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COOPERATIVE TRANSPORT BY SMALL TEAMS OF MOLECULAR MOTORS*

STEFAN KLUMPP

Center for Theoretical Biological Physics
University of California at San Diego
9500 Gilman Drive, La Jolla CA 92109-0374, USA
klumpp@ctbp.ucsd.edu

MELANIE J. I. MÜLLER and REINHARD LIPOWSKY

Max Planck Institute of Colloids and Interfaces
Science Park Golm, 14424 Potsdam, Germany

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Molecular motors power directed transport of cargoes within cells. Even if a single motor is sufficient to transport a cargo, motors often cooperate in small teams. We discuss the cooperative cargo transport by several motors theoretically and explore some of its properties. In particular we emphasize how motor teams can drag cargoes through a viscous environment.

Keywords: Molecular motors; diffusion; active transport; viscous load.

1. Introduction

Life is intimately related to movement on many different time and length scales, from molecular movements to the motility of cells and organisms. One type of movement which is ubiquitous on the molecular and cellular scale, although not specific to the organic world, is Brownian motion or passive diffusion: biomolecules, vesicles, organelles, and other subcellular particles constantly undergo random movements due to thermal fluctuations.[1] Within cells, these random movements depend strongly on the size of the diffusing particles, because the effective viscosity of the cytoplasm increases with increasing particle size.[2] While proteins typically diffuse through cytoplasm with diffusion coefficients in the range of $\mu m^2/s$ to tens of $\mu m^2/s$ and therefore explore the volume of a cell within a few minutes to several tens of minutes (for a typical cell size of a few tens of microns), a 100 nm sized organelle typically has a diffusion coefficient of $\sim 10^{-3}\mu m^2/s$ within the cell,[2] and would need ~ 10 days to diffuse over the length of the cell.

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For fast and efficient transport of large cargoes, cells therefore use active transport based on the movements of molecular motors along cytoskeletal filaments.[3–5] These molecular motors convert the chemical free energy released from the hydrolysis of ATP (adenosinetriphosphate) into directed motion and into mechanical work. They move in a directed stepwise fashion along the linear tracks provided by the cytoskeletal filaments. There are three large families of cytoskeletal motors, kinesins and dyneins which move along microtubules, and myosins which move along actin filaments. The filaments have polar structures and encode the direction of motion for the motors. A specific motor steps predominantly in one direction, the forward direction of that motor. Backward steps are usually rare as long as the motor movement is not opposed by a large force. Motor velocities are typically of the order of $1\,\mu$m/s, which allows a motor-driven cargo to move over typical intracellular distances in a few seconds to a few minutes. On the other hand, the force generated by a motor molecule is of the order of a few pN, which is comparable or larger than estimates for the viscous force experienced by typical (~ 100 nm sized) motor-driven cargoes in the cytoplasm.

A large part of our present knowledge about the functioning of molecular motors is based on *in vitro* experiments which have provided detailed information about the molecular mechanisms of the motors and which have allowed for systematic measurements of their transport properties.[3] In order to obtain such detailed information, the overwhelming majority of these experiments has addressed the behavior of single motor molecules. Within cells, however, transport is often accomplished by the cooperation of several motors rather than by a single motor as observed by electron microscopy[6,7] and by force measurements[8,9] and the analysis of cargo particle trajectories *in vivo*.[8–11] In order to understand the cargo transport in cells, it is therefore necessary to go beyond the single molecule level and to address how several motors act together in a team, in particular in cases where the cooperation of different types of motors is required such as bidirectional cargo transport. The latter situation, i.e. the presence of different types of motors bound to one cargo particle, is rather common and has been observed for kinesins and dyneins, kinesins and myosins as well as for different members of the kinesin family and even for members of all three motor families.[12,13]

In this paper, we review our recent theoretical analysis[14] of the cooperation of several motors pulling one cargo. We emphasize the ability of transport driven by several motors to deal with high viscosities and present an extended discussion of the case where a strong viscous force opposes the movement of the cargo particle. We also discuss how diffusion can be enhanced by motor-driven active transport and conclude with some remarks on the regulation of active transport.

2. Stochastic Modeling of Motor Cooperation

To study the cooperation of several molecular motors theoretically, we have recently introduced a model which describes the stochastic binding and unbinding of motors and filaments as well as the movements of the cargo particle to which these motors

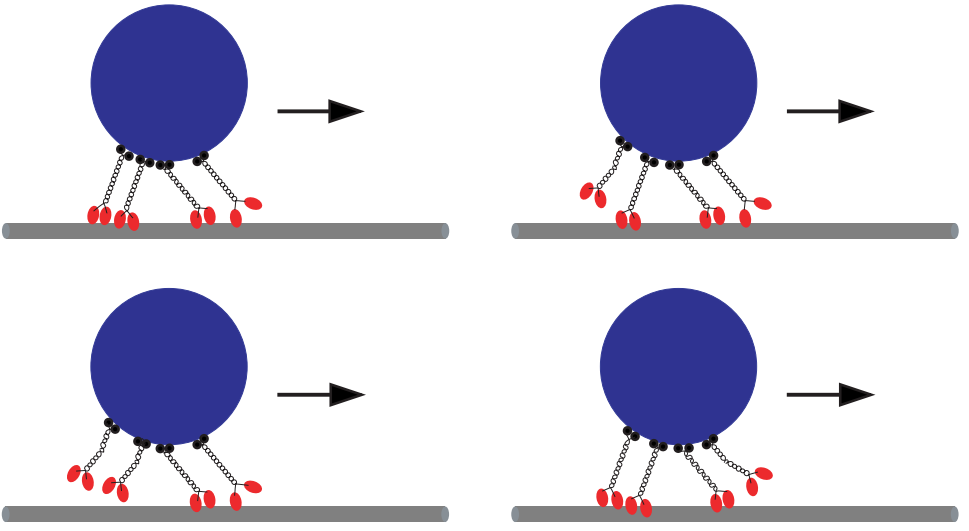

Fig. 1. A cargo particle is transported by $N = 4$ motors along a cytoskeletal filament. The number of motors which actually pull the cargo changes in a stochastic fashion due to the binding and unbinding of motors to and from the filament.

are attached.[14] The state of the cargo particle is described by the number n of motors bound to the filament. As shown in Fig. 1, this number changes stochastically between 0 and N, the total number of motors bound to the cargo, since motors bind to and unbind from the filament.[15] The model is therefore defined by a set of rates ϵ_n and π_n which describe the unbinding and binding of a motor, respectively, and which depend on the number n of bound motors, and by a set of velocities v_n with which the cargo particle moves when pulled by n motors.

In the simplest case, the motors bind to and unbind from the filament in a fashion independent of each other. In that case, the binding and unbinding rates are given by

$$\epsilon_n = n\epsilon \quad \text{and} \quad \pi_n = (N-n)\pi_{\text{ad}} \tag{1}$$

with the single motor unbinding and binding rates ϵ and π_{ad}, respectively. For non-interacting motors, the cargo velocity is independent of the number of pulling motors and given by the single motor velocity, $v_n = v$, as shown both by microtubule gliding assays and by bead assays for kinesin motors.[16–18] For this case we have obtained a number of analytical results.[14] In particular, the model indicates a strong increase of the average run length, i.e. the distance a cargo particle moves along a filament before it unbinds from it. For motors which bind strongly to the filament, so that $\pi_{\text{ad}}/\epsilon \gg 1$, the average run length is given by

$$\langle \Delta x_{\text{b}} \rangle \approx \frac{v}{\epsilon N} (\pi_{\text{ad}}/\epsilon)^{N-1} \tag{2}$$

and essentially increases exponentially with increasing number of motors. Using the single molecule parameters for conventional kinesin (kinesin 1), we have estimated

that run lengths in the centimeter range are obtained if cargoes are pulled by 7–8 motors.[14] As these long run lengths exceed the length of a microtubule (typically a few tens of microns), they can however only be realized if microtubules are aligned in a parallel and isopolar fashion and if cargoes can step from one microtubule to another as observed *in vitro* using aligned microtubules.[19] The increase of cargo run lengths with increasing number of motors has been observed in several *in vitro* experiments,[17,18,20] however it has been difficult to determine the number of motors pulling the cargo. One method to determine the motor number is to use a combination of dynamic light scattering measurements and comparison of measured run length distributions with theoretical predictions.[18]

If the cargo is pulled against an opposing force F, this force is shared among the bound motors, so that each bound motor experiences the force F/n. Under the influence of an external force, the single motor velocity decreases approximately linearly, $v(F) = v(1-F/F_s)$, and the unbinding rate increases exponentially, $\epsilon(F) = \epsilon \exp(F/F_d)$ as obtained from optical tweezers experiments.[21,22] The two force scales are the stall force F_s and the detachment force F_d. For a cargo pulled by several motors, the velocities and unbinding rates in the different binding states are then given by

$$v_n = v\left(1 - \frac{F}{nF_s}\right) \quad \text{and} \quad \epsilon_n = n\epsilon \exp\left(\frac{F}{nF_d}\right). \tag{3}$$

Since the velocity now depends on the number of bound motors, the velocity of the cargo changes every time a motor unbinds or an additional motor binds to the filament. The trajectory of the cargo therefore consists of linear segments with constant velocity, and the distribution of the instantaneous velocities has several peaks which become more and more distinct if the force F is increased. In addition, the sharing of the force induces a coupling between the motors which leads to cascades of unbinding events, since the unbinding of one motor increases the force and, thus, the unbinding rate for the remaining bound motors. Such unbinding cascades occur also in many other biophysical systems which have a similar unbinding dynamics, in particular they have been studied extensively for the forced unbinding of clusters of adhesion molecules.[23–25] For the motors, the most important consequence of this type of force-induced coupling of the motors is that an increase in force not only slows down the motors, but also decreases the number of bound motors. Therefore, the force-velocity relation given by the average velocity as a function of the load force is a nonlinear relation for cargoes pulled by several motors, although it is approximately linear for a single motor.[14]

Rather than being imposed by an optical laser trap or other force fields that can be directly controlled *in vitro*, an opposing force can also arise from other motors which pull the cargo into the opposite direction. The presence of two types of motors which move into opposite directions bound to the same cargo is commonly found in cells and is required for bidirectional transport in essentially unidirectional systems of filaments as they are typical for the microtubule cytoskeleton. In general, the two

types of motors interact both mechanically by pulling on each other *and* via biochemical signals or regulatory molecules. If there are only mechanical interactions our model predicts a tug-of-war-like instability: if the motors pull on each other sufficiently strongly, one species will win, and the cargo performs fast directed motion rather than being stalled by the pulling of motors in both directions. Since the number of motors pulling the cargo is typically small, the direction of motion will however be reversed from time to time with a reversal frequency which decreases as the motor numbers are increased.

3. Motor Cooperation in Viscous Environments

One universal force that is always experienced by molecular motors is the viscous drag caused by the medium through which the cargo is pulled. In water or aqueous solutions, however, the viscous drag of the cargo is usually negligible since it corresponds to a force of only a small fraction of the motor stall force. For example, a bead with diameter 1 μm which moves at 1 μm/s through water experiences a viscous force of 0.02 pN which is tiny compared to a motor stall force of a few pN. Therefore, *in vitro* experiments are hardly affected by the viscosity of the solution, and changes in motor number do not lead to a change of the cargo velocity unless the viscosity is increased to ~ 100 times that of water.[26]

In highly viscous environments, this is different: if the viscous drag force is of the same order of magnitude as the single motor stall force, the velocity can be increased if the number of motors which share this force is increased. The latter effect has been observed in microtubule gliding assays with high solution viscosity where for low motor density on the surface the velocity decreases as a function of the microtubule length, while for high motor density the velocity is independent of the microtubule length.[26]

For a cargo pulled by n motors, inserting the Stokes friction force $F = \gamma v$ (with the friction coefficient γ) into the linear force–velocity relation leads to[14]

$$v_n(\gamma) = \frac{v}{1 + \frac{\gamma v}{nF_s}} \approx \frac{nF_s}{\gamma}. \tag{4}$$

This equation shows that the velocity increases with increasing number of motors if $\gamma v/(nF_s)$ is not negligibly small compared to one. In particular, in the limit of high viscosity or large γv, for which the last approximation in Eq. (4) is valid, the velocity is proportional to the number n of pulling motors.[a] In a highly viscous environment, the cargo's velocity distribution therefore exhibits maxima at integer multiples of a minimal velocity. Similar velocity distributions have recently been

[a]Strictly speaking, this proportionality is only valid for small n. As a consequence of this, an increase of n to motor numbers large compared to $\gamma v/F_s$ will increase the consumption of ATP without substantially increasing the cargo velocity.

observed for vesicles and melanosomes in the cytoplasm, see Refs. 10 and 11.[b] To first order in γ^{-1} the motors experience the force $F_n \approx nF_s$ which implies that the force per bound motor is independent of the number of bound motors and that the motors behave as independent motors for large viscous force, however with an increased effective single motor unbinding rate $\epsilon \exp(F_s/F_d)$.

4. Active Diffusion: Motor-Driven Diffusive Movements

We have emphasized that large particles experience a strong viscous drag in the cytoplasm and that therefore Brownian motion is too slow to drive transport of large particles in the cell. While this observation suggests that active transport is necessary within cells, it does not imply that the active transport must necessarily be directed transport. Alternatively, active transport could also be used to generate effectively diffusive motion, which is faster than passive Brownian motion, e.g. if a cargo particle performs a sequence of active molecular motor-driven runs in random direction. We call this effectively diffusive motion, which depends on chemical energy, active diffusion.[27] In cells, active diffusion can be achieved either by (i) switching the direction of motion by switching between different types of motors which walk along a unipolar array of filaments or by (ii) a single type of motor and isotropic (e.g. bidirectional or random) arrangements of filaments. The first case is typical for microtubule-based transport: microtubules are often arranged in a directed fashion, either in radial systems emanating from a central microtubule organizing center with their plus ends pointing outwards or in unidirectional systems where microtubules are aligned in a parallel and isopolar fashion such as in axons.[c] Bidirectional movements along these unidirectional microtubule arrangements have been observed for a large variety of intracellular cargoes.[12,13] These movements are driven by a combination of plus end and minus end directed motors. On the other hand, actin-based movements are often of the second type, since the actin cytoskeleton usually forms an isotropic random mesh, on which, e.g. myosin V-driven cargoes perform random walks.[29,30] Active diffusion has also been observed for random arrays of microtubules in cell extracts.[31] Let us mention that these two types of active diffusion are highly simplified. More complex scenarios include bidirectional, but biased movements along microtubules and the switching of cargoes between microtubules and actin filaments.[12,13] *In vitro*, one can use various techniques such as chemically or topographically structured surfaces,[32] motor–filament self-organization,[33] and filament crosslinking on micropillars[34] to create well-defined patterns of filaments for active diffusion which may be useful to enhance diffusion in bio-nanotechnological transport systems.[27]

[b]The microtubule gliding assays of Ref. 26 with high viscosity and intermediate motor densities on the surface exhibit a large variability of the velocity, however discrete peaks have not been resolved in that experiment.

[c]For these two types of filament alignments, we have recently determined the stationary motor concentration profiles, see Ref. 28.

The maximal effective diffusion coefficient which can be achieved by molecular motor-driven active diffusion is given by

$$D_{\mathrm{act}} \approx vLP_{\mathrm{b}}. \tag{5}$$

where L is the length of essentially unidirectional runs, given by either the average run length before unbinding from filaments or the mesh size of the filament pattern, and P_{b} is the probability that the cargo is bound to a filament.[27] In order to obtain large effective diffusion one therefore has to make sure that the cargo particle is bound to filaments most of the time, and that it has an average run length which is comparable or larger than the pattern mesh size. One possibility to satisfy both conditions is to use a sufficiently large number of motors. A larger number of motors also decreases the probability of switching direction at an intersection,[29] so that unidirectional runs exceeding the mesh size can be achieved.

Since the motor velocity is rather insensitive to the viscosity for small viscosities, active diffusion is much less affected by the viscous drag of the solution than passive Brownian motion,[27] in particular if a cargo is pulled by several motors. For a cargo of size 100 nm, Brownian motion in water is characterized by a diffusion coefficient in the order of a few $\mu\mathrm{m}^2/\mathrm{s}$. An increase in viscosity by a factor of 10 leads to a decrease of the diffusion coefficient by that factor. The active diffusion coefficient, on the other hand can be estimated to be of similar size or slightly smaller (using $v \sim 1\mu\mathrm{m/s}$, $L \sim 1$–$10\ \mu\mathrm{m}$ and $P_{\mathrm{b}} \lesssim 1$), however the latter value is essentially unaffected by an increase of the viscosity by up to a factor 100 compared to the viscosity of water, since the viscous force which arises from the movement of such a bead is only a fraction of $\sim 10^{-3}$ of the motor stall force. The effect is increased if a cargo is pulled by several motors, since the viscous force γv starts to affect the motor movement only if it is of the order $\langle n \rangle F_s$. Therefore only viscosities which lead to viscous forces that exceed $\langle n \rangle F_s$ have a considerable effect on active diffusion.

5. Aspects of Control

In the preceding sections we have discussed how cells achieve cargo transport over large distances through a viscous environment by the cooperation of a small number of molecular motors. In principle, cells could also use a single motor which generates a larger force and binds more strongly to the corresponding filament rather than a team of motors, but motor cooperation appears to be preferred. The use of several motors has the advantage that the transport parameters can be easily controlled by controlling the number of pulling motors, e.g. by activating motors, by increasing their binding to filaments or by recruiting additional motors to the cargo.

The use of multiple weak bonds rather than a single strong bond in order to enable simple ways of control appears to be a general principle in cellular biology which applies to various cellular processes as diverse as the build-up of strong, but at the same time highly dynamic clusters of adhesion molecules[35] and the

binding of transcription factors to DNA where programmable specificity of binding is achieved by sequence-dependent binding of the transcription factor to a short stretch of DNA.[36]

If it is true that the main purpose of motor cooperation is the controllability of motor-driven movements, one may ask whether this function imposes constraints on the properties of the motors. For example, in order to both up- and down-regulate the cargo velocity or run length, it is clearly desirable to be able to both increase and decrease the number of pulling motors and, thus, to have an average number, $\langle n \rangle$, of pulling motors that is not too close to either 1 or N, the total number of motors, but rather is of the order of $N/2$.[d] The average number of pulling motors can be estimated[14] by $\langle n \rangle \sim N/[1 + \epsilon(F)/\pi_{\rm ad}]$, so that the requirement $\langle n \rangle \sim N/2$ implies $\epsilon(F)/\pi_{\rm ad} \sim 1$ or, for cargoes subject to a strong viscous force, $\pi_{\rm ad}/\epsilon \sim \exp(F_s/F_d)$. The latter condition represents a relation between the single motor parameters possibly imposed by a functional constraint related to motor cooperation. The parameters of conventional kinesin approximately satisfy this relation, which could however be coincidental. It would therefore be interesting to see whether this relation is also satisfied by the parameters of other motors. If this is not the case, these differences might provide hints towards functional differences between different types of motors.

6. Concluding Remarks

Cargo transport in cells is often carried out by small teams of molecular motors rather than by single motor molecules. We have described how one can analyze the transport by several motors in terms of a continuous-time Markov process which depends on single motor parameters as obtained from single molecule experiments during the last decade. Our theoretical approach is sufficiently versatile to be extended to more complex situations where additional molecular species are present such as the cooperation of two types of motors or the interaction of motors with regulatory proteins. In particular, it will be interesting to use this model to study control mechanisms of motor-driven transport.

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[d] As mentioned, in highly viscous environments the total motor number N itself may be fixed by the tradeoff between increased cargo velocity and efficient ATP usage, such that $\langle n \rangle \sim \gamma v/F_s$.

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