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A Novel Role for Retrograde Transport of Microtubules in the Axon

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Short microtubules move within the axon in both directions. In the past, it had been assumed that all of the short moving microtubules are oriented with their plus-ends distal to the cell body, regardless of their direction of movement. The anterogradely moving microtubules were posited to play critical roles in the establishment, expansion, and maintenance of the axonal microtubule array. There was no known function for the retrogradely moving microtubules. In considering the mechanism of their transport, we had assumed that all of the short microtubules have a plus-end-distal polarity orientation, as is characteristic of the long microtubules that dominate the axon. Here we discuss an alternative hypothesis, namely that the short microtubules moving retrogradely have the opposite polarity orientation of those moving anterogradely. Those that move anterogradely have their plus-ends distal to the cell body while those that move retrogradely have their minus ends distal to the cell body. In this view, retrograde transport is a means for clearing the axon of incorrectly oriented microtubules. This new model, if correct, has profound implications for the manner by which healthy axons preserve their characteristic pattern of microtubule polarity orientation. We speculate that pathological flaws in this mechanism may be a critical factor in the degeneration of axons during disease and injury, as well as in neuropathy caused by microtubule-active drugs. © 2012 Wiley Periodicals, Inc

Key Words: microtubule, axon, neuron, motor, kinesin, dynein, microtubule-severing protein, katanin, spastin, neuropathy, degeneration, axonal transport

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Introduction

It has long been recognized that microtubules in the axon are almost exclusively oriented with their plus-ends distal to the cell body [Heidemann et al., 1981; Baas and Lin, 2011]. Live-cell imaging with fluorescently tagged proteins that associate with the plus-ends of microtubules during bouts of rapid assembly (+tips such as EB1 and EB3) have made visualization of microtubule polarity orientation straightforward [Stepanova et al., 2003]. These studies confirm that axonal microtubules are nearly uniform in their plus-end-distal orientation, but also reveal the existence of a small number of microtubules with the reverse orientation. The number of reverse-oriented microtubules is higher early in axogenesis and also higher in the more plastic regions of developing axons, such as their growth cones and sites of impending branch formation [Stepanova et al., 2003; Hasaka et al., 2004]. Reverse-oriented microtubules are also transiently higher in axons undergoing phases of morphological change, for example, in response to growth factors [Qiang et al., 2010]. We hypothesize that the axon's nearly uniform microtubule polarity pattern is at constant risk of being corrupted. Live-cell imaging on developing axons has revealed that roughly 2/3 of the short mobile microtubules transit in the anterograde direction while the rest transit in the retrograde direction [Wang and Brown, 2002; He et al., 2005; Myers and Baas, 2007]. In the past, we had assumed that all of these short mobile microtubules are oriented with their plus-ends distal to the cell body, and this assumption has guided our hypotheses about which motors might potentially transport them [Baas et al., 2006]. We have been working to characterize the molecular motor proteins and related mechanisms that transport microtubules of the same orientation in two different directions in the axon. But what if the underlying assumption about their polarity orientation is wrong?

In this report, we put forth a different perspective that assigns a functionally critical role to the retrogradely moving short microtubules. In this view, the microtubules that

transit in the anterograde and retrograde directions all move with their plus-ends leading, which means that the microtubules moving in opposite directions have opposite polarity orientations. A model based on this premise is appealing for many reasons. The first is simplicity, as very complex mechanisms would be required for the axon to decide which microtubules of the same orientation should move in each direction, and what the proportions should be. Second, the model assigns a purpose to the retrograde movements, as they would represent a relentless clearing mechanism to drive reverse-oriented microtubules back to the cell body, thereby preserving the nearly uniform microtubule polarity pattern of the axon. Third, the model has profound implications for disease, as it suggests that preserving the microtubule polarity of the axon requires a great deal of ongoing effort by the axon. If the clearing mechanism fails, the axon would gradually lose its characteristic microtubule polarity pattern, resulting in abnormalities in the transport of innumerable cargoes that rely on a nearly uniformly oriented microtubule array in the axon.

Microtubules are Mobile in the Axon

It has now been a decade since the laboratory of Anthony Brown first reported the direct visualization of microtubule transport in axons [Wang and Brown, 2002]. For many years prior, the issue had been controversial, with several prominent laboratories and even textbooks declaring that axonal microtubules are completely stationary [see for example, Hirokawa et al., 1997]. This mistaken conclusion had been reached mainly on the basis of photobleach studies in which a small bleached zone was imposed on the microtubule array in the axon of a neuron into which fluorescent tubulin had been microinjected. The bleached zone was visualized every few minutes and was documented in several different reports not to move, leading to the conclusion that the microtubules in the axon do not move. The technical breakthrough by the Brown laboratory was to make a much longer bleached zone and visualize it every few seconds, rather than minutes. Rapidly moving fluorescent microtubules were observed to move through the bleached zone, with the fluorescent microtubules originating from both sides of the zone. Notably, microtubules were observed to move both anterogradely and retrogradely through the bleached zone, contrary to our own expectation that the microtubules would move only anterogradely. Also somewhat initially surprisingly, in all cases, the mobile microtubules were quite short, only a few microns in length. In agreement with the conclusions of the earlier photobleach studies, no movement was identifiable even with this improved regime in the case of the longer microtubules. These days, in our own laboratory, we are obtaining entirely similar results in experiments on neurons expressing tubulin tagged with green fluorescent protein (GFP) or mcherry [Hasaka et al., 2004; He et al., 2005; Ahmad et al., 2006; Myers and Baas, 2007; Qiang et al., 2010; Liu et al., 2010] (Fig. 1). Similar observations of bidirectionally mobile short microtubules have also been made in neurons expressing GFP-tagged tau [Konzack et al., 2007].

The details of the live-cell imaging have opened the door to greater insights into the mechanisms that regulate and drive microtubule transport in the axon. For example, until these observations were made, microtubules were generally considered to move slowly down the axon, slower than the rates of known molecular motors [Black and Lasek, 1980]. The imaging studies revealed that microtubules actually move at the fast rates of known motor proteins, but that their movement is intermittent and asynchronous. In addition, it became apparent from the imaging studies that the only microtubules that undergo rapid concerted transport in the axon are quite short, never exceeding about 7–10 μm in length. These observations have implications for the molecular motors that drive the short microtubules to move, and also for another category of proteins called microtubule-severing proteins that break the lattice of a long stationary microtubule into multiple short microtubules that can potentially move. We put forth a model called "cut and run" based on the severing of axonal

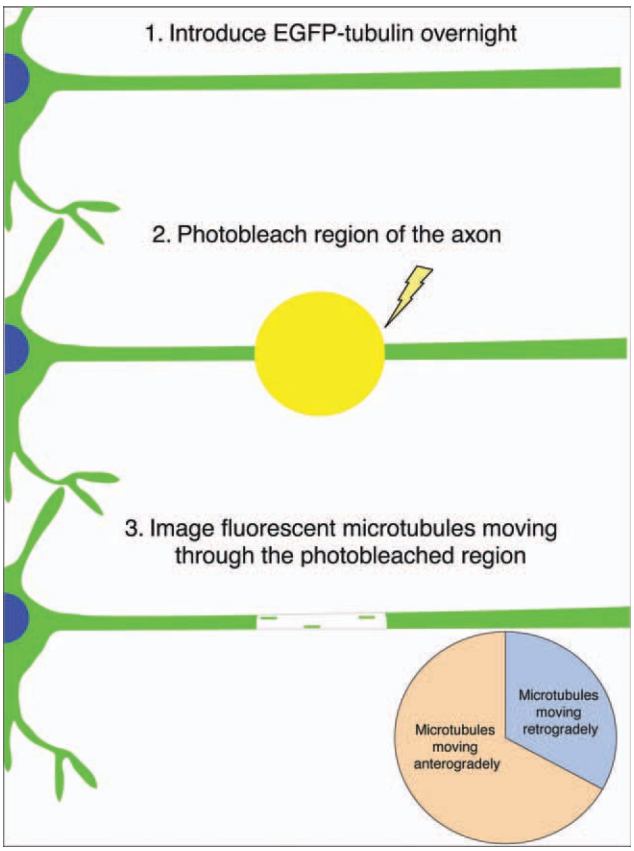

Fig. 1. Schematic illustration of the live-cell imaging paradigm for visualizing the transport of microtubules in the axon. For details, see text and Hasaka et al. [2004]; He et al. [2005]; Myers and Baas [2007]; Qiang et al. [2010]; Liu et al. [2010].

microtubules by enzymes such as katanin and spastin, and the transport of the resulting short microtubules by motors such as cytoplasmic dynein and members of the kinesin superfamily [Baas et al., 2005, 2006].

A body of previous work had implicated cytoplasmic dynein as the principal motor protein that transports microtubules with their plus-ends leading anterogradely down the axon [Dillman et al., 1996a,b; Ahmad et al., 1998]. Cytoplasmic dynein moves toward the minus end of a microtubule, so it makes sense that a microtubule would move with its plus-end leading, if the motor itself were effectively immobilized. Proof-of-principle for this comes from in vitro studies in which microtubules have been observed to move against dynein adhered to glass [Vale et al., 1992]. In the axon, the general idea is that a short microtubule would move with its plus-end leading if dynein's motor domain interacted with the short microtubule, while dynein's cargo domain interacted with a structure with greater resistance to movement. In theory, such a structure could be a long stationary microtubule, perhaps the actin cytoskeleton, or even a large membranous structure such as the endoplasmic reticulum. We depleted dynein heavy chain from neurons using RNAi and found that the frequency of anterograde movement of short microtubules was reduced [He et al., 2005]. However, the movement was not obliterated completely. The frequency of movement was halved in the anterograde direction, while the retrograde movement was unaffected. The rates of movement of those short microtubules still moving remained the same. Hence, we concluded that cytoplasmic dynein is one motor that transports short microtubules anterogradely, but that there is at least one other motor that transports them anterogradely and one or more other motors that transport them retrogradely.

The hunt was on for relevant kinesins. We figured that the most likely candidates were the so-called mitotic motors, which are known to regulate microtubule–microtubule sliding in mitosis [Baas, 1999]. However, we found that depleting two different mitotic motors (our most likely candidates) from neurons did not reduce the frequency of short microtubule transport in the axon, but rather increased it, and in both directions [Myers and Baas, 2007; Liu et al., 2010]. Thus the function of these two motors, namely kinesin 5 (also termed Eg5, kif11, KSP, or BimC) and kinesin-12 (also termed kif15 or KLP2) appears to be more akin to a brake on microtubule transport rather than a driver of it. While this is interesting and almost certainly important, the question remained: what are the motors that actually transport short microtubules in the axon?

What are the Motors?

With our studies on microtubule transport to date suggesting that cytoplasmic dynein accounts for less of the total transport than we had previously surmised and with our two best kinesin candidates apparently off the table, we began to ponder not only new ideas, but also the technical limitations of the approaches that we had used thus far. With regard to cytoplasmic dynein, the RNAi approach depleted roughly 85% of the protein, and hence we could not dismiss the possibility that the remaining 15% was still performing a great deal of work [He et al., 2005]. In fact, if we depleted more of the dynein, the growth and morphology of the axon, as well as the overall distribution of microtubules, became so abnormal that we could not have trusted the results of the microtubule transport assay to be meaningful [Ahmad et al., 2006]. With this in mind, it seems valid to keep on the table a one-motor model in which cytoplasmic dynein drives all of the microtubule transport in the axon. We originally doubted this possibility because if it were the case, we would have expected a partial dynein depletion to partially diminish the transport of microtubules in both directions in the axon, not just one direction. Interestingly, another study from our laboratory indicated that short microtubules can move anterogradely against either long microtubules or against the actin cytoskeleton, but can move retrogradely only against long microtubules [Hasaka et al., 2004]. Thus, one potential explanation for our findings is that dynein drives all of these movements, but that the transport of microtubules against actin is much more sensitive to a partial depletion of dynein than is the transport of microtubules against other microtubules (Fig. 2).

A model in which cytoplasmic dynein accounts for most or all of the microtubule movements in the axon is appealing because there is a growing body of evidence across cell types demonstrating that dynein imposes powerful forces on microtubules that configure them, transport them and organize them [Abal et al., 2002; Dehmelt et al., 2006; Wu et al., 2011; Gusnowski and Srayko, 2011]. Even so, another theme across cell types is redundancy of function, and it would be surprising for a system as sophisticated as a vertebrate neuron to rely exclusively on one protein when so many others could theoretically participate. We have shied away from the view that nonmitotic kinesins can transport microtubules in cells because these motors are optimized for vesicle transport rather than microtubule–microtubule interactions. However, even nonmitotic kinesins can transport microtubules when the motor is adhered to glass and it is probably unwise to underestimate the versatility of any kinesin. Recent studies from the Gelfand laboratory suggest that conventional kinesin (kinesin-1) can drive microtubule transport in *Drosophila* S2 cells [Jolly et al., 2010], thereby establishing precedent for motors usually considered to transport vesicles to also transport microtubules. Thus, the question remains open as to which motors transport microtubules in each direction in the axon.

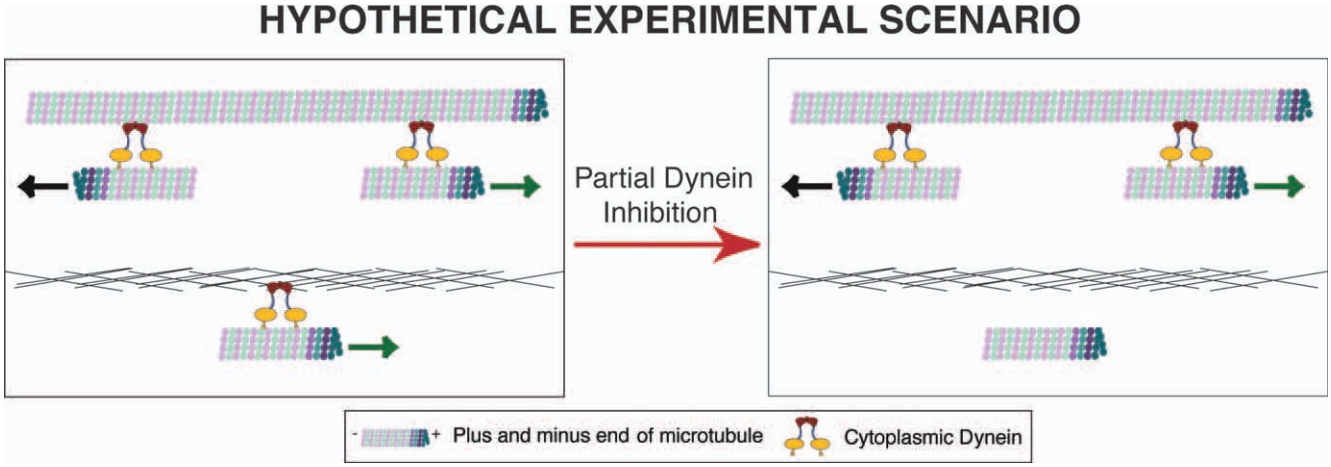

Fig. 2. Schematic illustration of a possible explanation for the results of previous studies on partial depletion of cytoplasmic dynein on axonal microtubule transport. Normally, in the axons of cultured neurons, 2/3 of the microtubule movements are in the anterograde direction while 1/3 are in the retrograde direction [Wang and Brown, 2002]. If dynein is partially depleted, the number of anterograde movements is halved while the number of retrograde movements in unaffected [He et al., 2005]. Other studies indicate that microtubules can be transported either by generating forces against long immobile microtubules or against the actin cytoskeleton [Hasaka et al., 2004]. The possibility presented in this schematic is that partial dynein depletion results in a preferential loss of the category of movement that occurs against the actin cytoskeleton.

Possible Scenarios

Based on our original assumption that all of the short mobile microtubules have their plus-ends distal to the cell body, we tentatively concluded that dynein and at least one kinesin transports microtubules anterogradely while other kinesins transport microtubules retrogradely. If the short microtubule was moving against anything other than another microtubule, the motor domain would presumably interface with the short microtubule while the cargo domain interacts with the nonmicrotubule structure. Almost all kinesins would thereby move microtubules with minus ends leading, except for the kinesin-14 family, which has the opposite directionality relative to the microtubule lattice than the other kinesins. Kinesin-14 would behave similarly to cytoplasmic dynein. This is called the sliding filament mechanism. If the short microtubule is moving relative to a long microtubule, the short microtubule could still move by the sliding filament mechanism, assuming that the motor domain was interacting with the short microtubule and the cargo domain was interacting with the long microtubule. Alternatively, the short microtubule could move as cargo, if the cargo domain were to interact with the short microtubule, while the motor domain moved along the long microtubule (Fig. 3). If the latter were the case, the motor would presumably move the short microtubule without regard to its polarity orientation. In other words, dynein and kinesin-14 would move short microtubules retrogradely while other kinesins would move the short microtubules anterogradely, irrespective of the orientation of the short microtubule. While others have argued in favor of this kind of cargo mechanism [Yamada et al., 2008], we disfavor it because it seems as if it would create a great deal of microtubule movement in the axon with no useful purpose, such as establishing or maintaining microtubule polarity orientation.

One possibility that we have considered is that perhaps a soup of available kinesins transports microtubules equally forward and backward, and that dynein is needed to tip the balance toward greater numbers of short microtubules moving anterogradely. This would fit the data on dynein inhibition, as depleting dynein causes the numbers of microtubules moving in each direction to be roughly equal. Assuming for a moment that this is correct and that all of the short mobile microtubules are oriented with plus-ends distal, the retrogradely moving microtubules would have to be transported by a sliding filament mechanism, while the anterogradely moving microtubules would have to be transported as cargo. If the retrogradely moving microtubules were minus end distal, this mechanism would not work. If the kinesin were carrying the short microtubules anterogradely as cargo, it would do so irrespective of their polarity orientation. So, while we would not underestimate the capacity of motor-driven transport for complexity, all of these machinations stretch credulity, at least in our minds. This is especially true when the question is posed as to why the neuron would go through all of this trouble without working toward functionally important goals, such as organizing the microtubule array.

As indicated at the onset of this article, we now favor a model in which the anterogradely moving microtubules have their plus-ends distal while the retrogradely moving

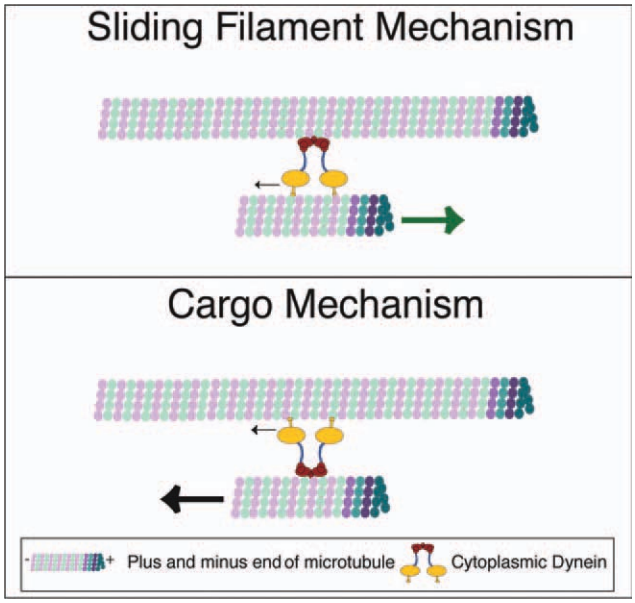

Fig. 3. Schematic illustration of the sliding filament mechanism, wherein the motor domain of cytoplasmic dynein interacts with the short mobile microtubule while the cargo domain interacts with the long stationary microtubule versus the cargo mechanism, wherein the motor has the opposite orientation relative to the short and long microtubules. In the sliding filament model, the short microtubule is transported with regard to its polarity orientation. In the cargo model, the short microtubule is simply transported along the long microtubule irrespective of the polarity orientation of the short microtubule. The same two mechanisms would theoretically work for kinesin motors as well. In the case of dynein and perhaps some kinesins, the actin cytoskeleton could substitute for the long microtubule in the sliding filament mechanism.

microtubules have the minus ends distal to the cell body (Fig. 4). We find this view appealing because all of the transport would then be highly purposeful—establishing a predominance of plus-end-distal microtubules in the axon via anterograde microtubule transport and preserving the pattern via retrograde microtubule transport. We should note that such an idea was originally proposed by the Jan laboratory, who documented an uptick in the numbers of mal-oriented microtubules in the axons of *Drosophila* neurons when dynein functions were impaired [Zheng et al., 2008].

The Axonal Microtubule Polarity Pattern is at Constant Risk of Corruption

If there were never any reverse-oriented microtubules arising in the axon, there would be no need to clear them out. However, the live-cell imaging experiments with +tips have now made it clear that minus-end-distal microtubules occasionally arise in the axon. They are small in number, but in theory, one of these mal-oriented microtubules could lead to more and more, as they are severed into short pieces that can then elongate. Given this, it makes sense that the axon would have to have a mechanism for ensuring that reverse-oriented microtubules do not get a foothold. The minus-end-distal microtubules could theoretically arise from the flipping of short microtubules, assuming that short microtubules can get extremely short relative to the axon's diameter. Such flipping may be particularly likely in broader flatter regions of the axon such as the growth cone of an elongating axon or sites of branch formation, which are also sites of especially active microtubule severing [Yu et al., 1994, 2008; Dent et al., 1999; Qiang et al., 2010] (Fig. 5). We have long believed that there is no local nucleation of entirely new microtubules in the axon [Baas and Heidemann, 1986; Baas and Ahmad, 1992], but others have pointed to the presence of microtubule-nucleating proteins in the axon that might suggest otherwise [Kuijpers and Hoogenraad, 2011; Stiess and Bradke, 2011]. Such local nucleation, if it exists, could create microtubules of either polarity orientation, and those with minus ends distal would have to be eliminated or cleared. Finally, it seems reasonable that with any system, potential errors can arise by imperfectly functioning mechanisms, and a system for self-correction would be highly valuable and perhaps even essential.

It is worth mentioning that dendrites of vertebrate neurons have mixed microtubule polarity orientation and in fact, this pattern is quintessentially important for their identity [Baas and Lin, 2011]. During dendritic development, the plus-end-distal microtubules appear first, followed by the orderly appearance of minus end distal microtubules, introduced by specific and tightly regulated mechanisms [Sharp et al., 1995, 1997]. Thus, a dendrite is not merely a flawed axon that fails at organizing its microtubule array. The fact that axons accumulating minus end distal microtubules do not become dendrites suggests that there is more to distinguishing the axonal and dendritic microtubule arrays than the presence of reverse-oriented microtubules.

Microtubule Polarity Flaws During Disease

Neurodegenerative diseases often involve flaws in axonal transport [Falnikar and Baas, 2009], which are usually attributed to abnormalities in the motor proteins or their regulators. Another possibility is that traffic jams arise due to microtubule polarity flaws in the axon. In other words, if the proportion of reverse-oriented microtubules exceeds some tolerable threshold level, the efficiency of axonal transport could be jeopardized. Local regions of polarity flaws could cause organelles to accumulate, and physically impede the transport of other organelles, leading to even

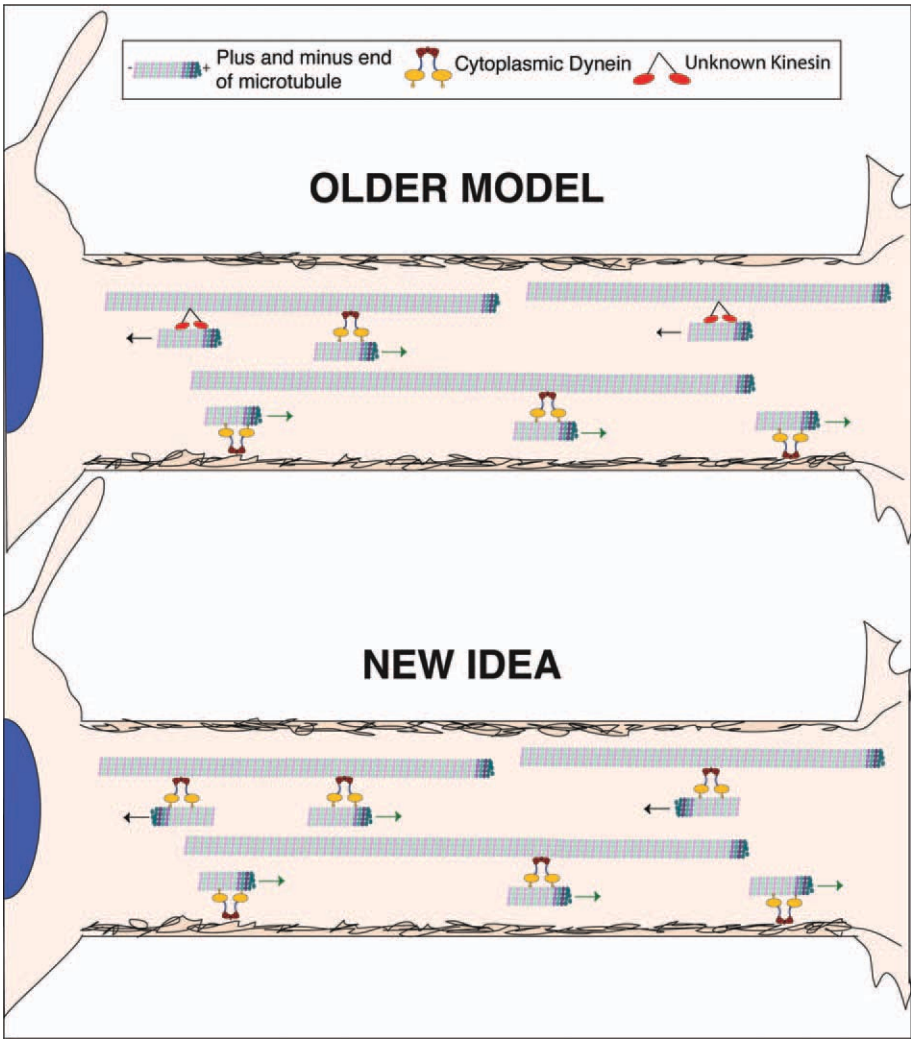

Fig. 4. Schematic illustrations comparing the previously proposed model for short microtubule transport in the axons versus a new model. In the old model, all of the short microtubules have a plus-end-distal polarity pattern and some of them are conveyed anterogradely by cytoplasmic dynein [Baas et al., 2006]. Other motors are necessary for the retrograde transport of the short microtubules and to contribute to their anterograde transport as well. In the newer model, the short microtubules moving anterogradely are oriented with their plus-ends distal while the short microtubules moving retrogradely are oriented with minus ends distal. In theory, with the new model, cytoplasmic dynein could account for all of the movement in both directions.

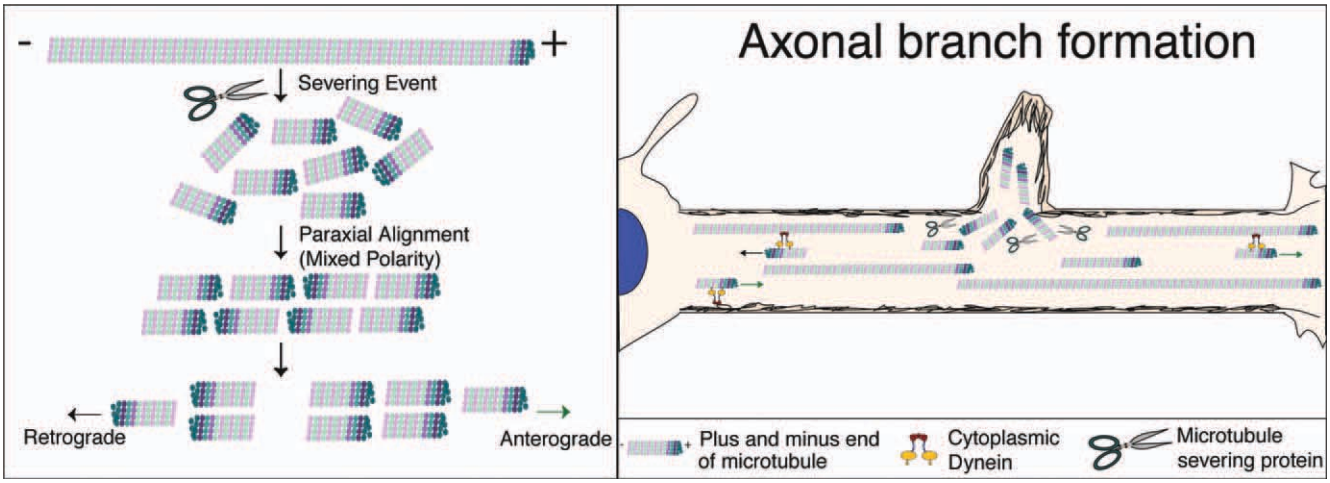

Fig. 5. Schematic illustration of how microtubule-severing events could contribute to the appearance of mal-oriented microtubules in the axon. Panel on the left shows how the severing of a long plus-end-distal microtubule results in a number of short microtubules that could tumble around, especially in broader flatter regions of the axon, to result in a mixture of short microtubules of both orientations [see Qiang et al., 2010]. According to the new model, illustrated in Fig. 4, the plus-end-distal short microtubules would then move anterogradely in the axon while the minus-end-distal microtubules would be cleared from the axon by moving retrogradely, back to the cell body. The panel on the right shows the necessity for microtubule severing in the formation of axonal branches, an event that could create minus-end-distal microtubules that subsequently need to be cleared.

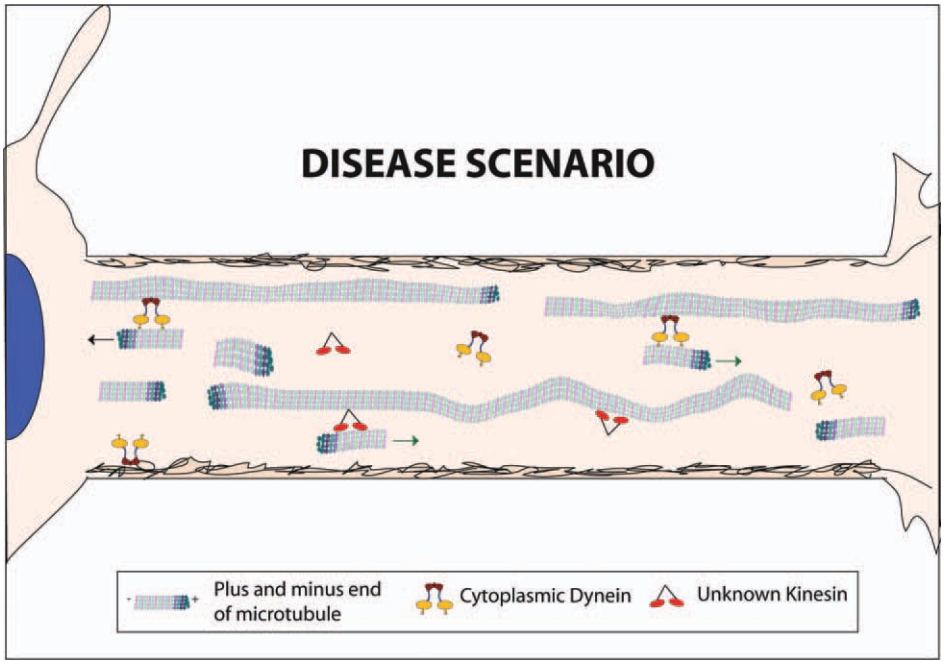

Fig. 6. Schematic illustration showing an example of how a disease scenario might result in flaws in microtubule transport, leading to flaws in microtubule polarity orientation in the axon. In turn, this would lead to flaws in the axonal transport of various cargoes.

greater problems [Kuznetsov and Avramenko, 2009]. We posit that flaws in microtubule polarity orientation could be an important and yet uninvestigated aspect of neurodegeneration (Fig. 6). In addition, when used for cancer treatment, microtubule-active drugs such as taxol are known to cause peripheral neuropathies [Carlson and Ocean, 2011]. This has generally been attributed to abnormal microtubule stabilization, but it is also possible that abnormally stimulating microtubule assembly causes the appearance of reverse-orientated microtubules in the axon, which in turn causes significant problems with the directionality of organelle transport along microtubules. Recent experimental evidence supports this view [Shamesh and Spira, 2010]. This point is important when considering the potential use of microtubule-active drugs for treatment of nerve injury [Hellal et al., 2011; Sengottuvel et al., 2011] and neurodegenerative diseases such as Alzheimer's [Trojanowski et al., 2005; Brunden et al., 2010, 2011]. For these various reasons, we would argue that understanding how the microtubule polarity pattern of the axon is preserved in the face of challenges will be essential for treating neurodegenerative diseases and also for taking appropriate caution when using microtubule-active drugs to treat disease and injury.

Moving Forward

The idea that all short microtubules move with their plus-ends leading is appealing in its simplicity, as well as the fact that it would be so functionally useful for the axon. Mechanistically, it would also be very simple, as one could envision cytoplasmic dynein doing the lion's share of the work, if not all of the work. One idea that we find appealing is that other motors have the capacity to transport microtubules and would indeed transport them, except that dynein is preferred and hence overrides the other motors. Thus, when dynein is depleted, the movement of microtubules does not cease, but the movement loses its appropriate characteristics because other motors have taken over what dynein should be doing (Fig. 7). This scenario would be consistent with what we have reported thus far on the impact of partial dynein depletion on microtubule transport [He et al., 2005] as with what the Jan laboratory has reported on the effects on *Drosophila* with dynein mutations, in which mal-oriented microtubules appear in the axon [Zheng et al., 2008]. In fact, such a scenario was proposed by the Jan laboratory as a likely explanation for their results. In our laboratory, we have also observed evidence for increased microtubule polarity flaws in axons of neurons in which we have more thoroughly depleted dynein (S. Lin and P.W. Baas, unpublished data).

Thus far, in this report, we have considered various scenarios that would be consistent with currently available data. Now the challenge is how we move forward experimentally to resolve exactly what is happening with regard to microtubule transport in the axon. In the past, we have argued that microtubule transport is important in the axon for many reasons, such as delivering new microtubules into the axon for the expansion of the microtubule array, for establishing the characteristic microtubule polarity pattern of the axon, and also because the same motor-driven forces that transport the short microtubules

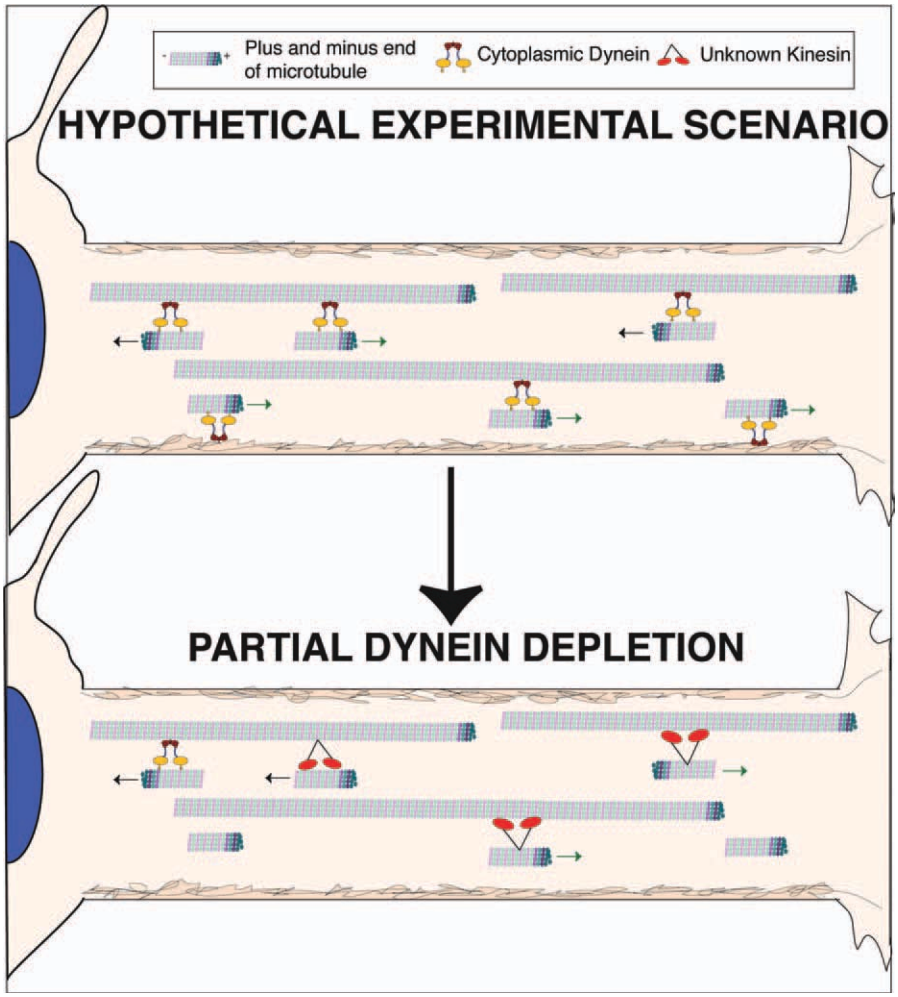

Fig. 7. Schematic illustration showing a possible scenario by which partial dynein depletion would result in changes in the movement of short microtubules with respect to their polarity orientation. In this scenario, when cytoplasmic dynein is depleted, other motors (as yet unidentified kinesins) that normally do not transport the microtubules, begin to do so.

impinge upon the long microtubules to regulate axonal navigation and to contribute to the balance of forces that determine whether the axon grows or retracts [Baas et al., 2006]. In addition to all of this, we now believe there is even greater urgency in resolving the issue mechanistically, because of the implications for disease and the potential side effects of microtubule-active drugs. The task will not likely be simple; however, as a growing body of evidence suggests that cells utilize motors in complicated ways [e.g., Ma and Chisholm, 2002; Mallik and Gross, 2004; Muller et al., 2008; Laib et al., 2009; Hendricks et al., 2009, 2010; Jolly and Gelfand, 2011; Steinberg, 2011]. Experimental complications include the usual sticky wicket of compensation, as gradual depletion of one motor might conceivably alter the profile of expression of other motors. Acute inactivation of motors, for example with function-blocking antibodies, would presumably dodge this kind of compensation, but has led to complicated results in the past [Brady et al., 1990], perhaps due to the presence of multiple motors simultaneously occupying a microtubule or cargo structure. As noted above, we strongly suspect that there is another type of compensation that might occur, with motors that normally do not transport microtubules starting to do so once the usual motor for transporting them is depleted or inactivated. In lieu of an approach with no shortcomings or complications, we suspect that it will take a coalescence of evidence from a variety of experimental approaches to unveil the motor-based mechanisms that underlie microtubule transport in the axon.

Probably the most important step in moving forward is to actually visualize the orientation of the moving microtubules in the axon. In addition to unequivocally resolving a key unknown factor, this kind of information would be required to evaluate the various mechanistic scenarios that remain on the table. For example, it would be fascinating to learn that depletion of dynein changes the microtubule array from one in which all of the mobile microtubules move with plus-ends leading to an array in which microtubules are moving in both directions with both polarity orientations or in different directions with different polarity orientations. How to do this

is not trivial, as the short mobile microtubules appear to be quite stable and hence do not display +tips at their plus ends. However, recent studies have shown that cells also express proteins that bind minus ends of microtubules [Meng et al., 2008; Goodwin and Vale, 2010; Meunier and Vernos, 2011], and the binding to the minus end does not depend on the ends being dynamic. On the contrary, the "minus tips" appear to confer stability to the microtubule, potentially making them ideal markers for revealing microtubule transport, if expressed as fluorescent fusion proteins.

Conclusions

We now favor the hypothesis that properly oriented (plus-end-distal) microtubules transit anterogradely down the axon, but that reverse-oriented (minus-end-distal) microtubules transit back to the cell body. The rare long minus-end-distal microtubules in the axon arise when short reverse-oriented microtubules elongate and become stationary before they can be cleared. This model is appealing because it only requires that short microtubules move with their plus-ends leading, regardless of their orientation. In terms of function, such a model is attractive because it gives purpose to the retrograde movement of microtubules, namely to prevent mal-oriented microtubules from accumulating in the axon, thereby preserving the microtubule polarity pattern that is so quintessential to the identity of the axon and its normal functioning. A mechanism for the relentless retrograde transport of mal-oriented microtubules would act as a self-repair mechanism to correct flaws in microtubule orientation that may occur when axons undergo plastic change, especially changes such as branch formation that involve a great deal of microtubule severing. Finally, this perspective justifies intensive study into the particulars of axonal microtubule transport, as it suggests that the axonal microtubule array is under constant risk of corruption, if the proper mechanisms are compromised during disease, injury, or treatment of patients with microtubule-active drugs. Studies are underway in our laboratory to advance our knowledge on these important issues.

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