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Coordination of Molecular Motors: From *in vitro* Assays to Intracellular Dynamics

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Summary

New technologies have emerged that enable the tracking of molecular motors and their cargos with very high resolution both *in vitro* and in live cells. Classic *in vitro* motility assays are being supplemented with assays of increasing complexity that more closely model the cellular environment. In cells, the introduction of probes such as quantum dots allows the high resolution tracking of both motors and vesicular cargos. The “bottom up” enhancement of *in vitro* assays and the “top down” analysis of motility inside cells are likely to converge over the next few years. Together, these studies are providing new insights into the coordination of motors during intracellular transport.

Introduction

Molecular motors drive a myriad of essential processes in the cell, including targeted delivery of vesicular cargos, localization of organelles and mRNAs, and chromosome and spindle dynamics during mitosis. The three classically described linear molecular motors, myosin, kinesin and dynein, carry their cargos along actin filaments (AFs) and microtubules (MTs) in these transport roles, in addition to their well-characterized functions in muscle contraction and flagellar beating. New single molecule fluorescence microscopy techniques and infrared optical traps have led to remarkable progress in understanding the transduction of metabolic energy into mechanical force and motion, using *in vitro* systems combining purified motors with their tracks on glass microscope slides (Box 1). Of course, transport in the cell occurs in an environment that is considerably more complex than is reflected in these simplified assays of motor function. Intersecting and bundled networks of cytoskeletal filaments, obstacles such as actin-binding or microtubule-associated proteins bound along motor tracks, and the coordination of multiple motor types bound to the same cargos are all likely to affect motility within the cell (Fig. 1). The gap between simplified *in vitro* assays of motor activity and the biology of intracellular transport *in vivo* is being bridged from both sides. Here we consider “bottom up” experimental approaches using purified components in motility assays modified to reflect elements of complexity present within cells, as well as “top down” studies of motors tagged with fluorescent reporters such as quantum dots (QDs) and introduced into cells. The results of these experimental studies on the coordination and collective properties of molecular motors can be compared to theoretical predictions, reviewed in this volume by Guérin et al. [1].

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Box 1

Modeling Intracellular Motility With *In vitro* Assays

A.*Gliding assays* for all three types of molecular motors (myosins, kinesins, and dynein) involve attaching the motor to a glass microscope slide and monitoring the translocation of either actin filaments or microtubules across the surface upon addition of ATP (Box Figure 1). Sliding velocity, ATP dependence and some indications of population dynamics can be obtained. **S*ingle motor*** assays involve an inverse configuration: the actin filament or microtubule is attached to the glass surface and the movement of the motor is monitored, either directly via a fused fluorescent tag such as GFP, organic fluorophore or a quantum dot (QD). This approach yields nanometer resolution, allowing the measurement of step size and angular changes during translocation. In ***single motor assays with beads***, motors are attached to small polymer spheres that are easily observed by differential interference constrast or phase microscopy. This configuration can be used in an optical trap to measure step size, processivity and stall force. Additionally, multiple motors of the same or different types can be bound to the same bead to study collective motor activity. **B.** Closer approximations of the cellular environment can be developed in ***crossed filament assays*** (Box Figure 1). In these assays, the translocation and/or switching of motors can be monitored through actin-actin (AF-AF), microtubule-microtubule (MT-MT), and ***microtubule-actin filament (MT-AF) intersections***. Other **obstacles** to motility can be bound to cytoskeletal filaments, such as the microtubule-associated protein (MAP), tau. While bead assays provide information on motor dynamics, monitoring ***vesicle motility*** *in vitro* provides insight into the coordinate regulation of motors bound to their natural cargos. **C.** Finally, direct measurements of motility in a cell, such as the neuron shown here, can be made by expressing GFP-labeled motors, or by introducing motors or probes into the cell through pinocytosis or endocytosis.

Processivity and Gating

The three major motor families, kinesins, dyneins, and myosins, show considerable variation in structure, speed, and number of steps taken per diffusional encounter with their cytoskeletal track (mechanical processivity). The paradigm is a highly processive, two-headed motor, such as kinesin-1, which drives cargos toward the plus end of microtubules. Cytoplasmic dynein is also a highly processive two-headed motor, but moves toward MT minus ends. Similarly, myosin-V and myosin VI move in opposite directions along actin filaments. Kinesin and dynein produce repeated steps along microtubules at the 8 nm periodicity of the tubulin dimers, although dynein can exhibit larger steps as well as reversals [2,3]. Myosin V takes 36 nm steps toward the barbed end of actin, corresponding approximately to the half-pitch of actin's double helix; whereas myosin VI exhibits pointed end directed steps of 20 – 40 nm [4]. Each of these examples has been shown to move with a "hand-over-hand" gait [5–8] in which both heads can bind simultaneously to their track. Their stride involves detachment and forward motion of the trailing head to become the leader (Fig. 2A→ B).

High processivity implies a mechanism to prevent both heads from dissociating from the track at the same time. For kinesin and myosin V, thermal fluctuations stretch the two heads apart to reach the next filament binding site at the completion of the step, leading to an intramolecular force that pulls the leading head backward and the trailing head forward (colored arrows in Fig. 2A and B). The intramolecular forces on the two heads are the most likely signals that cause the trailing head to detach while the leading head remains bound [9,10]. Steps in the biochemical or mechanical pathways are said to be gated by internal strain in the molecule. Besides the intramolecular force, physical interactions between the two heads (for instance

between the two ring-shaped heads of dynein if they are adjacent on the microtubule [11]) or structural changes in the filament could also mediate head-head communication.

How Do Multiple Motors Interact on a Cargo?

The same concepts potentially influence motions of a vesicle or filament transported by several motors mechanically coupled to each other through the cargo. The restraining force that stops motility, the stall force, is expected to increase with the number of motors, although not necessarily in proportion [12,13]. The distance a cargo is transported before it dissociates from its track is markedly lengthened by increased numbers of motors [14,15]. At typical transport velocities of artificial cargos *in vitro* and probably also in cells, the viscous drag of the environment is relatively small and the transport speed is determined mainly by the motor dynamics.

Coupling between several identical motors

By imaging GFP-labeled kinesins in a gliding filament assay at low surface density, and by observing swiveling of the MT when only one kinesin was bound, Leduc et al. [16] could identify intervals corresponding to one, exactly two, or higher numbers of motors. MTs bound to single kinesins exhibited 8.1 nm steps in this assay, in agreement with earlier studies of kinesin motility [17]. MTs driven by two kinesins, however, took twice as many steps per second, averaging 4.2 nm displacements per step, leading to the same velocity. The implication is that intermolecular forces transmitted through the microtubule did not greatly modulate the stepping dynamics, but reduced the step size for the ensemble as expected from the cargo-mediated strain in Fig. 1C→ D . Double-headed kinesin molecules assembled into pairs on an artificial scaffold did not move faster than single ones, also indicating low kinetic interaction through the cargo [18]. On the other hand, several single-headed kinesin molecules assembled onto a scaffold enhanced motility speed, indicating cooperation among single heads via the mechanical coupling [19].

Interaction between fast and slow motors

In many types of intracellular transport, motors with differing dynamic properties cooperate to move the same cargo. When several motors with different speeds are present, the aggregate velocity is expected to reach an intermediate value determined by a balance of forces between their individual force-velocity curves. Examples are the faster and slower isoforms of myosin II that are intermixed in individual muscle fibers [20], and the heterotrimeric kinesin-II and homodimeric OSM-3 (both members of the kinesin-2 family) that carry intraflagellar transport particles (IFTs) toward the tips of cilia [21]. In gliding filament assays using pairwise mixtures of myosin II isoforms differing over a 50-fold range of maximal velocities [22], or kinesin-related motors, kinesin II and OSM-3, differing 3-fold in velocity [23], intermediate velocities were observed. In both of these cases, a half-and-half mixture of the two isoforms translocated the filaments at speeds less than half-way between the individual velocities. This dominance of the slower isoform can be explained by changes in the detachment rate from the actin or MT at the end of the working stroke, so that a relatively small number of the slower motor produces a strong component of drag.

When the number of motors of each type is small enough that stochastic probabilities allow periods of complete detachment, the net velocity can be envisioned as an “alternating action” mechanism of one motor type and then the other. Or, the motors might be nearly continuously engaged and interact via forces transmitted between them through the cargo. In this situation, when one molecule steps forward on its track, the other bound motors not stepping at that moment are pulled forward by the cargo (Fig. 2C→ D). Their elasticity resists this motion thereby producing a differential, oppositely directed tensile component between the stepping

and non-stepping molecules (a “mechanical competition” model, Fig. 1D). The transition rates governing attachment and detachment may depend on force applied to the motor, similar to the gating described above for the individual heads [24]. For the two IFT kinesins, either mechanical competition in which the slower kinesin-II generated a drag force or else alternating periods of fast and slow velocity could explain the *in vitro* motility results. But evidence from *in vivo* studies of IFT transport with knockout constructs suggests that the two motors exert forces on each other in a mechanical competition [23].

In contrast, with mixtures of kinesin-1 and a mutant purposefully slowed 15-fold [25], the transport velocity was dominated by the faster wild-type motor. The active motor is able to dissociate the weaker mutant, possibly due to the known asymmetry of kinesin-MT dissociation rates under forward (plus-end directed) and backward (minus-end directed) forces [26]. Similarly, when a weak binding myosin, such as unphosphorylated smooth muscle myosin, was mixed with an active myosin isoform, the more strongly bound motor could dissociate the weaker one to maintain a high velocity ([22] and other papers cited therein).

Besides interactions mediated via forces through their load, motors associated with a cargo could potentially interact directly or share structural or modulatory components (Fig. 1E). Features of the cytoskeletal tracks could also affect the operation of nearby motors. For instance, muscle myosin shows cooperative attachment caused by the regulatory component, tropomyosin, that winds along the actin helix (Fig. 1E) [27]. The various mechanisms for interactions between multiple motors through shared cargos and substrates are presumably applicable to the regulation of directionality, velocity and processivity of transport in cells.

Obstacles and Crossovers

To further study motor interactions, *in vitro* assays have been developed with added complexity, such as filament intersections or filament-binding proteins. For example, ***kinesin-1*** motors were imaged by fluorescence as single molecules or bound in ensembles to polymer beads as they encountered MT-MT intersections. If the approach was along the overpass microtubule, motility was observed to continue through the microtubule intersection, without a switch or a dissociation [28], consistent with their highly directed motility parallel to the protofilament axis [29,30]. However, to a bead arriving at an intersection from an underpass, an overlying MT is an obstacle. It only takes one or a few kinesins at the front of the bead to switch the cargo onto the crossing microtubule, presumably due to kinesin’s high processivity. The methodical nature of kinesin motility helps to explain its interactions with the neuronal microtubule binding protein, tau. Encounters with patches of tau on microtubules caused individual kinesins to dissociate as if they were pried off of the MT, unable to walk around [13,31].

Dynein, consistent with its much more varied stepping dynamics [2,32], exhibits a less uniform response than kinesin at MT-MT intersections: passing, pausing, switching, dissociating and even reversing its direction [28]. The stepping and directional variability of dynein on individual bare MTs and at obstacles presumably also explains how dynein navigates around or through patches of tau better than kinesin [31]. However, lack of cooperation of the dynein motors becomes more apparent at higher motor densities, as the dynein-bound beads become tethered at MT-MT intersections under these conditions [28].

Myosin V passes and switches at branches and intersections of actin filaments [33]. During motility, a myosin V head stepping from the trailing to the leading position averages 72 nm, twice the distance of the molecule’s center (Fig. 1A → B, [5,33]). But this distance varies over a broad range, 50 – 95 nm, for individual steps, indicating flexibility, and the molecule sometimes tilts sideways around the filament and continues at a new azimuth [34]. The high

likelihood for switching at AF-AF intersections [35], suggests a random search for the target monomer unbiased by stiffness at the head-tail junction.

A novel finding made by Ali et al. [35] was that at a MT overpass, myosin V often switched tracks onto the microtubule and exhibited passive one-dimensional diffusive sliding along the MT. Myosin V is known to interact with microtubules in various ways [36], but the diffusive motion was unexpected. Myosin V and kinesin increase each other's processive run lengths, a kind of intermolecular cooperation that may enhance transport of cargos containing both motors [37]. Although these non-cognate filament interactions *in vitro* are greatly diminished at physiological salt concentrations, they may nevertheless be relevant in cells because several motors on a cargo could compensate for the weak individual affinity. These findings raise the interesting possibility that myosin searches along MTs for cargos, preparing to switch them to the actin filament network.

Mixtures of motor classes

Several different types of motors can be added to a quantum dot or polymer cargo enabling direct testing of the relative efficacy of the motors under competing loads or to facilitate switching onto another filament type. With mixtures of dynein/dynactin and myosin V on polymer beads *in vitro*, an apparent tug of war takes place at AF-MT intersections. The likelihood of switching onto an actin filament or MT is closely related to the maximum force and the density of each type of motor on the bead, the outcome being determined most noticeably by the relative force between the two motor types [38]. Surprisingly, whether the starting filament is the MT or actin and whether it's an underpass or overpass hardly affects the outcome, suggesting that the motors commonly interact with both filaments at the intersection. The tug of war model for interactions between motors at a filament intersection, or between two oppositely directed motors on a single filament, is similar to the mechanical competition model discussed above for two motor types with the same directionality that share a cargo. In these mechanisms, both types of motor act simultaneously, rather than alternating, one engaged, then the other.

These *in vitro* assays show that motors and cargos interact in a number of ways. Their mechanics, force, directionality and velocity, are coupled to each other via elastic connections with the cargo, leading to cooperative modulation of their stepping dynamics and directionality. Measurements of corresponding parameters in the cellular environment may indicate if the biophysical properties of the motors are similar and whether the same phenomena take place in the cell.

Motors Moving in Cells

The question of how *in vitro* measures of motor function compare to motility observed in the complex cellular environment may be addressed in two ways. First, the behavior of exogenous, labeled motors introduced into cells can be studied. In this experimental approach, motors move along the complex cellular cytoskeleton, operating independently of any association with intracellular cargos. Alternatively, the movement of native organelles and vesicles can be analyzed, usually by the introduction of a fluorescent probe such as a quantum dot taken up by endocytosis. In this experimental design, motors work collectively as they associate with endogenous cargo. As multiple motors of the same type or different types may be associated with the same cellular cargo, motility may be more complex. Motility of endogenous cargos is also more likely to be affected by the regulatory environment of the cell. Both approaches have been employed, and the results from these studies have many striking similarities to data obtained from *in vitro* studies.

Microtubule-based motility

The introduction of fluorescently labeled motors into cells has allowed the high resolution tracking of motors moving along the endogenous cytoskeleton under physiologically relevant conditions of ionic strength and ATP concentration. The velocities observed in these assays are remarkably similar to those observed *in vitro*. In a direct comparison, a truncated kinesin-1 construct labeled by the tandem fusion of three monomeric citrine tags was expressed in transfected COS cells. The cells were then either lysed to generate cytosolic extracts for *in vitro* experiments or imaged using total internal reflection fluorescence (TIRF) microscopy in live cell experiments [39]. Kinesin-1 moved processively in both assays, with similar velocities (0.8 μm/sec) and run lengths (0.8 to 1.2 μm/run). Kinesin motors bound to QDs and internalized into HeLa cells by osmotic lysis of pinocytic vesicles also exhibited velocities and run lengths similar to those observed for the same kinesin construct *in vitro* [40]. In contrast to these observations, in a study of kinesin-bound QDs internalized via lipid transfection [41], directed motility in the cell was frequently interrupted by pausing. This “stop-and-go” behavior differed significantly from observations with the same kinesin construct *in vitro*. Maximum velocities also differed, with cellular velocities up to twice that observed *in vitro* [41]. The differences observed among these studies merit further analysis, but may be due either to distinct properties of the specific kinesin constructs tested and their relative susceptibility to regulatory control within the cell, or to the various cell types used in these assays, which may contain cytoskeletal tracks with differing properties.

The overall effect of the complex organization of the cellular cytoskeleton on motility *in vivo* is still not clear. Motors may select particular tracks in the cell; for example kinesin-1 but not kinesin-2 or kinesin-3 preferentially moves along a subset of microtubules marked by post-translational acetylation [42,43]. Track-associated proteins such as microtubule-bound MAPs did not appear to affect kinesin in some studies [39] but may have affected kinesin in other work [41]. While these observations are in apparent contrast to the strong inhibition of kinesin by MAPs seen *in vitro* [13,31], there are a few caveats. First, following preferential tracks in the cell may allow motors to avoid filaments with associated roadblocks, such as microtubules heavily decorated with MAPs. Second, variability in the cellular complements of MAPs may explain some of the observed differences in motility; both the level of expression and the types of MAPs expressed vary from one cell type to another. For example, in neurons, increasing levels of tau expression led to a significant inhibition of kinesin-mediated organelle transport [44], in contrast to the apparent lack of inhibition of kinesin by MAPs seen in COS and HeLa cells, which do not normally express tau.

Actin-based motility

Myosin-V motors have also been introduced into cells and monitored with single molecule resolution. Both Nelson et al. [45] and Pierobon et al. [46] introduced single myosin-V motors into cultured cells (COS-7 and HeLa, respectively). Both groups found that single myosin molecules moved with velocities and step sizes similar to those measured *in vitro*. At short time scales (several steps) the introduced myosin-V motors moved processively. However, movement over longer time scales or distances was more random than directed. Monte Carlo simulations suggest that the apparent random walk is due to complexity of the actin network rather than reflecting a lack of processivity of the motor [45]. The random walk pattern of myosin-V-bound QDs moving near the cortex is best understood as a processive motor moving along a dense, intersecting, and randomly oriented cortical actin network.

Other studies have similarly suggested that myosin motor activity in the cell is influenced by the geometry of cytoskeletal tracks [47,48]. This has been shown most dramatically for myosins in a study by Brawley and Rock [49] in which fluorescently labeled myosins V, VI, and X were introduced into extracted COS, S2 and U2OS cells, which retained the overall organization of

the actin cytoskeleton seen in intact cells. The introduced myosins moved preferentially along some actin structures but not others. For example, myosin VI but not myosins V and X moved along stress fiber bundles, while myosin X was recruited to filopodia. Track switching was seen at actin filament intersections in regions with high filament density, similar to the results of Nelson et al. [45] in intact cells, as well as to the *in vitro* filament switching assays described above.

Motors on Cargos

Perhaps the major difference between purified motors studied *in vitro* and organelle movement within the cell is the association of motors with native cargos, which has the potential to affect motor function in several ways. For some motors, binding to cargo may drive dimerization, changing a relatively nonprocessive motor to a processive form that can drive transport effectively over longer distances. Cargo-induced dimerization was initially proposed for the kinesin motor KIF1A/Unc104 [50,51], although this model is not supported by more recent work [52]. Cargo-induced dimerization of myosin-VI was similarly controversial, but recent developments now favor this model [53]. Even for motors known to form stable dimers, cargo binding is likely to increase motor density, so that motors are functioning in arrays rather than individually. *In vitro* studies have shown that coupling multiple motors to a single artificial bead cargo can significantly increase stall force, directionality, and/or processivity [12,13, 54].

Further, intracellular cargos may recruit multiple types of motors, such as the oppositely oriented motors kinesin and dynein, or motors that move along distinct cytoskeletal filaments such as myosin and dynein. For at least some cargos, multiple types of motors remain stably bound to vesicles undergoing bidirectional motility [55–57]. Cargo binding may also affect motor coordination and/or regulation, for example through the activities of cargo-bound scaffolding proteins (reviewed in [58]).

Recent work suggests that motors of opposite polarity bound to the same cargo are required to activate bidirectional cargo transport. Ally et al. [59] found that the mislocalization of peroxisomes that occurs in S2 cells upon depletion of either kinesin-1 or cytoplasmic dynein was rescued by the expression of a peroxisome-targeted construct encoding an unrelated motor with the same polarity (Unc104 or Ncd, respectively). While the exogenous constructs restored organelle distribution and some features of bidirectional movement, it is unclear if a full restoration of wild type motility was achieved. However, this work does suggest a novel concept – that there is a mechanical coupling of oppositely-oriented microtubule-based motors, and that this coupling is required for activation of motility in either direction along the microtubule. This observation differs significantly from results of *in vitro* studies on purified motors bound to artificial bead cargos, so it is clear that more work is required to explore the mechanisms that lead to mechanical, biochemical, or regulatory coupling of motors in the cell.

How Many Motors?

Since *in vitro* studies have demonstrated that motility characteristics are strongly dependent on the number of motors simultaneously bound to a cargo, it is important to know how many motors are associated with endogenous vesicles and organelles moving in cells. A range of experimental approaches suggests that very few motors are actively engaged during transport along the microtubule. Both classic EM studies (reviewed in [14]) and force measurements on lipid droplets either in Drosophila embryos [60] or mammalian cells [61] have led to estimates that range from 1 to 7 actively engaged motors. Studies on purified axonal transport vesicles indicate that the *total* number of tightly associated motors is also low: ~1 kinesin-1 and 6 dyneins per vesicle [62]. Interestingly, if we consider the stall force measurements of ~6 pN for kinesin-1 [63] and ~1 pN for dynein [2], then the number of motors stably associated with

motile cargos approaches a balance of forces, as recently shown for endosomal motility in cell extracts from *Dictyostelium* [64]. This situation can be modeled as a tug-of-war [24]. If these motors are simultaneously engaged, then unproductive back-and-forth motility might result, such as that seen in many cellular experiments. However, either a temporary dominance of one motor, due to force-dependent dissociation [24], or a regulatory switch that preferentially activates or inhibits one motor population would result in directed movement. Identification of a small complement of microtubule motors tightly bound to a vesicle suggests an efficient regulatory scheme where small changes in the number of engaged motors can manifest in large changes in the directed motility of the cargo along the cellular cytoskeleton [62].

The number of myosin motors associated with intracellular cargo, or engaged with the actin filament track during active transport, is still unclear. For melanosomes, which move along both the microtubule and actin cytoskeletons in a highly regulated process [65], the number of cargo-associated myosin-V motors ranges from ~65 during aggregation to ~88 motors during dispersal, when myosin-V activity is key [66]. Estimates of myosin-V motors associated with axonal transport vesicles from immuno-EM studies range from 6 to 120, dependent on vesicle size, with many fewer kinesin motors detected on the same vesicles [67].

Tracking Vesicular Cargos in Cells

To examine the activities of motors moving endogenous biological cargos, high-resolution imaging has been performed using a range of reporters, including GFP-fusion proteins targeted to specific organelles [68], or internalized markers such as gold nanoparticles [69] or QDs, that allow tracking of vesicle motility with high spatial precision. QDs, either derivatized by attachment of specific ligands, such as nerve growth factor (NGF) [70–72] or nonspecific peptides (polyarginine [73]), or QDs that have been aggregated by crosslinking to an antibody [74], are taken up by endocytosis.

Tracking of QDs internalized into endosomes shows multiple forms of motility. Movement of QD-labeled endosomes near the membrane is consistent with myosin-driven motility along the actin cytoskeleton [74]. The observed centripetal velocities (0.3 – 0.8 μm/sec) and step sizes (15 and 29 nm) suggest this motility at the cell periphery is driven by myosin VI. Away from the cortex, endosomes often exhibit saltatory movements suggestive of a tug-of-war between oppositely oriented microtubule motors [62]. Directed runs of several microns are also observed, consistent with unidirectional movement of endosomes along the microtubule cytoskeleton [70,73].

For microtubule-based vesicular motility in the cell, a broad range of velocities is observed, centered at ~1 μm/s. Step size analysis shows minimum steps either toward or away from the cell center of 8 nm, the repeat length for motor binding sites along the microtubule [68,73, 74]. Plus end-directed steps are almost exclusively limited to 8 nm [68,69,74]. Analysis of minus end-directed motility reveals occasional larger steps of 16 and 24 nm that may occur consecutively during a run [69,73]. These cellular observations are consistent with the variable stepping of purified mammalian dynein observed *in vitro* [2].

Analysis of NGF-coupled QDs taken up by neurons through receptor-mediated endocytosis reveals long-range unidirectional motility directed toward the cell center [70]. While pausing was frequent, plus end-directed runs were rare. Imaging of NGF-QD motility in a cellular model of neurodegeneration indicates more frequent pausing, shorter runs, slower velocities, and generally less directed motility than seen in control neurons [72]. These observations suggest that transport properties are carefully regulated within the cell, and that transport may become mis-regulated during cellular stress responses.

Conclusions and Perspectives

The studies described here are closing the gulf between the "bottom up" approaches using *in vitro* assays with increasing complexity, and "top down" approaches that study motility in live cells with increasing temporal and spatial resolution. Intermediate methods have also been described, such as the isolation of cellular cargos with bound motors for *in vitro* studies of motility, and the "unroofing" of cells to make the cytoskeleton accessible to exogenously added motors. New geometries and technologies that reveal biophysical and cellular mechanisms are leading to rapid progress in understanding how motors operate in cells. Heisenberg said in a different context, "Science describes nature as exposed to our method of questioning." Expanding our ways of viewing biological transport will reduce the uncertainties about its mechanisms down to their natural limits.

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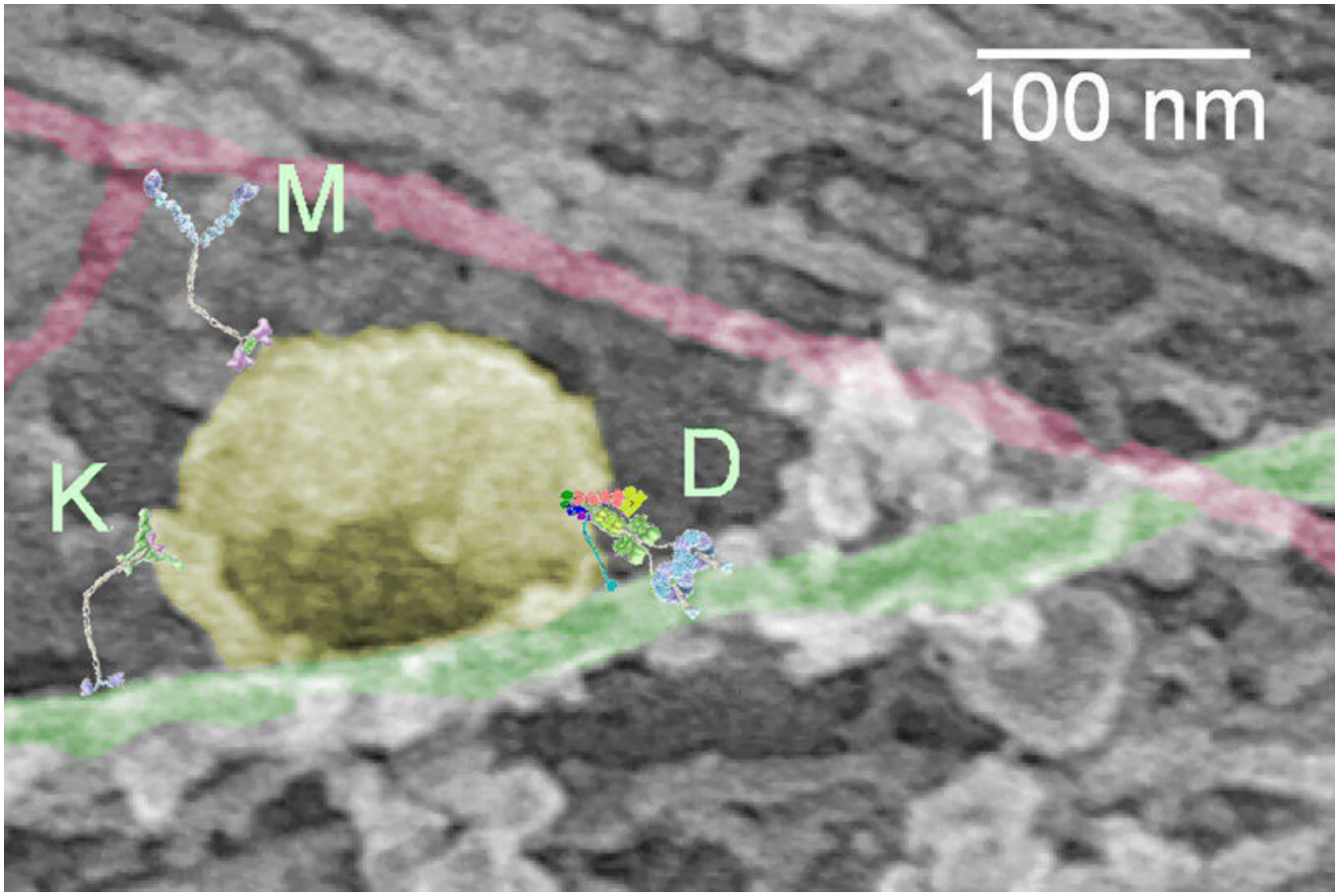


Figure 1. Multiple motor proteins, including dynein, kinesin, and myosin-V, drive vesicle motility along the complex cellular cytoskeleton

In this electron micrograph showing a platinum replica of an unroofed rat embryonic fibroblast, a vesicle pseudo-colored in yellow is bound along a microtubule (green), in the vicinity of an actin filament highlighted in red. Schematic structures of the motors dynein (D), kinesin (K), and myosin-V (M) are drawn approximately to scale. The authors gratefully acknowledge Tatyana Svitkina of the University of Pennsylvania for providing the electron micrograph.

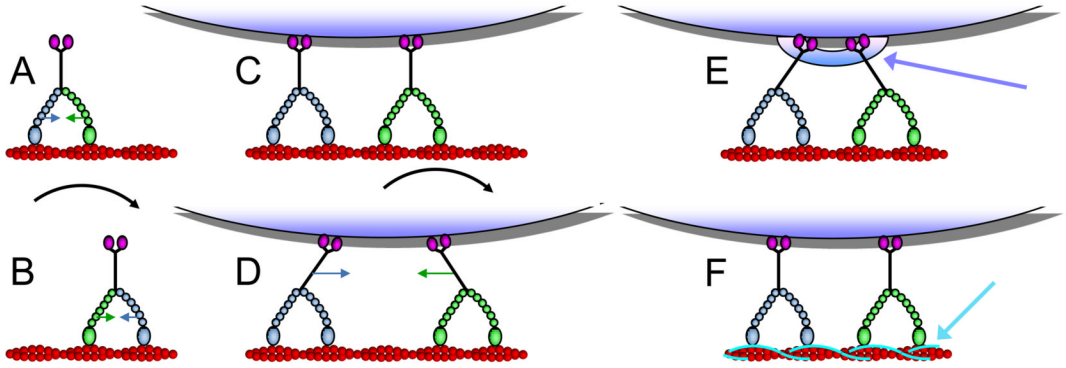
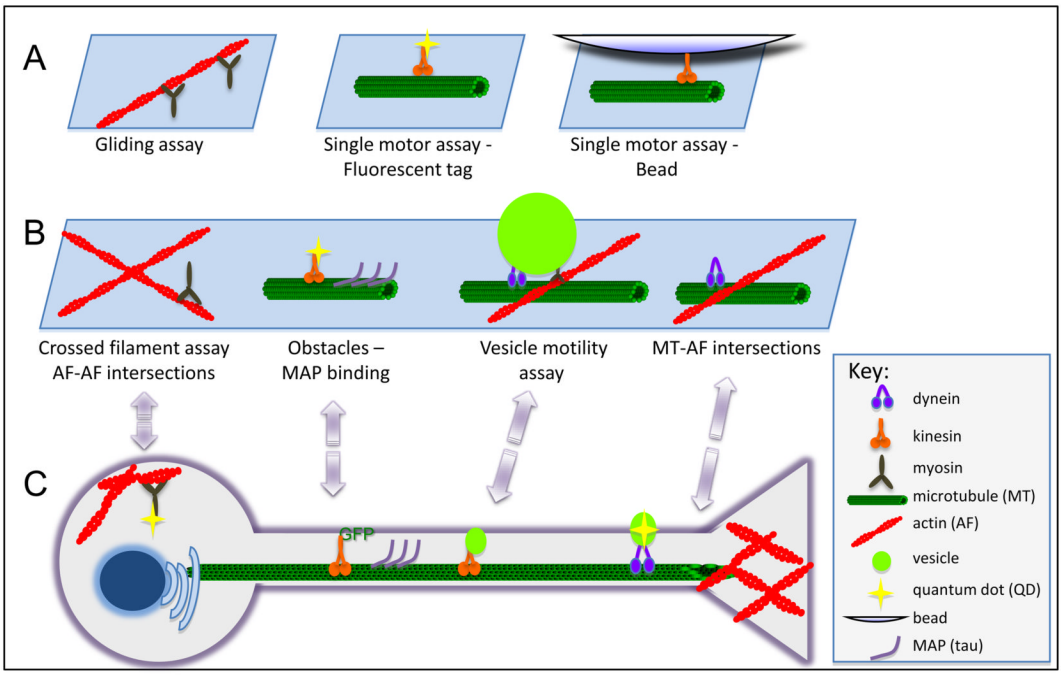


Figure 2. Mechanisms for interaction between the motor components

A, a double-headed motor, such as myosin V is bound to its track, actin (red), by both heads. The trailing head (blue) detaches and steps forward (A → B) to become the leading head. Due to stretch of the molecule required to reach the next binding site, the leading head is pulled backward (blue arrow in B) and the trailing head is pulled forward (green arrow in B). C and D, two myosin V molecules bound to the same cargo particle and to the same actin filament. When one of the motors (green) steps forward (C → D), the cargo moves half as far as the motor steps. Attachment and motor compliances strain the stepping motor backwards and the non-stepping motor (blue) forward (arrows in D). E, an extra regulatory component (blue arrow) may control the activity of the motors. F, a filament binding protein, such as tropomyosin (light blue arrow) drawn here or MAPs, might regulate motor-track interactions and cooperative behavior of nearby motors.



Box Figure 1.