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Cargo transport: molecular motors navigate a complex cytoskeleton

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Intracellular cargo transport requires microtubule-based motors, kinesin and cytoplasmic dynein, and the actin-based myosin motors to maneuver through the challenges presented by the filamentous meshwork that comprises the cytoskeleton. Recent *in vitro* single molecule biophysical studies have begun to explore this process by characterizing what occurs as these tiny molecular motors happen upon an intersection between two cytoskeletal filaments. These studies, in combination with *in vivo* work, define the mechanism by which molecular motors exchange cargo while traveling between filamentous tracks and deliver it to its destination when going from the cell center to the periphery and back again.

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Introduction

Cargo transport of organelles, secretory vesicles, and protein complexes by tiny molecular motors is an essential intracellular process. The importance of this process is emphasized by mutations to these motors lead to genetic diseases such as amyotrophic lateral sclerosis [1], paraplegia [2], and Griscelli syndrome type 1 [3]. Molecular motors that drive cargo transport along the cytoskeletal highway include myosins traveling along actin filaments and kinesin and cytoplasmic dynein motors traveling on microtubules (Figure 1). These motors share cargo transport duties and face the challenge of maneuvering through a complex cytoskeleton with numerous microtubule and actin filament intersections. How these motors navigate these obstacles and whether they work together to assure that cargo reaches its final destination is still unclear. In this review, we will highlight recent single molecule *in vitro* experiments that characterize the transport capacity of individual and small ensembles of molecular motors along constructed cytoskeletal networks. We will discuss how these results contribute to our understanding of intracellular cargo transport *in vivo*.

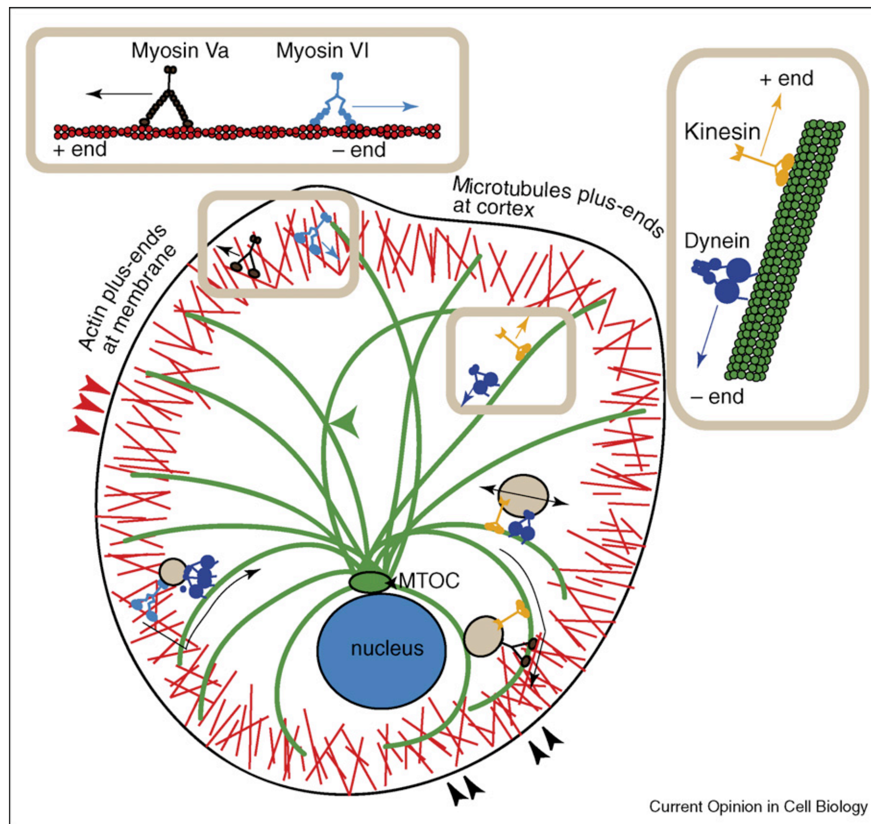
Cargo transport and track switching

Both the secretory and endocytic pathways require that vesicular cargo be transferred between actin and microtubule tracks. Ideally, microtubules originate from an organizing center near the nucleus and fan out with their plus ends toward the cell periphery. Cargos (e.g. secretory vesicles) carried by plus end directed kinesins are translocated along microtubules toward the cortex. Upon reaching the dense cortical actin meshwork the cargo is transferred to myosin Va for delivery to the cell membrane (Figure 1). The overall actin polarity within the cell cortex is directed with barbed or plus ends toward the cell membrane, the direction to which myosin Va walks.

In the reverse direction, during endocytosis, vesicles may be transported initially through the actin cortex by the minus end directed myosin VI. Cargo is then handed-off to minus end directed cytoplasmic dynein traveling on microtubules (Figure 1). As described, cargo transport involves switching between motor carriers and tracks at actin filament–microtubule intersections as observed *in vivo* for encapsulated viruses [4], melanosomes and peroxisomes [5••,6•,7], vesicles (human epidermal growth factor receptors HER2) [8••], and endocytosed quantum dots [9•].

Unlike the ideal actions outlined above, intracellular transport is plagued with impediments to motion brought about by the intracellular milieu and the motors themselves. For example, multiple motor types are known to be associated with individual cargos (Figure 1). Are their cargo transport activities coordinated or do these motors undergo a constant 'tug of war' [6•]? For example, Gross *et al.* [10] observed that the velocity of melanosomes transported along microtubules increases when the myosin Va is effectively inactivated by a dominant negative construct. This result suggests that kinesin-driven movement may be slowed by myosin Va bound to the same melanosome. Kural *et al.* [5••,6•] observed GFP-tagged peroxisomes moving bidirectionally on microtubules, suggesting that both kinesin and dynein are present and that for discrete periods of motion only one of the motor types dominates. Bidirectional excursions are the result of: (1) one of the motor types winning the tug of war, or; (2) coordinated dissociation of one motor type so that the opposite type can power motion. Another impediment to motion is the

Figure 1



Cartoon representation of motor proteins and vesicular cargo transport in the cell. Myosin family motors, myosin Va (dark brown) and myosin VI (light blue), walk along actin filaments (red) at the cortex. Myosin Va walks toward the F-actin plus end, which is oriented toward the membrane. Myosin VI walks toward the minus end of F-actin, toward the cell interior. Microtubule-based motors include the kinesin family motors (orange) and cytoplasmic dynein (violet). Kinesin motors walk to the plus ends of microtubules (green), which are oriented toward the actin cortex. Dynein motors walk toward the minus end of the microtubule, which is located at the microtubule-organizing center (MTOC, green) near the cell nucleus (blue). F-actin and microtubules cross at the cell cortex, as highlighted by black arrowheads (lower right). F-actin cross in the cortex, highlighted by the red arrowheads (left). Microtubules can intersect other microtubules highlighted by the green arrowhead (center). Vesicular cargo (tan) can bind to myosin VI and dynein to switch from actin-based to microtubule-based motion while being transported into the cell interior (lower left). Vesicles can bind kinesin and myosin Va to switch from microtubule-based to actin-based motion in order to be transported to the cell cortex (lower right). Vesicles traveling on microtubules can experience a tug of war from kinesin and dynein simultaneously bound (right).

cytoskeleton itself, which presents a crowded sea of physical barriers to forward motion, such as crossing filaments, associated proteins, or other motors with bound cargo all of which may limit the cargo's forward motion.

Similar questions relate to the specific mechanism by which the hand-off of cargo occurs at cytoskeletal track intersections. Is it a coordinated process or a tug of war, where the stronger or more numerous motor wins? Even more simply, what does a single kinesin or dynein motor do when it encounters a microtubule–microtubule intersection or when a myosin faces an actin–actin intersection? To understand how such motors maneuver through the cytoskeleton, the molecular structure and *in vitro* function of these molecular motors provide insight into the physical constraints that limit their maneuverability.

Inherent motor properties crucial for cargo transport

All three motor types described here (i.e. myosins, kinesins, and cytoplasmic dyneins) have two motor domains that hydrolyze ATP and convert chemical energy into force and motion. These two motor domains are highly coordinated so that the molecule steps processively in a hand-over-hand fashion, taking multiple steps before diffusing away from its track [11•,12–14]. Although, a single processive motor can, in principle, act as a cargo transporter, it is more likely that several motors are involved, assuring consistent intracellular cargo delivery. For example, Vershinin *et al.* showed that multiple kinesin motors working together *in vitro* can carry cargo far longer than a single motor [15••]. On the contrary, recent *in vivo* work suggests that only a single motor is actively engaged at any one time since stepwise cargo movements,

equaling the stride length of a single motor, were observed inside cells [5••,8••,16]. This follows given that two or more kinesin motors *in vitro* generate 4 nm or less step sizes when simultaneously interacting with the microtubule [17•]. However, single motor processivity is not an essential property for cargo transport provided there are sufficient numbers so that one motor remains in contact with the track at any point in time, as demonstrated by several single-headed or non-processive dimeric motors being able to transport cargo [18–20].

Recent findings suggest that motor processivity can be enhanced by the motor associating via a second binding domain, protein, or protein complex. KIF1A uses a second electrostatic binding domain to power single-headed processivity [19]. Melanophilin is an accessory protein that provides a tether for myosin Va motility [21]. Dynactin is a large complex that can dock dynein and kinesin-2 to cargo and enhances processivity by tethering the motors to the microtubule [22•]. These electrostatic tethers effectively prevent the free motor from diffusing away, allowing it to reattach and begin another processive run.

Single molecule optical trapping and total internal reflection fluorescence microscopy studies have determined the step size (more appropriately the stride length) for these motors [11•,12,13,23,24,25•,26•]. For kinesin and myosin Va, the step size is fairly constant (8 nm, kinesin; 36 nm, myosin Va) [11•,12,13,23], whereas cytoplasmic dynein and myosin VI may have variable step sizes (dynein, 4- 32 nm; myosin VI, 20–50 30 nm) [14,24,25•,27]. These stride lengths reflect both the structure of the motor and the track upon which it steps. For example, myosin Va and dynein dimerize at some distance from their individual motor domains, allowing the motors to skip multiple binding sites on its track before the lead motor domain completes its diffusive search and binds to the track. The dimerization domain for myosin Va is flexible enough so that the unbound leading head can freely explore its diffusional space before attaching to the actin filament [28••,29•,30•]. Kinesin, on the contrary, has a short link between the dimerization and motor domains, resulting in a more compact structure and thus less able to skip binding sites on the microtubule. These properties impact these motors' capacity to maneuver through a cytoskeletal intersection.

Another crucial property is the motor's maximum force generation. Through optical trapping experiments, kinesin can generate stall forces of 5–8 pN [31,32], twice that of myosin Va [33], and equal to or greater than cytoplasmic dynein [24,34,35]. These absolute forces may be a factor in cargo transport if tug of wars are common, or when cargos encounter intracellular obstacles that present a load that may impede motion.

In vitro maneuvering through intersections

A recent series of experiments take a bottom-up approach to build complexity by constructing a simple model of the cytoskeleton on a glass coverslip. By adhering isolated actin filaments and/or microtubules to form filament intersections, one can observe how a single motor, or a small ensemble of motors attached to a bead, navigates an intersection (Figure 2).

Myosin Va at actin–actin intersections

Using this approach, Ali *et al.*, determined that a single myosin Va can deal with an actin–actin intersection (Figure 2A) without hesitation [28••]. In fact, 48% of the time, a myosin Va molecule switched tracks, 37% terminated at the intersection, while 15% crossover [28••]. Given myosin Va's structure and large 36 nm stride length, one might have expected a higher crossover frequency assuming that myosin Va could easily step over the 7 nm physical barrier presented by the intersecting actin filament. A simple model was developed to explain the result in which myosin Va's inherent flexibility allows the leading head to undergo a limited three-dimensional, diffusive search [29•,30•]. It is assumed that all monomers within range are equally attractive. The intersecting filament presents four times as many monomers within reach of the myosin head. Thus, the motor chooses to switch filaments and turn more often than crossing over (Figure 2A), which may account for the 50% probability of melanosomes switching actin tracks *in vivo* [7]. In fact, the motor's flexibility is so great that it can take up to a 150° turn at an intersection and thus is easily capable of switching filaments at a 70° angle created by the ARP2/3 complex [28••].

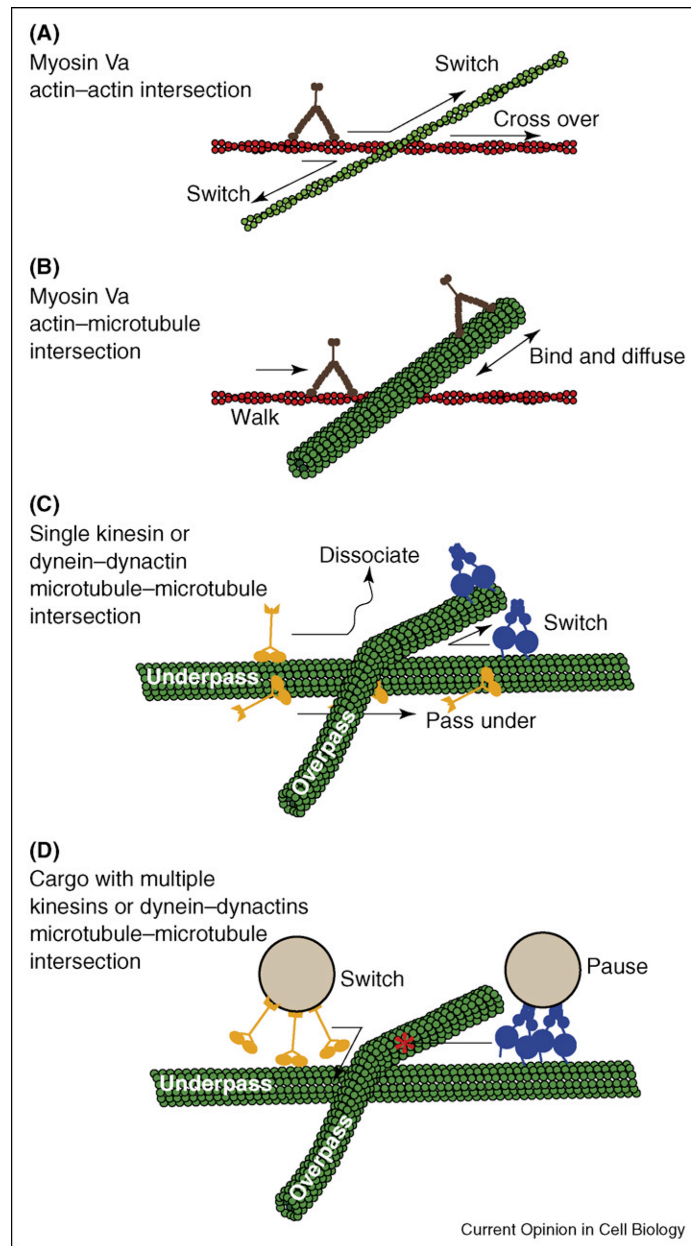
Myosin Va at actin–microtubule intersections

In this same study, Ali *et al.* also observed how myosin Va deals with an actin–microtubule intersection [28••]. Surprisingly, myosin Va motors can switch from actin to microtubules (Figure 2B). Once associated with the microtubule, the myosin will diffuse along the microtubule owing to an electrostatic interaction between the positively charged surface Loop 2 on myosin Va and the negatively charged tubulin E-hook. Ali *et al.* speculate that this interaction may allow the myosin Va to effectively pace along the microtubule, waiting to link up with cargo being transported by kinesin. A more intriguing possibility is that the myosin Va may enhance kinesin's cargo transport capacity through its electrostatic interaction with the microtubule and thus act as a tether preventing the cargo from diffusing away from its track.

Kinesin and dynein at microtubule–microtubule intersections

Two recent studies have examined how kinesins and dyneins manage at microtubule–microtubule intersections. Using kinesin-coated beads, Vershinin *et al.* observed that beads with three or more kinesins pause

Figure 2



Schematic diagram of *in vitro* assays with intersecting cytoskeletal filaments. Intersections have been created between two F-actin filaments, an F-actin and a microtubule, and two microtubules. **(A)** For actin–actin intersections, recent studies have observed myosin Va not only switching most frequently to the crossing filament but also passing over the intersecting F-actin (green). **(B)** The same study used the microtubule as an obstacle for myosin Va stepping, but found myosin Va was able to bind and diffuse along microtubules. **(C)** An interesting feature of microtubule–microtubule intersections is that they have 'underpass' and 'overpass' tracks. Single kinesins are able to transgress the intersection on the underpass track by going under the overpass bridge. Dynein–dynactin was most likely to switch between microtubules. **(D)** Two studies of beads decorated with kinesin at microtubule–microtubule intersections have shown that kinesin-coated beads switch frequently at the intersection. Dynein–dynactin-coated beads were found to pause at the intersection at high motor density but pass or switch at low motor density.

58% of the time at microtubule intersections with the remaining 42% switching between microtubules after deforming the microtubules [15••]. Interestingly, adding a very little tau, a microtubule-associated protein (MAP), increased the switching probability to 91% without any detectable microtubule deformations. This implies that tau, by blocking kinesin binding to the microtubule, reduces the chance that multiple kinesin motors will engage in a tug of war at the intersection.

A second study by Ross *et al.* characterized the behavior of single fluorescently labeled kinesin or dynein–dynactin molecules at microtubule–microtubule intersections [36••]. Given the 25 nm microtubule diameter, a microtubule–microtubule intersection creates an effective underpass and overpass (Figure 2C). In fact, single kinesins make their way easily through intersections whether traveling on the underpass or overpass microtubule, suggesting that kinesin is small enough to squeeze through the underpass (Figure 2C). However, dynein–dynactin was less likely to pass but would more easily switch from one microtubule to another at the intersection, reflecting dynein–dynactin's larger size and flexible nature. These authors then increased complexity of the experiment by decorating beads with dynein–dynactin or kinesin as did Vershinin *et al.* [15••]. Interestingly, kinesin-decorated beads switch microtubules at intersections much more frequently than single motors: approximately 66% of the time from an underpass and 33% of the time from an overpass. These statistics were quite stable for all decoration densities, in agreement with the results of Vershinin *et al.* [15••]. However, for dynein–dyanctin-decorated beads, as the decoration density increases, the percentage of beads that become moored at the intersection increases (Figure 2D). In fact at the highest dynein–dynactin concentrations, $\sim$100% of beads are stuck at the intersection, no matter if starting from the underpass or overpass microtubule. Since dynein–dynactin may be used to tether organelles in the cell at places of high microtubule density, this *in vitro* result may support this biological role for dynein–dynactin [36••].

Conclusions

With the advent of single molecule biophysical techniques, the recent flurry of *in vivo* and *in vitro* studies have provided significant insight to how molecular motors manage to transport and deliver cargo within the cell. However, many questions still remain that pose experimental challenges. For example, *in vitro* studies must build further complexity to characterize how cargo with mixed populations of actin and microtubule-based motors navigate through a well-defined three-dimensional array of microtubules and actin filaments. Such studies will help define the exchange and hand-off of cargo that occurs at microtubule–actin filament intersections near the cell cortex. If this is a coordinated process, then understanding the modes by which these motors are regulated will be crucial. This is not a trivial matter, since regulation can occur by autoinhibition [37••,38,39•,40], motor–motor interactions [41,42], and binding partners that exist either on the cargo or the track itself [15••,43]. Therefore, *in vitro* studies using endogenous, membrane-encapsulated cargos or organelles would be extremely enlightening to determine how motors and their regulating partners on cargo behave at cytoskeletal intersections. For the most part, *in vivo* studies have relied on the motion of the cargo to infer the motors' transport properties. The challenge will be to correlate the dynamics of cargo movement with that of the motor or motors themselves in real time. Although the challenges are great, building complexity *in vitro* and breaking down complexity *in vivo* will be the key to answering an extremely difficult question: what is involved in getting cargo within the cell from point A to point B?

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