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Axonal transport disruption in peripheral nerve disease:

From Jack's discoveries as a resident to recent contributions

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Abstract

Many neurodegenerative diseases and neuropathies have been proposed to be caused by a disruption of axonal transport. However, the mechanisms whereby impaired transport causes disease remain unclear. Proposed mechanisms include impairment in delivery of organelles such as mitochondria, defective retrograde neurotrophic signaling, and disruption of the synaptic vesicle cycle within the synaptic terminal. Simple model organisms such as the fruitfly, *Drosophila melanogaster*, allow live imaging of axonal transport to be combined with high-throughput genetic screens and are providing insights into the pathophysiology of peripheral nerve diseases.

Keywords

amyotrophic lateral sclerosis; axonal transport; dynactin; hereditary motor neuropathy; retrograde signaling

Introduction

The transport of electrical signals from the cell body to a synapse a meter away in a matter of milliseconds is a well-recognized phenomenon of our peripheral nerves. A much less appreciated feat that our nerves continuously perform is the coordinated bidirectional axonal transport of organelles, proteins, and other cargo from our feet to our spinal cord. Anterograde cargo are transported from the cell body to the synapse using the kinesin family of molecular motors, whereas retrograde cargo utilize the dynein/dynactin complex; however, the regulation of these motors and the coordination of axonal transport remains poorly understood.

How do cargo such as mitochondria know which direction to go along the nerve, and how is this regulated by metabolic demand at the synapse and along the axon? Neuronal organelles including synaptic vesicle precursors must be directed to travel into axons versus dendrites, and directed to the proper synapses or growth cones. Similarly, microtubules, neurofilaments, and other cytoskeletal proteins are constantly being turned over, such that

new filaments are continuously trafficked down axons to replace damaged ones. Furthermore, aged proteins and organelles such as synaptic vesicle proteins and mitochondria are shuttled back to the soma where they are recycled or degraded in lysosomes. Both the growth and maintenance of axons require neurotrophic signaling from synapses, trafficked along axons to the soma within endosomal vesicles. Somehow the transport of multiple different cargo is regulated spatially and temporally to balance supply with demand.

Given the complexity and importance of this process, it is not surprising that a disruption of axonal transport has been implicated in an increasing list of neurological diseases. Anyone who has ever been stuck on a freeway following an accident, had a package lost in the mail, or been the victim of a last minute plane cancelation certainly appreciates the value of reliable transportation. Thus, with aging, given that neurons generally do not regenerate, potholes in axonal roads may increase in number and become increasingly difficult to repair.

Retrograde Transport in Nerve Survival and Disease

During his neurology residency at Johns Hopkins, Jack Griffin made his first monumental scientific discovery: tetanus toxin is taken up at the neuromuscular junction (NMJ) and enters the CNS by retrograde axonal transport along peripheral nerves (Price et al., 1975; Griffin et al., 1976). Jack showed this with a series of elegant experiments, injecting radiolabeled toxin into distal muscles of the rat hindlimb and demonstrating intraxonal toxin distal to nerve crush with electron microscopy and autoradiography. Prior to this amazing discovery, it had been thought tetanus toxin was transported along blood vessels, lymphatics, or other intraneuronal extraaxonal structures, and it was thought unlikely to be retrogradely transported along axons (Zacks and Sheff, 1966; 1968). This discovery paved the way for a future understanding of the importance of retrograde transport of neurotrophic factors, signaling endosomes, and other organelles to neuron health.

While there had been good evidence for fast retrograde axonal transport, whether or not slow axonal transport of cytoskeletal proteins had a retrograde component was unknown, but the prevailing view at the time was that newly synthesized cytoskeletal proteins traveled unidirectionally in the anterograde direction. John Glass, working in Jack's lab, devised an elegant experiment to demonstrate that slow axonal transport of neurofilament and tubulin proteins indeed also occurs in a bidirectional fashion. Nerve ligation experiments like those described above were not feasible to demonstrate retrograde slow axonal transport in wild type animals, as the distal axon degenerated in 1–3 days following transection. To demonstrate retrograde slow axonal transport, John Glass utilized a mouse line that had markedly delayed Wallerian degeneration, known as the WldS or Ola mouse (Lunn et al., 1989; Glass and Griffin, 1991). Using these mice, they demonstrated that neurofilaments and tubulin proteins accumulated in the proximal end of the distal stump after ligation, suggesting that cytoskeletal components can redistribute in the retrograde direction via slow axonal transport (Watson et al., 1993; Glass and Griffin, 1994).

Axonal Transport Disruption in Toxic Neuropathies

Some of the primary tools used to study axonal transport in rodents are toxins. Jack discovered that the neurotoxin β,β' -iminodipropionitrile (IDPN) selectively disrupts slow axonal transport without affecting fast anterograde or retrograde axonal transport (Griffin et al., 1978; 1983). Jack was then able to use this as a model to understand how disruption of axonal transport causes neuropathy. Jack and others have shown that impairment of slow axonal transport causes a proximal accumulation of neurofilaments. Because neurofilaments regulate axonal diameter, this accumulation leads to a marked swelling of the axon. Subsequently, Jack showed the mechanism whereby exposure of the industrial toxin, acrylamide, causes neuropathy. Acutely, acrylamide causes decreased axonal transport, also causing proximal accumulations of neurofilaments and swelling (Gold et al., 1985). Chronic administration of both IDPN and acrylamide causes atrophy distal to the proximal axonal swellings that propagates distally over time and is reversible (Clark et al., 1980).

While these industrial toxins are rare causes of neuropathy today, chemotherapeutic drugs, in particular microtubule-binding drugs that disrupt mitosis such as taxane derivatives (paclitaxel) and vinka alkaloids (vincristine), are common toxic causes of neuropathy. Although the mechanism of neuronal toxicity is incompletely understood, taxoids are believed to cause axonal degeneration by disruption of anterograde axonal transport (Theiss and Meller, 2000; Yang et al., 2009). Peripheral neuropathy is the major dose-limiting toxicity of these otherwise effective chemotherapeutic regimens, and thus further investigation into the mechanisms of neuropathy caused by these drugs is needed.

Axonal Transport Disruption in Inherited Neuropathies and Neurodegenerative Diseases

The similarities of neurofilament accumulation observed in toxin-induced neuropathy models with those seen in the neurofibrillary tangle pathology of Alzheimer's disease led Jack and others to propose that axonal transport disruption might be a common pathogenic mechanism in many neurodegenerative diseases (Griffin and Watson, 1988). Indeed, over the last 20 years, increasing evidence has supported his hypothesis that axonal transport disruption is an early event in the pathogenesis of many neurodegenerative diseases (De Vos et al., 2008; Staff et al., 2011). For example, impairment of fast axonal transport has been proposed to be an early event in the pathogenesis of amyotrophic lateral sclerosis (ALS) (Sasaki and Iwata, 1996; Williamson and Cleveland, 1999; Ligon et al., 2005).

In 2003, Kurt Fischbeck and colleagues identified a mutation in the p150^{Glued} subunit of dynactin in a family with an autosomal dominant, slowly progressive lower motor neuron disease termed hereditary motor neuropathy type 7B (HMN7B) (Puls et al., 2003; 2005). The mutation is a G59S missense mutation in a highly conserved cytoskeleton associate protein Gly-rich (CAP-Gly) microtubule-binding domain, and causes the p150 protein to form aggregates and reduces binding to microtubules (Puls et al., 2005; Levy et al., 2006). Work from Erika Holzbaur and Elizabeth Fisher had previously shown that disruption of retrograde axonal transport in mice caused motor neuron degeneration, but this was the first direct evidence that disruption of dynein/dynactin leads to neurodegeneration in humans

(LaMonte et al., 2002; Hafezparast et al., 2003). Intriguingly, Phil Wong and colleagues overexpressed this mutant form of p150 in mice and this led to an ALS-like phenotype with ubiquitinated aggregates, motor neuron degeneration, progressive paralysis, and early lethality (Laird et al., 2008). Working with Jack, Mohammed Farah identified neurofilament accumulations within proximal motor axons in young animals and progressive axonal degeneration with aging, pathological hallmarks of the disease (Laird et al., 2008). More recently, mutations in kinesin and dynein have also been implicated in other inherited neuropathies (Riviere et al., 2011; Weedon et al., 2011; Harms et al., 2012), directly implicating these molecular motors in the development of neuropathy.

Investigations of Axonal Transport in *Drosophila* Models of Disease

Jack's mentorship, inspiration, and support have led me to my current position at Johns Hopkins as a physician-scientist studying axonal transport in peripheral nerve diseases. As a junior neurology resident at Hopkins, I met with Jack, the neurology chairman at the time, on multiple occasions to discuss my career plans. My Ph.D. was in *Drosophila* neurobiology, but I had intended to study mammalian models of neurologic disease during postdoctoral and residency training. I knew of the enormous potential that fruit flies had in the study of molecular neuroscience and neuropathology (Bilen and Bonini, 2005; Marsh and Thompson, 2006), but as a physician-scientist, I wanted to make an impact on the translation of neuroscience discoveries into new treatments for patients, something I believed I was specifically trained for and, according to Jack, was in high demand (Griffin, 2006). I was astounded to find that not only did Jack appreciate the value of *Drosophila* as a model system, but he also was a tremendous advocate for utilizing simple model systems in translational research (not coincidentally, his son Erik had just begun his postdoctoral training in a *Caenorhabditis elegans* lab at the time). Not only did Jack encourage me to stay in flies, but he was also a tremendous advocate for developing a *Drosophila* research program within the neurology department at Johns Hopkins.

Jack steered me towards my current scientific mentors, Jeff Rothstein and Alex Kolodkin, and this has led to the development of a project that benefited extensively from Jack's advice. Since the *Glued* gene and the dynein/dynactin complex are highly conserved in *Drosophila*, this appeared to be an excellent gene to use to model motor neuron degenerative disease in a simple genetic organism. Several groups had previously tried to model ALS in flies by expressing mutant SOD without a gross phenotype (Watson et al., 2008). I was encouraged by the recent success of other groups in modeling neuromuscular diseases in the fly (reviewed in Lloyd and Taylor, 2010), and we introduced the G59S mutation into the fly *Glued* gene. We were indeed able to identify phenotypes in our fly model that were consistent with motor neuron degeneration including aggregates of mutant proteins, adult-onset locomotor impairment and early lethality (Lloyd et al., 2012). Importantly, we can use fly genetics to screen the genome for modifiers of these phenotypes; such screens are underway and are identifying novel suppressors of motor neuron degeneration.

To determine how the G59S mutation in p150^{Glued} causes motor neuron degeneration, we have studied the larval peripheral nervous system, an excellent model for studying NMJ development and function (Lloyd and Taylor, 2010). *Drosophila* larvae are also an excellent

model system for investigating axonal transport (Martin et al., 1999). Mutations in dynein, dynactin, and kinesin cause a specific larval ‘tail-flip’ phenotype, and genetic screens aimed at identifying mutations that cause this phenotype have identified novel regulators of axonal transport (Bowman et al., 2000). Genetic disruption of retrograde transport in larval nerves causes focal axonal swellings that accumulate neuronal organelles such as endosomes (Fig. 1). Interestingly, expression of mutant proteins that cause neurodegenerative disease including mutant amyloid precursor protein (APP) and pathogenic polyglutamine proteins cause similar ‘axonal jam’ phenotypes in larval nerves (Gunawardena and Goldstein, 2001; Gunawardena et al., 2003). These data further suggest that axonal transport disruption may be a common pathogenic process in neurodegenerative diseases.

Genetically encoded fluorescent proteins that label specific vesicle populations or mitochondria can be expressed in subsets of larval neurons, and the transport of these organelles can be followed in real time with fluorescent microscopy. We and others have developed specialized chambers for imaging larval axons and NMJs over long periods of time (Pilling et al., 2006; Fuger et al., 2007). Recently, using a novel imaging method termed SPAIM (simultaneous photobleaching and imaging) to visualize single vesicles, Wong and colleagues demonstrated that dense core vesicles undergo long-range circulation from the axon initial segment to the distal-most NMJ bouton to distribute themselves evenly at synaptic boutons (Wong et al., 2012). Using this approach and system, we demonstrated that initiation of retrograde transport at the distal-most synaptic bouton, called the terminal bouton (TB), requires the CAP-Gly microtubule-binding domain of p150^{Glued} (Lloyd et al., 2012; Wong et al., 2012). p150^{Glued} is enriched at microtubule plus ends of TBs, and mutations that cause HMN7B disrupt the initiation of retrograde transport from TBs, but not along axons (Cronin and Schwarz 2012; Lloyd et al., 2012). Since the CAP-Gly domain preferentially binds microtubule plus ends, we hypothesize that HMN7B results from an inability of the p150^{Glued} CAP-Gly domain to capture and stabilize dynamically unstable microtubules, leading to a failure to initiate retrograde transport at TBs and progressive accumulation of dynein, endosomes, and other cargo (Fig. 2). Ultimately, with progressive disruption of p150^{Glued} function in larvae (and presumably, with aging in humans), the distal-most synaptic boutons retract, leading to a distal axonopathy.

Interestingly, mutations in nearby amino acids in the same CAP-Gly microtubule-binding domain of p150^{Glued} have been found to underlie a very different neurodegenerative disease called Perry Syndrome (Farrer et al., 2009). Patients with Perry Syndrome develop progressive degeneration of a specific subset of brainstem neurons causing central sleep apnea, parkinsonism, and depression, but no evidence of motor neuron pathology. Thus, an understanding of how these closely related but disparate mutations in the same domain of the same protein cause distinct neurodegenerative syndromes may shed light into the cell-type specificity of neurodegeneration. Our long-term goal is to use fruit flies to identify new genes that modulate axonal transport and neurodegeneration. We plan to use these models in high throughput genetic and pharmacologic screens in the fly to identify potential therapeutic targets and pharmaceutical compounds for the treatment of neuropathy and neurodegenerative disease.

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Figure 1.

(Top) Nerves of wild type (WT) *Drosophila* larvae. The synaptic vesicle marker Synaptotagmin (Syt) fused with GFP is normally targeted to the synapse and present at low levels in axons. (Bottom) Larvae depleted of the retrograde motor cytoplasmic dynein heavy chain (cDhc64c) by motor neuron-specific expression of RNAi leads to 'axonal jams' of Synaptotagmin (Syt-GFP) and other endosomal markers.

Figure 2.

Model for pathogenesis of distal axonopathy in HMN7B and possibly other motor neuron diseases. (A) Healthy NMJ synaptic boutons showing dynein-mediated retrograde and kinesin-mediated anterograde microtubule-based transport of cargo to and from the axon, respectively. At the distal-most bouton (terminal bouton), the CAP-Gly domain of p150^{Glued} binds the plus-end of microtubules and allows initiation of retrograde transport by activating the Dynein motor. (B) In our *Drosophila* model of HMN7B (G59S mutation in p150^{Glued} CAP-Gly domain indicated by asterisk), the earliest abnormalities are an accumulation of endosomal vesicles containing kinesin and dynein motor proteins within swollen terminal boutons. (C) We hypothesize that this terminal bouton defect leads to synaptic instability and presynaptic terminal retraction, leading to the denervation seen in patients with HMN7B and potentially other motor neuron diseases.