Co-operative transport by molecular motors

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Abstract

Intracellular transport is often driven co-operatively by several molecular motors, which may belong to one or several motor species. Understanding how these motors interact and what co-ordinates and regulates their movements is a central problem in studies of intracellular transport. A general theoretical framework for the analysis of such transport processes is described, which enables us to explain the behaviour of intracellular cargos by the transport properties of individual motors and their interactions. We review recent advances in the theoretical description of motor co-operativity and discuss related experimental results.

Introduction

The complex internal structure of cells depends, to a large extent, on active transport by molecular motors. In many cases, the transport of cellular cargos such as RNAs, protein complexes, filaments and organelles relies on the co-operative action of several molecular motors [1]. Furthermore, many cargos exhibit bidirectional movements that involve two motor species that move in opposite directions, for example, kinesin-1 and cytoplasmic dynein, or switch between microtubule-based and actin-based transport. How multiple motors are co-ordinated, in particular when the transport involves two or more species of motors, is currently an area of active research. In the present review, we discuss three different scenarios for co-operative transport from a theoretical perspective. The three cases are as follows: unidirectional transport by one team of motors, bidirectional transport by two teams of motors and transport on different tracks, involving both actin- and microtubule-based motors. All three cases have been studied extensively in recent years, both experimentally [2–7] and theoretically [8–10].

Theoretical approaches can contribute to the study of motor co-operation in several ways. One important objective of theory is to integrate the well-established properties of individual motors into comprehensive models for co-operative transport. The comparison of quantitative theoretical predictions and experiments can then provide insights into mechanistic details that are not directly accessible experimentally. For example, experiments usually trace the trajectory of a cargo, which may have a complex relationship with the movements of the individual motors, since several different motor configurations may lead to the same cargo behaviour. In this case, theoretical models can provide a link between the behaviour on the cargo level and the behaviour of the individual motors working collectively. In general, theory also provides a conceptual framework for the analysis of experimental results; even if it is not predictive in a quantitative manner, it can still suggest how to analyse

data. In the following, examples of the interplay between theory and experiment are provided, which we expect to be useful for gaining an improved understanding of co-operative intracellular transport.

Unidirectional transport

Even though a single motor molecule can power processive motion, transport in cells is often driven by more than one motor [1]. One advantage of cargo transport by several motors is a higher velocity if the cargo experiences a high viscosity [8,12]. Another advantage of co-operative transport is an increased run length compared with the run length of a single motor, which is typically $1\,\mu$m: if one motor unbinds, the cargo is still transported by the other motors and the unbound motor has a chance to rebind to the filament. In this way, cargos can be transported over typical cellular distances of tens of micrometres. Using a simple but rather general model that relates the parameters of cargo transport to the properties of the individual motors, we have derived a relationship between the run length and the number of motors pulling the cargo that indicates that the run length increases exponentially with the number of motors [8]. Qualitatively, an increase in the run length has been known for a long time [13,14], but quantitative experiments remain challenging, because it is difficult to determine the number of motors involved in the transport. Two previous studies of the run lengths of beads covered with different amounts of kinesin-1 have attempted to estimate the motor number on the basis of force measurements [15] or run length distributions [2]. While the observations from the latter study were consistent with the theoretical predictions, the former study found longer run lengths than expected.

However, in both studies only the average number of motors could be determined and the actual number of engaged motors varied from bead to bead. Furthermore, the precise geometric arrangement of the motors was not known. These difficulties have been overcome in a recent study that used synthetic complexes of two kinesin motors connected through a rigid DNA scaffold [3]. The dynamics

Figure 1 | Unidirectional transport by a team of identical motors

(**a**) Cargo transport by two identical motors. A mesoscopic description of cargo transport characterizes the state of the cargo by the number of active motors and specifies rates for the stochastic transitions between these states (binding/unbinding of a motor) [8]. (**b**) The state with two motors bound to the filament consists of different microstates with different relative positions of the motors and different strain forces between the motors. These microstates can be explicitly taken into account in more microscopic models (F. Berger, C. Keller, S. Klumpp and R. Lipowsky, unpublished work). (**c**) Magnified part of a simulated trajectory when the cargo is actively pulled by both motors. The continuous line is the trajectory of the cargo. The dotted lines are the effective distances of motors 1 and 2 from the cargo. The fluctuations of their distances arise from stochastic stepping of the individual motors.
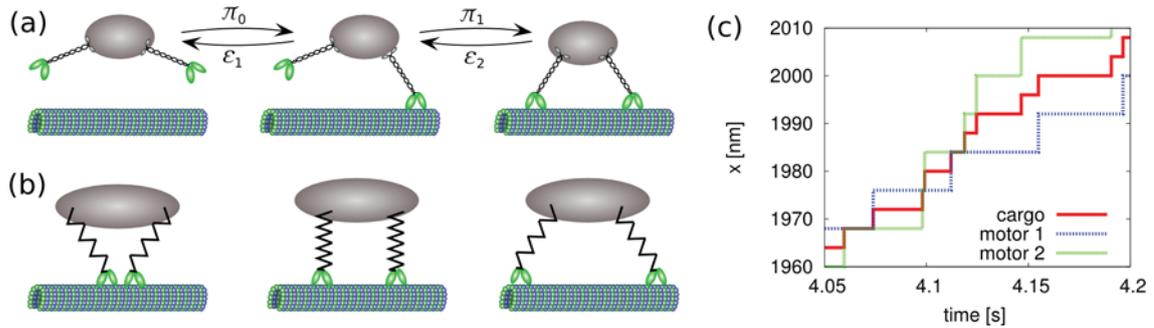
of this complex can be described by the cargo states shown in Figure 1(a) as introduced in [8]; each state of the cargo is characterized by the number of motors linking it to the filament. Transitions between these states correspond to binding and unbinding of a motor. The rates of unbinding transitions depend on the force experienced by the motors, since the unbinding rate, ε_1, of a single motor is exponentially force-dependent, $\varepsilon_1(F) = \varepsilon \exp(F/F_d)$ [16] with a force scale F_d, the detachment force, which is of the order of piconewtons. When a cargo is transported simultaneously by two motors, we can define an effective unbinding rate, ε_2, for the unbinding of one of the two motors as the inverse of the average time, during which both motors actively pull the cargo. If the motors unbind from the filament independently of each other, this effective unbinding rate would be the sum of the single unbinding rates $\varepsilon_2 = 2\varepsilon_1$ [8]. The experimental results, however, imply a higher unbinding rate, $\varepsilon_2 > 6\varepsilon_1$ [3], indicating that the motors interfere in such a way that they pull each other from the filament.

To address the origin of such interference and to study its influence on the collective transport by several molecular motors, we developed a discrete state model, which relates the single-motor properties to the effective unbinding rate (F. Berger, C. Keller, S. Klumpp and R. Lipowsky, unpublished work). This analysis indicates that the interference depends on the stochastic stepping of the motors and on the elasticity of the motor–motor coupling, see Figure 1(b). Because motor stepping is a stochastic process, the distance between the two motors fluctuates, see Figure 1(c). This change in distance induces a fluctuating strain force in the elastic linker between the motors. If this force exceeds the detachment force, F_d, the motor is likely to unbind. Thus the dynamics of the two-motor complex is governed by the competition between two main processes: spontaneous unbinding of the motors and the build-up of the strain force.

These two processes are governed by two different time scales: (i) the average binding time $t_2 = 1/\varepsilon_2 = 1/2\varepsilon_1$ during which two non-interfering motors remain simultaneously bound to the filament; and (ii) the time t_F it takes to build up a force between the motors that is comparable with the detachment force. Both quantities can be determined from microscopic models (F. Berger, C. Keller, S. Klumpp and R. Lipowsky, unpublished work). Now, we categorize the unbinding of the full system as spontaneous or induced, depending on which time scale is smaller. If the binding time t_2 is smaller than the time for producing a force comparable with the detachment force F_d, unbinding of a single motor is not predominantly driven by interference, but occurs spontaneously. Unbinding induced by motor interference occurs when $t_F < t_2$. In this case, large forces build up before any motor unbinds spontaneously.

The comparison of these time scales indicates that (i) the extent to which interference effects occur depends on the motor parameters and the details of the mechanical coupling between the motors, and (ii) elucidates why interference effects are seen in some models [17–19], but not in others [20,21]. For example, an important role is played by the elasticity of the linker [17], as indicated by a previous experiment [22]. With a stiffer linker, it takes less time to build up high strain forces between the motors and thus the time t_F is rather small. If the motor linkers are modelled as cables [20,21], no strain forces build up during compression of the linkers, resulting in a larger t_F. Here, theory provides a general framework to characterize different systems and can help one to infer individual motor properties from the collective behaviour. Our considerations emphasize that the interference depends on the details of the coupling of the motors, which may be different for different systems. Nevertheless, the observations of interference effects indicate that, while run lengths of cargo do increase with the number

Figure 2 | Bidirectional transport by two antagonist motor teams

(**a**) One dynein (left) pulls against two kinesins (right). (**b**) Typical trajectory of a cargo transported by a stochastic tug-of-war corresponding to bidirectional transport.

(a)

(b)
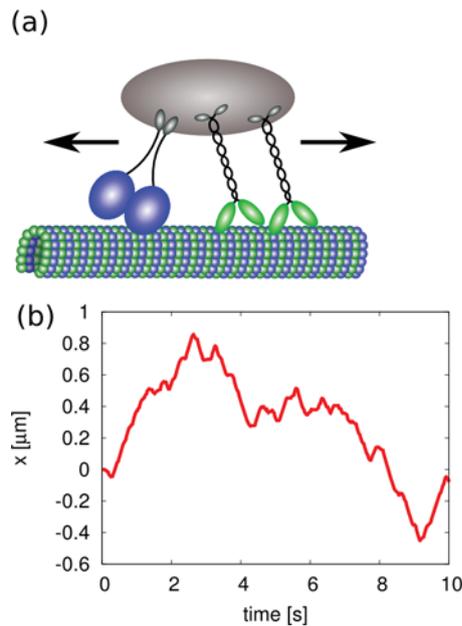
of pulling motors, the increase may be less pronounced than what is estimated based on non-interacting motors and that more motors than previously thought may be necessary for transport over typical cellular distances.

Bidirectional transport

Many intracellular cargos, e.g. mitochondria, pigment granules, endosomes, lipid droplets and viruses, move in a bidirectional manner, reversing direction every few seconds [23,24]. Bidirectional motion requires at least two types of motors that are attached to the cargo, e.g. kinesin-1 and cytoplasmic dynein, see Figure 2(a). Two mechanisms for bidirectional transport have been proposed [23,24]: (i) biochemical co-ordination by a hypothetical co-ordination complex which ensures that only one type of motor is active at any given time; and (ii) mechanical co-ordination through a tug-of-war between the motors, which pull on each other until one type of motor team wins and drags the cargo in its direction. Several recent experiments provide clear evidence for such mechanical interactions between the two motor teams [5,25,26]

We have developed a systematic theory for bidirectional transport based on a stochastic tug-of-war [9,27,28]. This theory depends on the known properties of individual motors and the assumption that the motor teams exert load forces on to each other, which are shared among the individual motors. One of the key features of this model is the occurrence of unbinding cascades: suppose that both motor teams, A and B, pull on the cargo with the same force. When one motor unbinds, say, from team A, the remaining A motors have to sustain all of the opposing force. Thus the force experienced by each individual A motor is increased, which increases the unbinding rate for the A motors. In this way, each motor team undergoes an unbinding cascade until all motors of that team are detached and the cargo is transported in the direction of the other team.

Our theoretical analysis could clarify some misconceptions associated with a tug-of-war. Previously, it was thought that the dominant state in a tug-of-war is a tie between the two teams, so that the cargo moves only very slowly, which would be inconsistent with the observed fast bidirectional transport. Our theory, however, shows that such blocked situations are resolved quickly through cascades of motor unbinding and that a stochastic tug-of-war leads to fast bidirectional transport for a large range of single-motor parameters [9], see Figure 2(b). Furthermore, cargo transport can easily be regulated by changing the single-motor parameters, which affects the competition between the two motor teams. Thus a tug-of-war can lead to net movement into one or the other direction, as found experimentally in bidirectional transport [29,30]. Our model for co-ordination through a stochastic tug-of-war has successfully been used to analyse and explain different patterns of transport in recent *in vitro* [5,6,26] and *in vivo* [5] studies.

Transport on different tracks

Long-range transport within a eukaryotic cell is typically microtubule-based, whereas short-range transport at the cell periphery is actin-based. To be able to switch from one transport system to the other without any interruption, actin motors as well as microtubule motors are attached simultaneously on the same cargo [31], see Figure 3(a). Switching between filament species depends on cellular regulation, on the cargo and on the number and type of motors [32–34]. It has recently been discovered that, when a cargo is transported on one kind of filament, say a microtubule, both microtubule and actin motors can interact with that filament. The microtubule motor is strongly bound and actively pulls the cargo, whereas the actin-based motor tethers the cargo to the filament, presumably via an unspecific electrostatic interaction. It has been found that myosin V can diffuse randomly on a microtubule [35] and that kinesin-1 has a weak affinity for actin [7]. A cargo transported by kinesin-1 and myosin V along a microtubule exhibits fast directed motion interrupted by diffusive events (see Figure 3b). During these diffusive events, kinesin is inactive but the cargo is still tethered to the filament by the myosin motor, which gives kinesin the chance to rebind. Overall, the run length of such a cargo particle is more than twice that of a cargo transported by a single kinesin alone [7]. Modelling this type of transport allowed us to deduce the single-motor binding and unbinding rates from the measured trajectories [10]. Because the affinity of myosin V for the microtubule is weak, no significant load forces on kinesin-1 are produced [10]. For this reason, this scenario is different from a tug-of-war. Using the deduced single-motor parameters, we can predict the transport of a

Figure 3 | Alternating transport on different tracks

(**a**) Cargo transported by the active microtubule-based motor kinesin and the actin-based motor myosin V, which diffuses on the microtubule. (**b**) The trajectory of such a cargo exhibits fast directed motion interrupted by diffusive events [10].
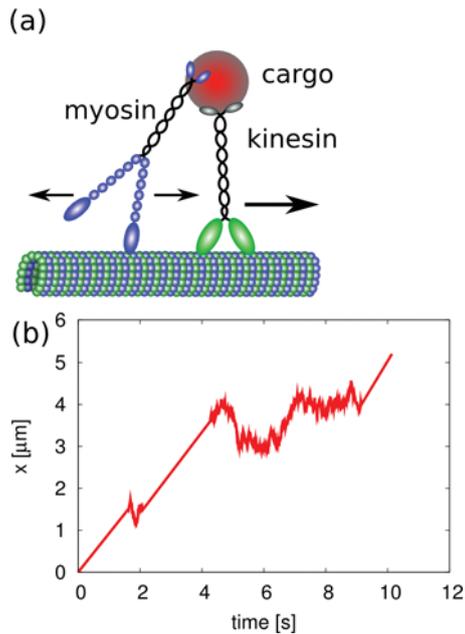
cargo with one active motor and several diffusive linkers. In this case, we find an exponential increase of the run length with the number of diffusive linkers [10].

Such an enhancement of motor processivity has also been observed in other systems. For example, the non-processive myosin motor Myo2p was shown to transport a cargo processively along an actin filament if the kinesin-related protein Smy1p was present on the same cargo [36]. Similarly, effects of diffusing linkers have also been discussed on the level of single-motor molecules for dynein and kinesin-3 [37].

The attachment of both kinesin and myosin to the same cargo should be advantageous, because the cargo is able to switch between actin and microtubule tracks. In addition, it has an increased run length not only on microtubule, as we discussed, but also on actin, as reported in [7]. However, it is still an open question as to whether this type of motor co-operation can be realized under physiological conditions [38].

Discussion and outlook

We have discussed different modes of co-operative transport: unidirectional transport, bidirectional transport and transport on different tracks. Bidirectional transport can be explained as a stochastic tug-of-war without a putative co-ordination complex. The enhanced run length of a cargo with passive and active motors can be understood from single-motor properties. Two unifying features of the different transport modes are (i) the stochastic binding and unbinding, which leads to fluctuating numbers of actively pulling motors, and (ii) a general increase in cargo processivity by motor

co-operativity. In all cases, little is known experimentally about the detailed behaviours of individual motors during collective transport, but our theory provides a general framework to address such problems and to deduce single-motor properties from experimental data. For example, in the case of unidirectional transport, different mechanisms for motor–motor interference can be studied and compared with new experimental data. In this way, our theory contributes to a quantitative understanding of cargo transport by several molecular motors.

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