargo)
Microtubule tethering	Motor switches to diffusive state	Both motors bound in diffusive state	Motor switches to moving state
Mechanical activation	Motor switches into inactive state	Either both motors detached, or bound and stalled	Thermal or motor-driven mechanical activation
Steric disinhibition	Motor switches into inhibited state	Either both motors detached, or attached but inhibited	Motor released from inhibited state

unavoidable. Furthermore, for some specific model systems, bidirectional cargo transport can be explained well both qualitatively and quantitatively using the tug-of-war model. However, for the large number of intracellular cargos that demonstrate the paradox of co-dependence among antagonistic motors, experiments are not consistent with the tug-of-war paradigm. These conflicts require the development of new models to describe bidirectional cargo transport in cells.

An important step towards defining new models is to generate experimentally testable model predictions. TABLE 1 lists the three mechanisms proposed above together with the tug-of-war model and defines three key characteristics for each; the termination of a unidirectional run, the description of the pause state and the resumption of movement after a pause. These characteristics provide the first qualitative model predictions that can be addressed experimentally. For instance, a preliminary model classification can be achieved by obtaining high temporal and spatial resolution measurements of cargo dynamics in the paused state and determining whether the data are best fit by a 'diffusive' model (characterized by a linear mean-squared displacement versus a time profile), a draw model (characterized by normally distributed experimental noise, resulting in a flat mean-squared displacement profile) or a kicking and screaming model (the data consist of a series of slow runs) (FIG. 3). The next steps are to build a mathematical framework (such as the one proposed in FIG. 6, although others are also possible), to use these models to generate quantitative experimental predictions, such as the switching rate, pause duration or other metrics, and then challenge these predictions with targeted experiments.

When modelling a biological system, there is always a trade-off between simplicity (parsimony) and

complexity. Although the Müller tug-of-war model contains 14 parameters, the model is actually quite parsimonious when considering the details of the underlying motors and the complex dynamics of the system that it is describing. It is likely that new bidirectional models will necessarily be more complex; however, because existing simpler models cannot account for the experimental data, this added complexity is warranted. Further biological features of intracellular transport that may affect cargo behaviour include microtubule heterogeneity (resulting from microtubule-associated proteins and tubulin post-translational modifications), cargo geometry (including cargo size, compliance of the motor–cargo linkage and the ability of cargo-bound motors to laterally diffuse and thus locally concentrate in the membrane) and regulatory proteins that alter motor activity and binding to cargo. Owing to the biological complexity, an important task is to delineate the specific details that have an impact on transport characteristics from those that only have minor roles and can be left out of the models. Fortunately, the influence of these variables can be addressed by combinations of knockdown and inhibition studies in cells, together with creative reconstitutions of bidirectional motility *in vitro*.

Different cargos (for example, lysosomes, secretory vesicles, mitochondria, lipid droplets and melanosomes) have different transport requirements and are in many cases transported by different sets of motors. Therefore, it is expected that different mechanisms may apply to different intracellular transport systems. By defining classes of bidirectional transport models and focusing on characterizing the key transport parameters that underlie the model behaviour, a combined experimental and computational approach to these problems should yield important new insights into this fascinating problem.

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Competing interests statement

The author declares no competing interests.