Polyglutamine Diseases and Transport Problems

Deadly Traffic Jams on Neuronal Highways

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The expansion of CAG repeats encoding glutamine (polyQ) causes, to date, 9 late-onset progressive neurodegenerative disorders, including Huntington disease, spinobulbar muscular atrophy, dentatorubral-pallidoluysian atrophy, and spinocerebellar ataxias 1, 2, 3, 6, 7, and 17. Although many studies using both knockout and transgenic mouse models suggest that a toxic gain of function is central to neuronal dysfunction, the exact mechanisms of neurotoxic effects remain elusive. Protein aggregations within neurons seem to be a common manifestation in almost all polyQ diseases, and such accumulations are perhaps major triggers of cellular stress and neuronal death. Recent data lead to the tantalizing proposal that disruption of axonal transport pathways within long, narrow-caliber axons could lead to protein accumulations that can elicit neuronal death, ultimately causing a neuronal dysfunction pathway observed in polyQ expanded diseases. Perhaps perturbations in transport pathways are an early event involved in instigating polyQ disease pathology.

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Our nervous system can be thought of as requiring a transport system composed of highways that transport essential cargoes from a central station, cell bodies in the spinal cord or brain, to places of need, nerve terminals or synapses, very much like a busy freeway system. Most materials within axons or synapses are synthesized within the neuronal cell body and are moved along lengthy axons to sites of function. Molecular motors, such as kinesin and dynein, are proteins that power cargo transport and use adenosine triphosphate hydrolysis to move vital cargoes on microtubule tracks (Figure 1). Within axons, organelles, vesicles, cytoskeletal proteins, signaling molecules, and other supplies from the cell body are transported by kinesin motors in the anterograde direction to nerve terminals and synapses, while signaling molecules and other components that need to be returned to the cell body from synapses are transported in the retrograde direction by dynein and some kinesin motors. Since transport of cargoes is required for cell viability, it is conceivable that disruption in this long distance transport system can lead to disease pathology observed in many neurodegenerative diseases, including polyglutamine (polyQ) diseases.

AXONAL TRANSPORT PATHWAYS AND NEURODEGENERATIVE DISEASE

The importance of the axonal transport system in disease states has been recently highlighted by several intriguing studies. These studies not only suggest that strangulation of the axon may lead to disease pathology but also implicate components of the axonal transport machinery as targets for the development of human disease. Mutations in KIF1Bβ result in Charcot-Marie-Tooth disease type 2A, which is characterized by progressive dysfunction of peripheral neurons, possibly owing to the reduced transport of synaptic vesicle precursors. A missense mutation in the neuronal kinesin heavy chain gene KIF5A results in hereditary spastic paraplegia, a condition that arises owing to...
axon degeneration of motor and sensory neurons at the distal ends of the longest axons of the central nervous system. In a dynamitin (a component of the dynactin complex that interacts with dynein) overexpressing mouse model, excess dynamitin disassembles dynactin and inhibits retrograde transport, suggesting that impediment of axonal transport is sufficient to cause motor neuron degeneration observed in amyotrophic lateral sclerosis. While mice with transgenic amyotrophic lateral sclerosis showed abnormalities in microtubule-based transport, with decreased rates of slow axonal transport and degeneration of motor neurons, the dynamitin transgenic mice demonstrate a late-onset progressive motor neuron degenerative disease. A mutation in the human dynactin gene DCTN1 causes human motor neuron disease, and missense mutations in cytoplasmic dynein heavy chain (Dnchc1) cause selective impairment of axonal retrograde transport, cell death, Lewy body–like inclusions, and progressive motor neuron degeneration. Together, these studies argue that axonal transport failure can be a causative feature in neurodegenerative disease, strengthening the proposal that disruption of axonal transport is an important determinant in the initiation and perhaps the progression of pathogenesis.

Axonal transport problems have also been implicated in Alzheimer disease (AD). Recent work from our laboratory demonstrates that the amyloid precursor protein (APP) can function as a kinesin-I receptor. In Drosophila overexpression of wildtype human APP or familial mutations responsible for AD (Swedish and London) cause axonal vesicle accumulations, which contain APP and trigger neuronal cell death. Secretases (β-secretase and presenilin [PS]) that are responsible for the generation of pathogenic amyloid-β (Aβ) appear to be present in APP vesicles. Perhaps axonal blockages containing APP vesicles are sites of Aβ production, and processing of APP within these sites leads to the dissociation of kinesin from APP vesicles leading to the failure of transport within narrow axons, thus triggering the induction of neuronal dysfunction or death.

Yet another link between AD and axonal transport problems comes from the observation that PS, a component of the γ-secretase complex, interacts with GSK3β. An enhancement in GSK3β activation and a deficiency in kinesin-1–mediated transport were observed in PS mutations that cause familial AD (FAD). Intriguingly, GSK3β phosphorylates kinesin light chains leading to the dissociation of kinesin from membranes. Perhaps the disassociation of kinesin from APP-PS–containing vesicles results in the failure of transport in FAD-PS mutants. Together, these observations suggest that the axonal transport pathway may be central to the pathogenesis observed in AD.

Disruptions in transport pathways could also be involved in the pathogenesis of Huntington disease (HD) and other polyQ expansion diseases. Since protein aggregates are a common feature in all polyQ diseases, it is conceivable that failure in the transport system may also result in polyQ pathogenesis. Herein we briefly highlight polyQ disease pathology and discuss several recent advances relating this pathology to possible transport problems.

PolyQ PROTEINS AND DISEASE PATHOLOGY

Polyglutamine repeat diseases are a class of hereditary neurodegenerative diseases caused by the expansion of CAG triplet repeats encoding a polyglutamine tract in the normal protein (Table). These disorders are progressive, dominantly inherited (except for spinobulbar muscular atrophy), typically begin in midlife, and result in severe neuronal dysfunction and neuronal cell death. The expanded trinucleotide repeats are unstable, with increased repeat length correlating with worsening of the disease phenotype. The polyQ expansion is believed to confer a toxic gain of function, perhaps causing an increased propensity for the mutant protein to misfold and aggregate. Although all 9 polyQ diseases are genetically distinct and can be characterized by their specific lesion distributions in the nervous system, recent studies indicate that except for spinocerebellar ataxia (SCA) types 2 and 6, the formation of intranuclear inclusions within neurons is a common hallmark of all 9 diseases. Nuclear inclusions have been found in neuronal populations susceptible to the disease process, and this has lead to the widespread belief that intranuclear aggregations are central to polyQ pathogenesis.

Clinically, polyQ disorders share several common features, including slow progression and late (adult) onset. They also exhibit anticipation, becoming earlier and/or more severe in succeeding generations, which is correlated with an intergenerational increase in repeat length. Each of the polyQ disorders affect specific but overlapping regions of the brain. The clinical pathologic features of spinobulbar muscular atrophy are relatively distinct, whereas that of dentatorubral-pallidoluysian atrophy...
phy overlaps HD, and SCAs (reviewed in Evert et al13). Involuntary movements, intellectual impairment, and emotional disturbances clinically characterize HD, while spinobulbar muscular atrophy is a rare progressive neuromuscular disorder characterized by proximal weakness, atrophy, and fasciculations. Dentatorubral-pallidoluysian atrophy is characterized by progressive dementia, myoclonic epilepsy, cerebellar ataxia, and choreathetotic movements. All SCAs exhibit variable degrees of cerebellar and brainstem degeneration accompanied by progressive cerebellar ataxia associated neurological signs including ophthalmoplegia, dementia, and extrapyramidal signs.

At the molecular level, polyQ disease proteins are expressed ubiquitously throughout the brain and other tissues, although the normal function of most of these proteins remains unknown (Table). Exceptions are the androgen receptor in spinobulbar muscular atrophy, the P/Q-type calcium channel subunit in SCA6, and, based on recent work the huntingtin protein. Except for SCA3, the polyQ tract is located toward the N-terminal region of the protein in all polyQ diseases. Apart from the polyQ tract, the polyQ proteins do not share any other common features. In the case of SCA3 and HD, cleavage of the mutant protein is thought to promote aggregation.

### Table. PolyQ Diseases

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Protein</th>
<th>Location/Expression</th>
<th>Function</th>
<th>Inclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Huntington disease</td>
<td>Huntingtin</td>
<td>Ubiquitous cytoplasmic/mostly in neurons</td>
<td>Axonal transport</td>
<td>Dystrophic striatal, corticostriatal neurons (CI and NI)</td>
</tr>
<tr>
<td>Spinocerebellar ataxia</td>
<td>Ataxin-1</td>
<td>Neuronal nuclei and peripheral tissue</td>
<td>?</td>
<td>Purkinje neurons (CI and NI)</td>
</tr>
<tr>
<td>Type 1</td>
<td>Ataxin-2</td>
<td>Purkinje cells</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Type 2</td>
<td>Ataxin-3</td>
<td>Ubiquitous cytoplasmic</td>
<td>?</td>
<td>Ventral pons (CI and NI)</td>
</tr>
<tr>
<td>Type 6</td>
<td>α11, Ca+ channel P/Q</td>
<td>Central nervous system</td>
<td>Homeostasis and signaling</td>
<td>Purkinje neurons (CI only)</td>
</tr>
<tr>
<td>Type 7</td>
<td>Ataxin-7</td>
<td>Ubiquitous nuclear</td>
<td>?</td>
<td>Cerebral cortex (CI and NI)</td>
</tr>
<tr>
<td>Type 17</td>
<td>TBP</td>
<td>Ubiquitous nuclear</td>
<td>TATA binding protein ?</td>
<td>Inferior olivary complex and cerebral cortex (NI)</td>
</tr>
<tr>
<td>Dentatorubral-pallidoluysian atrophy</td>
<td>Atrophin-1</td>
<td>Ubiquitous cytoplasmic</td>
<td>?</td>
<td>Dentate (NI)</td>
</tr>
<tr>
<td>Spinobulbar muscular atrophy</td>
<td>Androgen receptor</td>
<td>Motor neurons</td>
<td>Development and growth</td>
<td>Motor neurons (CI and NI)</td>
</tr>
</tbody>
</table>

Abbreviations: Ca+, calcium; CI, cytoplasmic inclusions; NI, nuclear inclusions; PolyQ, polyglutamine.

**VIEWS OF NEUROTOXIC EFFECTS IN PolyQ DISEASE**

Although little is known about the mechanism by which polyQ expansion leads to pathogenesis, one proposal is that misfolding of the mutant protein triggers a cascade of events, which ultimately leads to disease pathology (reviewed in Evert et al13). The misfolded protein may undergo proteolytic cleavage, interact with other proteins, self-aggregate, and these aggregates may later translocate into the nucleus. Although the propensity of polyQ proteins to aggregate is a common feature observed in all 9 polyQ expansion diseases, it remains unclear whether aggregates, which contain not only mutant protein but components of the ubiquitin-proteosome pathway, chaperones, transcriptional regulators, and other polyQ-containing proteins, are the cause of pathogenesis or the end result of a cascade of events. Indeed, Wanker14 postulates that aggregates themselves are neurotoxic even though the distribution of aggregates within the central nervous system does not completely match areas of neuronal loss.

Whatever the cause, most proposals for disease mechanism include the hypothesis that aggregates alone are a precondition of neurotoxic effects, and several models propose that dysfunction originates from aggregate formation (reviewed in Tarlac and Storey15): (1) Sequestration of cellular factors away from their usual locations into aggregates are proposed to compromise their function and cause toxic effects; (2) recruitment of transcription factors into aggregates are proposed to attenuate transcription factor function; and (3) accumulation of molecular chaperones and proteosomes into aggregates are proposed to limit their availability in the cell, leading to diminished clearance and harmful accumulation of misfolded or damaged proteins, eventually activating cellular stress response pathways and inducing apoptosis.

In contrast, however, many recent studies propose that neuronal dysfunction occurs before aggregate formation. Prior to the detection of aggregates, neuronal and behavioral problems were detected in knock-in HD mice with 94 CAG repeats,17 in SCA1 mice with 154 CAG repeats,18 and in SCA7 mice with 266 CAG repeats.19 Indeed, another model suggests that aggregation of polyQ proteins may initially function as beneficial “sinks” that activate degradation pathways, which may later become defective owing to overactivation, ultimately resulting in neuronal dysfunction.20 Since ubiquitin and components of the proteosome were observed to colocalize with aggregates, further aggregation of polyQ proteins might block the degradation pathway.
An important point of controversy is whether neuronal toxic effects observed in polyQ diseases result from nuclear or cytoplasmic events. The most common type of aggregates observed in polyQ disease are intranuclear, but these may occur later in disease progression owing to problems or failures in other pathways. Indeed, neuropil aggregates were observed with expanded polyQ repeats in the context of the androgen receptor in transgenic HD mice and in HD-affected patient brains before the onset of clinical problems. Similarly, Klement et al demonstrate that while nuclear localization of ataxin-1 is necessary, nuclear aggregation of ataxin-1 is not required for initial pathogenesis in transgenic SCA1 mice. Consistent with these findings are recent results demonstrating that both cytoplasmic and nuclear accumulation pathways can independently lead to neuronal dysfunction and that cytoplasmic accumulations precede nuclear inclusions. Thus, cytoplasmic accumulations may be the primary events in toxic effects. However, it is still unknown if protein cleavage plays a role, and if aberrant cleavage of N-terminal fragments containing pathogenic polyQ repeats are the cause of cytoplasmic inclusion formation and disease pathology. Perhaps the cytoplasmic accumulations observed within axons are sites of N-terminal cleavage, and these pathogenic N-terminal fragments then promote nuclear entry and activate transcriptional processes, which may lead to nuclear-mediated toxic events.

Viewed broadly, an intriguing feature of polyQ disease is that while disease-causing genes are widely expressed (Table), only neurons are affected. These observations raise the question of whether the specificity observed is due to the nature of the neuron, with its long narrow axonal and dendritic processes. Essential components must be transported over great distances in axons and dendrites along microtubule tracks for cell viability. Perhaps defects in this transportation system have long-term effects on polyQ disease pathology.

Can Axonal Transport Defects Cause PolyQ Disease Pathology?

Although genes encoding motor proteins may be key players in human diseases when mutated, it is possible that proteins that regulate or interact with motor proteins may also cause disease when mutated. Alternatively, abnormal interactions of proteins not normally functioning in transport could cause transport problems subsequently leading to neuronal defects. Indeed, the widespread occurrence of axonal (or dendritic) inclusions observed in polyQ diseases raises the possibility that perturbations of transport pathways are an early susceptibility factor in disease pathology. In fact, new work leads to the proposal that stalling of vesicles within narrow caliber axons triggers aggregate formation within axons, which could then initiate a cascade of events, resulting in neuronal death and dysfunction. There are 2 complementary aspects to this proposal: (1) disease-causing proteins may have normal functions in the axonal transport system and may cause axonal blockages when altered; and (2) “sticky” diseased proteins may physically block transport within narrow axonal processes, and also titrate normal proteins, instigating pathways that lead to subsequent neuronal problems.

Many observations support these proposals for HD. In 1997, Block-Galarza et al showed that huntingtin, the protein that causes HD, was transported both anterogradely and retrogradely in rat sciatic nerve axons. Immunolocalization studies in human and rat brains revealed cytoplasmic huntingtin within neurons, and biochemical analyses indicated that huntingtin was enriched in compartments containing vesicle-associated proteins. Recently our laboratory found that normal Drosophila huntingtin functions in the axonal transport pathway, perhaps to transport a subclass of vesicles. Although huntingtin associates with the axonal transport machinery is still unclear, it can be proposed that huntingtin may associate with motor proteins via HAP1, a protein that has been shown to interact with both huntingtin and the p150 subunit of dynactin, thereby enabling retrograde transport and perhaps anterograde transport. HAP1 itself is transported both anterogradely and retrogradely and also associates with vesicles and with microtubules. Intriguingly, mutations in the Drosophila HAP1-like protein, Milton, causes axonal transport defects and may function in the transport of mitochondria to synapses by binding to kinesin. Thus huntingtin and HAP1 may have vital roles in axonal transport and perhaps with dynactin they have a role in establishing bidirectional transport.

Pathologic evidence for axonal transport problems in HD comes from observations in transgenic HD...
mouse models and human patient brains. Several groups have demonstrated that dystrophic striatal and corticostriatal neurites in HD exhibit characteristics of blocked axons, namely, accumulations of vesicles and organelles in swollen axonal projections and termini in association with huntingtin aggregates.30,35 Huntingtin accumulations have been found in axons of striatal projection neurons in R6/2 and knockin mouse models of HD and in human patient brains.23 These striatal axonal inclusions are better correlated with striatal neuronal loss than the presence of nuclear inclusions. Intriguingly, the axonal pathology observed in striatal neurites is virtually identical to the phenotype of motor protein mutants in Drosophila and polyQ-induced axonal blockages found in Drosophila models of polyQ disease.25,26

Although little is known about how these phenotypes arise and whether these observations are an early indication of neurodegeneration or the initial step in a cascade of events that cause dysfunction, these findings together with the evidence for cytosolic localization of full-length huntingtin protein and its association with cytoskeletal and vesicular structures are compelling arguments for a role of axonal transport in the pathology of HD. Since mutant huntingtin was shown to interfere with its anterograde transport, contributing to the depletion of brain-derived neurotrophic factor in the striatum,28 perhaps normal huntingtin is required for efficient vesicle trafficking of cortical brain-derived neurotrophic factor. Indeed, a recent study supports this proposal.30 A similar mechanism can be proposed for other polyQ diseases. Expression of expanded polyQ repeats in the context of the androgen receptor also causes neuropil aggregates and alters the distribution of kinesin.23 Consistent with this, recent data from Szelenyi et al39 demonstrate a polyQ length-dependent inhibition of anterograde and retrograde transport in isolated squid axoplasm by truncated versions of huntingtin or the androgen receptor. In SCA6, axonal accumulations are observed that appear to contain accumulations of neurofilaments and other materials that fail to be transported.40 In addition, expression of different pathogenic polyQ proteins within Drosophila neurons causes axonal blockages, which increase with reductions in motor proteins and reduce the amount of motor proteins available for normal transport.25 These observations suggest that accumulations of disease proteins, perhaps those that are sticky, can physically block transport pathways and titrate motors proteins away from their normal functions within narrow-caliber axons or dendritic processes. Consistent with this idea, it was recently shown that cytoplasmic huntingtin aggregates trap or titrate polyQ proteins, which may further block transport pathways.21 Thus, the 2 pathogenic pathways suggested by the transport hypothesis (described earlier) may not be mutually exclusive.

Although transport problems cannot account for all aspects of polyQ disease pathology, they provide a plausible explanation for how failures in the transport system could result in neuronal loss. Future experiments should focus on investigating axonal transport problems in other polyQ diseases for which excellent animal models are available and for which human tissues are readily accessible. Since different motor proteins move a variety of cargos, including membrane organelles, protein complexes, complexes of nucleic acids, signaling molecules, neuroprotective and repair molecules and cytoskeletal complexes along microtubule tracks, the susceptibility of different neurons to different polyQ disease may result owing to the specialized function of the normal protein within those neurons. Thus, rigorous investigations are needed to elucidate the normal functions of proteins involved in polyQ diseases, in particular to determine if these are involved in the axonal transport pathway.

EXPERIMENTAL THERAPEUTICS AND THEIR EFFECT ON AXONAL TRANSPORT AND POLYQ DISEASE PATHOLOGY

Although it is still not understood how polyQ disease pathology is caused, several groups have focused on identifying chemical agents that modulate cell toxicity or aggregation in a variety of polyQ disease models including yeast, Caenorhabditis elegans, Drosophila, and mouse (reviewed in Bates and Hockly43). Current potential therapeutic interventions targeting specific molecular events include minocycline (a caspase 1 inhibitor), cystamine (an inhibitor of tissue transglutaminase), Congo red (an inhibitor of polyQ aggregation), and suberoylanilide hydroxamic acid (a hydroxamic acid inhibitor) (reviewed in Bates and Hocky43). It is still unknown if these compounds act on the target for which they were selected. While it is important to distinguish if transport failures are an early secondary problem as opposed to being the true initiating cause of polyQ disease, pharmacological interventions directed toward the transport process may be valuable in the development of therapies. An important first step is to test if any of the above compounds prevent axonal transport problems in the in vivo Drosophila model system, which exhibits specific axonal transport phenotypes within polyQ expressing neurons.25 Ultimately, it is critical to develop axonal transport assays in living patients.

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