Review

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Teamwork in microtubule motors

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Diverse cellular processes are driven by the collective force from multiple motor proteins. Disease-causing mutations cause aberrant function of motors, but the impact is observed at a cellular level and beyond, therefore necessitating an understanding of cell mechanics at the level of motor molecules. One way to do this is by measuring the force generated by ensembles of motors *in vivo* at single-motor resolution. This has been possible for microtubule motor teams that transport intracellular organelles, revealing unexpected differences between collective and single-molecule function. Here we review how the biophysical properties of single motors, and differences therein, may translate into collective motor function during organelle transport and perhaps in other processes outside transport.

Understanding cell mechanics in terms of force-generating molecules

Cytoskeletal motor proteins are mechanochemical enzymes that generate force to drive organelle transport [1], mitosis [2,3], beating of cilia and flagella [4], nuclear migration [5], oscillation and rotation [6], cytoplasmic streaming [7,8], biogenesis and maintenance of the endoplasmic reticulum (ER) and Golgi [9], and many other cellular processes [10]. Force originates from an ATP-driven displacement of approximately 10 nm in the motor, but the work done in an enzymatic cycle (= force generated × displacement) cannot exceed the energy available from an ATP molecule (~100 pN-nm; we assume one ATP used/cycle). This restricts the force of motors to <10 pN [11]. However, most motor-driven processes require a much larger force [12,13] and must therefore be driven by collective force from teams of motors. As an example, consider the cytoskeletal machinery that segregates chromosomes [2,3,14]. A combination of force from cell cortex-associated motors pulling on astral microtubules (MTs), MT motors (see Glossary) driving vesicular transport against viscous drag, interpolar motors tugging at overlapping MTs, and forces from polymerizing/depolymerizing MTs drives this process. Force-generating molecules are therefore dispersed across a scaffold containing elements of heterogeneous mechanical properties [14,15]. Despite progress using *in silico* [16] and *in vitro* [17] approaches, how individual molecules function within such heterogeneous cellular structures is hard to measure and interpret. The big question remains: can we understand cellular mechanics in terms of the force-generating molecules?

The classical function of MT motors, namely organelle transport, presents a system where the aforesaid heterogeneity of mechanical elements is reduced. This has allowed force probing of motor-driven organelles inside cells [18–25]. With valuable input from *in vitro* studies [26–34] and theoretical work [35–40], a clearer picture of teamwork during organelle transport is emerging [1,41–43]. This review discusses some fascinating differences between the single-molecule architecture and function of different MT motors and how these differences may take center stage during their collective function. We first discuss the single-molecule functions of motors, then summarize how these may be important for multiple motor-driven transport of cargoes. We end with an outlook of the exciting possibilities that lie ahead and with the hope that important clues to motor-driven processes outside transport will emerge from these findings.

Microtubule motors: not all the same

Most long-distance transport in cells is driven by kinesin and dynein motors, which carry organelles as cargo to MT plus and minus ends, respectively. How this transport is regulated has been hotly debated [1,32,36,38,42–47], is possibly specific to the organelle in question, and involves many motor-associated regulatory proteins [48–50]. We do not focus on this here; rather, we concentrate on core single-motor architecture and function and its implications for the cellular function of the motors. Because the same motors do many things inside cells, such understanding may reveal rules generic to multiple processes over which context-specific regulation can be layered.

Glossary

Catch-bond behavior: strengthening of receptor–ligand bond against separating force, possibly through allosteric changes in the binding partners.
Force–velocity (*F–V*) curve: velocity of a processive motor plotted as a function of load force; can be obtained from optical trap measurements.
Hand-over-hand mechanism: mechanism of motility where the two heads of a motor alternately take the leading position.
Load: opposing force applied against the motion of a motor; for example, by an optical trap or another motor.
Microtubule motors: enzymes that couple their chemical cycle to a mechanical cycle, thus generating force against microtubule filaments by hydrolyzing ATP.
Powerstroke: movement of a structural element (e.g., lever arm) in a motor to generate force against a filament.
Processive motor: a motor that takes multiple steps before detaching from its filament; for example, kinesin takes hundreds of steps.
Stall force: opposing force required to completely stop the movement of a motor – also equal to the maximal force produced by the motor.
Step/step size: distance between consecutive binding sites of a motor on the filament (e.g., 8 nm for kinesin).

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Kinesin-1 and cytoplasmic dynein are the best studied MT motors, at both the single-molecule and collective level. Our discussion therefore centers on these motors and refers to them as 'kinesin' and 'dynein', respectively, unless otherwise indicated. Kinesin's motor domain may have evolved from a G-protein ancestor [51] and contains an ATP and a MT-binding site in close proximity to each other. Small nucleotide-dependent conformational changes in the motor domain are amplified into steps by using a lever-like strategy. Kinesin-1 uses a 'hand-over-hand' mechanism for motility [52] and takes 8-nm steps irrespective of the opposing load. It is a highly processive motor, taking hundreds of steps before detachment. This processivity persists against load from an optical trap, where kinesin rarely takes a back step and generates an approximately 6 pN force robustly, irrespective of ATP levels [53,54].

Dynein is a different beast altogether. It belongs to an ancient ATPase associated with diverse cellular activities (AAA) family of proteins [55], with six AAA domains arranged in a ring in each bulky head (Figure 1). Each head may bind up to four ATPs in AAA1–4, with AAA1 as the primary ATP hydrolysis site. Unlike kinesin's coordinated hand-over-hand stepping, dynein stepping is stochastic or coordinated depending on the tension and distance between the two heads [56,57]. Dynein's force-generating arm, consisting of its flexible MT-binding stalk and linker-stem domains, is approximately 25 nm (Figure 1), an order of magnitude longer than kinesin's lever arm [55], making it unlikely that kinesin's lever-like mechanism would work for dynein. This has prompted suggestions that dynein may use a winch-like strategy to generate force against the MT [58,59], with the head rotating in the direction shown in Figure 1 to pull on the MT, which in turn propels the cargo towards MT minus-end. Yeast dynein has been investigated extensively [56,57,60] but does not function in organelle transport and differs in structure, force generation, velocity, and processivity from dynein in higher eukaryotes [61], which is the focus of this review. There appears to be consensus [22–24,26,61–64] that mammalian dynein generates a much smaller force (1–1.5 pN) than kinesin, although higher forces were also reported [65,66]. Further, dynein frequently enters a diffusive state [26,67,68], detaches easily and back-steps against load [26,69], is poorly processive [26], perhaps by design [70], and sidesteps/back-steps while walking [26,56,57,67]. Thus, single-dynein function appears to be weak and erratic compared with kinesin [11].

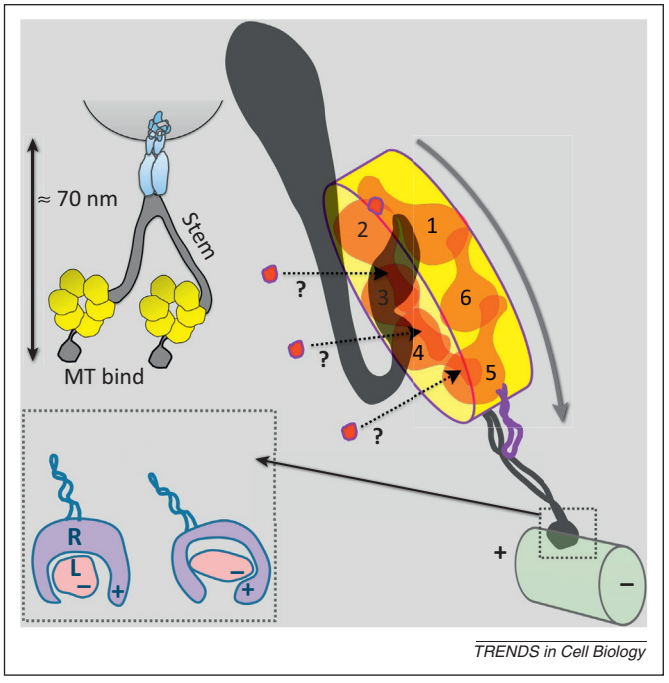

Figure 1. The dynein motor and possible adaptations for cooperative force generation. A dimeric dynein motor is shown attached to a cargo (gray half-sphere at top) through dynein intermediate and light chains (blue). The dynein ring in each head is shown as six ATPase associated with diverse cellular activities (AAA) domains (yellow). The stem and microtubule (MT)-binding domain are also shown. A magnified view of one dynein head is shown on the right. AAA domains (numbered 1–6) are arranged within the yellow outline of the ring. Each domain has a large and a small region, with close proximity between adjacent domains. The linker (dark gray) is a mechanically stiff element shown here in a strained state bent across the left face of the ring close to AAA2–4, curving to become the stem and then moving behind the ring to connect to the other stem (not shown). A coiled coil MT-binding stalk (gray) emerges between AAA4 and AAA5. The tip of this stalk contacts a MT (green tube) and is supported by a buttress (purple). ATP-binding sites are present in the interfacial regions between AAA1/2, 2/3, 3/4, and 4/5. The AAA1/2 interface is the primary ATP hydrolysis site and is shown with a bound ATP (red sphere). ATP could bind AAA2, 3, and 4 as dynein experiences load (possibility of binding denoted by a ?; the exact sequence of ATP binding and occupancy at different sites is unknown). The affinity of the linker to each module of AAA2, 3, and 4, and therefore its bending, could be dictated by the presence/absence of ATP at these sites. We speculate that load-induced ATP binding (broken arrows) at AAA2, 3, and 4 may enhance individual linker–AAA affinities, increasingly curving the linker to shorten dynein's step size under load and increasing its force in an ATP-dependent manner. Lower inset: A rough schematic of catch-bond adhesion to explain the tenacity of dynein under high load. A receptor (R) and ligand (L) are shown under zero force. Charged residues can exist on R and L (+ and −). R can represent the globular MT-binding domain of dynein and L can represent domains on the MT surface. R and L are weakly bound in this depiction. When tension develops along the MT binding stalk because dynein is straining against high load (force in direction of blue arrow), allosteric deformation in R and L could 'lock' them together, resulting in a catch-bond effect. This effect could be enhanced by deformation-induced proximity of opposing charged residues in R and L.

Single-molecule properties important for collective function

How do single-molecule properties manifest during collective force generation by motors? Because it is not yet possible to experimentally observe the dynamics of individual motors within a functioning team, our understanding relies on computer simulation of collective behavior using single-molecule properties. Because a motor's function is load dependent [22,54], the load shared by any motor in a team dictates how the motor (and therefore the team) will work [22,31,37]. Of particular value in simulations is the single-motor force–velocity (F–V) curve, which can be measured in an optical trap [54]. This quantifies how fast a motor moves against a load and could be a signature of how the motor will work in a team. Kinesin-1 is relatively insensitive to low/intermediate opposing loads, because of which its F–V curve is convex-up [54]. By contrast, dynein's velocity drops rapidly at low loads, resulting in a concave-up F–V curve – this behavior was attributed to dynein's gear-like response of reducing step size (and therefore velocity) against load [64,71]. This difference in F–V response appears important for collective function (see later).

To model the dynamics of collective motor function using single-molecule information, an initial geometric arrangement of motors on the cargo and a criterion for