

Molecular motors as cargo transporters in the cell—The good, the bad and the ugly

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Available online 5 June 2006

Abstract

Single molecule properties of the cargo transporting processive molecular motors myosin-V, kinesin-1, and cytoplasmic dynein have been reported. These different classes of motors are known to cooperate during intracellular transport, and multiple motors (of same or different types) are simultaneously present on a given cellular cargo. However, differences in function are observed between these classes of motors—they have different force production ability, have a different average run length and step along their respective filaments using different size steps. Overall, the robustness of the motion they generate could be different. Is this apparent heterogeneity important for intracellular transport? Here we present a brief discussion of how the properties of these motors might be adapted to their coordinated function in vivo.

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1. Introduction

Molecular motors are a key to establishing the structure and organization of the cell, and are therefore important for many biological processes. Thus, motors with different architecture and function have evolved [1–4]. Here we focus on long-distance cellular cargo transport, i.e. transport that occurs due to the cargo's association with “processive” molecular motors that can take hundreds of steps along the respective filament (microtubule (MT) or actin) before detachment. Some cargos moved by such motors are vesicles, mRNA, mitochondria, endosomes, virus particles, etc. This transport is crucial for the organization of the cell because it localizes selected organelles at appropriate spatio-temporal coordinates. This is typically achieved through the activity of three different classes of motors—cytoplasmic dynein, kinesin and myosin—that may function separately, or be in a large protein complex along with other regulatory proteins [5,6]. A general scheme of such transport in an interphase cell is schematized in Fig. 1. As can be seen, motors are specific about the filament they attach to, and their direction of motion along the filament after attachment.

There is an increasing body of evidence now that in vivo, a single cargo very often has *multiple* motors (possibly of different classes) attached to it, and actively transporting the cargo. In addition, there are a large

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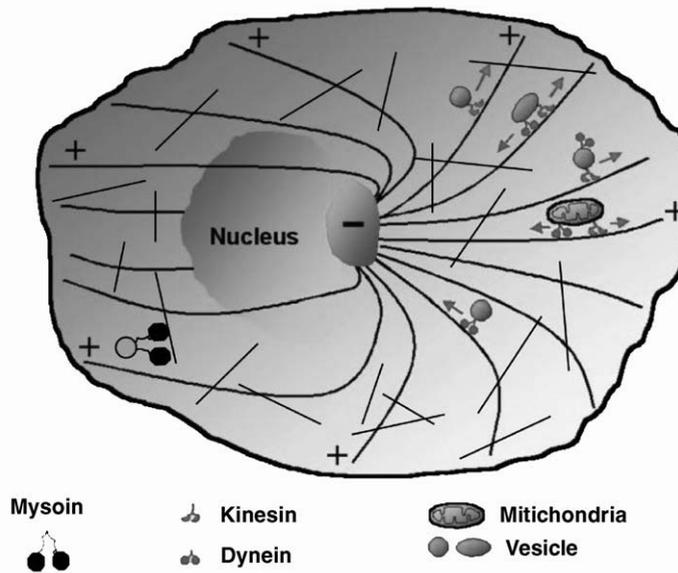


Fig. 1. Schematic of a typical interphase cell. The microtubules (thick lines) are shown in radial arrangement with the fast-growing plus ends at the cell periphery and minus end at the microtubule organizing center near the nucleus. Actin filaments (thin lines) are organized in a more random fashion. Various cargos are being transported by microtubule-based motor (kinesin and dynein) or an actin-based motor (myosin). Multiple motors, even of different kinds can attach to and transport a given cargo, usually in a bidirectional manner along the microtubule. Each cargo may have a complex of several motors and non-motor accessory proteins (details not shown) which could regulate the function of these motors.

number of non-motor accessory proteins (not all of which are known) present on the cargo. These proteins may be in the form of a complex [5,6], though the size and components of such a hypothesized complex are not yet known. The proposed role of this non-motor protein complex is to regulate the relative activity of different cargo-bound motors, and thereby determine net transport, or localization of that cargo. During transport of certain cargoes (e.g. pigment granules in melanophore [7]) a delicate coordination exists between transport on MTs versus actin. Pigment granules get “handed over” from MTs to actin during dispersion, and vice versa for aggregation [8,9]. Upon intake, phagosomes/endosomes have been shown to undergo a period of myosin-driven motion on actin near the periphery of the cell before getting on to MTs and subsequent inward transport via dynein/kinesin [10].

It is important to understand the properties of individual motors in the context of this interplay of classes of motors—is a class of motors designed to allow coordination with another class during different cellular processes? With single molecule data available on the three major cargo-transporting motors (kinesin-1, myosin-V and cytoplasmic dynein), we are now in a position to speculate on this issue. However, it should be clarified that because of their artificial environment and the use of purified motor proteins, motor protein function in *in vitro* single molecule measurements could be different from the “real world” scenario *in vivo*. The real world relevance of hypotheses based on *in vitro* function of motors can be verified only through subsequent measurements of cargo transport within a cell.

2. Molecular motor driven transport—the need for heterogeneity

Perhaps one of the important requirements of cellular transport is heterogeneity—many kinds of organelles of different shapes and sizes are transported in the cell as components of various cellular processes. The length and time scales over which such transport occurs could be quite different. Only a limited set of processive motors effects this transport, and it is known that motors of a particular kind can attach to many different

kinds of cargo. To generate this heterogeneity, it would then be desirable to “tune” the function of a motor to suit the process in question. Two factors could be important for such tuning:

- (1) Design and function of the motor at a single molecule level—how much variability in function does a single motor have? *In vitro* single molecule measurements are suited to answer this question because they reveal the function of a single motor working in isolation.
- (2) Association of the motor with other motor or non-motor proteins which regulate function—the larger this set of external regulators, more variability in function could be expected *in vivo*.

3. Kinesin and myosin

Kinesin-1 and myosin-V show similarity of the core motor domain, suggesting that they have some similarities in function [11]. Both these motors move in a hand-over-hand manner [12–14], with a fixed step size (8 nm for kinesin-1 and 36 nm for myosin-V). They exhibit robust unidirectional motion, with rare instances of pausing or backward motion [15–17]. They generate a relatively high force (~6 pN for kinesin-1 and 3 pN for myosin-V). Because of their simpler design, these motors could allow limited scope for regulation of function. Their function is modified to some extent through association with other proteins, or through self-inhibition in the absence of cargo in kinesin-1. The possibilities for regulation in these motors have been discussed in Ref. [4] and references therein.

4. Dynein—complexity and its implications

On the other hand, dynein appears to be a much more complex motor by construction [18,19]. The dynein head domain is much bigger (>500 kDa) than kinesin or myosin, and each head has a unique ring-like construction made up from multiple globular domains, where up to four ATP molecules can bind to a single head [20,21]. For yeast cytoplasmic dynein it has been shown that more than one of these ATP molecules could be hydrolyzed during the dynein cycle [22]. A possible biological importance of such complexity and multiple ATP binding sites is a proposed gear mechanism within the dynein head [23]. We have observed that in *in vitro* experiments using purified cytoplasmic dynein, application of a backward load using an optical trap induces a shortening of the step size of dynein, with consequent increase in force produced [23]. A recent report [24] of large *in vivo* step sizes in quantum-dot-labeled dynein-driven endosomes is interesting in this respect. While further research is required, it is possible that dynein gear changes do occur in the cell, depending on the local environment (viscosity, interaction with other classes of motors applying load, etc).

A theoretical model of dynein’s function based on Monte Carlo simulations has also been developed [25]. This allows prediction of the expected force–velocity curve for dynein, reflecting how both its step size and enzymatic cycle could depend on load. Comparing the force–velocity curve to that of kinesin-1, one concludes that dynein velocity is affected more strongly than kinesin on the application of load. Thus, if we assume that typical loads (e.g. due to viscous drag) that a cargo experiences *in vivo* are the same in different directions of travel, there should be a larger variation in velocities of minus-end-directed *in vivo* transport. While the extent to which this is actually observed *in vivo* remains to be determined, this is an interesting question as far as regulation of cellular traffic is concerned—are there any advantages in having increased variability in one direction of motion along MTs?

One implication of the complexity of dynein is poor function [4]—dynein is a weak motor, and might require additional factors such as dynactin to stay attached to the MT during motion and help dynein to move [26,27]. We have recently reported an analysis of the *in vitro* single molecule function of cytoplasmic dynein [28]. We find that dynein motors exhibit back and forth motion, but dynein was found to be able to exert force only when moving towards the MT minus-end. Thus, combined with an analysis of the properties of motion, in agreement with earlier studies [29–31] we suggest that the back and forth motion reflects diffusion on the MT [28]. An interesting observation is that a given motor often interconverts between a diffusive and a motile state. In the diffusive state, it can move backwards (towards

MT plus end), and this leads to a large variation in velocity of single dynein driven cargo. Approximately 19% of the net motion was found to be backward in this case. For kinesin-1 driven cargo, backward motion is extremely rare. The ability to switch into a diffusive state where the motor cannot generate force, but remains weakly bound to the MT could in principle help in avoiding traffic-jam situations where oppositely directed cargoes (driven by kinesin and dynein) get stuck on a MT because neither motor will yield to the other. To avoid this, a weaker dynein motor with the ability to enter into a weakly bound state is desirable because it could “step back” and let the kinesin driven cargo pass by.

5. Cooperative motion of multiple dynein motors—more the better

To better understand the significance of this mediocre function of dynein, and how dynein driven motion could be regulated in the cell, we investigated the *in vitro* motion of cargo driven by multiple dynein motors [28]. We performed experiments in which polystyrene beads were incubated in solution containing ATP with increasing dynein concentrations. The motion of such beads was then investigated using an optical trap and simultaneous video acquisition to determine the position of the bead–motor complex under conditions of load and also under no load (free motion). A bead would be captured in the trap and motors on the bead allowed to generate several stalls in the trap. After that the bead was released, and the subsequent free motion of motors was quantified by video tracking. From many such measurements of stall force, the constructed stall force histogram was found to have a periodicity of ~ 1 pN, which is the single dynein stall force. This multiplicity of the stall force histogram might indicate that multiple dyneins on the same cargo can cooperate during motion, since the forces of individual motors are additive (Fig. 2).

The additive nature of stall force also allowed us to use these experiments (where the stall force was measured just prior to free motion) to estimate the motion driven by a known number of active motors on the cargo. To quantify the free motion, as in the case of single dynein driven motion, a parsing algorithm was used. This program parsed the video track (position of bead along MT versus time) into segments of constant velocity with a certain tolerance for thermal fluctuations of the bead. We analyzed the histogram of velocities obtained for multiple video tracks of dynein driven motion, both in the single dynein and in the multiple dynein case. For multiple dynein driven motion, the properties of motion are significantly improved and the diffusive component of motion is reduced ($\sim 2\%$ net backward motion for multiple dynein motion). Since multiple dynein motors are known to drive *in vivo* cargoes, our results are directly relevant to an *in vivo* condition. It appears that the cell can regulate minus-end MT motion from the limit of single-dynein mediocre motion to robust multiple-dynein motion. If this is true, it would add to the variability of dynein function arising from the gear mechanism, as discussed earlier.

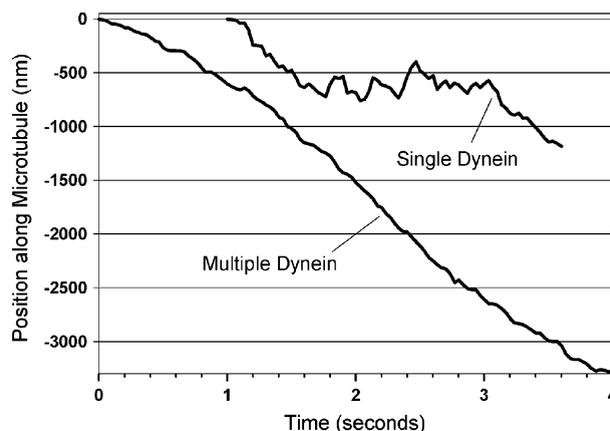


Fig. 2. Improvement in motion of polystyrene beads when driven by multiple dynein motors. Representative video tracks (30 frame/s) are shown. Single dynein runs are usually short, with pauses and segments of backward motion. A detailed analysis of such motion using many single dynein driven tracks indicates that the motor can enter a non-productive diffusive state. In contrast, the motion driven by multiple dyneins is usually long and robust.

6. Conclusion

Clearly, intracellular transport is a complex story with many players. How the function of this multitude of components is regulated in a precise manner by the cell will be an important direction of research for the future. Observations of motor function made under artificial *in vitro* conditions cannot obviously be directly used as conclusive evidence of *in vivo* function. However, we believe that based on the results of controlled *in vitro* experiments on complexes of motors, we can perhaps ask specific questions that motivate a next generation of *in vivo* measurements and ultimately lead to a better understanding of the complexity of cellular transport.

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