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Microtubule motors: moving forward on many fronts

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Abstract

Microtubule motors drive the movement of many different cargoes in eukaryotic cells. A combination of *in vitro* and *in vivo* approaches has led to a better understanding of their mechanism of action and function and are also revealing that the microtubule track itself may have an important role to play in directing cargo movement within the cell.

Introduction and context

When you look at a living eukaryotic cell by light microscopy, what strikes you is that everything in the cell is moving, often over considerable distances. Much of this motility is driven by microtubule motors, which use the energy from ATP hydrolysis to walk along microtubules, which are linear polymers made up of α/β-tubulin dimers. A wide range of cargoes, including membrane-bound organelles, chromosomes, mRNA, and microtubules themselves, are transported by these motors.

There are two families of microtubule motors: the dyneins and kinesins. Dyneins move toward the minus end of microtubules, which are found in the centre of cells such as fibroblasts, with cytoplasmic dynein-1 (referred to as dynein from now on) driving most minus end-directed membrane movement. Many kinesins, including the founding member kinesin-1, translocate toward the rapidly growing and shrinking plus ends, which are usually oriented toward the cell periphery. However, dynein regularly takes backward steps, at least when single purified motor molecules are analysed [1-3], and kinesin-1 can also step backward under a load [2,4]. This flexibility may help motors get around obstacles in the cell. Here, I review some recent key advances and controversies surrounding dynein and kinesin-1 function in the cell.

Major recent advances

One motor or more?

In vitro assays have proven extremely useful for the analysis of the force generation and stepping mechanisms of single motor molecules. However, in the cell, it is likely that multiple motor proteins work together to move each cargo. A key property of each motor is its processivity, which is the number of steps it takes along the microtubule before it dissociates. While single dynein or kinesin-1 molecules move artificial cargoes such as beads 1-2 μm along microtubules *in vitro*, this distance is considerably increased when multiple motors are attached to the bead [2,5]. The number of dynein molecules assembled onto messenger ribonucleoprotein (mRNP) particles is thought to influence both the rate and duration of their translocations in Drosophila embryos [6]. Similar conclusions have been reached for both kinesin-1- and dynein-driven peroxisome movement *in vivo* in Drosophila S2 cells [7]. Based on the existence of clear steps in the velocity histogram, Kural and colleagues [7] proposed that each active motor contributes approximately 1.2 μm/sec, meaning that 10 motors would be needed to generate the maximum rate of 12 μm/sec. However, this interpretation is complicated by the fact that microtubule sliding and buckling within the cytoplasm can also generate rapid peroxisome movement [8]. Moreover, a recent study of lipid droplets in Drosophila embryos demonstrated that the number of

kinesin-1 molecules engaged had no effect on either the length of movement or its rate [9]. Although the control of motor number may play a part in regulating cargo movement, it cannot be the only mechanism. Instead, associated proteins could modulate motor activity or the microtubule track itself could affect translocation efficiency. Both possibilities are discussed below.

Accessory proteins as regulators

It has long been thought that a dynein-associated complex called dynactin acts to improve dynein's processivity, making it similar to that of kinesin-1 [10]. Dynactin can bind to microtubules via two regions in its N-terminal domain of its p150 subunit: the CAP-Gly and basic domains [11-13]. Data from *in vitro* assays have suggested that the CAP-Gly domain may act as an anchor, while the basic domain allows p150 to 'skate' along the microtubule [12], providing an attractive mechanism for enhancing dynein's processivity.

This model has been brought into question by an *in vivo* study that used RNA interference (RNAi) knockdown to remove wild-type p150, which was replaced by p150 lacking its microtubule-binding domains [14]. This strategy revealed that microtubule binding was not needed for processive movement of peroxisomes or mRNPs [14]. This result has since been confirmed for dynein-dependent Golgi apparatus positioning [15]. The absence of p150's microtubule-binding regions did affect microtubule organisation, however [14], and the ability of dynactin to bind to microtubule plus ends [15]. Studies in *Saccharomyces cerevisiae* suggest that dynactin's microtubule function is critical when dynein needs to generate large amounts of force, such as when moving the nucleus into the bud neck, but that it is not required to drive microtubule sliding across the cell cortex [16]. Interestingly, natural splice forms of p150 that lack the microtubule-binding domains exist, suggesting that there may be subsets of dynactin complexes that perform different roles in the cell. That dynactin containing truncated p150 can still enhance dynein's processivity has been confirmed using recombinant dynein and dynactin purified from *S. cerevisiae* [16]. Perhaps the binding of dynactin p150 to the dynein intermediate chain is sufficient to improve dynein's function, even in the absence of dynactin-microtubule interactions: how this activation is achieved remains to be determined.

There is no known equivalent of the dynactin complex for kinesin-1, but instead the binding of kinesin-1 to cargo may activate the motor by unfurling it from its folded inactive cytosolic state, in which the C-terminal domain of the kinesin heavy chain (KHC) interacts with and inhibits the N-terminal motor domain [17-19]. It seems that both the heavy and light chains of kinesin-1 need to interact with cargo molecules or accessory factors for the motor to be fully activated [20,21]. Furthermore, the specific cargo protein that binds kinesin-1 may affect motor properties since vesicles containing alcadein-α move at a faster rate than those containing amyloid β-protein precursor [22]. Cargo-associated proteins may also toggle motors between active and inactive states since the direct binding of the mitochondrial protein Miro to the motor domain of kinesin-1 in the presence of calcium prevents kinesin binding to microtubules [23]. This may explain how changes in intracellular calcium caused by glutamate receptor activation lead to accumulation of mitochondria at synapses [24]. Likewise, the activity of the heterogeneous nuclear ribonucleoprotein Squid is needed to switch mRNP particles from a motile state to dynein-dependent anchoring in Drosophila oocytes [25].

In an interesting twist, kinesin-1 activity may be regulated by molecules that are associated with the microtubule track rather than the cargo since a microtubule-associated protein (MAP) named ensconsin is required for motility of full-length KHC in Drosophila embryo extracts, whereas its absence has no effect on a constitutively active truncated version of KHC [26]. One interpretation is that ensconsin somehow plays a part in the unfolding and activation of kinesin-1, which is a very surprising function for a MAP.

Not all tracks are the same

While ensconsin may promote kinesin-1 motility, other MAPs, including the neuronal MAP tau, have been shown to compromise motor function. Kinesin-1 is sensitive to much lower concentrations of tau than dynein, and tau tended to cause kinesin-1 detachment [5,27] whereas dynein tended to reverse when encountering a patch of tau [27]. The distribution of MAPs in a cell therefore might influence which motors use specific tracks effectively. This is supported by the apparent preference of trans-Golgi network (TGN)-derived secretory vesicles for a subclass of septin-2-associated microtubules in epithelial cells, where septin 2 is thought to promote motility by clearing those microtubules of MAP4 [28]. Interestingly, the Golgi apparatus itself can serve as a nucleation site for microtubules via the activity of the A-kinase anchoring protein (AKAP450) [29] and these Golgi-nucleated microtubules are stabilised at their plus ends by a microtubule plus end-binding protein, CLASP2 (cytoplasmic linker-associated protein 2) [30]. Whether these microtubules are preferential tracks for vesicles moving away from the Golgi apparatus and how these microtubules relate to septin-2-associated microtubules remains to be established.

The presence of septin 2 also marks out those microtubules that are modified by polyglutamylation [28], which along with acetylation, polyglycylation, and detyrosination form a series of reversible post-translational modifications that are found on subpopulations of microtubules [31]. There is accumulating evidence that these modified microtubules may be preferred tracks for kinesin-1 [31-34] because KHC has a higher affinity for microtubules containing acetylated and detyrosinated α-tubulin and for polyglutamylated/polyglycylated β-tubulin [33]. Intriguingly, the acetylated lysine is thought to be in the lumen of the microtubule, suggesting that changes in the interior can lead to alterations in the exterior of the microtubule lattice. In *Aspergillus nidulans*, however, kinesin-1 showed no preference for stable detyrosinated microtubules, whereas a kinesin-3 family member did [35]. Likewise, in mice lacking a subunit of tubulin polyglutamylase, it was a kinesin-3 rather than kinesin-1 or -2 that was mislocalised [36]. This suggests that the cell type and situation (and possibly even cargo) may influence which microtubules are preferred tracks.

Why might a choice of track be important? In a small non-polarised cell, it may not be that critical, but in a moving cell, it might be advantageous to be able to load vesicles budding from the Golgi onto microtubules that lead directly to the leading edge, thus facilitating cell extension and migration. Furthermore, when epithelial cells are undergoing polarisation and then maintaining that state, it may help to separate microtubules that lead to the apical versus the basolateral surface. In this regard, it is interesting that two different populations of microtubules have been observed in polarised MDCK cells [37] and that septin 2 expression is needed for these cells to polarise in the first place [28]. Distinctly modified microtubules may also underlie the initial differentiation of neurites into axons or dendrites by encouraging selective transport of certain cargoes, such as JIP1-containing structures, by kinesin-1 along a single neurite, leading to that neurite becoming the axon (for example, [33,38,39]). It is not yet clear what determines whether specific cargoes move preferentially into the mature dendrites or axon, but microtubule modification, MAPs, and plus end-binding proteins have all been suggested to play a part, along with the motor itself and the cargo to which it is bound [33,36,38-43].

Future directions

There are many questions left unanswered about how microtubule motors work in the cell. For example, the regulation of the activity of individual motors is still poorly understood. Moreover, understanding how multiple motors, particularly those of opposite polarity, work together when on a single cargo is a huge challenge. For example, do such motors engage in a tug-of-war to determine in which direction a cargo moves or are their activities switched on and off coordinately [44]? Here, we may well find that the answers vary according to the particular cargo. Recent *in vivo* studies have often led us to reassess what we thought we had learned about this issue from the use of single-molecule *in vitro* assays. It is an exciting time for motors though, as their roles in brain development and function, such as the requirement for kinesin-1 activity in learning, are becoming uncovered [45]. Their importance is further underscored by the extensive links between motor malfunction and disease [46-48].

Abbreviations

AKAP450, A-kinase anchoring protein 450; CAP, cytoskeleton associated protein; CLASP2, cytoplasmic linker-associated protein 2; Gly, glycine; JIP1, Jun NH_2-terminal kinase (JNK) interacting protein 1; KHC, kinesin heavy chain; MAP, microtubule-associated protein; MDCK, Madin Darby canine kidney; mRNP, messenger ribonucleoprotein; RNAi, RNA interference; TGN, trans-Golgi network.

Competing interests

The author declares that she has no competing interests.

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