

Axonal Transport and Neurodegenerative Diseases

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Introduction

The primary functions of the neuron are to receive, process, and transmit information. A typical neuron has multiple dendrites and a single elongated process, the axon. The dendrites and the cell bodies play a role in the collection and processing of information, and the axon is responsible for the transmission of information to other neurons via synapses. Whereas dendrites are usually in close proximity to the neuronal cell bodies and have some capacity for protein synthesis, axons can extend up to tremendous lengths, sometimes several feet, and are generally devoid of the protein synthetic machinery. As a result, axonal proteins are synthesized in the cell bodies and subsequently transported into the axons and synapses. This process is called axonal transport. Axonal transport is essential for the survival of axons and synapses, and it occurs throughout the life of the neuron.

The unique architecture of the axon combined with its dependence on the remotely located cell body for growth and maintenance makes it especially vulnerable to a variety of insults. Not surprisingly, axonal and synaptic defects are characteristic features of most neurodegenerative diseases, and it was long suspected that defects in axonal transport could play a major role in these diseases. Although correlative evidence linking axonal transport defects and neurodegenerative diseases was well known, recent experimental evidence from several laboratories suggests that many neurodegenerative diseases may be a direct consequence of altered or defective axonal transport. In this article, we first highlight the basic principles of the mechanisms of axonal transport. Then, we review some of the evidence that links axonal transport to neurodegenerative diseases, including pathogenic mutations in human genes that encode proteins involved in axonal transport and studies of animal models of disease showing impairments of axonal transport.

Basic Mechanisms of Axonal Transport

Although many specific details of axonal transport mechanisms are not fully understood, the basic principles are well established. In general, to move any cargo, two components are required – motors to

move the cargo and rails to transport it. In addition, motor proteins often require adaptor/linker proteins to attach the motors to the cargo. For the rails, the neurons use the structural network of the cell, the cytoskeleton. Most long-range transport is dependent on the microtubules, but actin filaments also play a role in short-range movements. Microtubules in axons are polarized (unlike dendrites), allowing a directional bias in the movement of the motors and their cargo. For the motors, the neurons have an abundance of small molecular machines, known as kinesins and dyneins, moving the cargo mainly anterogradely (away from the cell body to the axon tip) or retrogradely (toward the cell body), respectively (Figure 1). The anterograde transport consists of components required for maintaining synapses, cytoskeletal proteins required for maintaining axonal structure and function, as well as a variety of other proteins required for neuronal function, including mitochondria, various chaperones, and glycolytic enzymes. The retrograde transport consists mainly of endosomal/lysosomal organelles that carry degraded proteins back to the cell bodies for degradation and also neurotrophic factors required for neuronal survival. In reality, however, most transport is bidirectional, with a bias either in the anterograde or in the retrograde direction.

Our understanding of the mechanisms of transport has increased dramatically during the past few decades due to advances in the development of model systems to dissect basic transport mechanisms and advances in biochemistry and genetics that have led to the identification and characterization of the molecular motors and their cellular functions. Model systems developed to visualize the transport process revealed that the bulk of proteins are transported anterogradely in two broad, discrete groups. Whereas some of the proteins are transported rapidly, at rates of 100–400 mm day⁻¹ (1–5 μm s⁻¹), quickly reaching up to the tip of the axon, others move at rates that are several orders of magnitude slower, at approximately 0.2–5 mm day⁻¹ (0.0002–0.05 μm s⁻¹). These two components are called fast axonal transport and slow axonal transport, respectively. Whereas fast transport mainly comprises vesicular cargo, including synaptic proteins, ion channels, and other components, slow axonal transport is primarily composed of transported cytoskeletal proteins, mainly microtubules, neurofilaments, and actin, along with many additional cytosolic proteins involved in neuronal homeostasis. Visualization of slow transport of the cytoskeleton revealed that despite moving with overall distinct dynamics, both slow and fast transport use similar

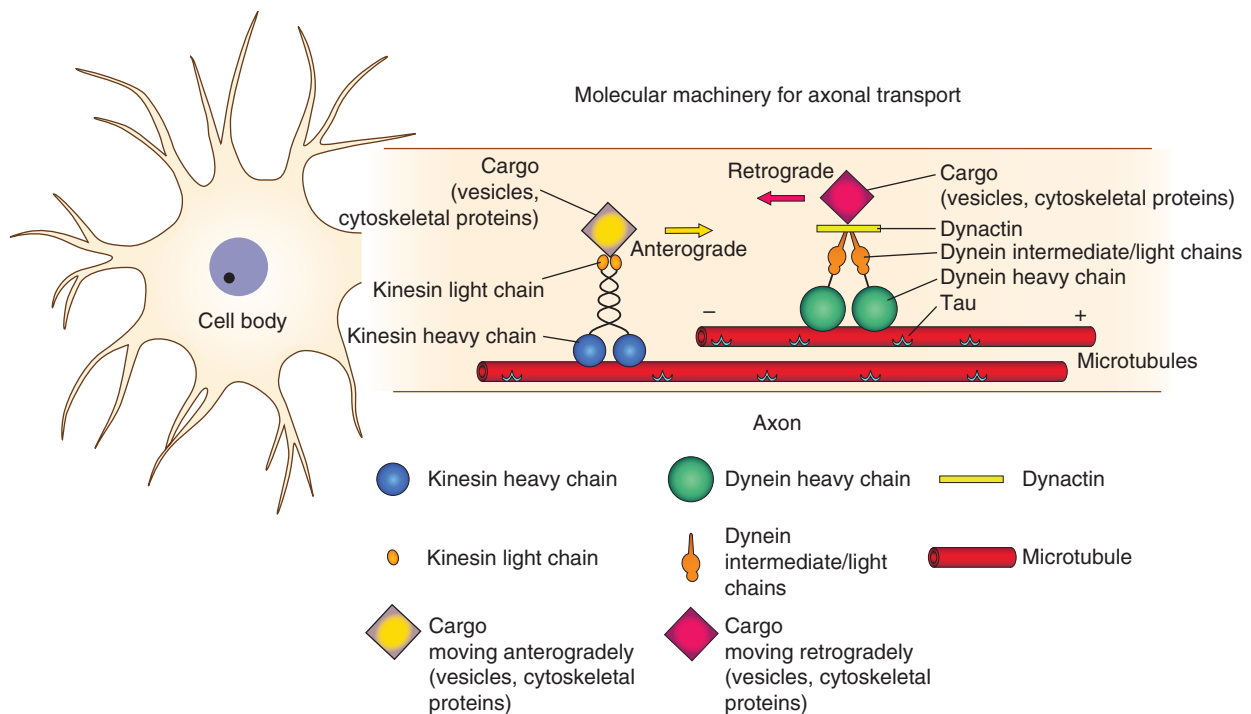


Figure 1 Basic principles of axonal transport. Cargo is transported anterogradely and retrogradely by the molecular motors kinesin and dynein, respectively. A variety of additional adaptor/linker proteins may also help in binding kinesin to the cargo (not shown). Dynactin, a multiprotein complex, links dynein to its cargo and is thought to increase the efficiency of the motor. Long-range movement occurs along cytoskeletal 'tracks,' mainly microtubules.

underlying mechanisms. These studies showed that slow cargo moves rapidly like fast cargo, but unlike fast cargo, it pauses for prolonged times during transit. Thus, the overall movement is infrequent and intermittent, leading to a slow overall rate of the transported cytoskeletal population. It has also been shown that slow and fast transport share similar molecular motors.

Progress in biochemistry and genetics has led to the identification of approximately 45 members of the kinesin superfamily (Kifs), grouped into 14 subfamilies, and two members of the cytoplasmic dynein family. Besides this large array of motor proteins, there are also a number of linker/adaptor proteins responsible for binding cargo to the motor proteins that have been identified. This heterogeneity is thought to play a major role in the recognition of specific motors to their cargo. Many excellent reviews on the subject are available.

Axonal Transport and Neurodegenerative Diseases

The following developments have dramatically highlighted the role of axonal transport disruptions in human neurodegenerative diseases: (1) the discovery of motor protein mutations in human neurodegenerative diseases, (2) axonal transport defects in animal

and *in vitro* cellular models of neurodegenerative diseases, and (3) newly discovered roles in axonal transport regulation for known pathogenic proteins involved in neurodegenerative diseases. With a focus on specific diseases, we discuss some of the recent findings linking axonal transport to neurodegenerative diseases.

Kinesin Mutation in Hereditary Spastic Paraplegia

Hereditary spastic paraplegias (HSPs), also known as familial spastic paraplegias, represent an autosomal dominant inherited group of neurodegenerative diseases. Patients present in their thirties or forties with symptoms in the lower limbs, with gradual proximal spread of symptoms. Neuropathologically, a distal axonopathy is seen, with severe degeneration and gliosis of the distal corticospinal tracts and relative sparing of the tracts in the brain stem and proximal cord. Due to the peculiar distal-to-proximal 'dying back' axonopathy observed in this disease, it had been hypothesized that dysfunctions in axonal transport leading to selective damage of the distant portions of the axons may be responsible for the pathogenesis of HSPs.

Indeed, a missense mutation in one of the genes encoding a major kinesin protein (the gene for kinesin heavy chain Kif 5a) was found in a family with HSP. The same mutation was found in all affected

members of the family, as well as in some presymptomatic members. This mutation occurs within a functional motor domain of the kinesin protein and a homologous mutation in yeast has been found to decouple kinesin binding to microtubules, highlighting the functional role of the kinesin mutation in the pathology of HSPs.

Dynactin Mutations in Distal Spinal and Bulbar Muscular Atrophy

Dynactin is a large protein complex linked to the retrograde motor dynein, and it is thought to link the motor to its cargo and/or increase the processivity of the motor. Animal models disrupting the dynein/dynactin complex develop late-onset motor neuron degeneration, and missense mutations in a dynein subunit cause progressive motor neuron degeneration in mice. Due to the central role of the dynein/dynactin complex in axonal transport, and based on data from the animal studies, it was hypothesized that mutations in the dynein/dynactin complex could play a role in neurodegeneration. Indeed, mutations in the gene encoding a subunit (p150^{Glued}) of the dynactin complex have been reported in a family with the neurodegenerative disease distal spinal and bulbar muscle atrophy (SBMA). In familial SBMA cases, the disease is transmitted in an autosomal dominant fashion and is manifested as a primary lower motor-type neuropathy with patients presenting in their thirties, often with breathing difficulty due to vocal fold paralysis, which later leads to weakness in the face and distal extremities. Neuron loss as well as inclusions containing dynein and dynactin were also seen in autopsy studies from one patient. The mutation reduces the binding affinity of dynactin to microtubules and also causes subtle defects in dynein function.

Kinesin Mutations in Charcot–Marie–Tooth Disease

Charcot–Marie–Tooth (CMT) disease comprises a heterogeneous group of inherited peripheral neuropathies characterized by motor and sensory deficits, often presenting in young adults as tingling, numbness, and loss of deep reflexes. The progression of the disease varies among individuals, with symptoms ranging from mild neuropathy to complete disability. Two basic forms can be recognized, with primary demyelinating (CMT1) or axonal (CMT2) types of degeneration predominating. Various genes have been implicated in CMT syndromes, including several genes known to play a role in myelination (PMP22 and MPZ) and genes for gap junction proteins (Connexin 32). However, in a remarkable series of studies, it was shown that mutations in a kinesin subunit protein (Kif 1B beta) can lead to an axonal type of CMT (CMT type 2A).

While studying animal models of kinesin knockout mice, it was found that heterozygous knockout mice for one of the kinesins, Kif 1B, developed progressive muscle weakness with normal motor nerve conduction velocities, symptoms resembling axonal type CMT, CMT type 2. Incidentally, the gene for CMT type 2A had been mapped to the same interval as the gene for Kif 1B, and genomic analysis of pedigrees with CMT type 2A revealed that these patients had a mutation in the Kif 1B gene. It was further shown that the mutant motor protein may not tightly bind to microtubules, thus suggesting a loss of function of the Kif 1B protein in patients with CMT type 2A.

Axonal Transport Defects in Alzheimer's Disease and Other Tauopathies

The story of the role of axonal transport in Alzheimer's disease (AD) is a rapidly developing one. Neuropathologically, the two hallmarks of AD are deposits of fibrillar A β into diffuse and neuritic plaques in the extracellular space, and filamentous accumulations of tau proteins as neurofibrillary tangles and neuropil threads within neurons and their processes. Amyloidogenic A β peptides are generated by proteolytic processing of the A β precursor proteins (APPs), conveyed by fast axonal transport. Several enzymes are involved in the proteolytic processing of APPs to A β , including the gamma- and beta-secretases (presenilins and BACE, respectively). Human mutations of both APPs and presenilins are seen in familial AD cases, highlighting the critical roles of these two proteins in AD. On the other hand, tau is a microtubule binding protein thought to stabilize microtubules *in vivo*.

The accumulation of tau in neuronal cell bodies and axons, as well as axonal swellings seen in AD, prompted the notion that axonal transport failure was important in the pathogenesis of this disease. Recent observations indicate that defects in axonal transport can occur long before the onset of severe symptoms in animal models of AD as well as in patients with AD. Interestingly, global reduction of axonal transport by reduction of kinesin levels can also exacerbate AD-type pathology in mouse models, further highlighting the role of diminished axonal transport in the pathogenesis of this disease.

The previous observations suggest that axonal transport defects play a role in the pathogenesis of AD. In addition, many key proteins directly involved in AD are also thought to play roles in the regulation of axonal transport. Studies of several different model systems have suggested that APP may act as a receptor for kinesin, thus proposing a direct link between a pathogenic protein and the axonal transport machinery. However, other studies have been unable to confirm

these results. It has also been proposed that APPs may be transported in a vesicular complex containing presenilins and BACE, the gamma- and beta-secretase enzymes, and altered processing of APPs within this complex can lead to exacerbation of AD. These findings suggest that misregulation of APPs, either directly from known APP mutations (as in familial AD) or indirectly via proteins associating/interacting with APPs, can lead to disruption of fast axonal transport in general, thus leading to axonal depletion of critical components and neurodegeneration.

Another interesting line of evidence highlighting the role of presenilins in the regulation of fast axonal transport in AD derives from studies of presenilin mutant mice. As mentioned previously, presenilins are proteins responsible for regulated proteolysis of APPs, and mutations in presenilins are seen in most cases of early familial AD. Several studies indicate that presenilins interact with glycogen synthase kinase-3 β (GSK-3 β). GSK-3 β is a kinase with many different roles, including the phosphorylation of kinesin light chains, and it has been shown that GSK-3 β -mediated phosphorylation of kinesin light chains led to detachment of the kinesin motor from the cargo, thus preventing further transport of cargo. By using transgenic presenilin mutant mice, it was also shown that mutant or absent presenilin increased GSK-3 β levels, thereby phosphorylating kinesin light chains, detaching the kinesins from their cargo, and impairing axonal transport.

Roles in axonal transport regulation have also been assigned to tau. Because tau binds to microtubules and is thought to stabilize them, it was proposed that dysfunctions in tau can destabilize microtubules and lead to a failure of axonal transport. Indeed, human tau mutations impair the ability of tau to bind to and stabilize microtubules, and tau overexpression in cellular models can lead to defects in axonal transport. In addition, the mechanisms causing the accumulation of tau in AD are also beginning to be understood. Defective axonal transport of disease-associated mutant tau has also been demonstrated in mouse models, providing experimental evidence for a mechanism of tau accumulation.

A final line of evidence suggesting a role for axonal transport defects in AD comes from studies of ApoE4, a gene whose allelic state is associated with an increased risk for AD. Mice expressing human ApoE4 exhibit defects in axonal transport, and the receptor for ApoE4, ApoER2, binds to JIP1/2, a protein that appears to mediate the binding of APPs to kinesin I. Thus, it can be postulated that overexpression of ApoE4 protein can lead to misregulation of JIP1/2-mediated binding of kinesin to APPs, leading to defects in fast axonal transport.

Stabilization of microtubules is also being explored as a therapeutic option in AD. The proof of principle for this concept was demonstrated in a study that showed that a microtubule-stabilizing drug (paclitaxel) could ameliorate the neurodegenerative phenotype in transgenic tau mice by offsetting the loss of tau function by stabilizing the microtubules and correcting the fast axonal transport defects in these mice.

Axonal Transport and Huntington's Disease

Neuropathologically, Huntington's disease is characterized by atrophy and degeneration of striatal neurons, with aggregates of pathological polyglutamine containing the protein huntingtin. Huntingtin is a predominantly cytoplasmic protein that associates with vesicles and moves in the fast axonal transport component. Although it has been known for several years that polyglutamine repeats within the huntingtin protein cause a gain of deleterious function leading to neurodegeneration, the exact pathogenic role of the repeats is unclear. By infusing huntingtin-harboring pathological polyglutamine repeats into a model for studies of fast axonal transport, it was shown that fast axonal transport was specifically inhibited by pathologically expanded polyglutamine repeats (but not normal proteins), along with inhibition of neurite extension in cultured cells. Furthermore, disruption of the *Drosophila* huntingtin gene also caused axonal transport defects. These findings lend support to a model in which aggregates of polyglutamine repeats disrupt fast axonal transport. Whether the disruption of axonal transport is a direct effect of the polyglutamine repeats or a secondary phenomenon remains to be established.

Axonal Transport and Amyotrophic Lateral Sclerosis

The histopathologic observation of prominent neurofilament-rich inclusions in the axons of spinal motor neurons of patients with amyotrophic lateral sclerosis (ALS) led to the hypothesis that disrupted axonal transport of proteins may play a role in the pathogenesis of the disease. However, the first direct evidence that axonal transport is disrupted in ALS awaited the development of transgenic mouse models of familial ALS based on expression of mutant superoxide dismutase (SOD-1) protein mutant mice that replicate several key aspects of ALS. Studies of these SOD-1 transgenic mouse models of ALS showed that the transport of neurofilament proteins was retarded in these animals, even before the mice were symptomatic, thereby implicating impaired axonal transport as an early deficit in ALS. Mutations in the p150 subunit of dynactin have also been reported in ALS patients.

Axonal Transport and Synucleinopathies

Synucleinopathies, also known as α -synucleinopathies, are a group of neurodegenerative disorders in which the primary pathology is the intracytoplasmic accumulation of α -synuclein primarily in neurons and, in some cases, glial cells. These disorders include Parkinson's disease, dementia with Lewy bodies, the Lewy body variant of AD, multiple system atrophy, and neurodegeneration with brain iron accumulation. In familial forms of Parkinson's disease, autosomal dominant missense mutations in genes encoding for α -synuclein are seen, suggesting a role of α -synuclein in the pathogenesis of these disorders. α -Synuclein is a highly conserved protein belonging to a multigene family that includes β -synuclein and γ -synuclein. α -Synuclein is strongly expressed in neurons, highly enriched in presynaptic terminals, and transported predominantly in the slow component. Axonal transport abnormalities of α -synuclein have been proposed in synucleinopathies, based on the observation that axonal α -synuclein pathology is pronounced in the disease and also on experimental evidence suggesting that α -synuclein may play a role in transport of presynaptic vesicles. Age-related retardation in the normal transport of α -synuclein was also seen in a study. Collectively, these studies propose a model in which age-related retardation of α -synuclein transport leads to accumulations of the protein over time, predisposing to the α -synuclein pathology in axons. Although these findings are interesting, many questions remain unanswered. The physiological role of synuclein is far from clear, and much work needs to be done to identify the role of synuclein in neurodegenerative disorders.

Conclusions and Future Directions for Research

A growing body of evidence implicates axonal transport defects in the etiology and pathogenesis of neurodegenerative diseases. Although motor protein defects in neurodegenerative diseases are direct evidence for this, it is likely that many other disease proteins are directly or indirectly linked to the complicated machinery of axonal transport. Thus, studies on the molecular mechanisms of transport are necessary to facilitate our understanding of the role of axonal transport in these diseases. Since pathogenic proteins in AD have roles in the regulation of axonal transport, and these proteins have been extensively studied, the time is perhaps ripe to uncover the links between AD and impaired axonal transport. With

greater understanding of the other neurodegenerative diseases, it is likely that many more links to axonal transport will be uncovered. Another exciting but largely neglected avenue of research is drug discovery efforts to counteract axonal transport impairments as therapeutic interventions for neurodegenerative diseases. Indeed, if axonal transport defects are shown to be part of a common mechanism of disease in many neurodegenerative disorders, the discovery of drugs that modulate axonal transport could result in the development of important therapeutic interventions for the treatment of these disorders.

See also: Axonal Transport Disorders; Axonal and Dendritic Transport by Dyneins and Kinesins in Neurons; Axonal Transport and ALS; Axonal Transport and Alzheimer's Disease; Axonal Transport and Huntington's Disease; Hereditary Spastic Paraplegia; Slow Axonal Transport.

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