Slow Axonal Transport and the Genesis of Neuronal Morphology

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ABSTRACT: The classic view of slow axonal transport maintains that microtubules, neurofilaments, and actin filaments move down the axon relatively coherently at rates significantly slower than those characteristic of known motor proteins. Recent studies indicate that the movement of these cytoskeletal polymers is actually rapid, asynchronous, intermittent, and most probably fueled by familiar motors such as kinesins, myosins, and cytoplasmic dynein. This new view, which is supported by both live-cell imaging and mechanistic analyses, suggests that slow axonal transport is both rapid and plastic, and hence could underlie transformations in neuronal morphology.

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INTRODUCTION

Neurons are arguably the most polarized cells in nature, and the cell type whose morphology is most intimately related to its specialized functions. Vertebrate neurons, such as the pyramidal neurons of the hippocampus or cerebral cortex or the large neurons that comprise peripheral sympathetic ganglia, extend a single thin axon that typically grows very long as well as multiple thick, tapering dendrites that remain comparatively short. Other neurons have their own distinctive morphologies, such as monopolar sensory neurons (from the dorsal root ganglia), which extend a single axon that bifurcates and no dendrites whatsoever. The Purkinje neurons of the cerebellum, by contrast, have unusually thick and prominent dendrites. At the tip of the growing axon is a specialized structure called the growth cone, which is highly responsive to environmental cues that direct activities such as turning, collapse, and consolidation. Axons and dendrites can both sprout and branch extensively during their development, and despite their impressive morphologies, can undergo substantial bouts of retraction, often disappearing entirely. Sprouting of dendrites continues well beyond birth, and axonal regeneration, while not as robust as clinicians would like, is certainly possible in the adult animal. A key priority of contemporary neuroscience is to better understand how neuronal morphogenesis is specified and orchestrated.

Neurons are rich in cytoskeletal elements, namely microtubules, actin filaments, and neurofilaments. These polymers play key architectural roles, and thereby contribute significantly to the acquisition of asymmetrical cellular morphologies. In addition, microtubules and actin filaments act as railways for the motor-based transport of subcellular organelles. Because motor proteins move along these filaments in one direction or the other with regard to the intrinsic polarity of the filament, distinct patterns of filament polarity orientation can generate asymmetries in the composition of each neuronal compartment (Black

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and Baas, 1989). Organelles generally move very short distances along actin filaments, but take much longer journeys along microtubules (Langford, 1995). The rapid and robust movement of organelles along microtubules within the axon is classically referred to as "fast axonal transport." Dendritic transport of this kind occurs as well, but is somewhat different given that dendrites contain microtubules of both polarity orientations while axons contain exclusively microtubules with their plus ends directed away from the cell body (Baas et al., 1988; Sharp et al., 1995). Actin-based organelle transport is particularly important in regions of axons and dendrites that are not rich in microtubules, such as dendritic spines or the leading edge of the axonal growth cone.

The other type of axonal transport, termed "slow axonal transport," has been more perplexing to understand, and therefore has received less attention over the years. Slow transport is the movement of the proteins that comprise the cytoskeleton itself, which also must be conveyed from their sites of synthesis within the cell body down the length of the axon. The original conception of slow transport was that microtubules, actin filaments, and neurofilaments are transported as an interconnected array of polymers that move continuously, very slowly (compared to the rate of fast transport), and exclusively anterogradely down the axon (Lasek, 1982). Little attention was given to any potential correlate for slow axonal transport within dendrites. This view of slow transport, which brings to mind a glacier inching along the landscape, is hard to understand mechanistically. Efficient movement within cells is usually generated by molecular motor proteins, and yet all known motors move at significantly greater rates than the documented rates of slow axonal transport. Moreover, it is difficult to imagine that rapid and highly plastic transitions in neuronal morphology could be the result of alterations in transport that is so slow. Today, however, there are new insights into slow transport based on contemporary live-cell imaging studies, as well as growing knowledge of molecular motor proteins. These new insights suggest the compelling possibility that slow axonal transport may not be a lumbering glacier after all, but more likely is a collection of short quick independent cytoskeletal movements regulated by familiar motors and cytoskeletal accessory proteins. Based on this, we propose that alterations in slow transport could be at the heart of the dramatic and rapid cytoskeletal reconfigurations that underlie changes in neuronal morphology. In this article, we explore the merits of this proposal.

SLOW TRANSPORT IS FAST

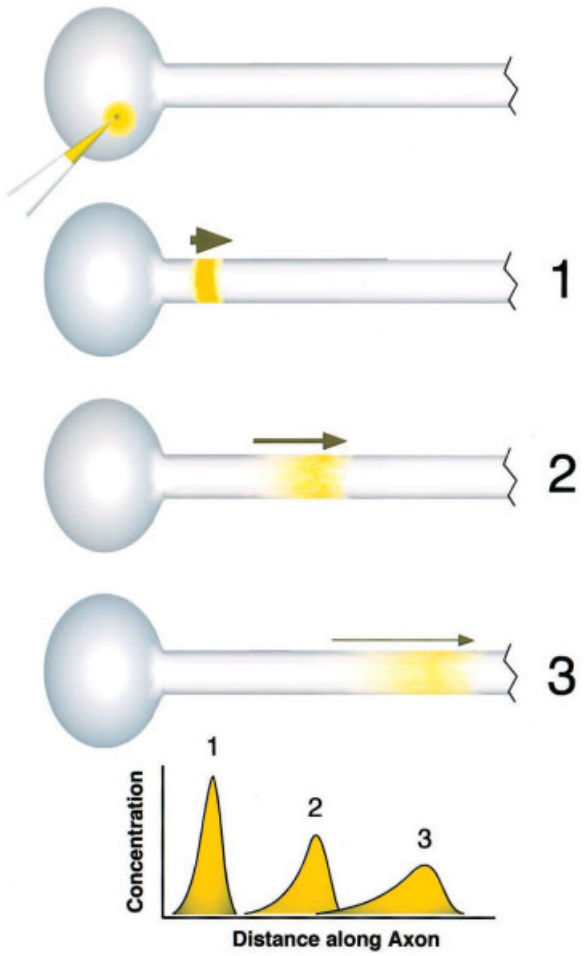
Slow transport was first appreciated in studies on whole animal nerves using isotopic labeling of newly synthesized proteins within a cluster of cell bodies whose axons comprised a nerve (reviewed in Lasek, 1988; Baas and Brown, 1997; Brown, 2000). In this technique, exposure of the cell bodies to radiolabeled amino acids results in the manufacture of labeled proteins, which are then conveyed down the length of the axon (Fig. 1). Because the cell bodies are not continuously exposed to the labeled amino acids, the labeled proteins appear as a pulse that travels down the axon as a wave (with a leading edge and a trailing edge). The rates of movement of different proteins are ascertained by cutting the nerve into pieces and separating and visualizing the proteins by electrophoresis and autoradiography. Membrane-associated proteins were shown to move quickly, as the "fast" component of axonal transport, while cytoskeletal and cytosolic proteins were shown to move significantly more slowly. Slow axonal transport was shown to consist of two subcomponents, the faster of which contains actin, actin-associated proteins, a small portion of the tubulin, and a huge array of cytosolic proteins that presumably bind to the actin "cytomatrix" (Black and Lasek, 1980). The slower subcomponent of slow transport contains most of the tubulin, the neurofilament proteins, and proteins known to associate with microtubules or neurofilaments. The waves of transported proteins spread as they moved down the axon, suggesting some asynchrony in the rates of movement of individual elements within each component (Fig. 1, bottom). However, the early interpretations of the work stressed the coherence over the spread, and it was proposed that cytoskeletal proteins are transported in the form of polymer-networks that move continuously down the axon at these slow rates.

The idea that cytoskeletal polymers gather together and move essentially in unison at a snail's pace down the axon was challenged in the early 1980's by several live-cell imaging studies that used a method that certainly should have been able to reveal such movement if it exists. The studies were performed on the axons of cultured neurons or the short axons of zebrafish or insects (see for example, Lim et al., 1990; Sabry et al., 1995). In these studies, the polymers were allowed to incorporate subunits that were either tagged with a fluorescent probe or a probe that fluoresces upon photoactivation. In either case, a small mark was made across the polymer array by either bleaching the fluorescent probe or activating the photoactivatable probe, and the mark was then observed for slow

synchronous movement (Fig. 2, left). The movement did not occur. While some authors concluded that cytoskeletal proteins do not move as polymers at all (Hirokawa et al., 1997), other authors pointed to the possibility that the polymers probably do move, but not as synchronously as once thought (Baas, 2000a, 2002). Additionally, in these studies, images were acquired at intervals of time on the order of several minutes apart, and thus faster movements would have been entirely missed. Indeed, ground-breaking new work has now shown that microtubules and neurofilaments both move as polymers, but move in short and intermittent bursts (Wang et al., 2000; Roy et al., 2000; Wang and Brown, 2001, 2002). These bursts of movement occur at rates similar to fast transport. These new studies employed much longer photomarks or natural gaps in the neurofilament array, with image acquisition every several seconds instead of minutes (Fig. 2, right). The vast majority of the polymers was shown to be in a "pausing" state at any given moment, while roughly 10% of them underwent these bouts of rapid movement. Interestingly, a small fraction of the polymer movements occurred in the retrograde direction. Thus the rate of slow transport reflects an average of infrequent fast movements, pausing, and even occasional backward retreats. These bouts of movement and nonmovement could certainly combine mathematically to appear in a large nerve as slow plodding progress of the cytoskeleton down the axon. The beauty of these observations is that they fully explain and reconcile the results of the earlier radiolabel studies with the results of the photomark studies, and also squarely place the rates and features of polymer movements within the parameters of known motor proteins. They also validate contemporary interpretations of the original radiolabel studies, which put more emphasis on the spread within the waves of movement (Lasek et al., 1993; Lasek, 1986), and an abundance of indirect data indicating rapid polymer movements within developing axons (see for example Yu et al., 1996; Slaughter et al., 1997).

SLOW TRANSPORT UTILIZES FAST MOTORS

In the early days of slow transport studies, it was generally assumed that neurons contain a highly spe-



COLOR

Figure 1 Early studies of slow axonal transport utilized pulse-chase experiments to determine the average transport properties of components of the cytoskeleton and cytoskeletal-associated proteins. Typically, radiolabeled precursors were introduced into the vicinity of neuronal cell bodies (diagrammatically shown as the microinjection of labeled precursor into the soma) and eventually became incorporated into the cytoskeleton as radiolabeled components and associated proteins that then moved through the axon. The average, overall progress of the radiolabel could be tracked by cutting nerves containing axons into pieces and analyzing the pieces using biochemical techniques (for example, scintillation counting and autoradiography of SDS-PAGE gels). By analyzing a number of axons at different times after introduction of the radiolabel, the average transport rates and the dispersions of different cytoskeletal components within the axon could be determined. The radiolabeled portion initially appeared as a sharp pulse, a relatively narrow and concentrated band within the axon, as a result of incorporation of the transient high titer of radiolabeled precursor into the axonal cytoskeleton (represented as a yellow band in the axon at time point 1). With increasing time (time points 2 and 3), the pulse moved distally through the axon as a result of slow axonal transport and also dispersed as a result of inhomogeneity of the transport process. These results are graphically illustrated at the bottom; the peak numbers correspond to the three time points.

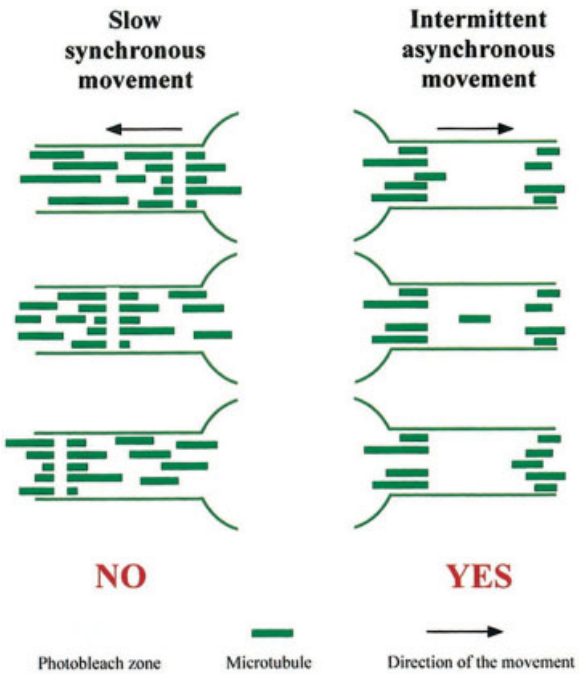


Figure 2 The older and newer approaches for analyzing slow axonal transport by photobleaching experiments are similar in that both involve photobleaching of regions of axons that were previously microinjected with fluorescently tagged protein subunits to generate uniformly labeled cytoskeletal polymer. (Variations include photoactivation instead of photobleach, introduction of fluorescent subunits at the single-cell stage of embryonic development, and expression of GFP-tagged subunits.) A critical difference between the approaches concerns the size of the photobleached region. The older protocol called for the photobleaching of a relatively narrow band of the axon; this band served as a fiduciary mark that could be followed if the cytoskeletal polymers moved en masse through the axon (represented by a gray band in the axons on the left side of the figure). This approach failed to obtain evidence for the en masse transport of cytoskeletal polymers. The figure displays the type of movement that was anticipated, but actually did not occur. On the other hand, recent successful experiments have utilized relatively broad spans of photobleached axon; these regions serve as a dark backdrop across which fluorescent polymers were seen to translocate (illustrated by the panels on the right side of the figure). The results indicate cytoskeletal polymers do not move slowly and synchronously, but rather translocate as individual filaments moving at velocities consistent with motor-driven activities.

cialized set of tools for transporting cytoskeletal polymers from the cell body down the length of the axon. At the time, the unknown mechanisms for moving the polymers were usually referred to as "the transport machinery," but later, it was concluded that such machinery must involve molecular motor proteins. In fact, one of the confounding features of a polymer-transport model was that it appeared to demand motor proteins with much slower rates than any known class of motor protein. This was particularly worrisome because the motors that move cytoskeletal polymers must be abundant along the length of the axon, and it seemed increasingly unlikely over the years that entirely new motors of such abundance were likely to be discovered, particularly with the genome projects progressing so rapidly. The recent live-cell observations indicating fast but intermittent transport of polymers alleviate this concern, and indicate that the motors for slow transport are actually fast motors, and hence are probably already identified motors such as cytoplasmic dynein and members of the myosin and kinesin families. In fact, it has already been demonstrated that such motors, when adhered to glass coverslips, can support the rapid movement of cytoskeletal polymers along the surface of the glass (for review see Vale, 1999). Presumably the motors are attached to substrates within the neuron with a greater resistance to movement than the polymer in question, and hence, the polymer moves. Another possibility is that one polymer moves as "cargo" along the length of another polymer, while a final possibility is that one polymer simply "piggy-backs" on another polymer that moves by one of the two other mechanisms.

There is growing precedent across cell types for the view that cytoskeletal polymers, particularly microtubules, are organized by forces generated by motor proteins. Particularly good progress has been made on the mitotic spindle, the most fundamental of all eukaryotic microtubule arrays (for review see Sharp et al., 2000b). Cytoplasmic dynein, a minus-end-directed motor, plays multiple roles in mitosis, including generating forces between the astral microtubules and the actin-based cell cortex to help separate the half-spindles during anaphase (Sharp et al., 2000a). If the astral microtubules were to lose their association with the centrosome, it is not difficult to imagine that the same dynein-based forces would propel them toward the leading edge of the cell and down the length of a cellular extension such as an axon (Baas, 1996, 1999, 2002). Interestingly, the vast majority of the cytoplasmic dynein that is anterogradely transported down the axon moves in the same rate component as the actin, suggesting that microtubules may move down the axon by pushing against the actin cytomatrix (Dillman et al., 1996). In this view, the cargo domain of the cytoplasmic dynein molecule is associated with the actin cytomatrix, leaving the motor domain available to interact with microtubules (Pfister, 1999). Such interactions could be intermittent, explaining why microtubules often pause in their movement and why microtubule transport is slower

on average than actin transport. Experimental support for this model has been provided in studies showing that disabling cytoplasmic dynein compromises axonal growth and curtails the movement of microtubules from the centrosome into developing axons (Ahmad et al., 1998).

Work on mitotic extracts has shown that it is also possible for cytoplasmic dynein to move microtubules relative to other microtubules (Heald et al., 1997). If this holds true in the axon as well, such forces could cause a microtubule to move either anterogradely or retrogradely, depending on which of the two microtubules has the lesser resistance to movement and which microtubule interfaces with the motor-domain of the dynein molecule. Other motors might also contribute to the movement of microtubules in the axon. The mitotic spindle utilizes a vast array of complementary and antagonistic motor-driven forces (Sharp et al., 2000a,b), and perhaps this is also true of the axonal cytoskeleton. Specific kinesin-related proteins have been identified in the mitotic spindle that are designed not for vesicle transport but specifically for force generation between microtubules and other cytoskeletal elements. These kinesin-related proteins were initially thought to be mitosis-specific because they appeared to be inactive during interphase. However, recent studies have revealed that these motors continue to be expressed in terminally postmitotic neurons, where they are prominent components of the microtubule arrays of axons or dendrites (Baas, 1999). The best-studied example is CHO1/MKLP1, originally discovered in the midzonal region of the mitotic spindle where microtubules of opposite polarity orientation overlap (Nislow et al., 1992). This motor was found to be necessary for the transport of a population of microtubules into dendrites oriented oppositely to those found in the axon (Sharp et al., 1997b; Ferhat et al., 1998b). Based on studies involving experimental depletion of CHO1/MKLP1 from developing neurons, we have proposed a model whereby the plus-end-distal microtubules are transported into the dendrite by cytoplasmic dynein (just as in the axon), thus providing a substrate against which the minus-end-distal microtubules can be transported by CHO1/MKLP1 (Yu et al., 2000). There is no detectable CHO1/MKLP1 in the axon, but other recent studies have demonstrated that axons (and dendrites) contain another mitotic motor protein called Eg5, which can also generate strong forces on microtubules (Ferhat et al., 1998a). A final means for moving microtubules appears to be particularly important in growth cones, where retrogradely moving actin filaments can carry microtubules along with them, using myosin-driven forces (Kabir et al., 2001; Schaefer et al., 2002). Current work in our laboratory is aimed at documenting the array of motor proteins that transport microtubules in different regions of the neuron.

There is far less experimental evidence relevant to the motors that transport actin and neurofilaments. Actin filaments are presumably transported by myosins. This has been shown to be true of the dramatic retrograde movement of actin filaments observed within nonmoving growth cones (Lin et al., 1996; Bridgman et al., 2001). However, little is known about the anterograde transport of actin down the length of the axon. The substrate the actin moves against is unknown, as are the particular configuration and polarity orientation of the moving filaments (although there is some evidence that axonal actin filaments may be of both polarity orientations; see Bearer and Reese, 1999). It is noteworthy that actin is configured differently within different domains of the axon. Actin is most concentrated in the cortex, just beneath the cell membrane, where the filaments are configured into a meshwork. Because the filaments within the array are not aligned paraxially, it is difficult to understand how myosin could move them along the long axis of the axon. Instead, myosin appears to produce contractile forces within the cortical actin that can actually cause axons to retract if unopposed (Ahmad et al., 2000; Gallo et al., 2002). The rest of the actin filaments appear to be aligned paraxially within the axonal shaft (Bearer and Reese, 1999), and these filaments may be better candidates for anterograde movement by myosins. Radiolabel studies have shown that both cortical and noncortical actin progress down the length of the axon (Heriot et al., 1985), but it seems possible that this is due to one population of moving filaments exchanging subunits with another population of nonmoving filaments. Obviously these ideas are highly speculative at present, and a great deal of work will be required to test their merit and elucidate precisely how motors transport actin within the axon and other regions of the neuron.

Neurofilaments do not interact directly with motors via their motor-domains, leaving two possibilities for how neurofilaments might be transported (Brady, 2000). One possibility is that the neurofilaments simply piggyback on moving microtubules. This model is attractive because it can explain why neurofilament transport rates are so extremely similar to microtubule transport rates. The other possibility is that neurofilaments are carried as cargo along the microtubules, in similar fashion to vesicular cargo. This model does not explain why microtubules and neurofilaments are conveyed in the same rate component of slow transport, but at present is supported by observations of kinesin association with neurofilaments and other

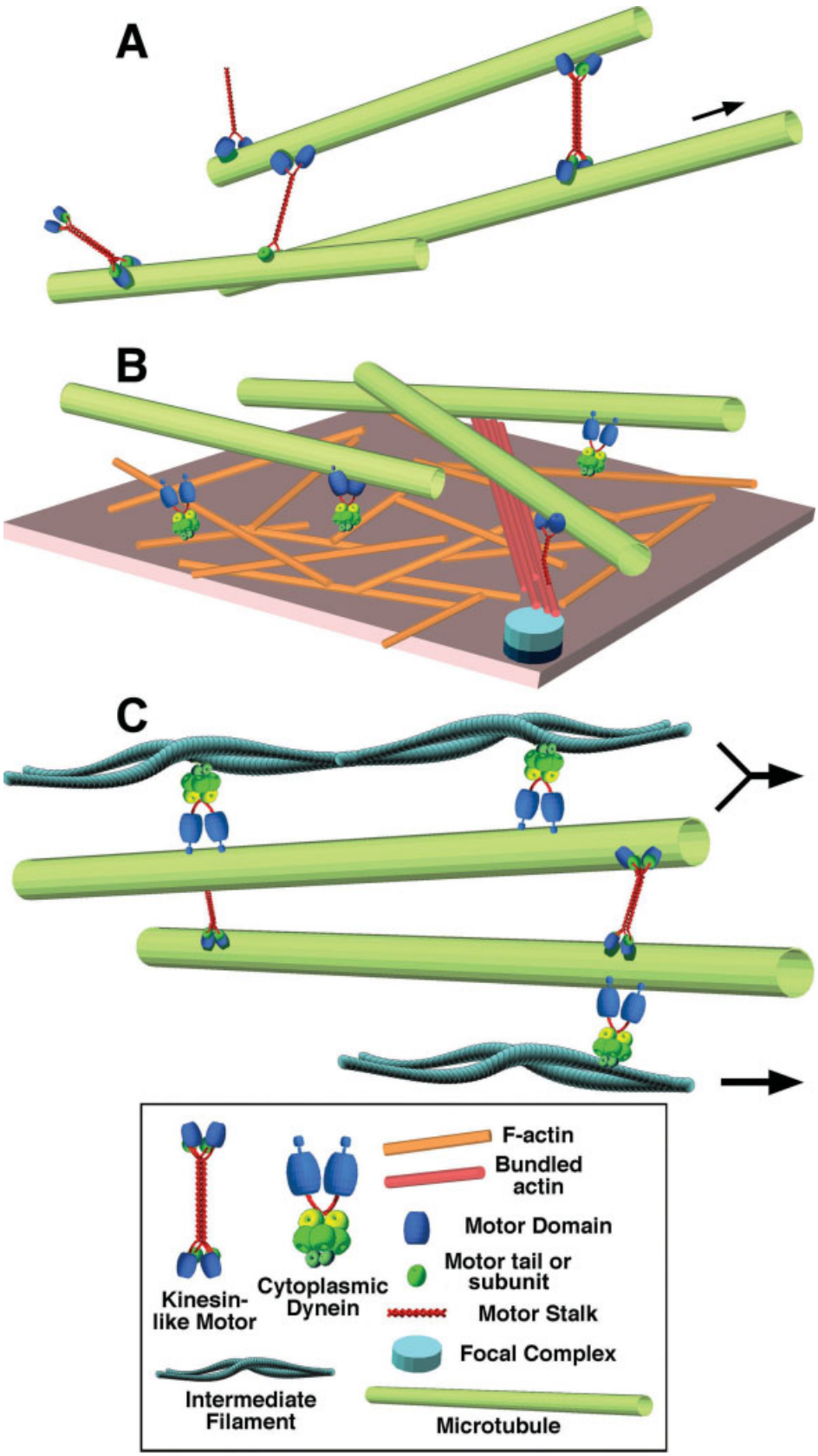

A
B
C
F-actin
Bundled actin
Motor Domain
Motor tail or subunit
Motor Stalk
Focal Complex
Microtubule
Kinesin-like Motor
Cytoplasmic Dynein
Intermediate Filament

Figure 3

types of intermediate filaments (Liao and Gundersen, 1998; Yabe et al., 1999; Prahlad et al., 1998). Kinesin, being plus-end-directed, would be the appropriate motor for moving neurofilaments anterogradely down the axon, if the movement were to occur along the microtubules rather than together with them.

Figure 3 schematically illustrates possibilities for the means by which microtubules, neurofilaments, and actin filaments may interact with motor proteins during slow axonal transport.

SLOW TRANSPORT AND POLYMER LENGTH

As suggested above (and discussed later in the article), it seems reasonable that the efficiency and rates of polymer transport are regulated in part by opposing motor forces. Several lines of evidence suggest that neurons can also regulate the movement of cytoskeletal polymers by altering their length. In recent live-cell work, all of the microtubules observed to move were at best only a few microns in length. This was the case in growth cones and branch points (Dent et al., 1999; Gallo and Letourneau, 1999), but also held true within the axonal shaft itself (Wang and Brown, 2002). Notably, we observed an inverse relationship between the length of a microtubule and its rate of movement (Dent et al., 1999), prompting us to conclude that shorter microtubules move quicker because there is less resistance to overcome. One obvious mechanism for regulating polymer length is through dynamics (addition and loss of subunits), but the principles of dynamic instability that regulate microtubules strongly favor the tendency of large numbers of short polymers to shift toward smaller numbers of longer polymers. In order for the polymers to become significantly shorter, additional regulatory molecules would presumably have to impinge upon the dynamics. Of course, one need only open a biology textbook to be reminded of the bewildering array of proteins that sever actin filaments and regulate their dynamics. With regard to microtubules, neurons contain a variety of factors that can promote assembly (such as CRMP2, see Fukata et al., 2002) or disassembly (such as SCG10, see Nixon et al., 2002). Even so, we suspect that neurons probably require a more direct means for fracturing long microtubules into short ones to enhance their speed, particularly in regions of the neuron participating in active growth. In support of this, we have documented evidence for particularly active microtubule severing in regions of new axonal branch formation using both serial reconstruction of electron micrographs and live-cell imaging (Yu et al., 1994; Dent et al., 1999) (Fig. 4).

Katanin is an ATPase with potent microtubule severing properties, initially studied in mitotic extracts. It consists of two subunits, one that severs microtubules and the other that targets the protein to the pericentriolar region of the centrosome (for review see McNally, 2000). As such, katanin was proposed to sever microtubules after their nucleation at the centrosome. In an experimental study, we determined that katanin is indeed required for microtubules to be "released" from the neuronal centrosome so that they can be transported into developing axons and den-

Figure 3 Microtubule motors can interact with a variety of "cargoes," including other cytoskeletal polymers, and exert a force that either tethers the filaments or moves one filament relative to another. (A) Analogous to the microtubule motors of a mitotic spindle, motors within neurons are believed to move and organize microtubules within axons and dendrites, leading to behaviors such as neurite extension, branching, turning, and retraction. Motors are shown spanning between microtubules to either bundle or translocate the polymers. (B) Microtubule motors are also believed to interact with heterologous components of the cytoskeleton, allowing the coordination of the different cytoskeletal systems. Strong circumstantial evidence indicates that neuronal microtubules interact with actin filaments (see text). Such interactions probably require participation of microtubule motors, and probably include the interactions between microtubules and cortical actin (shown as a network of orange filaments overlying the plasma membrane) and between microtubules and actin bundles (shown as a bundle of red filaments). (C) The translocation of intermediate filaments probably requires microtubules. Two models of microtubule-mediated transport of intermediate filaments are shown. First, intermediate filaments may be tethered to microtubules by motors capable of interacting with both filament systems; as microtubules translocate, intermediate filaments that "piggyback" on the microtubules are also translocated (indicated by the two-tailed arrow). Second, microtubule motors may carry intermediate filaments as cargo while moving along microtubules (bottom arrow). For simplicity, the numerous accessory proteins that are required for these activities have not been indicated.

drites (Ahmad et al., 1999). Interestingly, however, the story was not as simple as this. We also found katanin distributed throughout the entire neuron, not just at the centrosome, and we found that inhibition of katanin function was far more deleterious to axogenesis than would be predicted only on the basis of its function at the centrosome. In fact, the microtubules became exceedingly long throughout the cell body, and their excess length probably prohibited them from being transported in an orderly fashion into the developing axons. We suspect that katanin regulates microtubule length throughout the neuron, and is particularly important for breaking microtubules into smaller polymers in regions of active and rapid growth such as early branches, sprouts, and growth cones. It is documented from *in vitro* studies that katanin, if constitutively active, would sever microtubules until no polymer was left (McNally and Vale, 1993). For this reason, it seems clear that the neuron must have mechanisms for activating and deactivating katanin, and for doing so strategically and locally. Interestingly, a protein called spastin, known for

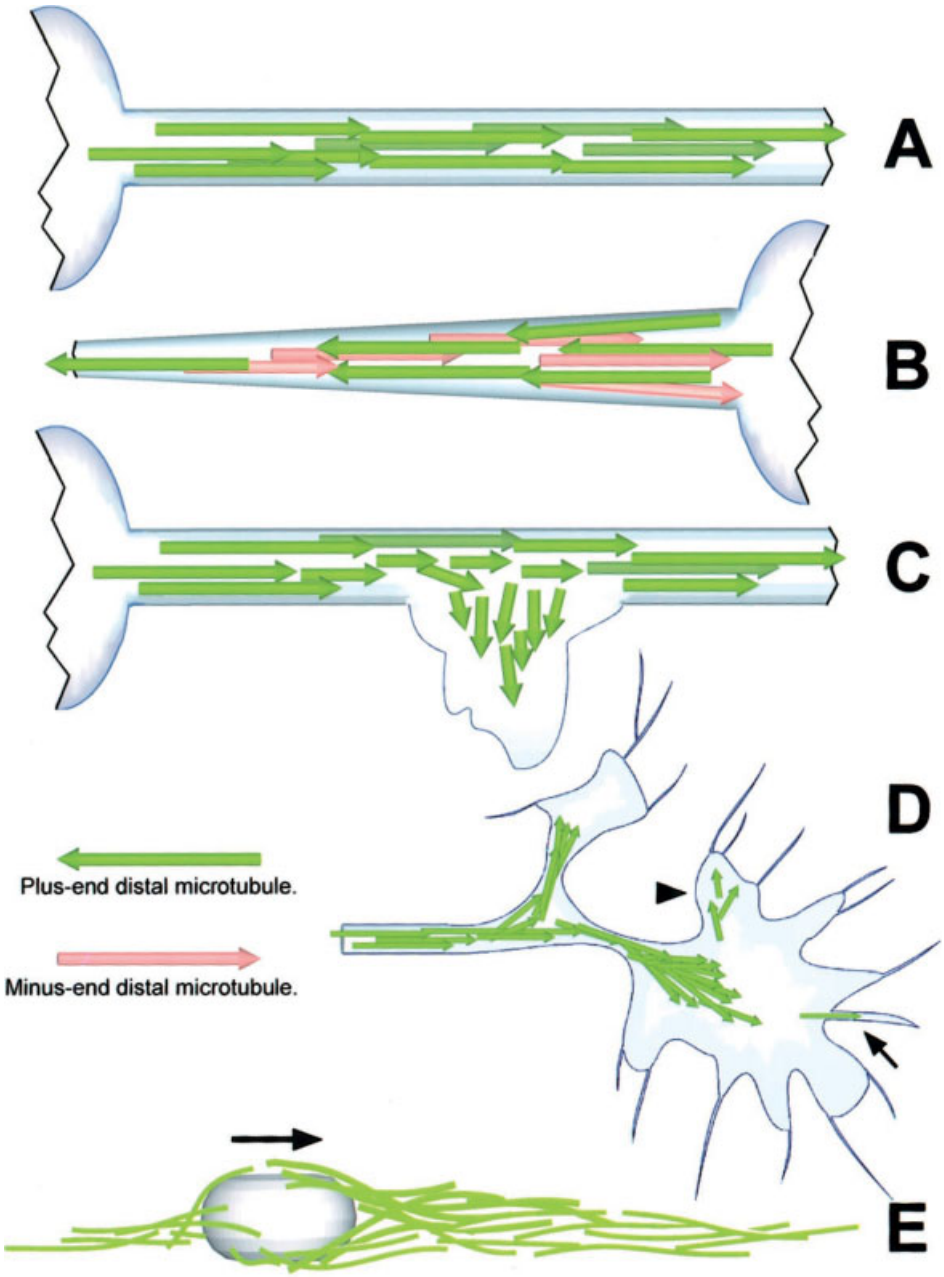


Figure 4

many years to be a target for mutations underlying clinical spastic neuropathies, has a great deal of homology with katanin, and has recently been shown to have similar microtubule severing properties (Errico et al., 2002).

Most of the neurofilaments observed to move have also been relatively short compared to the average length of the polymers documented within axons (see Wang et al., 2000; Wang and Brown, 2001; Roy et al., 2000). Notably, however, proteins that can depolymerize or sever neurofilaments physiologically are virtually unknown. It may be relevant to note that neurofilament proteins have been observed in a variety of forms after expression of tagged subunits in neuronal cells. These forms include filaments of various lengths, but also include "squiggles and dots" that have been proposed to be nonpolymeric precursors for the filaments (Shea, 2000). Alternatively, these structures could be rolled-up short filaments (Wang et al., 2000; Wang and Brown, 2001), or they might be artifacts of rapid abnormal expression. Notably, Goldman and colleagues have directly observed similar squiggles and dots of expressed intermediate filament proteins actively moving about the cytoplasm of non-neuronal cells and appearing to incorporate into the filament network (Prahlad et al., 1998; Chou and Goldman, 2000). While it is possible that these squiggles and dots represent a physiological "transport form" of the protein, it is also possible that they represent broken filaments that are able to use motor-driven mechanisms to recover from injury. In support of this latter idea, the squiggles and dots are far more abundant in cells that have been actively triturated than in cells that have not been disturbed for long periods of time. Additional studies will be required to ascertain whether the squiggles and dots are a normal transport form, a mode of recovery from injury, or simply iatrogenic.

SLOW TRANSPORT AND AXONAL RETRACTION

An older notion of slow transport was that cytoskeletal polymers are churned out from the cell body and pushed down the axon in a fashion that might bring to mind a pasta machine. The purpose of slow transport, in this and other earlier models, was not to remodel the cytoskeleton but to provide a relentlessly moving supply of polymer to replenish the axonal cytoskeleton during the life of the axon (Lasek, 1982). The contemporary observations suggest that the transport machinery is much more flexible, and can be locally regulated within the axon to play critical roles in plastic events underlying the remodeling of the axonal and dendritic arbors. Retraction is one such event. It is known that axonal arbors overgrow during the development of the nervous system, and a great deal of pruning occurs to achieve the final pattern of wiring. Examples of selective axonal retraction are seen in the

Figure 4 The microtubule arrays of neurons are organized in a manner characteristic for specific regions of neurons and for neurons displaying specific behaviors. Microtubules in axons are organized uniformly with their plus-ends distal to the cell body (A), while microtubules in dendrites have a mixed polarity—some microtubules are oriented with plus-ends distal and some with minus-ends distal (B). During the formation of a collateral branch, axonal microtubules at the branch site become significantly shorter (C) possibly as the result of microtubule severing by local activation of the homogeneously distributed enzyme, katanin. Microtubule shortening may be a necessary prelude to the motor-driven translocation of microtubules into the nascent branch. Within growth cones, microtubules are usually confined to a central region. However, individual microtubules can be observed to invade the actin-rich transition and peripheral zones (D). Because microtubule movements within these zones closely follow actin distributions and movements, these cytoskeletal systems might interact in part via motors (as well as other non-motor crosslinking proteins) that can associate with both systems (for example, arrow indicates a microtubule invading a growth cone filopodium along an actin bundle track). Growth cone turning is a necessary behavior for the proper navigation of an axon to its target, and is typified by the invasion of microtubules into a protrusive region of the growth cone in coordination with changes in actin organization (arrowhead). For simplicity, only microtubules are shown. Migrating neurons must transport the relatively massive organelles within the cell body, like the elliptical nucleus shown in (E), over a substratum. Microtubules of migrating neurons are arranged as a cagelike network around the nucleus and are interleaved with microtubules filling the rather short leading extension. If the microtubules were tethered by motors, the network could act as a coherent unit to transmit the tractile forces generated in the leading extension back to the microtubule-associated organelles in the cell body, allowing these structures to be dragged along with the leading extension.

withdrawal of all but one axon from each muscle fiber (Gan and Lichtman, 1998) and the withdrawal of occipital neuronal axons projecting to the spinal cord (Stanfield et al., 1982). Until recently, the prevailing view was that axonal retraction involves large-scale disassembly of the cytoskeletal polymers (Song and Poo, 1999). However, there are several reasons to question this view (discussed in Baas and Ahmad, 2001). For example, experimental depolymerization of microtubules can certainly cause axons to retract, but the morphology of such retracting axons is completely different from that of axons retracting physiologically. The former produces a thin withering axon with beads along its length, while the latter produces sinusoidal bends along the retracting axon, no beads, a thickened diameter during retraction, and a trailing streak at the tip of the shortening axon. In addition, it is well documented that global actin disassembly does not cause axons to retract, but actually inhibits their retraction (for discussion see Baas and Ahmad, 2001). Thus it does not appear to make sense that physiological retraction of axons would be induced by a wholesale loss of cytoskeletal polymers. Alternatively, might axonal retraction be a consequence of alterations in slow transport?

In a recent article, we investigated changes in the microtubule array during axonal retraction induced by nitric oxide in cultures of vertebrate sensory neurons (He et al., 2002). We found that there is no detectable loss of microtubules during retraction, at least in the early phases of retraction, and that pharmacologic inhibition of microtubule disassembly did not prevent the retraction from occurring. During retraction, the microtubules retreat backward, and become coiled and bent to accommodate the shape of the shortening axon. These observations indicate backward movement of microtubules. However, rather than a direct reversal of slow transport, it appears that the microtubule array is dragged backward, not as individual polymers, but as a mass of polymers. Axonal retraction is known to require actomyosin-based contractility (Ahmad et al., 2000), suggesting that this contractility can overwhelm any other forces that tend to drive the axon to grow, including slow anterograde transport. Interestingly, we have observed that inhibition of cytoplasmic dynein not only stops axons of cultured neurons from growing as expected, but it also causes axons to retract (Ahmad et al., 2000). Recent studies have shown that inhibition of cytoplasmic dynein in *Drosophila* causes even mature axons to break free of their synapses and retract (Eaton et al., 2002). While the explanation for this phenomenon remains uncertain, one plausible explanation is that the dynein-driven transport of microtubules against the actin cytomatrix might attenuate the contractility of the actomyosin system that would otherwise cause the axon to retract (Ahmad et al., 2000). Thus, either decreases in dynein-forces or increases in myosin-driven forces could shift an elongating axon to a bout of retraction.

These observations regarding axonal retraction shed new light on the complexity of slow transport, suggesting that the forces that drive cytoskeletal polymers to move forward could play additional roles as well. In particular, these forces appear to integrate the cytoskeleton into a functional unit, such that a shift in one type of force can produce dramatic effects on more than one of the polymer systems. This view also fits well with the live-cell observations suggesting a great deal of nonmovement of polymers at any given moment. It may be that forces are at play to prevent movement of the polymers as well as to generate movement, the former being akin to an isometric exercise. Such an interplay of motor-driven forces is precisely what occurs within the mitotic spindle (Sharp et al., 2000a,b), and it is provocative to speculate that a similar scenario exists for the axon.

SLOW TRANSPORT, NEURONAL MIGRATION, AND GROWTH CONE MOTILITY

During the development of the nervous system, the cell bodies of many neurons migrate over fairly substantial distances to arrive at their ultimate destinations. In these cases, the cell body generally extends a "leading process" in the direction of movement, and also displays a "trailing process" on its opposite end (Rakic et al., 1996). These two processes do not elongate significantly, but rather appear to guide the cell body in its movements. Once the cell body has arrived at its final destination, the leading process can then elongate away from the cell body and become a bona fide axon. Recent studies have begun to reveal new and interesting molecules, such as Lis1 and doublecortin, which are crucial for the cytoskeleton to participate in neuronal migration (for review see Feng and Walsh, 2001). Both of these molecules influence microtubules, suggesting that differences in the microtubule array may distinguish a migrating cell body from a stationary cell body. Stable microtubules are organized into a "cage" that surrounds the nucleus of the neuron (Letourneau and Wire, 1995) (Fig. 4). In migratory neurons, this cage appears to be dragged along with the microtubules that are propelled forward into the leading process. Although the precise functions of the relevant molecules are not entirely

clear, it appears that doublecortin is a microtubule stabilizer (Lin et al., 2000) and hence is probably important for organizing the microtubule cage. Lis1, on the other hand, is a regulator of cytoplasmic dynein (Smith et al., 2000). A possibility that we find compelling is that the same slow transport mechanisms that would otherwise convey individual microtubules from the cell body down the elongating axon are responsible for moving the entire microtubule array as a "unit" in migrating neurons. In this view, a key difference between a migrating neuron and a stationary neuron with an elongating axon is the degree of crosslinking within the microtubule array. Consistent with this view, we have recently determined that migrating neurons are particularly rich in KIF15, a mitotic kinesin-related protein thought to crosslink microtubules together and oppose their movement as individual polymers (Buster et al., 2003).

Once the cell body has become stationary, the motility becomes focused on the growth cone. The growth cone is the motile tip of the axon; it can forage over long distances within the embryo and is exquisitely sensitive to internal and external pathfinding cues. As the cone moves, the axon is elongated between it and the stationary cell body. Over a decade ago, Dennis Bray (1987) posed the question regarding growth cones: are they pushed or do they pull? In other words, does the motility of the growth cone arise from the ongoing anterograde transport of the cytoskeleton within the axon pushing it along, or does the motility arise from some sort of contractility of the cone itself, causing a "pull" on the axon? Experimental studies have since shown that growth cones are powerful machines of traction. They do indeed pull (Lamoureaux et al., 1989), and they do so in an actin-dependent manner (Forscher and Smith, 1988). However, does this necessarily mean that slow transport has nothing to do with growth cone motility? A broad definition of slow transport would include any motor-driven movement of cytoskeletal polymers, and it is clear that myosins are necessary to produce a retrograde flow of actin filaments within stalled growth cones (see earlier). When the growth cone is motile, this same myosin-driven movement of actin presumably contributes to the traction forces necessary for movement (Lin and Forscher, 1995; Lin et al., 1996) as does the contraction of individual filopodia (Heidemann et al., 1990). In addition, the fact that actin is the dominant cytoskeletal element within the motile growth cone is consistent with the fact that actin moves within the faster of the two subdivisions of slow transport (Lasek, 1982). It is also worth noting that axons can still grow when actin is pharmacologically depleted, and appear to do so by the pushing of the microtubules and neurofilaments anterogradely (Letourneau et al., 1987). This suggests that anterograde polymer transport may not be the only or even the most frequent means by which growth cones move, but could be quite important under certain circumstances. Interestingly, in some phases of rapid axonal growth, the tip of the axon is not heralded by a particularly dramatic growth cone (Bovolenta and Mason, 1987; Mason and Erskine, 2000), suggesting that a pushing mechanism may be more dominant than a pulling mechanism at certain points in development. (This contrasts with the more familiar appearance of a growth cone from a growing axon, shown in Fig. 4, in which most of the microtubules extend into the central region of the cone, with individual microtubules occasionally invading the actin-rich peripheral region and filopodia.) Also, it is worth noting that microtubules have been directly observed to move within motile growth cones (Tanaka and Kirschner, 1991; Dent et al., 1999), and have been observed to form looped bundles within stalled growth cones (Dent et al., 1999), presumably because microtubule movement continues to occur even when the cone itself is not moving. Thus we would contend that a broader and more contemporary definition of slow transport is indeed relevant to growth cone motility.

SLOW TRANSPORT IN DENDRITES

As noted earlier, the original conception of slow transport focused only on axons, and did not consider whether or not similar events occur within dendrites. Most dendrites are relatively short and hence do not face the same challenge as axons in terms of the delivery of proteins over distances where diffusion would be insufficient. Even so, dendrites require a highly organized cytoskeleton, and it seems reasonable to surmise that motor-driven forces are equally important in dendrites as axons or mitotic spindles. As noted earlier, studies from our laboratory have shown that dendrites have a pattern of microtubule polarity orientation that is distinct from that of the axon (Fig. 4), and that this appears to be due to an additional motor-driven force on the microtubules generated by the kinesin-like protein called CHO1/MKLP1 (Sharp et al., 1997b). Interestingly, when a large fragment of this motor is expressed in insect ovarian cells, the cells generate elongated dendrite-like processes with nonuniformly oriented microtubules (Sharp et al., 1996). On the other hand, when a large fragment of a motor that transports microtubules with a similar directionality to cytoplasmic dynein is expressed, the

cells generate elongated axon-like processes with uniformly oriented microtubules (Sharp et al., 1997a). These results suggest that the transport properties of the microtubule-based motors can account for both the distinctive polarity patterns of microtubules in axons and dendrites, and also, in turn, for distinctive features of their morphologies. On the basis of our findings, we have proposed that the dynein-driven anterograde transport of plus-end-distal microtubules is partially dampened by the CHO1/MKLP1-driven anterograde transport of minus-end-distal microtubules, thus accounting in part for the short stout morphology of the dendrite. In support of this idea, we have shown that depleting CHO1/MKLP1 from dendrites relieves the drag on the dynein-driven forces, causing plus-end-distal microtubules to thrust forward and minus-end-distal microtubules to retreat back to the cell body (Yu et al., 2000). As a consequence, the dendrite elongates and gradually takes on axonal characteristics. This phenomenon could be viewed as "taking the brakes off" of slow transport, thus causing the dendrite to lose its identity and become an axon. There is also more KIF15 in dendrites compared to axons, which presumably contributes as well to a dampening of the slow transport of microtubules (Buster et al., 2003). We would conclude that shifts in the balance of motor-driven forces largely determine whether an axon or a dendrite grows, retracts, or stalls, and also specify key features of the morphology of each type of process. Indeed, there are several examples in the literature of dendrites and axons exchanging identities (Hall et al., 1997; Bradke and Dotti, 2000; Hayashi et al., 2002), presumably due at least in part to alterations in microtubule transport.

CONCLUDING REMARKS

In this article we have discussed recent observations that suggest that slow transport is actually a collection of fast polymer movements regulated by a variety of motors as well as the length and other features of the polymers. This contemporary view of slow transport is entirely consistent with the results of classic radiolabel studies as well as the live-cell imaging studies that have been performed over the past several years. In addition, this view offers an explanation for slow transport that is consistent with growing knowledge of the molecules and mechanisms that regulate the cytoskeleton across cell types. It is provocative on this basis to consider that changes in neuronal morphology might be orchestrated by changes in slow transport. For example, just as in mitosis, shifting the balance of motor-driven forces on the cytoskeletal polymers could dramatically alter the rates, frequency, and directionality of polymer movements. Changing the lengths of the polymers could also have dramatic effects on their movements. These changes in polymer transport could make an axon grow faster, cause it to retract, transform an immature process into a dendrite, cause branches to form, establish distinctive features of axonal and dendritic morphology, and dramatically affect the motility of both cell bodies and growth cones. In addition, slow transport offers a potentially untapped avenue by which axonal regeneration after injury might be augmented (Baas, 2000b). To date, most considerations of cytoskeletal reorganization during neuronal morphogenesis have focused on non-motor mechanisms that influence the dynamics, stability, and organization of the polymers. While not refuting the contribution of these mechanisms, we propose that slow transport may also be critically important for these changes, and that more attention should be focused on the molecular basis of slow transport as a potential means for regulating neuronal morphogenesis.

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REFERENCES

Ahmad FJ, Echeverri CJ, Vallee RB, Baas PW. 1998. Cytoplasmic dynein and dynactin are required for microtubule transport into the axon. J Cell Biol 140:391–402.

Ahmad FJ, Hughey J, Wittmann T, Hyman A, Greaser M, Baas PW. 2000. Motor proteins regulate force interactions between microtubules and microfilaments in the axon. Nature Cell Biol 2:276–280.

Ahmad FJ, Yu W, McNally FJ, Baas PW. 1999. An essential role for katanin in severing microtubules in the neuron. J Cell Biol 145:305–315.

Baas PW. 1996. The neuronal centrosome as a generator of microtubules for the axon. In: Pedersen RA, Schatten GP, editors. Current Topics in Developmental Biology. New York: Academic Press, p 281–298.

Baas PW. 1999. Microtubules and neuronal polarity: lessons from mitosis. Neuron 22:23–31.

Baas PW. 2000a. Strategies for studying microtubule transport in the neuron. Microsc Res Tech 48:75–84.

Baas PW. 2000b. Elaboration of the axonal microtubule array during development and regeneration. In: Kalb RG, Strittmatter SM, editors. Neurobiology of Spinal Cord Injury. Totowa, NJ: Humana Press, p 155–168.

Baas PW. 2002. Microtubule transport in the axon. Internat Rev Cytol 212:41–62.

Baas PW, Ahmad FJ. 2001. Force generation by cytoskeletal motor proteins as a regulator of axonal elongation and retraction. Trends Cell Biol 11:244–249.

Baas PW, Brown A. 1997. Slow axonal transport: the polymer transport model. Trends Cell Biol 7:380–384.

Baas PW, Deitch JS, Black MM, Banker GA. 1988. Polarity orientation of microtubules in hippocampal neurons: uniformity in the axon and nonuniformity in the dendrite. Proc Natl Acad Sci USA 85:8335–8339.

Bearer EL, Reese TS. 1999. Association of actin filaments with axonal microtubule tracts. J Neurocytol 28:85–98.

Black MM, Baas PW. 1989. The basis of polarity in the neuron. Trends Neurosci 12:211–214.

Black MM, Lasek RJ. 1980. Slow components of axonal transport: two cytoskeletal networks. J Cell Biol 86:616–623.

Bovolenta P, Mason C. 1987. Growth cone morphology varies with position in the developing mouse visual pathway from retina to first targets. J Neurosci 7:1447–1460.

Bradke F, Dotti CG. 2000. Differentiated neurons retain the capacity to generate axons from dendrites. Curr Biol 10:1467–1470.

Brady ST. 2000. Neurofilaments run sprints not marathons. Nature Cell Biol 2:E43–E45.

Bray D. 1987. Growth cones: do they pull or are they pushed? Trends Neurosci 10:431–434.

Bridgman PC, Dave S, Asnes CF, Tullio AN, Adelstein RS. 2001. Myosin IIB is required for growth cone motility. J Neurosci 21:6159–6169.

Brown A. 2000. Slow axonal transport: stop and go traffic in the axon. Nature Rev Mol Cell Biol 1:153–155.

Buster DW, Baird D, Yu W, Solowska-Baird J, Kress M, Baas PW. 2003. Expression of the Mitotic Kwesin Kif15 in postmitotic neurons: implications for neuronal migration and development. J Neurocytol 32:79–96.

Chou Y-H, Goldman RD. 2000. Intermediate filaments on the move. J Cell Biol 150:F101–F105.

Dent EW, Szebenyi G, Callaway JL, Baas PW, Kalil K. 1999. Reorganization and movement of microtubules in axonal growth cones and developing interstitial branches. J Neurosci 19:8894–8908.

Dillman III JF, Dabney LP, Pfister KK. 1996. Cytoplasmic dynein is associated with slow axonal transport. Proc Natl Acad Sci USA 93:141–144.

Eaton BA, Fetter RD, Davis GW. 2002. Dynactin is necessary for synapse stabilization. Neuron 34:729–741.

Errico A, Ballabio A, Rugarli EI. 2002. Spastin, the protein mutated in autosomal dominant hereditary spastic paraplegia, is involved in microtubule dynamics. Hum Mol Genet 11:153–163.

Feng Y, Walsh CA. 2001. Protein-protein interactions, cytoskeletal regulation and neuronal migration. Nature Rev Neurosci 2:408–416.

Ferhat L, Cook C, Chauviere M, Harper M, Kress M, Lyons GE, Baas PW. 1998a. Expression of the mitotic motor protein Eg5 in postmitotic neurons: implications for neuronal development. J Neurosci 18:7822–7835.

Ferhat L, Kuriyama R, Lyons GE, Micales B, Baas PW. 1998b. Expression of the mitotic motor protein CHO1/MKLP1 in postmitotic neurons. Eur J Neurosci 10:1383–1393.

Forscher P, Smith SJ. 1988. Actions of cytochalasins on the organization of actin filaments and microtubules in a neuronal growth cone. J Cell Biol 107:1505–1516.

Fukata Y, Itoh TJ, Kimura T, Ménager C, Nishimura T, Shiromizu T, Watanabe H, Inagaki N, Iwamatsu A, Hotani H, et al. 2002. CRMP-2 binds to tubulin heterodimers to promote microtubule assembly. Nature Cell Biol 4:583–591.

Gallo G, Letourneau PC. 1999. Different contributions of microtubule dynamics and transport to the growth of axons and collateral sprouts. J Neurosci 19:3860–3873.

Gallo G, Yee Jr HF, Letourneau PC. 2002. Actin turnover is required to prevent axon retraction driven by endogenous actomyosin contractility. J Cell Biol 158:1219–1228.

Gan WB, Lichtman JW. 1998. Synaptic segregation at the developing neuromuscular junction. Science 282:1508–1511.

Hall GF, Yao J, Selzer ME, Kosik KS. 1997. Cytoskeletal changes correlated with the loss of neuronal polarity in axotomized lamprey central neurons in situ. J Neurocytol 26:733–753.

Hayashi K, Kawai-Hirai R, Ishikawa K, Takata K. 2002. Reversal of neuronal polarity by conversion of dendrites into axons in neonatal rat cortical neurons in vitro. Neuroscience 110:7–17.

He Y, Yu W, Baas PW. 2002. Microtubule reconfiguration during axonal retraction induced by nitric oxide. J Neurosci 22:5982–5991.

Heald R, Tournebize T, Habermann A, Karsenti E, Hyman A. 1997. Self assembly in Xenopus egg extracts: respective roles of centrosomes and microtubule self-organization. J Cell Biol 138:615–628.

Heidemann SR, Lamoureaux P, Buxbaum RE. 1990. Growth cone behavior and production of traction force. J Cell Biol 111:1949–1957.

Heriot K, Gambetti P, Lasek RJ. 1985. Proteins transported in slow components a and b of axonal transport are distributed differently in the transverse plane of the axon. J Cell Biol 100:1167–1172.

Hirokawa N, Terada S, Funakoshi T, Takeda S. 1997. Slow axonal transport: the subunit transport model. Trends Cell Biol 7:384–388.

Kabir N, Schaefer AW, Nakhost A, Sossin WS, Forscher P. 2001. Protein kinase C activation promotes microtubule advance in neuronal growth cones by increasing average microtubule growth lifetimes. J Cell Biol 152:1033–1044.

Lamoureaux P, Buxbaum RE, Heidemann SR. 1989. Direct evidence that growth cones pull. Nature 340:159–162.

Langford GM. 1995. Actin- and microtubule-dependent organelle motors: interrelationships between the two motility systems. Cur Opin Cell Biol 7:82–88.

Lasek RJ. 1982. Translocation of the neuronal cytoskeleton and axonal locomotion. Phil Trans R Soc Lond B 299:313–327.

Lasek RJ. 1986. Polymer sliding in axons. J Cell Sci Suppl 5:161–179.

Lasek RJ. 1988. Studying the intrinsic determinants of neuronal form and function. In: Lasek RJ, Black MM, editors. Intrinsic Determinants of Neuronal Form and Function. New York: Alan R. Liss, Inc., p 1–58.

Lasek RJ, Paggi P, Katz MJ. 1993. The maximum rate of neurofilament transport in axons: a view of molecular transport mechanisms continuously engaged. Brain Res 616:58–64.

Letourneau PC, Shattuck TA, Ressler AH. 1987. "Pull" and "push" in neurite elongation: observations on the effects of different concentrations of cytochalasin B and taxol. Cell Motil Cytoskeleton 8:193–209.

Letourneau PC, Wire JP. 1995. Three-dimensional organization of stable microtubules and the Golgi apparatus in the somata of developing chick sensory neurons. J Neurocytol 24:207–223.

Liao G, Gundersen GG. 1998. Kinesin is a candidate for cross-bridging microtubules and intermediate filaments. Selective binding of kinesin to detyrosinated tubulin and vimentin. J Biol Chem 273:9797–9803.

Lim SS, Edson KJ, Letourneau PC, Borisy GG. 1990. A test of microtubule translocation during neurite elongation. J Cell Biol 111:123–130.

Lin CH, Espreafico EM, Mooseker MS, Forscher P. 1996. Myosin drives retrograde F-actin flow in neuronal growth cones. Neuron 16:769–782.

Lin CH, Forscher P. 1995. Growth cone advance is inversely proportional to retrograde F-actin flow. Neuron 14:763–771.

Lin PT, Gleeson JG, Corbo JC, Flanagan L, Walsh CA. 2000. DCAMKL1 encodes a protein kinase with homology to doublecortin that regulates microtubule polymerization. J Neurosci 20:9152–9161.

Mason C, Erskine L. 2000. Growth cone form, behavior and interactions in vivo: retinal axon pathfinding as a model. J Neurobiol 44:260–270.

McNally F. 2000. Capturing a ring of samurai. Nature Cell Biol 2:E4–E7.

McNally FJ, Vale RD. 1993. Identification of katanin, an ATPase that severs and disassembles stable microtubules. Cell 75:419–429.

Nislow C, Lombillo VA, Kuriyama R, McIntosh JR. 1992. A plus-end-directed motor that moves anti-parallel microtubules in vitro localizes to the interzone of mitotic spindles. Nature 359:543–547.

Nixon AB, Grenningloh G, Casey PJ. 2002. The interaction between RGSZ1 and SCG10 attenuates the ability of SCG10 to promote microtubule disassembly. J Biol Chem 277:18127–18133.

Pfister KK. 1999. Cytoplasmic dynein and microtubule transport in the axon: the actin connection. Mol Neurobiol 20:81–91.

Prahlad V, Yoon M, Moir RD, Vale RD, Goldman RD. 1998. Rapid movements of vimentin on microtubule tracks: kinesin-dependent assembly of intermediate filament networks. J Cell Biol 143:159–170.

Rakic P, Knyihar-Csillik E, Csillik B. 1996. Polarity of microtubule assemblies during neuronal cell migration. Proc Natl Acad Sci USA 93:9218–9222.

Roy S, Coffee P, Smith G, Liem RKH, Brady ST, Black MM. 2000. Neurofilaments are transported rapidly but intermittently in axons: implications for slow axonal transport. J Neurosci 20:6849–6861.

Sabry J, O'Connor TP, Kirschner MW. 1995. Axonal transport of tubulin in Ti1 pioneer neurons in situ. Neuron 14:1247–1256.

Schaefer A, Kabir N, Forscher P. 2002. Filopodia and actin arcs guide the assembly and transport of two populations of microtubules with unique dynamic parameters in neuronal growth cones. J Cell Biol 158:139–152.

Sharp DJ, Brown HM, Kwon M, Rogers GC, Holland G, Scholey JM. 2000a. Functional coordination of three mitotic motors in Drosophila embryos. Mol Biol Cell 11: 241–253.

Sharp DJ, Kuriyama R, Baas PW. 1996. Expression of a kinesin-related motor protein in Sf9 cells induces them to extend dendrite-like processes with nonuniform microtubule polarity orientation. J Neurosci 16:4370–4375.

Sharp DJ, Kuriyama R, Baas PW. 1997a. Expression of a minus-end-directed motor protein induces Sf9 cells to form axon-like processes with uniform microtubule polarity orientation. J Cell Sci 110:2373–2380.

Sharp DJ, Rogers GC, Scholey JM. 2000b. Microtubule motors in mitosis. Nature 407:41–47.

Sharp DJ, Yu W, Baas PW. 1995. Transport of dendritic microtubules establishes their nonuniform polarity orientation. J Cell Biol 130:93–104.

Sharp DJ, Yu W, Ferhat L, Kuriyama R, Rueger DC, Baas PW. 1997b. Identification of a motor protein essential for dendritic differentiation. J Cell Biol 138:833–843.

Shea TB. 2000. Microtubule motors, phosphorylation, and axonal transport of neurofilaments. J Neurocytol 11–12: 873–887.

Slaughter TS, Wang J, Black MM. 1997. Transport of microtubules from the cell body into the axons of cultured neurons. J Neurosci 17:5807–5819.

Smith DS, Niethammer M, Ayala R, Zhou Y, Gambello MJ, Wynshaw-Boris A, Tsia L-H. 2000. Regulation of cytoplasmic dynein behavior and microtubule organization by mammalian Lis1. Nature Cell Biol 2:767–775.

Song HJ, Poo MM. 1999. Signal transduction underlying growth cone guidance by diffusible factors. Curr Opin Neurobiol 9:355–363.

Stanfield BB, O'Leary DDM, Fricks C. 1982. Selective collateral elimination in early postnatal development restricts cortical distribution of rat pyramidal tract neurones. Nature 298:371–373.

Tanaka EM, Kirschner MW. 1991. Microtubule behavior in the growth cones of living neurons during axon elongation. J Cell Biol 115:345–363.

Vale RD. 1999. Millennial musings on molecular motors. Trends Cell Biol 9:M38–M42.

Wang L, Brown A. 2001. Rapid intermittent movement of

axonal neurofilaments observed by fluorescence photobleaching. Mol Biol Cell 12:3257–3267.

Wang L, Brown A. 2002. Rapid movement of microtubules in axons. Curr Biol 12:1496–1501.

Wang L, Ho C-L, Sun D, Liem RKH, Brown A. 2000. Rapid movements of axonal neurofilaments interrupted by prolonged pauses. Nature Cell Biol 2:137–141.

Yabe JT, Pimenta A, Shea TB. 1999. Kinesin-mediated transport of neurofilament protein oligomers in growing axons. J Cell Sci 112:3799–3814.

Yu W, Ahmad FJ, Baas PW. 1994. Microtubule fragmentation and partitioning in the axon during collateral branch formation. J Neurosci 14:5872–5884.

Yu W, Cook C, Sauter C, Kuriyama R, Kaplan PL, Baas PW. 2000. Depletion of a microtubule-associated motor protein induces the loss of dendritic identity. J Neurosci 20:5782–5791.

Yu W, Schwei MJ, Baas PW. 1996. Microtubule transport and assembly during axon growth. J Cell Biol 133:151–157.