

Organizational Dynamics, Functions, and Pathobiological Dysfunctions of Neurofilaments

Thomas B. Shea, Walter K.-H. Chan, Jacob Kushkuley,
and Sangmook Lee

Abstract Neurofilament phosphorylation has long been considered to regulate their axonal transport rate, and in doing so it provides stability to mature axons. We evaluate the collective evidence to date regarding how neurofilament C-terminal phosphorylation may regulate axonal transport. We present a few suggestions for further experimentation in this area, and expand upon previous models for axonal NF dynamics. We present evidence that the NFs that display extended residence along axons are critically dependent upon the surrounding microtubules, and that simultaneous interaction with multiple microtubule motors provides the architectural force that regulates their distribution. Finally, we address how C-terminal phosphorylation is regionally and temporally regulated by a balance of kinase and phosphatase activities, and how misregulation of this balance might contribute to motor neuron disease.

1 Introduction

The orderly assembly of initially soluble subunits to form the complex network collectively referred to as the cytoskeleton presents a formidable challenge to any cell. Neurons, unlike other cells, must selectively transport cytoskeletal elements over distances that vastly exceed their perikarya into their axons. The cytoskeleton of the axon differs considerably from that of the perikaryon, and even from that of dendrites. Key differences include the large number of longitudinally oriented neurofilaments (NFs), which are enriched in C-terminal modification by

T.B. Shea (✉), W.K.-H. Chan, J. Kushkuley, and S. Lee
Departments of Biological Sciences and Biochemistry, Center for Cellular
Neurobiology and Neurodegeneration Research, University of Massachusetts
Lowell, One University Avenue, Lowell, MA, 01854
e-mail: Thomas_Shea@uml.edu

phosphorylation. For decades, the network of crosslinked, fibrous images observed via electron and conventional fluorescence microscopy, coupled with pioneering but simple radiolabeling techniques, led us to consider the axonal cytoskeleton as a support mechanism that, once assembled, was relatively inert. However, the advent of fluorescently conjugated cytoskeletal proteins and the ability to manipulate kinase activities *in situ* have revealed that (1) the axonal cytoskeleton is an incredibly dynamic structure, and that (2) the “crosslinks” are often more like handshakes along a moving reception line than permanent bolts between girders.

Development and maintenance of a functional nervous system is by definition dependent upon orderly elaboration and maintenance of the axonal array, which in turn is critically dependent upon organization of the axonal cytoskeleton. Although it is clear that compromise in axonal cytoskeletal dynamics can foster a range of mental and neuromuscular disorders throughout life, many fundamental processes that regulate axonal cytoskeletal organization remain the subject of controversy. One such controversial area is the role of NF phosphorylation in axonal transport.

The nature of this controversy, as discussed herein, is in part due to the complexity of NF phosphorylation, which involves the action of multiple kinases and hierarchical phosphorylation of multiple loci on NF subunits. In addition, however, a significant portion of the controversy arises from semantics and from different laboratories using approaches that highlight different aspects of NF dynamics.

Neurofilaments (NFs) are among the most abundant constituents of the axonal cytoskeleton. NFs have classically been considered to consist of three subunits, termed NF-H, NF-M, and NF-L, corresponding to heavy, medium, and light in reference to their molecular mass (Julien and Mushynski 1998). More recently, it has been demonstrated that an additional neuronal intermediate filament, alpha-internexin, is actually a fourth subunit (Yuan et al., 2006b). The C-terminal regions (“sidearms”) of NF-H and NF-M contain multiple phosphorylation sites (Julien and Mushynski 1998) and protrude laterally from the filament backbone when phosphorylated (Sihag et al. 2007). Phosphorylation of these C-terminal sidearms regulates the interactions of NFs with each other and with other cytoskeletal structures, and, in doing so, it mediate the formation of a cytoskeletal lattice that supports the mature axon (Nixon 1998; Pant and Veeranna 1995; Sihag et al. 2007). A considerable body of evidence, spanning several decades, from a number of laboratories using diverse systems and approaches, supports the notion that phosphorylation of C-terminal sidearms, in particular those of NF-H, regulates NF axonal transport (Ackerley et al. 2003; Collard et al. 1995; DeWaegh et al. 1992; Hoffman et al. 1983; Jung and Shea 1999; Jung et al. 2000a, b; Lewis and Nixon 1988; Komiya et al. 1987; Marszalek et al. 1996; Nixon 1993; Shea et al. 1993; Tu et al. 1995; Watson et al. 1993; Yabe et al. 2001a, b; Zhang et al. 1997; Zhu et al. 1998). Given the wealth of supporting information for this role, it was perhaps to be expected that the appearance of a report suggesting that NF-H played no role in the regulation of NF transport would stimulate debate, among which included a challenge to the extent to which their data supported this unanticipated claim (Rao et al. 2002, 2003; Shea et al. 2003; Yuan et al. 2006a, b). Herein, we evaluate the collective evidence to date for and against a role for NF-H C-terminal phosphorylation in regulation of NF axonal transport, and present a few suggestions for further experimentation in this area.

2 C-Terminal Phosphorylation Regulates NF Axonal Transport

A considerable amount of the prior evidence presented to support a role for NF-H C-terminal phosphorylation in the regulation of NF axonal transport was correlative rather than experimental (Archer et al. 1994; Hoffman et al. 1983; Lewis and Nixon 1988; Nixon and Logvinenko 1986); that is, regional and/or developmental slowing of transport rates were regionally or temporally associated with the increases in NF-H C-terminal phosphorylation. Conversely, however, simultaneous analyses within the same optic axons of transport rates of differentially phosphorylated forms of NF-H revealed that the least phosphorylated NF-H variants (which migrated at 160 kDa on SDS-gels) transported twice as fast as NF-H subunits phosphorylated to the extent that they migrated on SDS-gels at 200 kDa. The subset of these 200 kDa NFs that displayed a unique phospho-epitope recognized by the developmentally delayed monoclonal antibody RT97, and enriched in those NFs undergoing the slowest transport (Lewis and Nixon 1983; Yabe et al. 2001a, b), underwent a further twofold slower transport (Jung et al., 2000a, b). This fourfold range of transport rates displayed simultaneously within the same axons by differentially phosphorylated NF-H subunits in the absence of any experimental manipulation supports a role for NF-H C-terminal phosphorylation in regulation of NF axonal transport. Moreover, direct manipulation of phosphorylation state within these optic axons *in situ* by regional application of a phosphatase inhibitor within optic axons *in situ* both increased RT97 immunoreactivity and decreased the rate of NF transport without decreasing the transport of other cargo (Jung and Shea 1999). These latter experimental studies provide more than correlative evidence for a role of C-terminal NF phosphorylation in regulation of transport rate. The direct association of RT97 with slower-transporting NFs in these latter studies also argues against dismissing prior regional and developmental correlations between NF phosphorylation due to their correlative nature. Finally, overexpression of NF-H dramatically slowed NF axonal transport *in situ* (Collard et al. 1995; Marszalek et al. 1996).

Additional attempts to address the role of C-terminal phosphorylation in NF dynamics included deletion of NF-H, which resulted in an increased rate of NF transport (Jung et al. 2006; Zhu et al. 1998). However, this was accompanied by a significant increase in axonal microtubules (Zhu et al. 1998). As NFs undergo anterograde axonal transport, at least in part via the microtubule motor kinesin (Xia et al. 2003; Yabe et al. 1999, 2000), it can therefore be effectively argued that the observed increased rate of transport is due to increased availability of transport machinery, and perhaps also due to an increased NF-M content, because NF-M is thought to contribute to the association of NFs with kinesin (Yabe et al. 2000). Selective deletion of the sidearm, which does not invoke a compensatory increase in axonal microtubules, or NF-M (Rao et al. 2002), is indeed a more refined approach towards the investigation of any role for NF C-terminal sidearms than complete subunit deletion. To accomplish this, Rao and colleagues inserted a gene expressing a truncated, tail-less NF-H into mice, in which the endogenous full-length NF-H had been deleted.

However, this approach is still encumbered by compensatory increases in phosphorylation of the NF-M C-terminal sidearm. Most strikingly, this compensatory increase includes the de novo appearance of RT97 immunoreactivity on NF-M, indicating a degree of reciprocity between NF-H and NF-M (Rao et al. 2002; Sanchez et al. 2000; Tu et al. 1995), which may affect NF transport. In this regard, those NFs from NF-H mice bearing RT97 immunoreactivity on NF-M were selectively excluded from a standard kinesin motor preparation (Shea and Chan 2008), which is analogous to the exclusion from this motor preparation of NFs from normal mice that bear RT97 on NF-H (Jung et al. 2006). As the above correlative and experimental evidence suggests a relationship between C-terminal phosphorylation events that generate RT97 immunoreactivity with regulation of NF transport rate, compensatory phosphorylation of the NF-M C-terminal sidearm to foster RT97 immunoreactivity confounds interpretation of the impact of NF-H C-terminal deletion on NF transport in the same manner as the compensatory increase in microtubules and in NF-M subunits that accompany deletion of the entire NF-H molecules. Studies involving deletion of the C-terminal region of NF-M (Rao et al. 2003) similarly cannot effectively address the impact of NF-M C-terminal phosphorylation on transport regulation due to the presence of intact NF-H (Jung et al. 2006).

Studies of the impact of C-terminal deletion on NF transport in situ have thus far been confined to relatively early periods following the administration of radiolabel (3–14 days; Rao et al. 2002, 2003; Shea et al. 2003; Yuan et al. 2006a, b). There are two major flaws in this approach. The first problem is that the effect of C-terminal phosphorylation is confined to the trailing aspect of the wave, *not* the leading edge. “Extensively phosphorylated” NFs are those that exhibited a progressive slowing of transport rate, while nonphosphorylated NFs, instead, maintained a constant rate (Jung et al. 2000a, b). In these studies, extensively phosphorylated NF-H were confined to the most proximal segments, while the leading edge, which is completely lacking phospho-dependent RT97 immunoreactivity, continued to progress along axons. The second problem, directly related to the first, is that it is far too early at 3–14 days to attempt any definitive examination of the impact of phosphorylation on NF retention along axons. The slowest-moving NFs, which include those that partition within the so-called “stationary phase” retained for extend periods (e.g., 1 year; Nixon and Logvinenko 1986), are those that are the most enriched in C-terminal phosphorylation (Lewis and Nixon 1988). While C-terminal phosphorylation can clearly foster retention of some NFs along axons, the lack of C-terminal phosphorylation sites should not be expected to increase transport of the leading edge of the moving wave, *because NFs in this region are not phosphorylated even in intact NF-H* (Jung et al. 2000a, b). The failure of “truncated” NFs to speed up should not be extrapolated to indicate that they would also exhibit a subsequent failure to slow down; this possibility can only be addressed instead by long-term pulse-chase analyses, of the sort previously utilized to demonstrate that phosphorylation indeed slows transport of NFs comprised of full-length subunits (Lewis and Nixon 1988; Nixon and Logvinenko 1986).

Additional compelling experimental evidence supporting a role for NF-H C-terminal phosphorylation in the regulation of NF axonal transport comes from

Miller and colleagues (Ackerley et al. 2003), who demonstrated the transfection of cultured cortical neurons with constructs expressing site-directed mutated forms of NF-H along with nonmutated “wild-type” NF-H, each of which was tagged with green fluorescent protein to allow real-time monitoring of axonal transport within living neurons. In these analyses, seven consensus sites, known to be phosphorylated within the C-terminal region of NF, were mutated to alanine (to prevent phosphorylation), or aspartate (to mimic phosphorylation), generating “constitutively nonphosphorylated” and “constitutively phosphorylated” NF-H, respectively. Analyses of transport rates demonstrated that NFs containing constitutively non-phosphorylated NF-H transported faster than those containing wild-type NF-H, while NFs containing constitutively phosphorylated NF-H transported slower than those containing wild-type NF-H (Ackerley et al. 2003).

Site-directed mutagenesis of the NF-H C-terminal sidearm also interfered with the effects of kinases known to regulate NF transport. p24/44 MAP kinase-mediated NF phosphorylation is essential for anterograde NF transport (Chan et al. 2004), while phosphorylation by Cdk5 inhibits it (Ackerley et al. 2003; Shea et al. 2004). Constitutive phosphorylation of the C-terminal NF-H sidearm prevented the inhibition of anterograde NF axonal transport that normally accompanies MAP kinase inhibition, and prevented the acceleration of NF transport, which normally accompanies cdk5 inhibition (Ackerley et al. 2003; Shea et al. 2004). Finally, site-directed mutagenesis altered the association of NFs bearing these mutations with kinesin: mutation of C-terminal cdk5-consensus sites to alanines increased the association of NFs with kinesin as compared to wild-type NFs, while mutation to aspartate decreased their association (Shea and Chan 2008). These data further support a role for C-terminal NF-H phosphorylation in the regulation of NF transport, and can be considered to represent a somewhat more refined approach than truncation of a substantial length of the sidearm (Rao et al. 2003).

3 NF Transport and Residence Time Along Axons is Regulated by a Combination of Microtubule Motors and C-Terminal NF-H Phosphorylation

Two interrelated, well-described phenomena provide mechanisms by which C-terminal NF phosphorylation can impact NF axonal transport: phospho-mediated dissociation of NFs from their anterograde transport system and phospho-mediated NF–NF associations that compete with transport. Studies from several laboratories demonstrate that the microtubule motors kinesin and dynein mediate anterograde and retrograde NF axonal transport, respectively (He et al. 2005; Motil et al. 2006a, b; Prahlad et al. 2000; Shah et al. 2000; Theiss et al. 2005; Yabe et al. 1999, 2000; Xia et al. 2003). In this regard, C-terminal phosphorylation of NF-H progressively restricts association of NFs with kinesin to the point where RT97-reactive NFs do not associate with kinesin at all (Yabe et al. 1999), but instead demonstrates selective affinity for dynein (Motil et al. 2006a, b); restriction of binding to an

anterograde motor provides a mechanism by which C-terminal phosphorylation can slow NF axonal transport, especially when coupled with promotion of binding to a retrograde motor. The ability of kinesin and dynein (motors which undergo so-called “fast” axonal transport) to translocate cargo such as NFs (the majority of which undergo so-called “slow” transport) has been validated by the demonstration that NFs undergo a series of rapid excursions at a fast rate, interspersed with prolonged pauses, which averages out to slow transport (Wang et al. 2000; Roy et al. 2000). This also accounts for the ability of some NF populations to undergo rapid transport.

In addition to regulation of motor protein association, C-terminal NF phosphorylation promotes NF–NF associations, leading to the generation of NF “bundles” (Yabe et al. 2001a, b). So-called “bundled” NFs underwent transport and/or exchange at a rate of at least 2× slower than did the surrounding “individual” NFs (Yabe et al. 2001a). RT97 immunoreactivity is selectively concentrated within the bundles, and phosphatase inhibition increases bundle size within axonal neurites. NFs containing the above constitutively phosphorylated C-terminal NF–H are also selectively concentrated within the bundles, while the constitutively nonphosphorylated forms are selectively excluded (Chan et al. 2005). Notably, this is the opposite of their respective affinity with kinesin, which underscores the reciprocal influence of NF phosphorylation on anterograde transport and NF–NF associations that can lead to bundle formation. These analyses in culture are consistent with the findings of Lewis and Nixon (1988) that RT97-reactive NFs were the slowest-moving NFs within axons in situ. Phospho-mediated NF–NF associations are likely to generate a “macro-structure” that precludes effective transport (Shea and Flanagan 2001; Shea and Yabe 2000). Whether the formation of NF–NF associations requires additional phosphorylation events beyond those that restrict motor binding is not clear as yet. Nevertheless, as phospho-NFs can bind to each other, this gives rise to an additional competing force, unique to the extensively phosphorylated NFs, and not present for nonphosphorylated/less phosphorylated NFs that can interfere with NF-motor associations.

The dynamics of moving and pausing NFs in terms of their association and dissociation with motor systems have been mathematically modeled, and this model agrees with the published data from several in vivo systems (Brown et al. 2005; Craciun et al. 2005). This model describes a pool of NFs associated with motors and a pool that is dissociated from motors but can readily reassociate with them. While this model did not present any definitive regulatory factors, the above studies would suggest that NF C-terminal phosphorylation is one factor that regulates the shift of NFs between these two pools. In further support of this notion, comparative analyses revealed that, when they were actually moving, constitutively phosphorylated NFs underwent transport at the same rate as the constitutively nonphosphorylated NFs; however, constitutively phosphorylated NFs paused more frequently than the constitutively nonphosphorylated NFs (Ackerley et al. 2003). The model presented for NF transport dynamics by Brown and colleagues (Brown et al. 2005; Craciun et al. 2005; Trivedi et al. 2007) also describes a third “pool” of NFs, which are dissociated from motor systems and are restricted in some capacity from entering

the pool of NFs, which can reversibly associate with motors; the above studies would identify this latter pool as those NFs that have formed NF–NF associations to the extent that they are incapable of undergoing transport at least pending dissociation of these inter-NF linkages. Shea and Yabe (2000) presented a model of how continued formation of NF–NF associations could “trap” some NFs within the central region of a bundle. Any NF localized within a bundle would be restricted from reassociation with motors pending (1) dissociation and removal of sterically interfering NFs, (2) dissociation of any direct NF–NF associations of the given NF itself (which may require dephosphorylation), (3) availability of a cargo-free motor, and (4) association of the NF with that motor (which may also require an additional dephosphorylation event). This concept of steric hindrance of bundled NFs from reassociating with motors has been given experimental support by ongoing studies from our laboratory (Chan et al. 2007) in which some NFs dissociated from bundles by calcium chelation readily underwent kinesin-dependent association with MTs.

Brown and colleagues have recently expanded their modeling to include an additional “stationary” phase (Trivedi et al. 2007), which, like the earlier *in vivo* analyses of Lewis and Nixon (1988) and our analyses in differentiated neuroblastoma (Yabe et al. 2001a), is comprised of the slowest-moving “population” of NFs. Our earlier model of how phosphorylation can regulate NF transport (Shea and Flanagan 2001; Shea and Yabe 2000) can be expanded according to the more recent transport model of Brown and colleagues (Brown et al. 2005; Craciun et al. 2005; Trivedi et al. 2007), and integrated with it as follows.

Let us consider that nonphosphorylated NFs, which cannot form NF–NF bundles, can be characterized as either being on their motor or off their motor. By contrast, the distribution of NFs that have undergone key phosphorylation events not only encompasses these two states, but also includes a third condition: NFs that have undergone bundling, which restricts them from motor-dependent transport (Shea and Flanagan 2001; Shea and Yabe 2000). For simplicity, we will not further subdivide these populations into groups such as NFs that are associated with the motor, but with the motor “off track” as included in the model by Brown and colleagues (Brown et al. 2005; Craciun et al. 2005; Trivedi et al. 2007). Similarly, we will not distinguish between simple NF–NF associations and association of NFs with a bundle of NFs, but will instead group all NFs that have formed NF–NF associations as one population. Also for simplicity, we will assume that a given NF can move between/among available states with equivalent probability. Accordingly, nonphospho NFs would be expected to be associated with their motor 50% of the time, and dissociated from their motor the remaining 50% of the time. Phospho-NFs, by contrast, would be associated with their motor 33.3% of the time and dissociated from their motor 33.3% of the time (but not necessarily associated with other NFs), and associated with one or more other NFs for the remaining 33.3% of the time. As NF transport is contingent upon motor association and the rate of transport is dependent upon the frequency of motor association (Blum and Reed 1989), the selective decreased probability of phospho-NFs association with their anterograde transport system (33.3% vs. 50% for nonphosphorylated NFs) immediately suggests that they would undergo decreased overall anterograde transport.

Notably, the dynamics of NF bundling can contribute to dramatic additional slowing of net transport of phospho-NFs; once embedded within a bundle, a given NF must rely on elimination of all surrounding NFs before it can dissociate from the bundle and be available to resume motor association. Notably, if each such step is reversible, an “embedded” NF is equally likely to toggle back and forth between levels within a bundle, as opposed to consistently progressing towards the surface and dissociating from the bundle. Finally, even if a given NF dissociate from the bundle and is available for motor association, any passing motors may already be occupied with cargo (Shea and Yabe 2000). Both of these latter considerations would impose additional reductions in net NF transport rate.

These multiple, phospho-dependent dynamics could easily generate the wide range of transport rates observed for NFs in the various systems where studied, and strongly support a role for C-terminal NF phosphorylation in the slowing of NF axonal transport, and selective retention of phospho-NFs along axons for extended periods after less phosphorylated variants have translocated the full length of the axon (Jung et al. 2000a; Lewis and Nixon 1988; Nixon and Logvinenko 1986; Yabe et al 2001a).

It could be argued that phosphorylation could progressively slow NF axonal transport and generate the observed resident population of NFs by fostering longer periods of dissociation of NFs from their anterograde motor without invoking the need for bundling. However, simple dissociation, by phosphorylation either of NFs or of kinesin/linker proteins, is apparently inadequate in and of itself to generate a long-lasting resident NF population, as inhibition of p42/44 MAP kinase, known to be essential for anterograde NF axonal transport, did not simply curtail anterograde NF transport and leave NFs “in place” along axons. Rather, it resulted in bulk retrograde NF transport (Chan et al. 2004). Retrograde NF transport had been observed within axons under normal conditions, and could be highlighted by manipulations such as photobleaching regions of axons (which revealed that the bleached zone filled in from distal as well as proximal edges) or axonal transection (which, by blocking continuous anterograde flow of radiolabeled subunits, allowed retrograde NF transport to be observed distal to the transection); however, NFs undergoing retrograde transport were a minority as compared to those undergoing anterograde transport (Koehnle and Brown 1999; He et al. 2005; Roy et al. 2000; Wang et al. 2000; Motil et al. 2006a; Yabe et al. 1999, 2000). In studies where NF particle movement was quantified, 69–77% of moving NFs/NF particles underwent anterograde transport (Wang and Brown 2000; Roy et al. 2000; Chan et al. 2004). Individual particles were clearly capable of movement in either direction, as some were observed to change directionality of movement (Roy et al. 2001; Wang and Brown 2001; Chan et al. 2004), and inhibition of MAP kinase (required for anterograde NF transport) caused a “flip” of $76.7\% \pm 9\%$ particles moving in an anterograde direction to $75.6\% \pm 12\%$ moving in a retrograde direction (Chan et al. 2004). Bulk retrograde NF transport following inhibition of anterograde transport as seen by Chan et al. suggested that virtually all axonal NFs were subject to retrograde forces at all times, but net retrograde translocation was in some way

counterbalanced and, in the case of most of the moving wave, outweighed by anterograde forces. Studies from several laboratories demonstrated that dynein mediated retrograde NF transport, confirming the existence of an opposing motor force (He et al. 2005; Motil et al. 2006a, b; Shah et al. 2000; Theiss et al. 2005). In addition to mediating retrograde transport, dynein also propels MTs in an anterograde direction via cargo-based interactions with actin filaments, and/or larger MTs, and, in doing so, delivers MTs into axons (Baas et al., 2006). NFs interacting with such MTs would therefore undergo dynein-based translocation into axons as MT cargo (Motil et al. 2006a).

Notably, however, the entire NF bundle retracted following inhibition of anterograde transport, while the axon itself did not retract, nor was overall axonal transport altered. This finding challenges the notion that NFs within the bundle underwent slower transport because the bundle itself was simply too large to be translocated (Lewis and Nixon 1988; Yabe et al. 2001a, b). Rather, it suggests that the maintenance of this macrostructure along the axons was also in some manner dependent upon the counterbalance of opposing motor forces. In this regard, our prior studies demonstrate that dynein preferentially associated with phospho-NFs (Motil et al. 2006a, b). Notably, this is the opposite of what had been observed for kinesin, which preferentially associated with nonphosphorylated NFs (Yabe et al. 2000). These findings suggested that a drag imparted by dynein could be responsible for the selective slowing in the transport rate of phospho-NFs, while allowing less/nonphosphorylated NFs to continue transporting at relatively faster rate. This was perhaps to be anticipated, as the most highly phosphorylated NFs are selectively incorporated into bundles (Yabe et al. 2001a, b). In addition, however, we observed that manipulation of dynein activity influenced the bundle. Dynein-mediated slowing of transport, or cessation of transport due to simultaneous binding of kinesin and dynein, may facilitate or even be required for the formation of the divalent cation-mediated associations of phospho-NFs that lead to bundling. We interpret these data to indicate that both motors are likely to be critical for NF bundle formation. This could occur simply by increasing residence time of NFs within axons, which could foster an increase in NF C-terminal phosphorylation, including those events critical for NF–NF associations. This line of reasoning is consistent with the hypotheses of Glass and Griffin (1994), who suggested that retrograde NF transport was one mechanism contributing to increased residence time of NFs along axons. As NFs contain contiguous phosphorylated and nonphosphorylated domains (Brown 1998; Chan et al. 2005), NFs in various overall phosphorylation states may retain the capacity to associate simultaneously with both motors.

As C-terminal phosphorylation promotes NF association with dynein (Motil et al. 2006a, b), the bulk of axonal NFs are highly phosphorylated (Pant and Veeranna 1995), and equivalent amounts of NFs/NF particles are capable of translocation in either direction following kinase manipulation (above), the question remains as to why only a minority of axonal NFs undergo retrograde transport under normal conditions. The answer may lie in phospho-mediated NF bundling itself. Those NFs that are capable of association with dynein (i.e., those that have undergone

extensive C-terminal phosphorylation) are also the ones that form/associate with NF bundles. Thus, few NFs are freely available to undergo retrograde transport at any given time due to their tendency to form NF–NF associations leading to bundling. The major function of dynein with regard to NFs may not be to generate retrograde NF translocation, but rather to provide a counterbalance to anterograde transport. This line of reasoning is supported by the observation of robust retrograde transport within growth cones, which lack NF bundles (Chan et al. 2002). The potential importance of a balancing force provided by dynein activity in balancing the effects of kinesin was demonstrated by the generation of aberrant NF aggregates within axons following overexpression of dynamitin. When cells were transfected with a construct expressing dynamitin, those cells exhibiting modest expression of exogenous dynamitin displayed a net shift of NFs towards the distal end of neurites. However, those cells displaying robust expression of exogenous dynamitin displayed large NF aggregates within central and distal axonal regions. Finally, those cells displaying the most extreme levels of exogenous dynamitin displayed NF aggregates within the most proximal regions of axons (Motil et al. 2006a,b). These observations support the notion that the opposing force generated by dynein is an important regulator of orderly anterograde transport, and that an imbalance in motor activities may foster the development of NF spheroids characteristic of human motor neuron disease (discussed more fully below). Notably, shifting of the entire bundle following inhibition of motors is consistent with the notion that the cytoskeleton may be able to undergo some degree of transport “en bloc”.

In efforts to understand the biomechanics underlying this situation, we considered whether hypotheses and experimental evidence already advanced for another major axonal cytoskeletal constituent, microtubules (MTs) could also be relevant to NFs. MTs of varying lengths are observed along axons, but they display different motilities. Relatively short MTs can be observed to translocate, while long MTs remain stationary for extended periods. Both kinesin and dynein mediate translocation of relatively short MTs, which translocate along the longer MTs. In this case, a given motor would interact with a short MT via its cargo domain, interact with the longer MT via its MT-binding domain, and “walk” the short MT along the longer MT, and possibly the actin cortex. It seemed unlikely that these motors would somehow selectively interact with shorter rather than longer MTs, yet the longer MTs did not exhibit movement. This prompted the suggestion that multiple motors exerting anterograde and retrograde forces canceled each other out, and, more importantly, exerted a crosslinking effect. Just like with short MTs, a given motor would interact with one MT via its cargo domain, and an adjacent MT via its MT-binding domain. However, if both MTs were long, then multiple motors of differing directionality would interact with both of these MTs, and essentially cancel each other out (Ahmad et al. 2000; Baas et al. 2006). In contrast to MTs, however, crosslinking of NFs by either kinesin or dynein is unlikely because, while NFs are known cargo for both motors, there is

no evidence that they act as tracks. Accordingly, a single motor can interact with an NF via its cargo domain, but it could not interact with an adjacent NF via its MT-binding domain. However, retrograde movement of the bundle following inhibition of MAP kinase confirms that dynein has a functional association with the bundle. We, therefore, hypothesize that the MT-binding domains of bundle-associated motors are associated with adjacent MTs, and that the bundle maintains its normal distribution along axons via crosslinking to adjacent MTs. Notably, the MT array may also be tethered to the actin cortex, which may contribute to NF transport and distribution (Jung et al. 2004).

4 NF Phosphorylation State and Localization in Neuropathological Conditions: NFs May Contribute to Motor Neuron Disease by Sequestering Motor Proteins and/or Mitochondria

One hallmark of affected motor neurons in amyotrophic lateral sclerosis ALS/MND is the accumulation of filamentous “spheroids” within proximal axons (Julien and Mushynski 1998; Rao and Nixon 2003; Sihag et al. 2007). Spheroids are comprised of disorganized neurofilaments NFs displaying epitopes normally found exclusively on NFs within distal axonal regions. The pattern of NF-H phosphorylation in spinal tissue from ALS patients is identical to that of normal individuals; one interpretation is that perikaryal/proximal axonal phospho-NF spheroids are comprised of normally phosphorylated NFs that are simply mislocated (Bajaj et al. 1999). We hypothesized some time ago that precocious activation of one or more critical NF kinases could perturb association of NFs with their anterograde motor and promote aberrant accumulation by fostering precocious NF–NF associations (Shea and Flanagan 2001). Several experimental manipulations support this possibility. The NF kinases cdk5 and p38MAP kinase each phosphorylate NFs to generate epitopes in common with NF spheroids and are associated with ALS (Ackerley et al. 2004; Bajaj et al. 1999; Strong et al. 2001). Overexpression of MEKK-1, which in turn overactivated the stress-activated /c-jun terminal kinase, inhibited translocation of NFs into growing axonal neurites and fostered accumulation of axonal-specific phospho-NF epitopes within perikarya (DeFuria et al. 2006). Notably, deletion of the C-terminal region of NF-H delayed motor neuron pathology in a murine model of ALS (Lobsiger et al. 2005). Finally, as described earlier, injection of the phosphatase inhibitor okadaic acid invoked rapid de novo accumulation of RT97 immunoreactivity within perikarya and proximal axons of optic pathway (areas in which this degree of phospho-NF immunoreactivity is normally excluded (Jung and Shea 1999; Sanchez et al. 2000)), and simultaneously slowed NF transport within murine retinal ganglion cells in situ (Sanchez et al. 2000). This finding confirms that the

kinase(s) that generate extensive C-terminal NF phosphorylation are present and active within perikarya and proximal axons. This not only suggests that the extent of C-terminal NF phosphorylation within these regions is dependent upon a balance of kinase and phosphatase activities, but also supports the notion that decreased compensatory phosphatase activity could contribute to aberrant NF phosphorylation and mislocation.

A phospho-dependent decrease in association of NFs with kinesin (Yabe et al. 2000), a phospho-dependent increase in association of NFs with dynein (Motil et al. 2006a, b), and/or a phospho-dependent increase in association of NFs with each other (Shea et al. 2004; Yabe et al. 2001a) - in short, all of the phospho-dependent dynamics discussed thus far - could contribute to perikaryal accumulation of phospho-NFs, either by inhibiting anterograde NF transport, enhancing a retrograde “pull,” and/or by inducing precocious NF–NF associations within perikarya instead of axons. Which of these forces is the major factor leading to accumulation of NFs within perikarya/proximal axons remains to be determined, but it is likely that they each contribute to some degree. Notably, NF spheroids accumulate kinesin and dynein (Toyoshima et al. 1998a, b), which may represent an apparent futile attempt of these motors to translocate NFs that are “trapped” within the spheroids. Motor entrapment could ultimately impair overall axonal transport, including that of non-cytoskeletal elements (Collard et al. 1995). Resultant increased residence time of NFs within perikarya may contribute further to their aberrant phosphorylation (Black and Lee 1988) in a deleterious feedbackloop. Even a subtle shift in the balance of NF kinases and phosphatases may, over time, generate the extent of NF mislocalization that accompanies motor neuron disease (Motil et al. 2006b). Similarly, comparisons of axonal transport to models of traffic modeling suggests that a critical imbalance in association of NFs with their transport motors could generate a long-lasting “pile up” of NFs (Shea and Beaty 2007). Importantly, these analyses also suggest that restoration of appropriate motor balance could diminish continued delivery of NFs into these and perhaps deplete NF accumulations, as has been shown for experimentally induced NF aggregates in cultured cells (Shea et al. 1997).

As perikaryal NF accumulations contain NFs that are apparently normally phosphorylated, but mislocated (Bajaj et al. 1999; Strong et al. 2001), and as NF phosphorylation affects both transport and NF–NF associations, one speculation arising from these collective findings is that delaying extensive phosphorylation until NFs are longitudinally oriented within the lateral confines of the axon promotes orderly bundling, rather than generation of spheroids (Chan et al. 2007).

Notably, translocation of mitochondria into axons is dependent upon kinesin, and, as NFs bind mitochondria (Dubey et al. 2007; Leterrier et al. 1994; Wagner et al. 2003), the NF content of axons may provide a scaffold to maintain axonal mitochondrial distribution. These latter considerations provide a further mechanism by which impaired transport, including that of NFs, may contribute to motor neuron disease (De Vos et al. 2008).

5 Conclusions and Future Directions

In summary, a considerable body of literature amassed over the past few decades from a number of laboratories using a variety of experimental models and approaches provides insight into the mechanisms by which C-terminal NF phosphorylation may regulate NF axonal transport. The identification of responsible motors and the concurrent demonstration that phosphorylation both interferes with association of NFs with their anterograde motor, as well as promotes the NF–NF associations that correlate with the slowest-moving NFs, provide mechanisms that support these earlier hypotheses. While the approach of sidearm deletion (Rao et al. 2002, 2003; Yuan et al. 2006a, b) may indeed represent an improvement over deletion of the complete NF-H subunit, it unfortunately remains encumbered by some of the same compensatory mechanisms (e.g., NF-M hyperphosphorylation) that confounded interpretation following full-length NF-H deletion (Jung et al. 2006; Zhu et al. 1998). Of interest would be to examine the impact of NF-H C-terminal deletion on the association of NF isoforms with the slowest-moving NF population (i.e., the so-called stationary cytoskeleton) that is retained for extended periods along optic axons in long-term radiolabeling analyses (Nixon and Logvinenko 1986).

Beyond the simple consideration of C-terminal phosphorylation as regulating NF axonal transport lies the task of sorting out what is likely to be a series of hierarchical phosphorylation events, with distinct events regulating various aspects of NF dynamics. While phosphorylation events that induce the RT97 epitope are clearly associated with a number of characteristics of NF “maturation,” there are no data to indicate that all NFs bearing the RT97 epitope have the identical phosphorylation state. One series of phosphorylation events, accompanied by generation of the RT97 epitope, may promote association with dynein, while additional phosphorylation events (for which we have no distinct antigenic marker as yet) may be required to promote NF–NF bundling. By contrast, some C-terminal phosphorylation events (e.g., those that induce the SMI-31 epitope), which occur prior to those that generate the RT97 epitope (Sanchez et al. 2000), are apparently compatible with kinesin association.

While we (Chan et al. 2007) and others (Gou et al. 1998) have demonstrated a role for divalent cations such as calcium in NF bundling, simultaneous association with dynein and kinesin – not uncommon for cargo of these motors (Martin et al. 1999) – may be an integral aspect of NF bundling in a manner analogous to the demonstration that competing forces generated by kinesin and dynein exert an architectural role in the maintenance of the axonal cytoskeletal lattice (Ahmad et al. 2000; Baas et al. 2006). As NFs contain contiguous phosphorylated and nonphosphorylated domains (Brown 1998; Chan et al. 2005), NFs in various overall phosphorylation states may retain the capacity to associate simultaneously with both motors. Differential phospho-dependent association with kinesin and dynein could contribute to the wide range of anterograde transport rates observed for axonal NFs. Elucidation of the order of phosphorylation by C-terminal kinases, and which events mediate which of the above aspects of NF dynamics, remain important areas of investigation.

Acknowledgments This review was supported by the National Science Foundation.

References

- Ackerley S, Thornhill P, Grierson AJ, Brownlees J, Anderton BH, Leigh PN, Shaw CE, Miller CJ (2003) Neurofilament heavy chain side-arm phosphorylation regulates axonal transport of neurofilaments. *J Cell Biol* 161:489–495
- Ackerley S, Grierson AJ, Banner S, Perkinson MS, Brownlees J, Byers HL, Ward M, Thornhill P, Hussain K, Waby JS, Anderton BH, Cooper JD, Dingwall C, Leigh PN, Shaw CE, Miller CC (2004) p38alpha stress-activated protein kinase phosphorylates neurofilaments and is associated with neurofilament pathology in amyotrophic lateral sclerosis. *Mol Cell Neurosci* 26:354–364
- 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. *Nat Cell Biol* 2:276–280
- Archer DR, Watson DF, Griffin JW (1994) Phosphorylation-dependent immunoreactivity of neurofilaments and the rate of slow axonal transport in the central and peripheral axons of the rat dorsal root ganglion. *J Neurochem* 62:1119–1125
- Chan W K-H, Yabe JT, Pimenta AF and Shea TB (2002) Growth cones contain a highly-dynamic population of neurofilament subunits. *Cell Motil Cytoskel* 54:195–207
- Baas PW, Nada CV, Myers KA (2006) Axonal transport of microtubules: the long and short of it. *Traffic* 7:490–498
- Bajaj NPS, Al-Sarraj ST, Leigh PN, Anderson V, Miller CCJ (1999) Cyclin dependent kinase 5 (cdk5) phosphorylates neurofilament heavy (NF-H) chain to generate epitopes for antibodies that label neurofilament accumulations in amyotrophic lateral sclerosis (ALS) and is present in affected motor neurons in ALS. *Neuro Psychopharm Biol Psychiatr* 23:833–850
- Black MM, Lee VM-Y (1988) Phosphorylation of neurofilament proteins in intact neurons: demonstration of phosphorylation in cell bodies and axons. *J Neurosci* 8:3296–3305
- Blum JJ, Reed MC (1989) A model for slow axonal transport and its application to neurofilamentous neuropathies. *Cell Motil Cytoskel* 12:53–65
- Brown A (1998) Contiguous phosphorylated and non-phosphorylated domains along axonal neurofilaments. *J Cell Sci* 111:455–467
- Brown A, Wang L, Jung P (2005) Stochastic simulation of neurofilament transport in axons: the “stop-and-go” hypothesis. *Mol Biol Cell* 16:4243–4255
- Chan WK-C, Dickerson A, Otriz D, Pimenta A, Moran C, Malik K, Motil J, Snyder S, Pant HC, Shea TB (2004) Mitogen activated protein kinase regulates neurofilament axonal transport. *J Cell Sci* 117:4629–4642
- Chan WK-H, Yabe JT, Pimenta AF, Ortiz D, Shea TB (2005) Neurofilaments can undergo transport and cytoskeletal incorporation in a discontinuous manner *Cell Motil Cytoskel* 62:166–179
- Chan WK-H, Kushkuley J, Leterrrier J-F, Eyer J, Shea TB (2007) Calcium-mediated “bridging” of phosphorylated neurofilaments (NFs) promotes NF-NF association and inhibits NF-microtubule association: a mechanism for selective slowing of axonal transport of phospho-NFs. *Mol Biol Cell* 18 (Suppl) 2349
- Collard JF, Côté F, Julien JP (1995) Defective axonal transport in a transgenic mouse model of amyotrophic lateral sclerosis. *Nature* 375:61–64
- Craciun G, Brown A, Friedman A (2005) A dynamical system model of neurofilament transport in axons. *J Theor Biol* 237:316–322
- DeFuria J, Chen P, Shea TB (2006) Divergent effects of the MEKK-1/JNK pathway on NB2a/d1 differentiation: some activity is required for neurite outgrowth and maturation but overactivation inhibits both phenomena. *Brain Res* 1123:20–26

- De Vos KJ, Grierson AJ, Ackerley S, Miller CC (2008) Role of axonal transport in neurodegenerative diseases. *Annu Rev Neurosci* 31:151–173
- DeWaegh SM, Lee VM, Brady ST (1992) Local modulation of neurofilament phosphorylation, axonal caliber, and slow axonal transport by myelinating Schwann cells. *Cell* 68:451–463
- Dubey M, Chaudhury P, Kabiru H, Shea TB (2007) Tau inhibits anterograde axonal transport and perturbs stability in growing axonal neurites in part by displacing kinesin cargo: neurofilaments attenuate tau-mediated neurite stability. *Cell Motil Cytoskel* 65:89–99
- Gou JP, Gotow T, Janmey PA, Leterrier JF (1998) Regulation of neurofilament interactions in vitro by natural and synthetic polypeptides sharing Lys-Ser-Pro sequences with the heavy neurofilament subunit NF-H: neurofilament crossbridging by antiparallel sidearm overlapping. *Med Biol Eng Comput* 36:371–387
- 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. *J Cell Biol* 168:697–703
- Hoffman PN, Lasek RJ, Griffin JW, Price DL (1983) Slowing of the axonal transport of neurofilament protein during development. *J Neurosci* 3:1694–1700
- Jung C, Chylinski TM, Pimenta A, Ortiz D, Shea TB (2004) Neurofilament transport is dependent on actin and myosin. *J Neurosci*. 24:9486–9496
- Julien J-P, Mushynski WE (1998) Neurofilaments in health and disease. *Prog Nuc Acid Res Mol Biol* 61:1–20
- Jung C, Shea TB (1999) Regulation of neurofilament axonal transport by phosphorylation in optic axons in situ. *Cell Motil Cytoskel* 43:230–240
- Jung C, Lee S, Ortiz D, Zhu Q, Julien J-P, Shea TB (2006) Phosphorylation of the high molecular weight neurofilament subunit regulates the association of neurofilaments with kinesin in situ. *Brain Res* 141:151–155
- Jung C, Yabe JT, Shea TB (2000a) Hypophosphorylated neurofilament subunits undergo axonal transport more rapidly than more extensively phosphorylated subunits in situ. *Cell Motil Cytoskel* 47:120–129
- Jung C, Yabe JT, Shea TB (2000b) C-terminal phosphorylation of the heavy molecular weight neurofilament subunit is inversely correlated with neurofilament axonal transport velocity. *Brain Res* 856:12–19
- Jung CJ, Shea TB (2004) Neurofilament subunits undergo more rapid translocation within retinas than in optic axons. *Mol Brain Res* 122:188–192
- Jung C, Chylinski TM, Pimenta A, Ortiz D, Shea TB (2004) Neurofilament transport is dependent on actin and myosin. *J Neurosci*. 24:9486–9496
- Koehnle TJ, Brown A (1999) Slow axonal transport of neurofilament protein in cultured neurons. *J Cell Biol* 144:447–458
- Komiya Y, Cooper NA, Kidman AD (1987) The recovery of slow axonal transport after a single intraperitoneal injection of beta, beta'-iminodipropionitrile in the rat. *J Biochem (Tokyo)* 102:869–873
- Lasek RJ, Paggi P, Katz MJ (1992) Slow axonal transport mechanisms move neurofilaments relentlessly in mouse optic axons. *J Cell Biol* 117:607–616
- 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
- Leterrier JF, Rusakov DA, Nelson BD, Linden M (1994) Interactions between brain mitochondria and cytoskeleton: evidence for specialized outer membrane domains involved in the association of cytoskeleton-associated proteins to mitochondria in situ and in vitro. *Microsc Res Tech* 27:233–261
- Lewis SE, Nixon RA (1988) Multiple phosphorylated variants of the high molecular mass subunit of neurofilaments in axons of retinal cell neurons: characterization and evidence for their differential association with stationary and moving neurofilaments. *J Cell Biol* 107:2689–2701
- Lobsiger CS, Garcia ML, Ward CM, Cleveland DW (2005) Altered axonal architecture by removal of the heavily phosphorylated neurofilament tail domains strongly slows superoxide dismutase 1 mutant-mediated ALS. *Proc Natl Acad Sci USA* 102:10351–10356

- Martin M, Iyadurai SJ, Gassman A, Gindhart JG Jr, Hays TS, Saxton WM (1999) Cytoplasmic dynein, the dynactin complex, and kinesin are interdependent and essential for fast axonal transport. *Mol Biol Cell* 10:3717–3728
- Marszalek JR, Williamson TL, Lee MK, Xu ZS, Hoffman PN, Becher MW, Crawford TO, Cleveland DW (1996) Neurofilament subunit NF-H modulates axonal diameter by selectively slowing neurofilament transport. *J Cell Biol* 135:711–724
- Motil J, Chan W K-H, Dubey M, Chaudhury P, Pimenta A, Chylinski TM, Ortiz DT, Shea TB (2006a) Dynein mediates retrograde neurofilament transport within axonal neurites and anterograde delivery of NFs from perikarya into axons: regulation by multiple phosphorylation events. *Cell Motil Cytoskel* 63:266–286
- Motil J, Dubey M, Chan W K-H, Shea TB (2006b) Inhibition of dynein but not kinesin induces focal accumulation of neurofilaments in axonal neurites. *Brain Res* 1164:125–131
- Nixon RA (1993) The regulation of neurofilament protein dynamics by phosphorylation: clues to neurofibrillary pathology. *Brain Pathol* 3:29–38
- Nixon RA (1998) The slow transport of cytoskeletal proteins. *Curr Opin Cell Biol* 10:87–92
- Nixon RA, Logvinenko KB (1986) Multiple fates of newly synthesized neurofilament proteins: evidence for a stationary neurofilament network distributed non-uniformly along axons of retinal ganglion cell neurons. *J Cell Biol* 102:647–659
- Pant HC, Veeranna (1995) Neurofilament phosphorylation. *Biochem Cell Biol* 73:575–592
- 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 M, Nixon RA (2003) Defective neurofilament transport in mouse models of amyotrophic lateral sclerosis: a review. *Neurochem Res* 28:1041–1047
- Rao MV, Campbell J, Yuan A, Kumar A, Gotow T, Uchiyama Y, Nixon RA (2003) The neurofilament middle molecular mass subunit carboxyl-terminal tail domain is essential for the radial growth and cytoskeletal architecture of axons but not for regulating neurofilament transport rate. *J Cell Biol* 163:1021–1031
- Rao MV, Garcia ML, Miyazaki Y, Gotow T, Yuan A, Mattina S, Ward CM, Calcutt NA, Uchiyama Y, Nixon RA, Cleveland DW (2002) Gene replacement in mice reveals that the heavily phosphorylated tail of neurofilament heavy subunit does not affect axonal caliber or the transit of cargoes in slow axonal transport. *J Cell Biol* 158:681–693
- 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
- Sanchez I, Hassinger L, Sihag RK, Cleveland DW, Mohan P, Nixon RA (2000) Local control of neurofilament accumulation during radial growth of myelinating axons in vivo: selective role of site-specific phosphorylation. *J Cell Biol* 151:1013–1024
- Shah JV, Flanagan LA, Janmey PA, Leterrier J-F (2000) Bidirectional translocation of neurofilaments along microtubules mediated in part by dynein/dynactin. *Mol Biol Cell* 11:3495–3508
- Shea TB, Beaty WJ (2007) Traffic jams: models for neurofilament accumulation in motor neuron disease. *Traffic* 8:445–447
- Shea TB, Chan W K-H (2008) Regulation of neurofilament dynamics by phosphorylation. *Eur J Neurosci* 27:1893–1901
- Shea TB, Flanagan L (2001) Kinesin, dynein and neurofilament transport *Trends Neurosci* 24:644–648
- Shea TB, Yabe JT (2000) Occam's razor slices through the mysteries of neurofilament axonal transport: can it really be so simple? *Traffic* 1:522–523
- Shea TB, Fischer I, Paskevich PA, Beermann ML (1993) The protein phosphatase inhibitor okadaic acid increases axonal NFs and neurite caliber, and decreases axonal MTs in NB2a/d1 cells. *J Neurosci Res* 35:507–521
- Shea TB, Wheeler E, Jung C (1997) Aluminum inhibits neurofilament assembly, cytoskeletal incorporation and axonal transport: dynamic nature of aluminum-induced perikaryal neurofilament accumulations as revealed by subunit turnover. *Mol Chem Neuropathol* 32:17–39
- Shea TB, Jung CJ, Pant HC (2003) Does C-terminal phosphorylation regulate neurofilament transport? Re-evaluation of recent data suggests that it does. *Trends Neurosci* 26:397–400

- Shea TB, Yabe JT, Ortiz D, Pimenta A, Loomis P, Goldman RD, Amin N, Pant HC (2004) CDK5 regulates axonal transport and phosphorylation of neurofilaments in cultured neurons. *J Cell Sci* 117:933–941
- Sihag RK, Inagaki M, Yamaguchi T, Shea TB, Pant HC (2007) Role of phosphorylation on the structural dynamics and function of types III and IV intermediate filaments. *Exp Cell Res* 313:2098–2109
- Strong MJ, Strong WL, Jaffe H, Traggert B, Sopper MM, Pant HC (2001) Phosphorylation state of the native high-molecular-weight neurofilament subunit protein from cervical spinal cord in sporadic amyotrophic lateral sclerosis. *J Neurochem* 76:1315–1325
- Theiss C, Napirei M, Karl-Meller K (2005) Impairment of anterograde and retrograde neurofilament transport after anti-kinesin and anti-dynein antibody microinjection in chicken dorsal root ganglia. *Eur J Cell Biol* 84:29–43
- Toyoshima I, Kato K, Sugawara M, Wada C, Masamune O (1998a) Kinesin accumulation in chick spinal axonal swellings with beta,beta'-iminodipropionitrile (IDPN) intoxication. *Neurosci Lett* 249:103–106
- Toyoshima I, Sugawara M, Kato K, Wada C, Hirota K, Hasegawa K, Kowa H, Sheetz MP, Masamune O (1998b) Kinesin and cytoplasmic dynein in spinal spheroids with motor neuron disease. *J Neurol Sci* 159:38–44
- Trivedi N, Jung P, Brown A (2007) Neurofilaments switch between distinct mobile and stationary states during their transport along axons. *J Neurosci* 27:507–516
- Tu P-H, Elder G, Lazzarini RA, Nelson D, Trojanowski JQ, Lee VM-Y (1995) Overexpression of the human NFM subunit in transgenic mice modifies the level of endogenous NFL and the phosphorylation state of NFH subunits. *J Cell Biol* 129:1629–1640
- Wagner OI, Lifshitz J, Janmey PA, Linden M, McIntosh TK, Leterrier JF (2003) Mechanisms of mitochondria–neurofilament interactions. *J Neurosci* 23:9046–9058
- Wang L, Ho C-I, Sun D, Liem RKH, Brown A (2000) Rapid movements of axonal neurofilaments interrupted by prolonged pauses. *Nature Cell Biol* 2:137–141
- Watson DF, Glass JD, Griffin JW (1993) Redistribution of cytoskeletal proteins in mammalian axons disconnected from their cell bodies. *J Neurosci* 13:4354–4360
- 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
- Yabe JT, Chan W, Shea TB (2000) Phospho-dependent association of neurofilament proteins with kinesin in situ. *Cell Motil Cytoskel* 42:230–240
- Yabe JT, Chylinski T, Wang F-S, Pimenta A, Kattar SD, Linsley M-D, Chan W K-H, Shea TB (2001a) Neurofilaments consist of distinct populations that can be distinguished by C-terminal phosphorylation, bundling and axonal transport rate in growing axonal neurites. *J Neurosci* 21:2195–2205
- Yabe JT, Pimenta A, Shea TB (1999) Kinesin-mediated transport of neurofilament protein oligomers in growing axons. *J Cell Sci* 112:3799–3814
- Yabe JT, Wang F-S, Chylinski T, Katchmar T, Shea TB (2001b) Selective accumulation of the high molecular weight neurofilament subunit within the distal region of growing axonal neurites. *Cell Motil Cytoskel* 50:1–12
- Yuan A, Nixon RA, Rao MV (2006a) Deleting the phosphorylated tail domain of the neurofilament heavy subunit does not alter neurofilament transport rate in vivo. *Neurosci Lett* 393:264–268
- Yuan A, Rao MV, Sasaki T, Chen Y, Kumar A, Veeranna, Liem RK, Eyer J, Peterson AC, Julien JP, Nixon RA (2006b) Alpha-internexin is structurally and functionally associated with the neurofilament triplet proteins in the mature CNS. *J Neurosci* 26:10006–10019
- Zhang B, Tu P, Abtahian F, Trojanowski JQ, Lee VM-Y (1997) Neurofilaments and orthograde transport are reduced in ventral root axons of transgenic mice that express human SOD1 with a G93A mutation. *J Cell Biol* 139:1307–1315
- Zhu Q, Lindenbaum M, Levavasseur F, Jacomy H, Julien J-P (1998) Disruption of the NF-H gene increases axonal microtubule content and velocity of neurofilament transport: Relief of axonopathy resulting from the toxin b'b'-iminodipropionitrile. *J Cell Biol* 143:183–193

Cell Biology of the Axon

Koenig, E. (Ed.)

2009, XVI, 360 p. 50 illus., 22 illus. in color., Hardcover

ISBN: 978-3-642-03018-5