Transport of neurofilaments in growing axons requires microtubules but not actin filaments

ARTICLE *in* JOURNAL OF NEUROSCIENCE RESEARCH · FEBRUARY 2005

Impact Factor: 2.59 · DOI: 10.1002/jnr.20399 · Source: PubMed

4 AUTHORS, INCLUDING:

Subhojit Roy
University of California, San Diego
35 PUBLICATIONS **1,025** CITATIONS
SEE PROFILE

Scott T Brady
University of Illinois at Chicago
166 PUBLICATIONS **10,819** CITATIONS
SEE PROFILE

Mark M Black
Temple University
64 PUBLICATIONS **4,574** CITATIONS
SEE PROFILE

Journal of Neuroscience Research 79:442–450 (2005)

Transport of Neurofilaments in Growing Axons Requires Microtubules but Not Actin Filaments

Franto Francis,[1] Subhojit Roy,[2] Scott T. Brady,[3] and Mark M. Black[1]★

[1]Department of Anatomy and Cell Biology, Temple University School of Medicine, Philadelphia, Pennsylvania
[2]Department of Pathology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania
[3]Department of Anatomy and Cell Biology, University of Illinois School of Medicine, Chicago, Illinois

Neurofilament (NF) polymers are conveyed from cell body to axon tip by slow axonal transport, and disruption of this process is implicated in several neuronal pathologies. This movement occurs in both anterograde and retrograde directions and is characterized by relatively rapid but brief movements of neurofilaments, interrupted by prolonged pauses. The present studies combine pharmacologic treatments that target actin filaments or microtubules with imaging of NF polymer transport in living axons to examine the dependence of neurofilament transport on these cytoskeletal systems. The heavy NF subunit tagged with green fluorescent protein was expressed in cultured sympathetic neurons to visualize NF transport. Depletion of axonal actin filaments by treatment with 5 μM latrunculin for 6 hr had no detectable effect on directionality or transport rate of NFs, but frequency of movement events was reduced from 1/3.1 min of imaging time to 1/4.9 min. Depolymerization of axonal microtubules using either 5 μM vinblastine for 3 hr or 5 μg/ml nocodazole for 4–6 hr profoundly suppressed neurofilament transport. In 92% of treated neurons, NF transport was undetected. These observations indicate that actin filaments are not required for neurofilament transport, although they may have subtle effects on neurofilament movements. In contrast, axonal transport of NFs requires microtubules, suggesting that anterograde and retrograde NF transport is powered by microtubule-based motors.

Key words: neurofilaments; axons; microtubules; actin filaments; nerofilament transport

Neurofilaments (NFs) are actively transported in growing axons (Brown 2000; Wang et al., 2000; Roy et al., 2000). Wang et al. and Roy et al. demonstrated that NFs move anterogradely or retrogradely in the axon at a range of rates that varies from ≤0.1 μm/sec to ≥2 μm/sec, with an average of 0.6–0.7 μm/sec. A striking feature of NF movement is that it is highly intermittent, with NFs alternating between bouts of relatively rapid movement and prolonged pauses with relatively little movement. When integrated over time, including periods of active transport and pauses, the rate is quite slow, ~0.01 μm/sec, and agrees well with the average rates of NF protein transport observed in mature axons with classic radioisotopic labeling methods.

Vesicle transport in many cell types may use both actin filament and microtubule (MT) systems (for review see Langford, 2002; see also Nascimento et al., 2003). For example, in axons, MTs and MT motors mediate longitudinal movements that translocate cargo over long distances between the cell body and axon tip, whereas actin and actin motors mediate movements over comparatively short distances, positioning transported structures within discrete subcompartments within the axon. Coordination between these different motor systems may be mediated in part by direct interactions between actin and MT motors (Huang et al., 1999). In considering the mechanisms of intermediate filament transport, current perspectives on vesicle transport provide precedent for the view that both the MT and the actin systems contribute to NF transport behavior. In this regard, several studies have examined the movement of intermediate filament proteins in a variety of cell types, with the general consensus that these proteins can move in various forms using MTs and MT motors (for review see Helfand et al., 2003a). Similarly, studies of mutant mice lacking myosin Va have revealed abnormalities of NF organization in axons. The purpose of the present studies is to delineate further the role of the MT and actin systems in NF polymer transport in growing axons. We have applied a pharmacologic approach targeting either MTs or actin filaments to a system for visualizing NF translocation in living axons. The results show that NF transport is absolutely dependent on MTs, but not on actin filaments.

★Correspondence to: Dr. Mark M. Black, Department of Anatomy and Cell Biology, Temple University School of Medicine, 3400 N. Broad Street, Philadelphia, PA 19140. E-mail: mark.black@temple.edu

Received 14 September 2004; Revised 9 November 2004; Accepted 11 November 2004

Published online 5 January 2005 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jnr.20399

MATERIALS AND METHODS

Materials

Culture media were obtained from Gibco BRL (Grand Island, NY). Supplements for culture media were obtained from Gibco BRL or Sigma (St. Louis, MO), except for nerve growth factor (NGF), which was purchased from Invitrogen (Carlsbad, CA). Other reagents were obtained from Sigma unless otherwise indicated.

Cell Culture and Infection With Green Fluorescent Protein-Neurofilament H

Experiments reported here used sympathetic neurons dissociated from the superior cervical ganglia of 1–5-day-old rat pups. Neurons were grown on glass coverslips in 35-mm plastic tissue culture dishes as described previously (Brown et al., 1992). Neurons were dissociated from superior cervical ganglia of rat pups by using sequential treatments with collagenase and trypsin, followed by trituration (Black and Kurdyla, 1983). For some experiments, neurons were cultured and infected with green fluorescent protein-neurofilament H (GFP-NFH)-containing adenovirus exactly as described by Roy et al. (2000). However, more typically, we employed the following modifications of these procedures. Neurons were plated in serum-free N2 medium (Brown et al., 1992) onto glass coverslips pretreated with poly-L-lysine (1 mg/ml in borate buffer). Two hours after plating, the neurons were infected with GFP-NFH adenovirus for 5 hr. The infected construct was modified for constitutive expression rather than inducible expression (Szebenyi et al., 2002). After infection, the medium was replaced with fresh serum-free medium, and the cultures were maintained in this medium for ~24 hr. During this time, the neurons were well attached but either had no axons or extended relatively short processes. To induce rapid axon growth, neurons were transferred to L15-based medium containing matrigel and 5% fetal calf serum. Matrigel was purchased from Collaborative Biomedical Products (Bedford, MA) and was used at a 1:400 dilution of the stock supplied by the company. By the following morning, approximately 12 hr later, the neurons had long branched axons that continued elongating with time in culture. Experiments were performed 18–48 hr after matrigel addition, when the neurons had long branched axons. Compared with the culture and infection conditions described by Roy et al. (2000), these modified conditions result in earlier expression of GFP-NFH and less axon fasciculation.

Pharmacologic Manipulations

Latrunculin (Molecular Probes, Eugene, OR) was used to disrupt actin filaments, whereas vinblastine and nocodazole (both purchased from Sigma) were used to disrupt axonal MTs. Latrunculin was dissolved in dimethylsulfoxide (DMSO) at a concentration of 1 mM, vinblastine was dissolved in methanol at a concentration of 1 mM, and nocodazole was dissolved in DMSO at a concentration of 5 mg/ml. Appropriate volumes were added to culture medium to obtain the desired final concentrations.

Immunofluorescence Procedures

Cultured neurons were processed for immunofluorescent staining of MTs and actin filaments. Briefly, cultures were rinsed twice with phosphate-buffered saline (PBS) and then fixed by incubation for 10 min in PEM (80 mM PIPES, 5 mM EGTA, 1 mM $MgCl_2$, pH 6.8) containing 0.2% Triton X-100, 4% paraformaldehyde (EM Sciences, Gibbstown, NJ), and 0.15% glutaraldehyde (Polysciences, Warrington, PA). After fixation, cells were rinsed with PBS, further permeabilized by incubation in 0.5% Triton X-100 in PBS, quenched, and then blocked as described by Tint et al. (1998). We have shown previously that this fixation/extraction procedure preserves axonal MTs and actin filaments very well while at the same time extracting unassembled tubulin from axons (Black et al., 1996; Tint et al., 1998). MTs were revealed by using a mouse monoclonal antibody against alpha-tubulin (DM1 alpha; provided by Dr. V. Gelfand, University of Illinois; Blose et al., 1984). Actin filaments were revealed by staining with TRITC-phalloidin. Staining procedures were as described by Tint et al. (1998).

Image Acquisition and Analysis

Immunostained neurons were observed via epifluorescence microscopy with a Zeiss Axiovert inverted microscope (Carl Zeiss, Inc., Thornwood, NY), and images were obtained with a Princeton Instruments cooled CCD camera (Roper Scientific, Trenton, NJ) equipped with a back-thinned chip or with a SensiCam cooled CCD camera (Cooke Corporation, Auburn Hills, MI). The details of the imaging system, live cell imaging, and imaging of immunostained neurons were as described by Roy et al. (2000). NF transport was observed in natural gaps within the axonal NF array as described by Roy et al. (2000) or in photobleached gaps as described by Wang and Brown (2001). To quantify MT and actin filament staining in control and drug-treated axons, neurons were imaged with a ×40/1.3 N.A. plan neofluar oil-immersion objective, and the segmented mask procedure was used to quantify the intensity of tubulin and actin filament staining along the length of individual axons (Brown et al., 1992).

RESULTS

NF Transport Continues in Axons Deficient in Actin Filaments

Actin filaments were disrupted with latrunculin. Initial experiments examined various doses and treatment times to identify conditions that reduced actin filament staining of axons to negligible amounts. We found that relatively prolonged treatments at high doses were required to achieve this goal. Figure 1 shows actin filament staining in axons of control neurons and axons of neurons treated with 5 μM latrunculin for 6 hr. With these conditions of drug treatment, only trace staining for actin filaments was apparent in axons. Treatment times of 1, 2, or 4 hr reduced actin filament staining compared with controls, with increasing time in drug resulting in progressively less actin filament staining (data not shown). However, even after 4 hr of incubation, axonal staining with phalloidin was notably greater than that after 6 hr in drug. Quantitative analyses revealed that TRITC-phalloidin staining within the axon shaft was reduced by ~88% after 6 hr in 5 μM latrunculin. Specifically, the intensity of control axon staining with TRITC-phalloidin was 2,847 ± 1,192 ADU/μm axon (mean ± SD, n = 12),

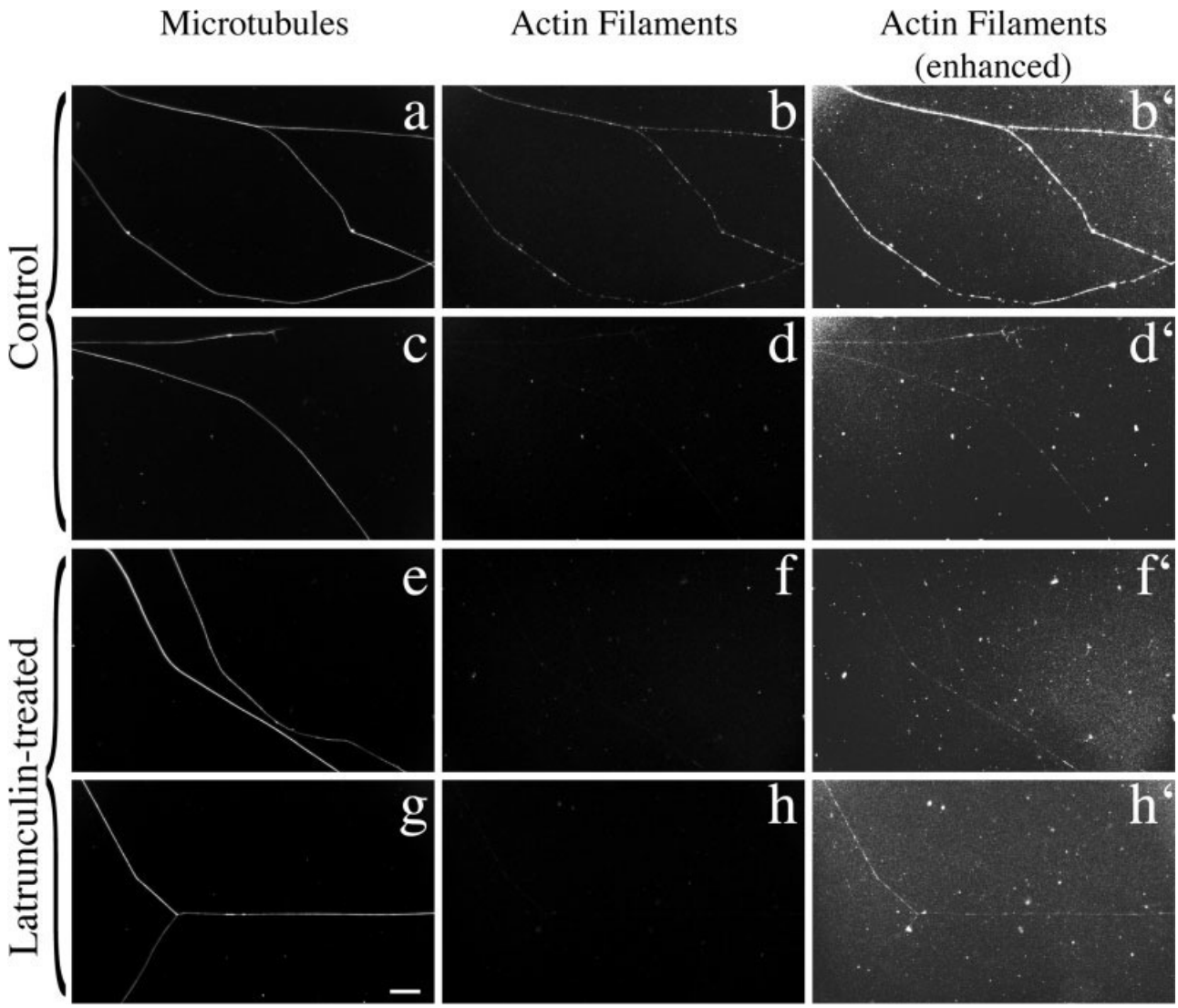

Fig. 1. Latrunculin treatment depletes axons of actin filaments. Neurons cultured for 2 days were treated with or without 5 μM latrunculin for 6 hr, extracted, fixed as described in Materials and Methods, and then doubly stained with an antibody to tubulin and with TRITC-phalloidin. The top two rows (**a–d′**) depict control axons, and the bottom two rows (**e–h′**) depict latrunculin-treated axons. Tubulin staining is shown in a, c, e, and g; the remaining panels show TRITC-phalloidin staining. To evaluate nonspecific fluorescence resulting from TRITC-phalloidin, staining was performed in the absence (b,b′,f,f′) or presence (d,d′,h,h′) of a tenfold excess of unlabeled phalloidin. Panels b′, d′, f′, and h′ are strongly enhanced versions of b, d, f, and h, respectively. Enhancement was performed by using the curves function in Adobe Photoshop as follows. The image of the control axon stained with TRITC-phalloidin without unlabeled phalloidin was adjusted so that most of the axon signal was saturated. This same adjustment was then applied to the remaining images to reveal the faint fluorescence associated with the axons. Then, all of the images were further enhanced by using the brightness contrast adjustment, applying the same transformation to each image. Note that including excess unlabeled phalloidin during staining reduced TRITC-phalloidin staining of control axons to negligible levels, comparable to the level seen in latrunculin-treated axons (compare b′ with d′ and f′). However, excess unlabeled phalloidin had no discernible effect on TRITC-phalloidin staining of latrunculin-treated axons (compare f′ with h′). Thus, 6 hr with 5 μM latrunculin effectively depletes axons of actin filaments. Scale bar = 17.8 μm.

whereas, for the latrunculin-treated axons, staining intensity was 351 ± 108 ADU/μm axon (n = 12); the difference between these values is significant at $P < 0.0001$ (*t*-test). We note that MT staining in the latrunculin-treated axons was significantly increased compared with controls (6,412 ± 1,718 ADU/μm vs. 9,294 ± 2,275 ADU/μm, $P = 0.002$). Thus, as has been reported previously, disruption of the actin and myosin systems can influence MT polymer levels (Dennerll et al., 1988; Yvon et al., 2001).

To evaluate the extent to which the residual TRITC-phalloidin staining in latrunculin-treated cells

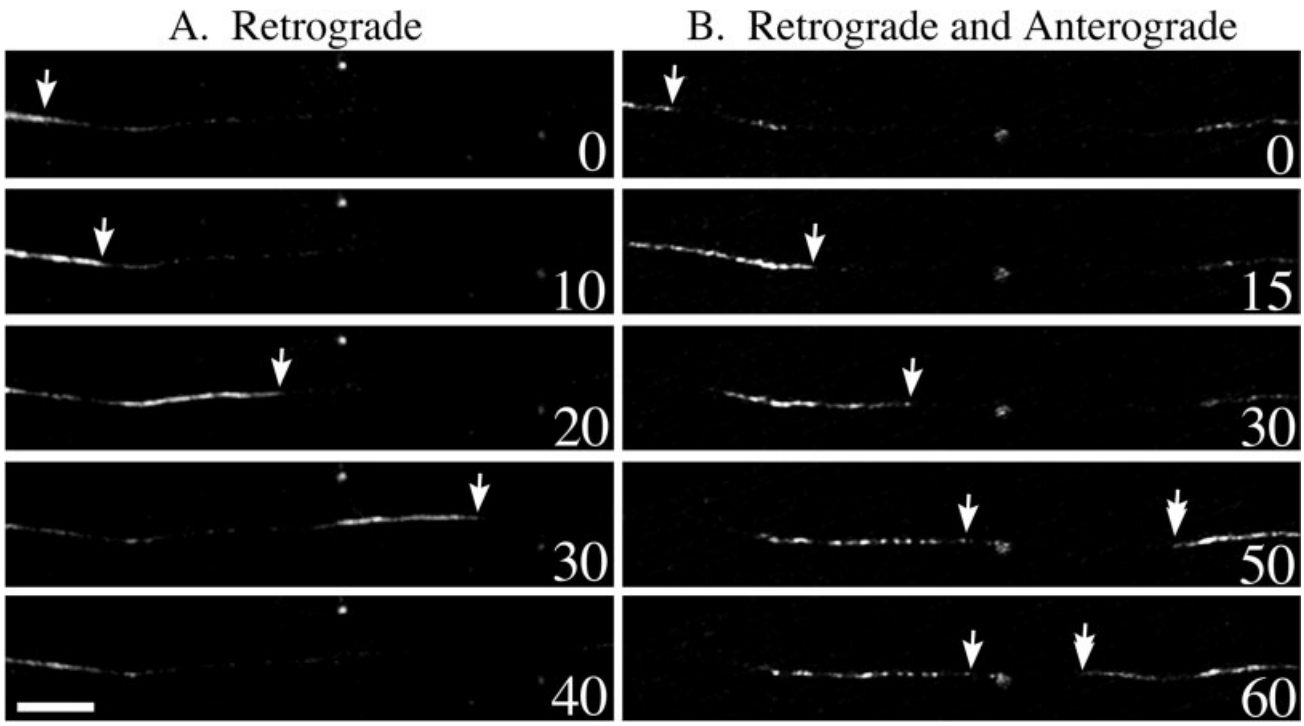

Fig. 2. Neurofilament transport in actin filament-depleted axons. Selected frames from sequences of two axons treated with 5 μM latrunculin for 6 hr. Elapsed time in seconds is indicated in the lower right of each frame, and the arrows show the front of moving NFs. The images in **A** show the movement of a retrogradely transported NF, which has an average transport rate of 1.8 μm/sec. In **B**, a retrogradely transported NF (single-headed arrows) translocates through the gap over a period of 30–40 sec at an average rate of 1.97 μm/sec and then pauses for the duration of the sequence. A second NF moves through the gap in the anterograde direction (double-headed arrows). This NF is first seen at 50 sec, and it moves steadily through the gap over the subsequent 45 sec at an average rate of 0.89 μm/sec. The figure shows the transport of this NF only over the first 10 sec of this period. Scale bar = 8.3 μm.

is due to actin filaments vs. nonspecific processes, TRITC-phalloidin staining was performed in the presence or absence of a tenfold excess of unlabeled phalloidin. The inclusion of excess unlabeled phalloidin will selectively block specific staining by TRITC-phalloidin, thereby revealing the glow resulting from nonspecific processes. As can be seen in Figure 1, the unlabeled phalloidin reduced TRITC-phalloidin staining of control neurons to negligible levels, comparable to that seen in latrunculin-treated neurons stained normally. In contrast, TRITC-phalloidin staining of latrunculin-treated neurons was not detectably diminished by the presence of unlabeled phalloidin. Collectively, these observations indicate that the trace TRITC-phalloidin fluorescence apparent in drug-treated neurons is largely nonspecific. Therefore, we conclude that 6 hr in 5 μM latrunculin effectively depletes axons of actin filaments.

NF transport occurs in latrunculin-treated cells, and it resembles that of control neurons in many respects. For the experiments reported here, neurons were imaged for 10 min to visualize NF transport, and we detected NF movement in 74% of all control neurons imaged. The corresponding value for latrunculin-treated neurons was 82% (18 of 22 axons), which is very similar to that obtained with control axons. Figure 2 shows examples of NF transport in two latrunculin-treated neurons, and Tables I and II show NF transport data obtained from the population of neurons studied. Basically, actin filament depletion had little if any effect on directionality and rate of NF transport. The only parameter examined that showed a notable change was the frequency of movement (Table I), which was decreased in latrunculin-treated cells compared with controls; on average, we detected a moving NF once every 3.1 min in control neurons, a value that agrees well with the frequency observed in previous studies on NF transport (Roy et al., 2000; Wang et al., 2000), but only once every 4.9 min in actin filament-depleted neurons. Entirely similar results were also obtained when neurons were cultured as described by Roy et al. (2000). These experiments examined NF transport in 4–5-day-old neurons, and actin filament depletion required treatment with latrunculin for ≥15 hr. With these extreme conditions of drug treatment, anterograde and retrograde NF transport was observed and was relatively normal in terms of directionality and rate, with a decrease in frequency comparable to that described above (data not shown).

We note that the average transport rates observed for NF polymer transport in both the anterograde and the retrograde directions, ~1 μm/sec (Table II), were slightly

TABLE I. Effects of Actin Filament Depletion on the Directionality and Frequency of NF Transport

	No. of moving NFs (anterograde/retrograde)	Total imaging time (min)	Frequency[a]	Total no. of axons	No. of sequences without NF movement
Control	36 (17/19)	111	3.1	14	3
Latrunculin	39 (21/18)	190	4.9	22	4

[a]The frequency of movement was calculated by dividing the total imaging time by the number of moving NFs, yielding a number for the amount of imaging time required on average to detect a moving NF (the larger the number, the lower the frequency).

TABLE II. Effects of Actin Filament Depletion on the Rate of NF Transport*

	Average NF transport rates		Maximum NF transport rates	
	Anterograde (mean ± SD)	Retrograde (mean ± SD)	Anterograde (mean ± SD)	Retrograde (mean ± SD)
Control	0.99 ± 0.43	1.15 ± 0.41	1.46 ± 0.51	1.99 ± 0.71
Latrunculin	0.96 ± 0.33	1.55 ± 0.74	1.43 ± 0.47	2.51 ± 1.03
P (*t*-test)	>0.8	>0.08	>0.8	>0.1

*Rates are in μm/sec. These data are based on analyses of 17 retrogradely transported NFs and 12 anterogradely transported NFs in control axons and 11 retrogradely transported NFs and 22 anterogradely transported NFs in latrunculin-treated axons. Latrunculin treatment had no statistically significant effect on the transport rates of NF in axons.

faster in the present studies compared with our previous work (0.6–0.7 μm/sec; Roy et al., 2000). The basis for this difference is unknown but may relate to differences in the culture conditions. In this regard, the culture conditions used here result in relatively high rates of axon extension (Slaughter et al., 1997), whereas growth in the L15-based medium described by Roy et al. (2000) results in slower rates of axon extension. Also, in the present studies, NF transport was assayed after much shorter periods of axon growth compared with the procedures used by Roy et al. (2000). As a result, even though rates of axon growth were faster in the present studies, overall axon length was much shorter than that described by Roy et al. (2000).

Microtubule Depletion Halts NF Transport

To evaluate whether MTs are required for NF transport, neurons expressing GFP-NFH were treated with drugs that cause MT depolymerization and then imaged to reveal NF transport. In one series of experiments, we used vinblastine, which very effectively promoted MT depolymerization (Fig. 3). After 3 hr in 5 μM vinblastine, axonal staining for MTs was negligible, and quantitative analyses indicated that MTs staining, normalized to axon length, was reduced by ≥98%. In contrast, vinblastine treatment had no obvious effect on actin filament staining (Fig. 3). In parallel cultures, neuron viability was assayed by using the Live/Dead Viability/Cytotoxicity Kit (Molecular Probes). The conditions of vinblastine treatment in these experiments had no detectable effects on cell viability (data not shown). In 92% of vinblastine-treated neurons (46 of 50) imaged, NF movements were not detected at all. In the remaining axons, only very limited movements were detected, resulting in an overall reduction of NF transport of ≥95% in the population of axons studied (Table III). In some experiments, neurons were fixed immediately after imaging using conditions that preserve MTs and remove unassembled tubulin and then stained to assess MT levels in the imaged axons. Because MT depletion renders axons quite fragile, some of the axons were lost during processing. However, in the axons that remained (12 of 24), no MT staining was apparent, and, in these same axons, no NF transport was detected.

In a separate series of experiments, we used nocodazole to disrupt axonal MTs. This drug depolymerizes MTs via mechanisms entirely different from those of vinblastine (Dustin, 1984) and thus provides a useful control for possible MT independent effects of vinblastine on NF transport. Neurons were treated with 5 μM nocodazole for 4.5–6 hr. This resulted in a dramatic decrease in MT staining in the axons, but not quite as extensive as that observed with vinblastine (data not shown; see also Baas and Black, 1990; Baas et al., 1991). The degree of MT loss in these experiments reduced anterograde and retrograde NF transport to an extent comparable to that seen with vinblastine (Table IV). Thus, we conclude that NF transport, both anterograde and retrograde, is absolutely dependent on MTs.

DISCUSSION

The principal goal of the present experiments was to determine the cytoskeletal requirements of NF polymer transport in growing axons. Recent work with a variety of cell types has revealed considerable movement dynamics

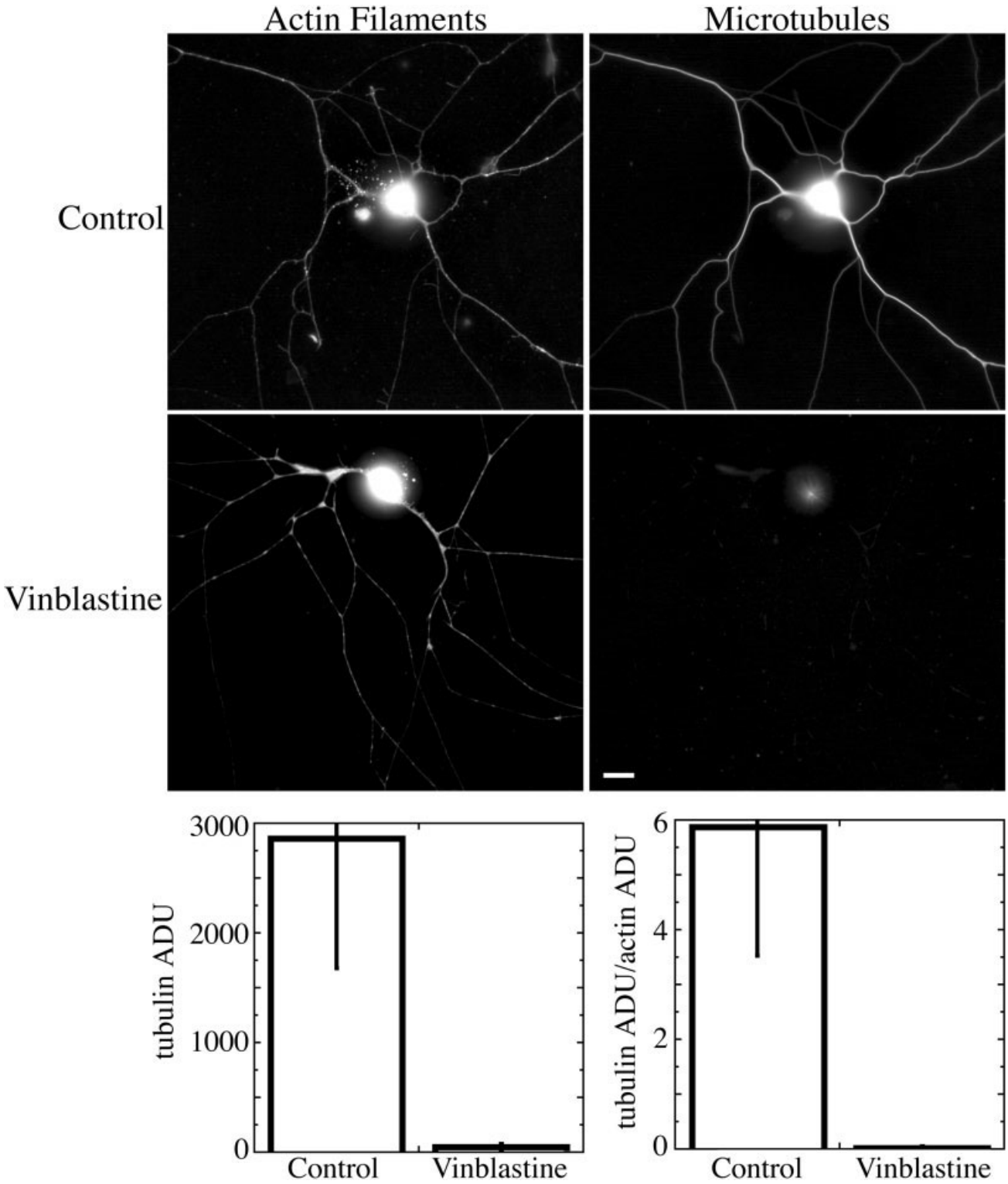

Fig. 3. Vinblastine treatment depletes axons of microtubules. Images of a control neuron and a neuron treated with 5 μM vinblastine for 3 hr showing actin filament and microtubule staining. Note that axonal staining for MTs is reduced to negligible levels after vinblastine treatment. The histograms show the results of quantitative analyses of the tubulin staining in control and drug-treated neurons. The histogram at left compares MT staining/μm axon [in analogue-to-digital units (ADU)] in control and vinblastine-treated cells, whereas the histogram at right compares MT staining normalized to actin filament staining in control and vinblastine-treated neurons. By both methods of analysis, MTs are reduced to negligible levels, and the difference between control and drug-treated axons is highly statistically significant (*t*-test, $P < 0.0001$).

TABLE III. Effects of Vinblastine Treatment on NF Transport

	No. of moving NFs (anterograde/retrograde	Total imaging time (min)	Frequency[a]	Total no. of axons	No. of sequences without NF movement
Control	86 (49/37)	356	4.1	41	12
Vinblastine	5 (2/3)	489	97.8	50	46

[a]The frequency of movement was calculated as described in Table I. Vinblastine treatment reduced NF transport frequency by more than 95% in the population of axons studied, and, in most axons, no movement was detected at all.

TABLE IV. Effects of Nocodazole Treatment on NF Transport

	No. of moving NFs (anterograde/retrograde)	Total imaging time (min)	Frequency[a]	Total no. of axons	No. of sequences without NF movement
Control	34 (16/18)	123	3.6	15	3
Nocodazole	1 (0/1)	162	162	17	16

[a]The frequency of movement was calculated as described in Table I. Nocodazole treatment reduced NF transport frequency by more than 95% in the population of axons studied, and, in most axons, no movement was detected at all.

of intermediate filament proteins (for review see Helfand et al., 2003a). These proteins in either particle form or short filaments undergo vigorous translocation that contributes to the overall configuration of the intermediate filament array. Studies on the mechanisms of this movement have revealed that it is MT dependent, and this is true for both particle and filamentous forms of intermediate filament proteins (for review see Helfand et al., 2003a). The present results expand these observations by showing that the transport of NF protein polymers in growing axons is MT dependent. The essential observation is that depletion of axonal MTs halts NF transport in both the anterograde and the retrograde directions. This result was obtained with two different drugs that depolymerize axonal MTs via entirely different mechanisms. Thus, like the movement of intermediate filament proteins in nonneuronal cell types (Helfand et al., 2003a) and nonpolymeric forms of NF proteins reported for various neuron model systems (Prahlad et al., 2000; Shea and Flanagan, 2001; Helfand et al., 2003a), NF polymer transport in elongating axons requires MTs.

In contrast to the absolute dependence of NF polymer transport on MTs, actin filaments are largely if not entirely dispensable for the longitudinal translocation of NFs in growing axons. By using latrunculin to deplete axons of actin filaments, we observed NF polymer transport that was indistinguishable from that of control axons in both directionality and rate. Although these observations are unequivocal, interpretation depends on the extent to which actin filaments are depleted from the axons by latrunculin. Detailed analyses of latrunculin-treated axons indicate that at most only trace amounts of actin filaments remain. However, the data are not sufficient to conclude that all actin filaments are disrupted. Therefore, it is formally possible that actin filaments are essential for NF polymer transport in axons and that the residual actin filaments remaining after latrunculin treatment are sufficient to maintain this function. We believe that this is unlikely for the following reasons. Depletion of MTs with either vinblastine or nocodazole brings NF polymer transport to a halt, even though normal amounts of actin filaments remain in the axon. If actin and myosin normally transport NF polymers in axons, then this movement should be apparent in axons depleted of MTs. The inability to detect such movement indicates that it is too small to resolve with our system. Our system can resolve longitudinal movements that are $\geq$0.3 μm/5 sec, and typically NF translocation occurs much faster than this (Wang et al., 2000; Roy et al., 2000; see also Table II). Thus, we conclude that the actomyosin system does not power the longitudinal movement of NFs in growing axons.

Although the available evidence indicates that actin filaments are not involved in powering the transport of NFs in axons, it is possible that the actin system contributes to NF transport behavior. In this regard, the frequency of NF transport events was reduced in latrunculin-treated cells. For control axons, we detect moving NFs on average once every ~3 minutes of imaging time, whereas, in actin-depleted axons, the frequency is reduced by ~65% to once every 5 min of imaging time (Table I). NF transport is characterized by relatively brief periods of rapid movement interspersed with prolonged quiescent periods with little or no movement (Wang et al., 2000; Roy et al., 2000). Actin disruption had no detectable effect on NF transport rate during the periods of active translocation. Thus, the decrease in frequency of NF movement events caused by actin disruption presumably reflects an increase in the duration of the quiescent periods. When averaged over time, this will have the effect of slowing NF transport. The relevance of this effect of actin filament

disruption on mechanisms of NF transport is difficult to evaluate. It may simply be an indirect consequence of cytoplasmic disorganization resulting from prolonged actin filament disruption. However, it is well documented that actin and myosin influence the MT-based transport behavior of membrane-bound structures and that disruption of actin filaments can alter this behavior (Brady et al., 1984; Huang et al., 1999; Langford, 2002; Lalli et al., 2003; Nascimento et al., 2003). Furthermore, mice lacking myosin Va have abnormal NF organization (Rao et al., 2002). These considerations together with the present findings of reduced NF transport frequency in actin filament-depleted axons are consistent with the possibility that actin filaments together with one or more of the myosin motors normally influence MT-based NF transport. Further work will be required to define the nature of this influence.

The dependence of NF transport on MTs focuses attention on a role for MT motors in powering this movement. Two general classes of MT motors exist, the kinesin superfamily and the dyneins. Most kinesins move their cargoes along MTs in the anterograde direction, whereas dyneins move their cargoes in the retrograde direction. NFs move in both the anterograde and the retrograde directions. Thus, a straightforward view is that one or more members of the kinesin superfamily mediate anterograde transport, whereas dynein mediates retrograde transport (Shea and Flanagan, 2001). Other possibilities have also been suggested in which dynein might mediate transport in both directions (Roy et al., 2000). Recent work supports the former view. Specifically, we have demonstrated that retrograde NF polymer translocation in axons is mediated via dynein (He et al., 2005). By using siRNA treatment to deplete dynein from axons, we observed that anterograde NF transport continued unabated, whereas retrograde transport was diminished or halted depending on the extent of dynein depletion. The motors for anterograde NF transport in our system have not been identified. However, from several nonneuronal systems, immunochemical evidence has been presented that kinesin-1 (conventional kinesin) may mediate anterograde movement of intermediate filament proteins along MTs (for review see Helfand et al., 2003a), and similar studies with neuronal systems have argued in support of a role for kinesin-1 in intermediate filament protein transport (Prahlad et al., 2000; Xia et al., 2003; Helfand et al., 2003b). However, detailed kinetic analyses of kinesin transport in pulse-chase studies failed to detect kinesin-1 moving with NF proteins (Elluru et al., 1995) and subcellular fractionation of brain tissue under conditions that preserve membrane interactions recover up to 95% of the kinesin-1 in various membrane fractions, with no detectable NF protein (Tsai et al., 2000). The picture is further complicated by reports that other kinesins might interact with NFs (Shah et al., 2000), raising the possibility that multiple members of the kinesin superfamily contribute to NF transport behavior. Further work, directly assessing kinesin perturbations on NF transport in axons is required to define fully the role of kinesin superfamily members in NF transport behavior.

ACKNOWLEDGMENTS

We thank Theresa Slaughter for her assistance in all phases of this work and Irina Tint for many helpful discussions. The work reported here was supported by grants from the NIH to M.M.B. and S.T.B.

REFERENCES

Baas PW, Black MM. 1990. Individual microtubules of the axon consist of distinct domains that differ in stability and composition. J Cell Biol 111:494–509.

Baas PW, Brown A, Slaughter T, Black MM. 1991. Microtubule dynamics in axons and dendrites. J Neurosci Res 30:134–153.

Black MM, Kurdyla J. 1983. Microtubule-associated proteins of neurons. J Cell Biol 97:1020–1028.

Black MM, Slaughter T, Moshiach S, Obrocka M, Fischer I. 1996. Tau is enriched on dynamic microtubules in the distal region of growing axons. J Neurosci 16:3601–3619.

Blose SH, Meltzer DI, Feramisco JR. 1984. 10-nm Filaments are induced to collapse in living cells microinjected with monoclonal and polyclonal antibodies against tubulin. J Cell Biol 98:847–858.

Brady ST, Lasek RJ, Allen RD, Yin HL, Stossel TP. 1984. Gelsolin inhibition of fast axonal transport indicates a requirement for actin filaments. Nature 310:56–58.

Brown A. 2000. Slow axonal transport: stop and go traffic in the axon. Nat Rev Mol Cell Biol 1:153–156.

Brown A, Slaughter T, Black MM. 1992. Newly assembled microtubules are concentrated in the proximal and distal regions of growing axons. J Cell Biol 119:867–882.

Dennerll TJ, Joshi HC, Steel VL, Buxbaum RE, Heidemann SR. 1988. Tension and compression in the cytoskeleton of PC-12 neurites II: quantitative measurements. J Cell Biol 107:665–674.

Dustin P. 1984. Microtubules. Berlin: Springer-Verlag.

Elluru R, Bloom GS, Brady ST. 1995. Fast axonal transport of kinesin in the rat visual system: functionality of the kinesin heavy chain isoforms. Mol Biol Cell 6:21–40.

He Y, Francis F, Myers KA, Yu W, Black MM, Baas PW. 2005. Role of cytoplasmic dynein in the axonal transport of microtubules and neurofilaments. Submitted for publication.

Helfand BT, Chang L, Goldman RD. 2003a. The dynamic and motile properties of intermediate filaments. Annu Rev Cell Dev Biol 19:445–467.

Helfand BT, Loomis P, Yoon M, Goldman RD. 2003b. Rapid transport of neural intermediate filament protein. J Cell Sci 116:2345–2359.

Huang JD, Brady ST, Richards BW, Stenolen D, Resau JH, Copeland NG, Jenkins NA. 1999. Direct interaction of microtubule- and actin-based transport motors. Nature 397:267–270.

Lalli G, Gschmeissner S, Schiavo G. 2003. Myosin Va and microtubule-based motors are required for fast axonal retrograde transport of tetanus toxin in motor neurons. J Cell Sci 116:4639–4650.

Langford GM. 2002. Myosin-V, a versatile motor for short-range vesicle transport. Traffic 3:859–865.

Nascimento AA, Roland JT, Gelfand VI. 2003. Pigment cells: a model for the study of organelle transport. Annu Rev Cell Dev Biol 19:469–491.

Prahlad V, Helfand BT, Langford GM, Vale RD, Goldman RD. 2000. Fast transport of neurofilament protein along microtubules in squid axoplasm. J Cell Sci 113:3939–3946.

Rao MV, Engle LJ, Mohan PS, Yuan A, Qiu D, Cataldo A, Hassinger L, Jacobsen S, Lee VM, Andreadis A, Julien JP, Bridgman PC, Nixon RA. 2002. Myosin Va binding to neurofilaments is essential for correct myosin Va distribution and transport and neurofilament density. J Cell Biol 159:279–290.

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.

Shah JV, Flanagan LA, Janmey PA, Leterrier JF. 2000. Bidirectional translocation of neurofilaments along microtubules mediated in part by dynein/dynactin. Mol Biol Cell 11:3495–3508.

Shea TB, Flanagan LA. 2001. Kinesin, dynein and neurofilament transport. Trends Neurosci 24:644–648.

Slaughter T, Wang J, Black MM. 1997. Microtubule transport from the cell body into the axons of growing neurons. J Neurosci 17:5807–5819.

Szebenyi G, Smith GM, Li P, Brady ST. 2002. Overexpression of neurofilament H disrupts normal cell structure and function. J Neurosci Res 68:185–198.

Tint I, Slaughter T, Fischer I, Black MM. 1998. Acute inactivation of tau has no effect on dynamics of microtubules in growing axons of cultured sympathetic neurons. J Neurosci 18:8660–8673.

Tsai M-Y, Morfini G, Szebenyi G, Brady ST. 2000. Modulation of kinesin-vesicle interactions by Hsc70: implications for regulation of fast axonal transport. Mol Biol Cell 11:2161–2173.

Wang L, Brown A. 2001. Rapid intermittent movement of axonal neurofilaments observed by fluorescence photobleaching. Mol Biol Cell 12: 3257–3267.

Wang L, Ho CL, Sun D, Liem RKH, Brown A. 2000. Rapid movement of axonal neurofilaments interrupted by prolonged pauses. Nat Cell Biol 2:137–141.

Xia CH, Roberts EA, Her LS, Liu X, Williams DS, Cleveland DW, Goldstein LS. 2003. Abnormal neurofilament transport caused by targeted disruption of neuronal kinesin heavy chain KIF5A. J Cell Biol 161:55–66.

Yvon A-M, Gross DJ, Wadsworth P. 2001. Antagonistic forces generated by myosin II and cytoplasmic dynein regulate microtubule turnover, movement, and organization in interphase cells. Proc Natl Acad Sci U S A 98:8656–8661.