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Cytoplasmic Dynein and Microtubule Transport in the Axon: The Action Connection

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Abstract

The neuron uses two families of microtubule-based motors for fast axonal transport, kinesin, and cytoplasmic dynein. Cytoplasmic dynein moves membranous organelles from the distal regions of the axon to the cell body. Because dynein is synthesized in the cell body, it must first be delivered to the axon tip. It has recently been shown that cytoplasmic dynein is moved from the cell body along the axon by two different mechanisms. A small amount is associated with fast anterograde transport, the membranous organelles moved by kinesin. Most of the dynein is transported in slow component b, the actin-based transport compartment. Dynactin, a protein complex that binds dynein, is also transported in slow component b. The dynein in slow component b binds to microtubules in an ATP-dependent manner in vitro, suggesting that this dynein is enzymatically active. The finding that functionally active dynein, and dynactin, are associated with the actin-based transport compartment suggests a mechanism whereby dynein anchored to the actin cytoskeleton via dynactin provides the motive force for microtubule movement in the axon.

Index Entries: Dynein; dynactin; actin; microtubule; axonal transport; motor protein.

Introduction

The neuron overcomes significant transport challenges in order to establish and maintain its axon. The axon extends a long distance from the cell body, and contains most of the neuronal cell volume, yet it has no machinery for protein synthesis. The axon is supported by a cytoskeleton consisting of neurofilaments, actin filaments, and microtubules *(1)*. All of the microtubules are oriented with their plus ends directed toward the synapse *(2,3)*. Newly synthesized material is transported from the cell body along the

axon (the anterograde direction) in three distinct components that move at different rates *(4–8)*. Membranous organelles and their associated proteins make up the fast component, traveling at ~100–200 mm/d. Tubulin and neurofilament proteins move at the slowest rate, ~0.1 mm/d, in slow component a. Actin, spectrin (fodrin), glycolytic enzymes, and the other axonal proteins are transported at ~1–10 mm/d in slow component b. In contrast, transport from the axon back toward the cell body (the retrograde direction) consists of membranous organelles moving along microtubules at ~100 mm/d.

The two fast axonal transport mechanisms are the best characterized *(9–11)*. Membranous organelles are transported along microtubules in the anterograde direction by the kinesin family of motor proteins, which move material toward the plus ends of microtubules *(12,13)*. Retrograde transport utilizes a different motor protein, cytoplasmic dynein, which moves cargo toward the minus ends of microtubules *(14)*. Axonal membranous organelle transport therefore requires at least two different motor proteins, kinesin for the anterograde direction, and dynein for the retrograde direction. Because there is no protein synthesis in the axon, the utilization of two motor proteins produces a regulatory problem. The function of cytoplasmic dynein is to move material from the axon terminal toward the cell body. Because dynein is synthesized in the neuronal cell body, it must be transported to the axon terminal. This article will discuss the recent characterization of the anterograde axonal transport of dynein and the implications of the finding that dynein is a constituent of slow axonal transport.

Cytoplasmic dynein is a large multi-subunit mechanochemical ATPase. The holoenzyme is ~1500kDa and is made up of two identical ~530 kDa heavy chains, as well as intermediate chains of 74 kDa, light intermediate chains of 50–60 kDa, and light chains of 8–20 kDa *(15,16)*. The two heavy chains each bind microtubules and hydrolyze ATP to generate motile force. The 74 kDa intermediate chains are involved in binding dynein to its cargo *(17–19)*. The functions of the light intermediate chains and light chains are unknown, however, they are physically associated with either the intermediate chains or the heavy chains and are likely involved in cargo binding *(20–22)*. Some mutations of the 8 kDa light chain in *Drosophila* are lethal and the phenotypes of other mutations include axonal guidance and synaptogenesis defects *(23–25)*.

Anterograde Axonal Transport of Cytoplasmic Dynein

Initial experiments to analyze the role of dynein in axonal transport utilized immunocytochemistry to analyze the location of dynein in ligated nerves *(26)*. When nerves are ligated for short periods of time, anterograde and retrograde membranous organelles accumulate on the proximal and distal sides, respectively, of the ligation. Immunocytochemistry or biochemical methods can then be used to analyze these two distinct organelle populations *(27,28)*. When this technique was employed on mouse saphenous nerves, dynein was found to accumulate on both sides of the ligation *(26)*. This was interpreted as indicating that cytoplasmic dynein associates with membranous organelles that move in both directions. This suggested that inactive dynein was transported to the nerve terminal on anterogradely moving membranous organelles (which are presumably moved by the kinesin family of motor proteins).

To analyze more comprehensively the anterograde transport of cytoplasmic dynein, we used a biochemical method, pulse chase analysis, in the rat optic nerve *(27a)*. When radiolabeled amino acids, ^{35}S- cysteine and methionine, are provided to retinal ganglion cells by intravitreal injection, the labeled amino acids taken up by the retinal ganglion cells are incorporated into newly synthesized proteins. The radiolabeled proteins are then transported along the axons of the rat optic nerve in the

three anterograde rate components. These experiments identified two pools of dynein moving in the anterograde direction. In agreement with the immunocytochemical experiments we found that dynein is transported in association with the small vesicles of fast component *(28a,29)*. However only a small amount was associated with these organelles. Most (~90%) of the cytoplasmic dynein was transported in association with the actin transport component, slow component b. No dynein was transported in slow component a. Subsequent segmental analysis of the slow component b transport component demonstrated that the transport kinetics of the various dynein polypeptides are identical, indicating that they are moving as part of the holoenzyme, not as individual subunits *(27, 28a, 29)*.

Analysis of the polypeptide composition of the dynein from the two anterograde transport pools demonstrates that the two pools of dynein contain unique subunits. Dynein associated with the fast component (membranous organelles) contains only one intermediate chain gene product and one light intermediate chain gene product, whereas the dynein associated with the actin transport complex (slow component b) contains several different intermediate chain and light intermediate chain gene products. Because these polypeptides are important for dynein binding to cargo, the different subunits may specify the association of dynein with the two distinct anterograde transport mechanisms.

The Association of Dynein with Actin in Slow Component b: Dynactin and Spectrin

The finding that much of the anterogradely moving dynein is transported in slow component b raises the issue of how it is associated with this actin-transport complex. One candidate linker protein is dynactin. Dynactin is a large multi-subunit protein complex. The backbone of the complex is a filament composed of actin-related protein 1 (Arp 1) *(30,31)*. The Arp1 sequence contains binding sites for many proteins that bind and cross-link actin, including spectrin and myosin, and Holleran and coworkers have shown that spectrin coimmunoprecipitates with dynactin *(32–34)*. Electron microscopic observations show that spectrin crosslinks actin filaments of a similar size as the dynactin Arp 1 filament *(35)*. One end of the Arp1 filament is capped by actin-capping protein and it has been proposed that the other end is capped by a unique polypeptide, p62 *(31)*. Attached to the Arp1 filament are two copies of the largest subunit, p150^{Glued}, the product of the *Drosophila* gene *Glued (36)*. The functions of the p50 and p24 dynactin subunits are unknown *(37–39)*.

Dynactin was initially identified as a protein that stimulated the dynein-generated movement of membranous organelles in vitro *(40)*. Subsequent work suggests that dynactin is involved in the binding of dynein to most of its cargoes *(30,32,41)*. It has been shown that the p150^{Glued} subunit binds the dynein 74 kDa intermediate chain and to microtubules *(42,43)*. In neurons there is an alternative splice isoform, p135^{Glued}, which lacks the microtubule binding site, but retains the dynein intermediate chain binding site *(44)*. Individual molecules of the dynactin complex are homodimers of either the p150^{Glued} or the p135^{Glued} isoforms. Genetic evidence from *Drosophila* and several fungal species suggest that dynein and dynactin interact in vivo, and dynein mutations suppress the phenotype of *Glued* (dynactin) mutants in *Drosophila (45–48)*. Finally, overexpression of the p50 dynactin subunit in cultured cells disrupts the dynactin complex, alters the localization of both dynactin and dynein in cultured cells, and interferes with dynein-based motility *(37,49–51)*. Recent studies suggest that spectrin may link dynactin, and thus dynein, to Golgi and other organelles *(52–55)*.

The anterograde axonal transport properties of dynactin in the rat optic nerve are similar to those of dynein *(28a,29)*. There is a small amount of dynactin associated with fast axonal

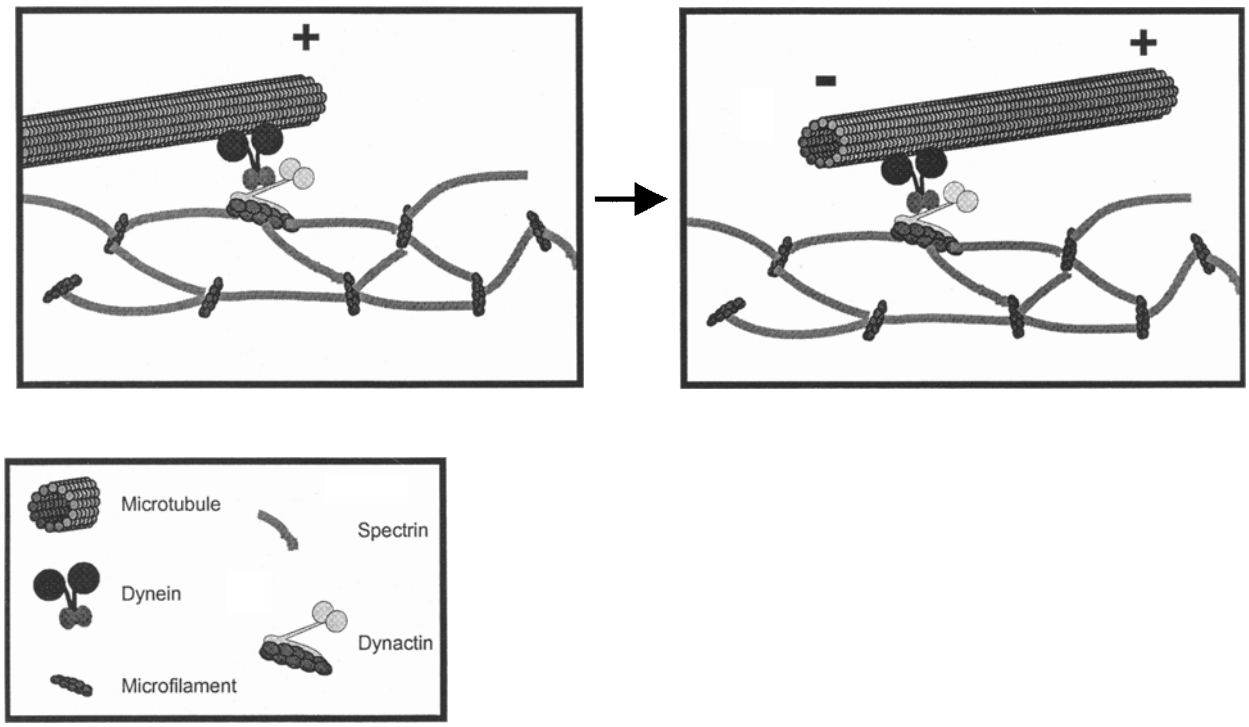

Fig. 1. Model for dynein linked to the spectrin-actin network generating axonal microtubule movement. Left panel, Dynactin, actin, and spectrin are incorporated into a network. Dynactin binds dynein and dynein binds a microtubule. The components are labeled in the lower panel. To show the structure of dynactin, it is drawn slightly larger than the actin filaments. Right panel, At a short time after the image in the left panel, the dynein has moved the microtubule; note that the plus end of the microtubule is leading.

transport, but most of the dynactin is transported with actin in slow component b. The fast and slow pools of dynactin can also be distinguished because they contain different isoforms of the p150^{Glued} subunit, and the p135^{Glued} isoform, which binds dynein, not microtubules, is found in slow component b. Affinity binding methods demonstrate that slow component b dynein binds to the p150^{Glued} dynactin subunit in vitro. Because it has long been known that a brain isoform of spectrin, fodrin, is transported in slow component b *(56)*, spectrin is a good candidate to link the dynactin to the actin cytoskeleton (Fig. 1).

It has previously been shown that the wave of radiolabeled slow component b proteins moving along the axon consists of subsets of proteins *(57)*. One set of polypeptides is found at the front of the slow component b wave. These proteins are thought to be the components of a continuously moving slow component b transport structure. The other proteins are found along the entire the slow component b wave. These proteins are thought to move more slowly because they bind to the transport structure with low affinity and thus spend some time dissociated from the structure. Although both dynein and dynactin are transported in slow component b, a kinetic analysis suggests that dynactin is moving slightly faster that dynein (Fig. 3 in ref. *29*). The relative rates of transport of dynein and dynactin are therefore consistent with the hypothesis that dynactin is part of the basic transport structure, whereas dynein spends some time dissociated from dynactin and thus the transport structure.

A more conclusive demonstration of a role for dynactin and spectrin in associating dynein with actin filaments will require further experimentation. Although interactions between pairs of individual proteins have been demonstrated biochemically—for example, a dynein-dynactin interaction, a dynactin-spectrin interaction, and a spectrin-actin interaction—the simultaneous interaction of these components has not been demonstrated in a single experimental system. In addition, immunolocalization data (electron microscopic, or immunofluorescence) showing the involvement of dynactin with the actin cytoskeleton in vivo is needed. However, it has been observed that both dynein and dynactin are located with actin in the cell cortex in fungal species and nematodes *(48,58,59)*. Based on those observations and on genetic studies, it has been proposed that dynactin and dynein in the cortex are involved in a microtubule-based positioning of the nucleus or mitotic spindle and their movement during cell division *(60–62)*.

The Role of the Cytoplasmic Dynein Associated with Anterograde Membrane Transport

The role of the two pools of dynein found moving in the anterograde direction in rat optic nerve is the subject of active investigation. However, the current data provides insight into their potential roles and functions. As indicated previously, the two pools of dynein are distinguishable by their intermediate chain subunits *(29)*. There are two genes for the cytoplasmic dynein intermediate chain (IC74) and five alternatively spliced mRNA products have been identified *(42,63)*. The dynein found moving with the membranous organelles in fast anterograde transport contains only the IC74-2C polypeptide (the product of gene number 2, alternative splice variant C). The IC74-2C mRNA and protein are found in all cells and tissues and thus appear to be ubiquitous. In many cultured cell lines, IC74-2C is the only dynein intermediate chain found *(63)*. Dynein containing IC74-2C is thus sufficient for constitutive dynein-based microtubule minus-end directed transport. It is therefore probable that the dynein associated with anterograde fast transport is destined to become the retrograde transport motor. If this is the case, the regulation of membranous organelle movement in axons may be similar to that found in the neuronal cell body.

Role of the Cytoplasmic Dynein Found in Slow Component b

Most of the dynein moving in the anterograde direction in the optic nerve is found in slow component b. Slow component b dynein has little or no IC74-2C. Instead, the other intermediate chain isoforms are found in this transport component *(29)*. These additional IC74 isoforms are found only in certain cells and tissues, and two of them are neuron-specific. The expression of these isoforms is regulated during rat brain development and they are first detected during neuritogenesis *(63,64)*. These observations suggest that the dynein found in slow component b might have a special role in the neuron, and in particular, the axon. Consistent with this idea, when rat pheochromoctyoma PC-12 cells are induced to differentiate axon-like processes by treatment with nerve growth factor, the expression of these intermediate chains is also stimulated (Salata et al., submitted). Dynein may be transported in slow component b in order to sequester it from membranous organelles, as a regulatory mechanism to prevent dynein activation during anterograde transport. Such prematurely activated dynein would move back to the cell body with the wrong cargo. Slow component b dynein would be recruited onto the correct cargo organelles only by an appropriate signal. Because the intermediate chains of slow component b dynein are different from those of dynein in fast component, they could be involved in axon-specific regulation. A second possibility is that slow component b dynein may have an active role to play in anterograde

axonal transport, for example, as a motor for microtubule movement in the axon (described in detail below). These two hypotheses are not mutually exclusive. Although slow component b dynein might function as the motor for microtubule movement, it could also be recruited to function as the retrograde membranous organelle motor. The current challenge is to identify which of the two pools of cytoplasmic dynein moving in the anterograde direction is utilized as the motor for retrograde transport of membranous organelles in the axon.

Model for Dynein-Generated Axonal Microtubule Movement

The polarity of microtubules in the axon, with the plus ends oriented toward the terminal is consistent with the idea that dynein is responsible for their movement. There is also considerable evidence that microtubules are actively transported in the axon *(65–67)*, although some investigators suggest that the transport unit is tubulin oligomers *(68–70)*. Recently, Ahmad and coworkers *(51)* found that disruption of the dynactin complex prevents the movement of microtubules into and along the axon. Because dynactin is required for dynein function, this implicates dynein in microtubule movement in the axon. Microtubule movement has been observed in real time in cultured fibroblasts. Keating and coworkers observed single microtubules detach from the centrosome and move with their plus ends leading *(71)*. This observation is also consistent with the suggestion that dynein is the motor for microtubule movement.

In order for a dynein molecule to move a microtubule, it must be anchored relative to the microtubule. The model system for dynein-generated movement of microtubules is the flagellum *(72)*. Flagellar dyneins move one microtubule relative to another because they have two microtubule-binding domains, both motor and cargo binding *(73)*. A flagellar dynein intermediate chain forms a stable bond with the cargo microtubule *(74)*. The heavy chain contains the motor domain. The binding of the motor domain to a second microtubule is coupled to the hydrolysis of ATP and allows this microtubule to move relative to the other *(73,75,76)*. However, sliding of one microtubule relative to another is not the mechanism used to move axonal microtubules. Microtubules are transported in slow component a, but dynein is not found in slow component a. Dynein is found in slow component b, associated with the actin cytoskeleton, an appropriate foundation for microtubule movement *(29)*. A model for how dynein, anchored to the actin cytoskeleton, might move a microtubule is diagrammed in Fig. 1. Dynein and dynactin are associated with an actin-spectrin network. Dynactin is crosslinked into the network by an actin-binding protein, such as spectrin. The dynactin binds cytoplasmic dynein to the actin network. The dynein binds to a microtubule, hydrolyzes ATP, and moves the microtubule down the axon with its plus end pointing toward the terminal. Consistent with the hypothesis, slow component b dynein binds microtubules in vitro and releases from the microtubules when Mg^{2+}-ATP is added.

Implications of the Model for the Axonal Transport of Microtubules

As McQuarrie and coworkers observed, transporting the motor for microtubule movement in slow component b (that moves faster than the microtubule) explains several observations about variations in slow axonal transport *(77)*. In particular the variations in the rate of microtubule transport (slow component a) in different nerves, and at different ages, and in different species, could be accounted for by varying any of the parameters of the dynein-microtubule interaction (discussed later in greater detail) *(78–82)*. Furthermore, if dynein bound to, but did not release from, a microtubule, it could explain why some tubulin is found in slow com-

ponent b in some nerves. It has been suggested that some of the material moving in slow component b is used to build or replace cytoskeletal elements in the axon *(83,84)*. That is, some slow component b proteins are deposited along the axon as the slow component b wave passes. A stationary population of dynein and dynactin anchored to an actin network would be ideally suited to generate the force necessary to move microtubules along the axon.

In an in vitro motility assay, dynein is attached to a glass coverslip and, in the presence of ATP, moves microtubules at the rate of 1–2 μ/s *(85,86)*. In the proposed model (Fig. 1) the axonal microtubules are moving much slower than dynein moves microtubules in vitro. There are several reasons why this might be the case. The relative positions of the cytoskeletal elements may not often allow force generation, for example, the microtubules might be to: far away from the dynein anchored to the actin network. The microtubules may be impeded by other organelles in the axon. For example, when Keating and coworkers observed microtubules moving in cultured fibroblasts, they observed one bend and curve when its forward movement was obstructed *(71)*. Also, axonal microtubules are dynamic; that is, their length continuously changes by alternating cycles of polymerization and depolymerization *(65,68,87)*. Therefore dynein may bind primarily short pieces of microtubules and thus interact with them only briefly. This possibility is consistent with the reports that short oligomers, and not long microtubules, are transported *(68)*. In addition the relative anterograde transport kinetics of dynein and dynactin suggest that dynein dissociates from the dynactin (and the actin network) for some time period; such dynein would not be able to generate force to transport microtubules. Finally, there may be other levels of dynein regulation; for example, cytoplasmic dynein may not be a processive motor, but may be designed to release microtubules after brief movements *(88,89)*. Under any of these conditions, microtubules would spend short periods moving at a fast rate and long periods not moving, so on average they would be transported at slow rates. This hypothesis is consistent with the observed motile behavior of fluorescently labeled mRNA in cultured cells *(90)*. The RNA movement is microtubule-dependent, and when RNA is moving it does so at fast transport rates. However, RNA spends considerable periods of time not moving, so its net velocity is that of slow transport.

One potential difficulty with this model is the role of dynactin in the axon. The dynactin p150^{Glued} subunit binds microtubules. So, it might be expected that p150^{Glued} would crosslink the actin to microtubules and that the two cytoskeletal elements would move at the same transport rate. Investigation of the microtubule binding of slow component b dynactin from rat optic nerve showed that it did not bind microtubules in vitro *(29)*. Therefore, the properties of slow component b dynactin from rat optic nerve are consistent with the observed protein transport. Interestingly, it is reported that the microtubule binding of dynactin is regulated *(29)*. Regulation of dynactin microtubule binding could explain how, in some nerves such as the sciatic nerve, some tubulin is transported in slow component b and some actin is found in slow component a.

Conclusion

Neuronal cytoplasmic dynein is transported toward the axon terminal in both fast component and slow component b. The dynein in the two transport components can be distinguished by their different intermediate chains. The association of dynein, dynactin, spectrin, and actin in the same transport component suggests a model for dynein-based microtubule movement in the axon. This model is consistent with the existing descriptions of axonal transport, and the model has more general implications for all cells. The observed properties of dynein and dynactin in the rat optic nerve suggest that the two proteins provide a mechanism to both link the actin filaments and microtubules and to move the two polymers relative to one another.

When dynactin is linked to the actin filament system it could bind to microtubules and thereby crosslink the two filament networks. When the dynactin binding to microtubules is "turned off" and dynein is added to the network, then movement is generated between the two filament polymers.

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