Review

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The emerging role of the first 17 amino acids of huntingtin in Huntington's disease

Abstract: Huntington's disease (HD) is caused by a poly-glutamine (polyQ) domain that is expanded beyond a critical threshold near the N-terminus of the huntingtin (htt) protein, directly leading to htt aggregation. While full-length htt is a large (on the order of ~350 kDa) protein, it is proteolyzed into a variety of N-terminal fragments that accumulate in oligomers, fibrils, and larger aggregates. It is clear that polyQ length is a key determinant of htt aggregation and toxicity. However, the flanking sequences around the polyQ domain, such as the first 17 amino acids on the N terminus (Nt17), influence aggregation, aggregate stability, influence other important biochemical properties of the protein and ultimately its role in pathogenesis. Here, we review the impact of Nt17 on htt aggregation mechanisms and kinetics, structural properties of Nt17 in both mono-meric and aggregate forms, the potential role of posttrans-lational modifications (PTMs) that occur in Nt17 in HD, and the function of Nt17 as a membrane targeting domain.

Keywords: amyloid; huntingtin; Huntington's disease; lipid binding; polyglutamine; posttranslational modifications.

Introduction

Huntington's disease (HD), a fatal neurodegenerative disorder, is caused by an expansion of a polyglutamine

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(polyQ) tract near the N-terminus of the huntingtin (htt) protein (Figure 1) (1). This polyQ expansion directly leads to htt aggregation into fibrils and a variety of other aggre-gates (2–4). While the exact mass of htt is dependent on the size of the polyQ domain, full-length htt is approxi-mately 350 kDa and is generally accepted to contain 3144 amino acids (Figure 1A). The polyQ domain begins at the 18th residue of htt and is contained in exon 1. Several lines of evidence suggested that N-terminal fragments comparable to exon 1 are directly involved in HD (5–10). The polyQ domain is flanked by the first 17 amino acids at the N-terminus (Nt17) of the protein and by a polyproline (polyP) stretch on its C-terminal side (Figure 1B). Down-stream of exon 1 are a number of HEAT repeats that are 40-amino acid-long sequences that are involved in pro-tein-protein interactions (11). Mutant htt is detected in patients' brains predominantly as microscopic inclusion bodies in the cytoplasm and nucleus (8); however, htt is also associated with a variety of membranous organelles, including mitochondria, endoplasmic reticulum, tubu-lovesicles, endosomes, lysosomes and synaptic vesicles (12–16). The precise mechanisms by which htt aggregates are toxic to neural cells, leading to the extensive cellular destruction that is the hallmark of HD, remain unclear. As a result, there is a pressing need to understand structural factors that modulate htt aggregation, contribute to patho-genesis and could potentially serve as therapeutic targets.

There are currently nine diseases related to polyQ expansions in proteins that are broadly expressed, and the nature of the proteins that contain the polyQ domain and their associated pathologies differ substantially (17). That is, each mutant polyQ protein causes a distinct neurodegenera-tive disease that is associated with different populations of affected neurons. One of the key aspects of these diseases is that there is a threshold length of the polyQ domain required for disease with a tight correlation between both the age of onset and severity of disease with the increasing length of the polyQ expansion. Specifically for HD, polyQ repeat lengths shorter than 35 do not result in disease, 35–39 repeats may elicit disease, 40–60 repeats cause adult onset and juvenile forms of HD are associated with repeats >60

Figure 1: Schematic representation of various features of the huntingtin (htt) protein.
(A) Full-length huntingtin protein with HEAT repeat sites (dark gray). The end of exon 1 is denoted by the black arrow. (B) Primary sequence of htt exon 1 indicating Nt17 (green), polyglutamine (polyQ) (purple), and the proline-rich region (gray). (C) Theoretical three-dimensional structure of Nt17 indicating hydrophilic (red) and hydrophobic (blue) faces of an amphipathic α-helix. (D) View down the barrel of the α-helix showing relative hydrophobicity of each residue (same color scheme as panel C), as well as the sites of reported posttranslational modification.

(18–20). This dependence and other observations suggest a toxic gain of function associated with expanded polyQ domains associated with these diseases.

While it is clear that polyQ expansion is central in HD, the context of the polyQ domain within htt can also play an important role. That is, the protein sequences directly adjacent to the polyQ domain can strongly influence the aggregation process both mechanistically and kinetically. This review focuses on the Nt17 domain and its potential role in HD. We will begin by reviewing the role of htt aggregation in HD from a general perspective and how Nt17 influences this specifically. Then, we will explore structural features of Nt17 that are of potential importance in HD. This will be followed by a discussion of the potential impact of posttranslational modifications (PTMs) that occur in Nt17. Finally, as Nt17 functions as a membrane targeting domain (14), we will end by discussing its role in htt aggregation on lipid membranes.

Microscopic inclusion bodies comprised of aggregated htt is a hallmark of HD

Over 40 years ago, abnormal nuclear structures were observed in HD brains (21); however, it was not until the development of the first mouse model of HD that it became apparent that these inclusions were composed of fibrillar aggregates of htt (8, 12). In these models, the expression of htt exon 1 with an expanded polyQ tract was sufficient to cause a progressive neurological phenotype in transgenic mice. More recent studies demonstrated that N-terminal fragments similar to exon 1 are detected in knock-in mouse models of HD that express full length htt (9). While the major hallmark of HD is the formation of intranuclear and cytoplasmic inclusion bodies of aggregated htt (22), the role of these intraneuronal aggregates in the etiology of HD remains controversial. For instance, in an early mouse model, intranuclear and cytoplasmic inclusion bodies preceded behavioral abnormalities (8); however, other studies indicate that inclusion body formation may protect against toxicity associated with soluble forms of htt by a sequestration mechanism (23–27).

Due to the direct correlation between htt aggregation and pathogenesis with polyQ length (28), it has been hypothesized that the aggregation of htt mediates neurodegeneration in HD. Due to the complexity of htt aggregation (as will be discussed in the next section), there is no clear consensus on the aggregate form(s) that underlie toxicity, and there likely exist bioactive oligomeric aggregates undetectable by traditional biochemical and electron microscopic approaches that precede symptoms. While identification of the toxic specie(s) of htt that trigger

neurodegeneration in HD remains elusive, such species might exist in a diffuse, mobile fraction rather than in inclusion bodies (24). It has also been reported that a thioredoxin-polyQ fusion protein could exhibit toxicity in a meta-stable, β-sheet-rich monomeric conformation (29), suggesting that polyQ might adopt multiple monomeric conformations, only some of which may be toxic. Consistent with such a scenario, molecular dynamic simulations and circular dichroism spectropolarimetry (CD) indicate that polyQ domains can adopt a heterogeneous collection of collapsed conformations that are in equilibrium prior to aggregation (30–33).

## The complexity of polyQ-mediated aggregation in htt

It has been demonstrated, biochemically and biophysically, that htt fragments containing expanded polyQ tracts readily form detergent-insoluble protein aggregates with characteristics of amyloid fibrils (28, 34). Synthetic peptides comprised of expanded polyQ domains flanked by short sequences to provide solubility also aggregated into amyloid-like fibrils, confirming that polyQ aggregates into amyloid-like fibrils (30, 35). Furthermore, the rate of aggregation into fibrils is highly correlated with polyQ length in synthetic peptides and htt exon 1 fragments (36). This correlation between aggregation rate and polyQ length has been recapitulated in cell culture models expressing htt fragments (27, 37, 38). While it is clear that htt with expanded polyQ tracts assembles into fibrils, htt also forms spherical and annular oligomeric structures (4, 39, 40). It is therefore evident that htt can form a variety of aggregate structures; however, the precise mechanisms by which these structures induce toxicity and neurodegeneration are unclear. Precise characterization of the possible aggregation pathways and the resulting heterogeneous mixtures of aggregates is a vital step in answering these important questions.

Extensive research has been performed to determine the kinetics of htt aggregation and to determine specific aggregate species on and off pathways to fibril formation (36). In this regard, several aggregation pathways have been proposed for the formation of fibrils of polyQ-containing proteins. Two of the more prominent aggregation schemes are: 1) re-arrangement of a monomer to a thermodynamically unfavorable conformation that directly nucleates fibril formation (41, 42) and 2) the formation of soluble oligomeric intermediates that slowly undergo structural re-arrangement into a β-sheet rich

structure leading to fibrils (2, 43, 44) (Figure 2). The precise nature of the oligomeric aggregates associated with the second scheme can be quite heterogeneous (45, 46), and there could be a variety of oligomeric species that are off-pathway to fibril formation. While polyQ peptide fibrils share many classical features associated with amyloids, initial reports using pure polyQ peptides supported the nucleation-elongation model for the formation of polyQ fibrils (30, 35). Subsequent studies have suggested other potential mechanisms associated with the potential of smaller aggregates of polyQ proteins appearing prior to nucleation of fibril formation (43, 47). Small oligomers, displaying various degrees of stability, of polyQ peptides with various glutamine lengths were observed by atomic force microscopy (2). Studies of polyQ peptides that were interrupted by other amino acids further support the formation of oligomers (44). While many oligomers were observed to directly initiate fibril formation when individually tracked in solution, many fibrils appeared to form without any obvious oligomeric precursors (2). Such observations suggest that these aggregation pathways are not mutually exclusive, making the situation considerably more complicated. Further atomic force microscopy studies of a variety of htt-exon 1 fragments have shown that aggregation reactions result in heterogeneous mixtures of distinct aggregate morphologies in a polyQ length- and concentration-dependent manner (2). Novel oligomers of htt exon 1 have also been identified in an *in vitro* mammalian system (45). Furthermore, distinct, polymorphic aggregates of htt have been observed (48), and X-ray crystallography has demonstrated that monomeric htt adopts multiple conformations (49, 50). These studies point to the complex nature of polyQ aggregation, necessitating the use of techniques capable of extracting quantitative data concerning relative amounts of specific aggregates forms.

Flanking sequences, including Nt17, play a prominent role in htt aggregation

It has been proposed that the protein composition surrounding expanded polyQ domains associated with different diseases, and concomitant protein interactions that vary due to protein context, will help to explain, at least in part, the striking cell specificity that is observed in each disease. In fact, the impact of protein context on the aggregation of polyQ domains has been demonstrated in numerous studies and systems (51–57). Broadly,
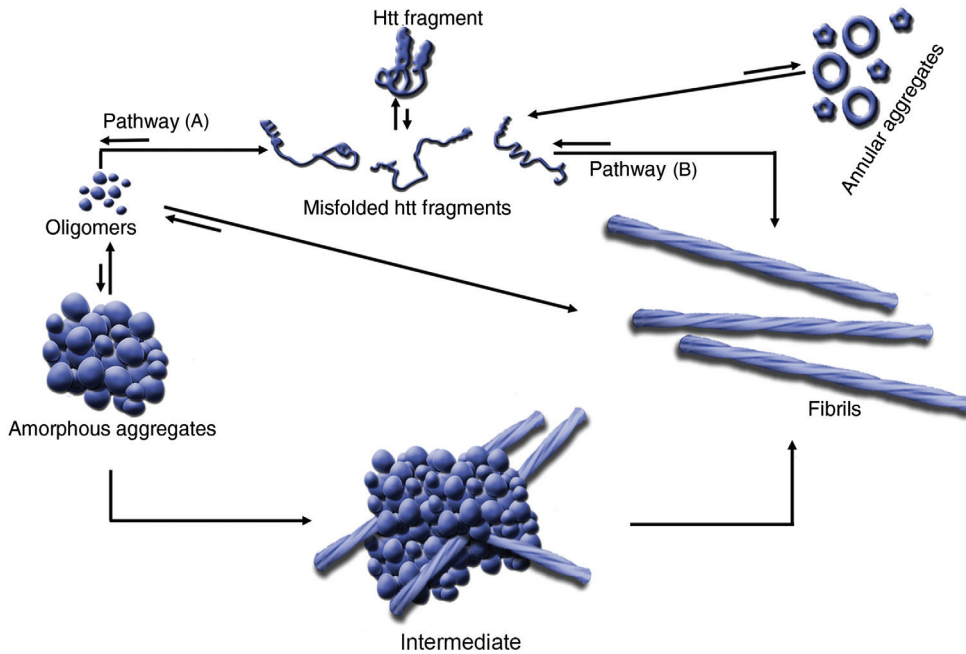
Figure 2: A schematic model for huntingtin (htt) aggregation.
A native monomer can sample a variety of distinct, misfolded monomer conformations, with the relative number and stability of these conformers potentially being polyglutamine (polyQ) length-dependent. Some misfolded monomers likely lead to aggregates, such as annular aggregates, that likely are off-pathway to fibril formation. There appear to be two generic aggregation pathways toward the formation of fibrils structures. (A) One of these pathways proceeds through oligomeric intermediates, some of which may be facilitated by Nt17. The size and stability of oligomeric aggregates can vary widely. A major structural transition must occur within an oligomer to initiate fibril elongation. (B) The other pathway to fibrils is a direct monomer to fibril transition. Oligomerization can also lead to the formation of large amorphous aggregates. All of these higher order aggregates may accumulate together to form the large inclusions that are a hallmark of Huntington's disease (HD).

these studies suggest a potentially critical role of flanking sequences to the polyQ structure and to potential mechanisms of aggregation. For example, the addition of a 10-residue polyP sequence to the C-terminus of a polyQ peptide, similar to that seen in htt, alters both aggregation kinetics and conformational properties of the polyQ tract (58). By inducing polyproline II (PPII)-like helix structure that propagates into the polyQ domain, flanking polyP sequences also inhibit the formation of β-sheet structure in synthetic peptides, extending the length of the polyQ domain necessary to initiate fibril formation (59). The polyP domain interacts with vesicle trafficking proteins (i.e., HIP1, SH3GL3, and dynamin) in a manner that may function to sequester these proteins into htt inclusion bodies, leading to neuronal dysfunction (60). Furthermore, the polyP flanking sequence has proven to be an effective target for manipulating the aggregation pathway. The anti-htt antibody MW7, which is specific for the polyP region, suppresses aggregation of htt *in vitro*, promotes proteasomal turnover of htt, and reduces toxicity when expressed as a single chain variable fragment (scFv) in cellular models of HD (46, 61–63).

A potential mechanism by which Nt17 influences toxic htt aggregation is that the formation of abnormal aggregates of mutant htt is directly influenced by this domain. Indeed, Nt17 has been implicated in driving the initial phases of htt exon 1 interaction (14, 64–67). Several mechanisms have been proposed that are mediated by Nt17. Most agree that the initial phase of aggregation when Nt17 is present begins with self-association of this domain, resulting in the formation of small, α-helix rich oligomers (68–70). As such, it appears that Nt17 promotes fibril formation via the oligomer mediated pathway. In a cellular environment, pathogenic htt exon 1 oligomer populations do not appreciably change even with recruitment of monomers into inclusion bodies, suggesting that the formation of oligomeric species mediated by Nt17 is the rate-determining step (71, 72). Since oligomers are widely considered to be the toxic species in HD, the promotion of oligomerization by Nt17 could have potentially important consequences of htt-related toxicity. PolyQ peptides that do not have the N-terminal flanking sequence tend not to form oligomeric intermediates and as a result, proceed directly to fibrillar aggregates (69); although,

some pure polyQ-peptide oligomers have been observed to form when aggregating on a surface (2, 73). The addition of a myc-tag preceding Nt17 in full htt exon 1 reduces the formation of oligomers *in vitro*, without changing the rate of fibril formation when compared to exon 1 proteins of similar polyQ length that lacked this myc-tag (74), suggesting that the ability of Nt17 to promote oligomerization can be interfered with by steric hindrances. Such findings underscore the critical importance of protein context to the rate of aggregation, aggregate stability, protein-protein interactions, lipid-protein interactions and cellular localization.

Nt17 structure in monomeric and aggregated htt

The Nt17 domain can form an amphipathic α-helix (AH) that is conserved in at least some aggregate forms of htt (Figure 1C) (66, 75, 76). There are several important biophysical properties associated with AHs (77), but in particular, AHs are often associated with binding of lipid membranes (78, 79). Briefly, an AH consists of a predominately hydrophilic face and a predominately hydrophobic face. AHs have been shown to have several functional properties such as preferentially sensing and binding highly curved membranes by detecting defects induced by curvature (80). Due to their ability to weakly bind membranes, their interaction with membranes is easily regulated. Nt17 has been shown to sequester truncated htt exon 1 peptides to regions of curvature on supported lipid bilayers (81). The structure of an AH is compatible with the formation of a variety of helix bundles in an aqueous environment that is driven by maximizing the interaction of the hydrophobic faces of adjacent helices (82, 83). Due to this, the association of Nt17 via the formation of interacting AHs has been proposed to play a role in the initial stages of htt aggregation.

The Nt17 primary sequence contains three positively charged lysine residues at positions 6, 9, and 15 (Figure 1B). Residues 6 and 15 lie at the boundary between the hydrophilic and hydrophobic faces in a theoretical AH that extends the entire length of Nt17 (Figures 1C–D). Additionally, two methionine, two serine, and two glutamic acid residues also reside on the hydrophilic face. The hydrophobic face consists of two phenylalanine residues, three leucine residues, and an alanine residue, with a leucine and a phenylalanine at each side of the boundary. It would appear that residues in the boundary region are critical in lipid membrane binding and intermolecular interaction.

Simulation of lipid membrane association show K6 and K15 are strong hydrogen bond donors with lipid bilayers (84). Similarly, K6 was found to be protected in an aggregated state by solution phase deuterium exchange mass spectrometry (MS) (85). These studies point to critical interactions involving amphipathic helices that can potentially be involved in the formation of the previously mentioned oligomeric precursors to fibril formation that are mediated by Nt17.

While there is little doubt that Nt17 is capable of forming an AH, under what conditions Nt17 adopts this structure in monomeric htt and the related consequences for aggregation are of critical importance. While the first crystal structures of htt exon 1 demonstrated that the polyQ domain is conformationally flexible, Nt17 was fully helical, with the helix extending into the polyQ domain for some crystal structures (50). Atomistic simulation in explicit aqueous solvent, CD, and solution nuclear magnetic resonance spectroscopy (NMR) all point to transient helical properties of Nt17 in the monomer (67, 69, 86, 87). Separate analytical ultracentrifugation and CD studies further support that Nt17 is only transiently helical, and only forms the full, stable AH upon association with a binding partner (71). That is, predominately random coil monomeric Nt17 exists in solution in equilibrium with an α-helix rich tetrameric species that may act as a critical nucleus for amyloid formation. The analytical ultracentrifugation experiment showed the propensity for Nt17 to form tetramers, while concentration-dependent CD showed an increase in α-helicity for isolated Nt17 aggregates (71). Nt17-containing polyQ peptides that did not tetramerize are hypothesized to serve as a pool from which monomer addition can occur during fibril elongation. Computational predictions of Nt17's free energy landscape in aqueous solution suggest that its most populated monomeric structure is a random coil that can interconvert, on a microsecond time-scale, with several helical conformers (86). Other atomistic simulations suggest that Nt17 exists in a two-helix bundle at 300 K and transiently exists as a straight helix (67).

Compelling evidence demonstrates that the α-helix is conserved in fibrils (66, 85, 88). Solid state NMR (ssNMR) and Fourier transform infrared spectroscopy studies confirm that the α-helix is conserved upon aggregation with polyQ segments above n=30 (66). A strong IR absorbance at 1655 cm^{-1}, indicative of α-helical character, from both small aggregates of Nt17 as well as htt exon 1 model peptides was observed in Fourier transform infrared spectroscopy experiments. Additionally, ssNMR chemical shifts and precise intrapeptide Nuclear Overhauser Effect measurements indicate residues 4–11 are in an α-helical

arrangement within a fibril structure, while residues 17–19 have a clear β-sheet structure (66). The location of Nt17 within fibrils appears to be on the periphery of the amyloid structure, as shown by ssNMR (66) and MS (85); however, pre-aggregated htt exon 1 inefficiently binds to lipid membranes, suggesting that Nt17 is tightly bound, or otherwise unavailable, in the final amyloid structure (89). The unavailability of Nt17 may be due to it being highly ordered in the fibril structure, as was observed by electron paramagnetic resonance (88). Analysis of these electron paramagnetic resonance results suggested that Nt17 could potentially make up the core of some fibrils. However, Nt17 appeared to undergo specific solvent-coupled motions in mature fibrils, as demonstrated by quantitative ssNMR measurements of residue-specific dynamics (90). Such solvent exposure and structural flexibility could allow for Nt17 to facilitate the interaction of htt aggregates with their environment. Such discrepancies in the location of Nt17 could suggest the formation of polymorphic fibril structures under different experimental systems and conditions (91). Still, these studies provide substantial evidence for the helical nature of Nt17 within amyloid fibrils.

The importance of Nt17 as a potential therapeutic target

Since Nt17 has been implicated in the formation of the toxic intermediate, it represents a novel structural target to inhibit (or alter) aggregate formation, thereby hopefully alleviating htt-mediated toxicity. A potential strategy would be targeted removal of the Nt17 tract all together; a concept that has been explored *in vitro* by cleaving Nt17 via proteolysis (42, 71). A tryptic proteolytic fragment of $Nt17Q_{37}P_{10}K_2$ ($SFQ_{37}P_{10}K_2$) exhibited aggregation kinetics and morphologies similar to $K_2Q_{37}K_2$, a simple polyQ peptide that lacks both flanking sequences (42). Similarly, a $MFQ_{37}P_{10}K_2$ tryptic analogue was shown to have similar kinetics to the true tryptic fragment (91). *In vitro*, these studies are a simple means of studying Nt17-mediated aggregation, but do not represent a likely means of treatment *in vivo*, due to the difficulty of selective proteolysis.

Perhaps a more practical means of inhibiting N-terminal aggregation is through structural complexation, either by a chaperone or an antibody-antigen that interacts directly with this domain (92–97). Interestingly, free Nt17 (lacking any polyQ) has been used to inhibit Nt17-mediated formation of $Nt17Q_{37}P_{10}K_2$ peptides *in vitro* (76, 91). The mechanism by which Nt17 inhibits amyloid formation appears to be identical to the mechanism by which

nucleation occurs, with the caveat that Nt17 alone does not contain an amyloidogenic polyQ tract. This increases the distance between adjacent polyQ tracts and the energy for polyQ fibrillization to occur (76). It has also been known for some time that molecular chaperones can modulate htt aggregation (98, 99). Many of these molecular chaperones, including Hsc70 (100) and Tric (94, 95), have been shown to bind directly to Nt17.

Another potential therapeutic strategy that has been extensively studied is the use of antibodies; unlike peptide therapeutics and inhibitors, antibodies are specific only for the target antigen. Several antibodies exist for htt exon 1, each recognizing distinct domains and potentially distinct monomers or aggregates (46, 101–104). In the past decade, intracellular antibodies, or intrabodies, for Nt17 have been developed that inhibit htt exon 1 fibrillization and cytotoxicity *in vitro* and cellular htt model, two of which are scFv and V_L 12.3.(92, 93, 105). When co-transfected with htt exon 1 (104Q) into COS-7, BHK-21, and HEK-293 cells, C4 scFv was shown to decrease aggregate formation relative to control groups that were not transfected with the intrabody (105). C4 scFv bound to htt exon 1 structures of non-pathogenic length (25Q), as well, which indicates that the polyQ conformation does not play a role in intrabody binding, and that the most likely binding target is Nt17 (105). The second intrabody, V_L 12.3, also targets the Nt17 domain; however, unlike C4, V_L 12.3 consists of only a single chain of the typical antibody structure. The efficacy of this antibody is highly increased after removal of disulfide bonds within the antibody structure (92, 93). Interestingly, an X-ray crystal structure of the antibody-antigen complex revealed that Nt17 was helical in the binding pocket of the intrabody (92), which either suggests that the binding pocket induces helicity in the proposed random structure of Nt17, or that the intrabody only recognizes helical Nt17. Intrabodies that were co-expressed with a pathogenic GFP-fused htt exon 1 (97Q) in HEK293 mammalian cells caused a dramatic decrease in htt inclusion bodies relative to controls. The V_L 12.3 intrabody was at least as effective in retarding aggregate formation as the C4 intrabody (92), as shown by fluorescence immunomicroscopy. These studies show that structural inhibition of Nt17 is certainly a viable means of inhibiting aggregation and, potentially, htt exon 1 toxicity.

PTMs in Nt17

Nt17 also contains numerous sites that can be posttranslationally modified, with some sites associated with

multiple potential modifications (Figure 1D) (106–108). These PTMs appear to have a profound effect on htt function and translocation (109–111), as well as toxicity associated with htt containing expanded polyQ domains (106, 107, 112–114). Most prevalent forms of PTMs occurring in Nt17 are phosphorylation (65, 114–117), acetylation (118), ubiquitination (119), SUMOylation (106, 112), and removal of the N-terminal methionine (114). Other htt modifications such as palmitoylation (120) and transglutamination (121) have also been observed.

The htt Nt17 domain has three potential phosphorylatable sites: threonine 3 (T3), serine 13 (S13), and serine 16 (S16) (full-length htt can also be phosphorylated at serine residues downstream from the N terminal domain). Numerous studies have reported that htt phosphorylation is associated with reduced levels of mutant htt toxicity (108, 114, 116, 117, 122–124). Mutating T3 to aspartate to mimic phosphorylation enhances the propensity of htt to aggregate in cultured cells and resulted in reduced lethality and neurodegeneration in *Drosophila* models of HD (114). Htt containing phospho-serine residues at 13 and 16 aggregate in a similar fashion to serine to aspartate phosphomimetic mutations, demonstrating that serine phosphomimetic mutations are valid mimics for studying the impact of htt phosphorylation on aggregation. Using a variety of combinations of serine phosphorylation mimics, it was established that serine to aspartate mutations significantly slows the formation of htt aggregates and reduces the stability of the fibril structure (65, 117). When S13 and S16 were mutated to aspartate in the Nt17 domain of transgenic mice that expresses full length htt with 97Q, visible htt inclusions within the brain were reduced, and HD-like behavioral phenotypes were reduced at 12 months of age (117). Altering the phosphorylation state of htt (specifically at S13) indirectly can also impact expanded htt-related toxicity, as casein kinase 2 inhibitors reduced S13 phosphorylation with a subsequent enhancement of htt-related toxicity in high content live cell screenings (125). Furthermore, ganglioside, GM1, triggers htt phosphorylation at S13 and S16, diminishing htt toxicity while restoring normal motor behavior in transgenic mice (115). Collectively, these findings further support the notion that Nt17 plays an important role in htt aggregation and, more specifically, that altering the phosphorylation state of Nt17 may be a viable therapeutic strategy.

Htt can also undergo SUMOylation, ubiquitination, and acetylation on specific lysine residues within Nt17. These potential sites include lysine residues at position 6, 9, and 15. SUMOylation, specifically, has been implicated in both cell and animal models to be involved in HD pathogenesis (106, 112). SUMOylation at lysine 6 (K6)

and lysine 9 (K9) stabilizes htt exon 1 fragments, reducing aggregation in cultured cells and exacerbating toxicity in *Drosophila* HD models (106). Rhes, a small G protein, preferentially binds mutant htt over wild type and acts as a SUMO E3 ligase (126). The subsequent SUMOylation by Rhes increases soluble levels of htt and enhances neurotoxicity (126). Ubiquitination is known to compete for the same lysine residues as SUMOylation, but functionally, it is associated with tagging proteins for degradation by the ubiquitin proteasome system, thereby reducing the toxicity of the mutant htt (127). However, the presence of ubiquitin in neuronal intranuclear inclusions in the HD striatum and cortex shows that the detoxification mechanisms by ubiquitination are incomplete for most proteins (12). Proteomic mapping by MS verified within Nt17 that K9 is appreciably acetylated in mammalian cell lysates (128). While the role of K9 acetylation has not yet been fully evaluated, acetylation of lysine 444 (K444) has been implicated in autophagic removal of mutant htt for clearance, leading to neuroprotection in the *Caenorhabditis elegans* HD model (118). Perhaps, acetylation within Nt17 plays a similar role, but the impact of acetylation within Nt17 on aggregation and toxicity of htt has not been fully elucidated.

Finally, PTMs can occur in tandem, directly affecting other PTMs. For instance, htt SUMOylation can be modulated by phosphorylation, explaining why S13 and S16 phosphomimics in htt were found to modulate SUMO-1 modification both in cells and *in vivo* (116, 117). SUMOylation is a transient process, and Nt17 lysines are predicted as low probability SUMO sites. Therefore, one way to enhance Nt17 SUMO-1 modification is to use serine phosphomimetic mutants (112). Interestingly, these phosphomimics also promote K6 htt SUMOylation, which may represent one way to regulate K9 acetylation (112). SUMOylation and ubiquitination can also influence each other by competing for the same Nt17 lysine 6, 9 and 15 (106), implying that one modification could be used to control or enhance the other. Understanding how Nt17 PTMs impact htt and modulate each other could lead to therapeutic intervention with potential implications to HD.

Nt17 mediates htt/lipid interactions with potential implications for toxicity

Another potential role of Nt17 in htt-related toxicity is that Nt17 directly dictates whether, and to what extent, toxic protein aggregates interact with cellular and subcellular compartments, such as organelles. These interactions

would be mediated by the ability of Nt17 to bind membranes comprised predominately of lipids and could play an important role in htt trafficking. These membranes may also be direct targets of htt aggregates, that is, htt aggregates may alter membrane structure and stability as part of a molecular mechanism of toxicity. A considerable number of observations directly and indirectly support such notions. Htt has been implicated in the transport of lipid vesicles (endocytic, synaptic or lysosomal), especially along microtubules (129–132). Expanded polyQ-conferred nuclear localization of htt appears to require additional htt sequences such as Nt17 (133), and Nt17 itself has been implicated to function as a nuclear export signal (134). Htt also associates with acidic phospholipids (135) which could play a role in nucleating aggregation. Furthermore, htt localizes to brain membrane fractions (136) which are primarily comprised of lipids.

There are a variety of membrane-associated functions attributable to htt in which the lipid-binding properties of Nt17 may play a role. These functions include cellular adhesion (137), motility (138, 139), cholesterol and energy homeostasis (140), and as a molecular scaffold for coordination of membrane and cytoskeletal communication (135) as well as facilitating microtubule-dependent vesicle transport (141). Nt17 facilitates the trafficking of htt exon 1 to membranes associated with the endoplasmic reticulum (ER), autophagic vacuoles, mitochondria, and Golgi (14, 142). Membrane curvature sensing, facilitated by AH structural characteristics of Nt17, may play a mechanistic role in these functions (81). Additional evidence that Nt17 directly interacts with lipid membranes was provided by the observation that the binding of htt exon 1 to large unilamellar vesicles composed of phosphatidylcholines or a mixture of phosphatidylcholines and phosphatidylserines enhanced the helical content of Nt17 (14). These changes in helical content were blocked by changing the eighth residue of Nt17 to a proline (14). A structural transition from random-coil in solution to an α-helical structure upon binding lipid bilayers was validated by high-resolution ssNMR structural studies (143, 144). Nt17 in synthetic polyQ peptides was required for synthetic polyQ peptides to bind model brain lipid extract membranes (73). Synthetic polyQ peptides with a variety of flanking sequences readily form fibrils in solution or solid surfaces, such as mica, but lipid membranes stabilize discrete oligomeric aggregates of polyQ peptides that contain Nt17 (73). However, both Nt17 and a C-terminal polyP domain were required for polyQ peptides to destabilize membrane structure, leading to leakage. Computational studies further support the important role of Nt17 in htt/lipid interactions and also provide evidence that the presence of both Nt17 and polyP enhance the interaction of htt with bilayers (84). The cooperative effect of Nt17 and the polyP domain in disrupting membranes may be particularly important in light of observed interactions between these two domains in a cellular environment that is altered with increasing polyQ length (145). The binding and disruption of lipid membranes by full htt exon 1 constructs can be partially inhibited by the addition of N-terminal tags, further supporting a prominent role of Nt17 in these phenomena (89).

In terms of a potential role in htt-related toxic mechanisms, the accumulation of monomeric htt and/or prefibrillar aggregates directly binding lipid membranes has been shown to disrupt bilayer integrity and to induce mechanical changes in the membrane (89, 146). Another factor regulating the interaction of htt with lipid membranes mediated by Nt17 is the previously discussed PTMs, which are known to be involved in htt translocating to specific organelles (14, 147). Additional affinity-based components for the interaction of other AHs with lipid bilayers also play a role, including electrostatics (148, 149), partitioning of lipid components (150–152), or the presence of other specific recognition motifs (153, 154). The availability of Nt17 to interact with lipid membranes could also play an important role in cell to cell transport of htt aggregates. This may prove particularly important with the realization that there are non-cell-autonomous toxic effects associated with HD (155–159).

Expert opinion

The collective evidence reviewed here strongly supports the conclusion that there is a need to identify, at a mechanistic level, the precise role Nt17 may play in modulating the formation of toxic aggregates, as well as influencing interactions with subcellular surfaces. Future studies aimed at further elucidating the role of Nt17 in the formation and stabilization of relevant aggregate species and potential toxic mechanisms involved in HD may prove particularly useful. Such information is important because it could lead to better defined therapeutic targets and strategies to treat HD in addition to the polyQ tract.

Highlights

– Due to the complexity of htt aggregation and diversity of potential aggregate forms, there is no clear consensus on the underlying toxic specie(s) most relevant to HD; however, there likely exists a variety of bioactive aggregates ranging from oligomers to fibrils.

- Precise characterization of htt aggregation and resulting aggregate structure may prove vital, and such studies will require the use of a variety of biochemical and biophysical techniques.
- Several studies have suggested a critical role of the Nt17 domain in htt in modifying the rate of aggregation, relative aggregate stability, specific protein-protein interactions, lipid-protein interactions, and cellular localization.
- A potential mechanism by which Nt17 influences toxic htt aggregation is by promoting the formation of oligomeric aggregates of htt.
- PTMs occurring in Nt17 can have a profound effect on htt function and translocation, as well as modifying the toxic gain of function associated with mutant htt.
- Targeting Nt17 through a variety of strategies, i.e., antibodies, small peptides, PTMs, can effectively inhibit or alter aggregation of mutant htt, suggesting that such strategies may represent viable therapeutic strategies.
- Nt17 directly dictates whether, and to what extent, htt interacts with cellular and subcellular membranes, and htt aggregation may alter membrane structure and stability as part of a variety of molecular mechanism associated with toxicity.

List of abbreviations

AH	amphipathic α-helix
CD	circular dichroism spectropolarimetry
Htt	huntingtin protein
HD	Huntington's disease
HEAT	Huntingtin, elongation factor 3 (EF3), protein phosphatase 2A (PP2A), and the yeast kinase TOR1
MS	mass spectrometry
NMR	nuclear magnetic resonance spectroscopy
polyQ	polyglutamine
polyP	polyproline
PTMs	posttranslational modifications
scFv	single chain variable fragment
ssNMR	solid state nuclear magnetic resonance spectroscopy

References

1. The Collaborative Huntington's Disease Research Group. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell 1993; 72: 971–83.

2. Legleiter J, Mitchell E, Lotz GP, Sapp E, Ng C, DiFiglia M, Thompson LM, Muchowski PJ. Mutant huntingtin fragments form oligomers in a polyglutamine length-dependent manner in vitro and in vivo. J Biol Chem 2010; 285: 14777–90.

3. Vonsattel JP, Myers RH, Stevens TJ, Ferrante RJ, Bird ED, Richardson EP. Neuropathologoical classification of Huntingtons disease. J Neuropath Exp Neur 1985; 44: 559–77.

4. Poirier MA, Li HL, Macosko J, Cai SW, Amzel M, Ross CA. Huntingtin spheroids and protofibrils as precursors in polyglutamine fibrilization. J Biol Chem 2002; 277: 41032–7.

5. Kim YJ, Yi Y, Sapp E, Wang Y, Cuiffo B, Kegel KB, Qin Z-H, Aronin N, DiFiglia M. Caspase 3-cleaved N-terminal fragments of wild-type and mutant huntingtin are present in normal and Huntington's disease brains, associate with membranes, and undergo calpain-dependent proteolysis. Proc Natl Acad Sci USA 2001; 98: 12784–9.

6. Cooper JK, Schilling G, Peters MF, Herring WJ, Sharp AH, Kaminsky Z, Masone J, Khan FA, Delanoy M, Borchelt DR, Dawson VL, Dawson TM, Ross CA. Truncated N-terminal fragments of huntingtin with expanded glutamine repeats form nuclear and cytoplasmic aggregates in cell culture. Hum Mol Genet 1998; 7: 783–90.

7. Hoffner G, Island M-L, Djian P. Purification of neuronal inclusions of patients with Huntington's disease reveals a broad range of N-terminal fragments of expanded huntingtin and insoluble polymers. J Neurochem 2005; 95: 125–36.

8. Davies SW, Turmaine M, Cozens BA, DiFiglia M, Sharp AH, Ross CA, Scherzinger E, Wanker EE, Mangiarini L, Bates GP. Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. Cell 1997; 90: 537–48.

9. Landles C, Sathasivam K, Weiss A, Woodman B, Moffitt H, Finkbeiner S, Sun B, Gafni J, Ellerby LM, Trottier Y, Richards WG, Osmand A, Paganetti P, Bates GP. Proteolysis of mutant huntingtin produces an exon 1 fragment that accumulates as an aggregated protein in neuronal nuclei in Huntington disease. J Biol Chem 2010; 285: 8808–23.

10. Sathasivam K, Neueder A, Gipson TA, Landles C, Benjamin AC, Bondulich MK, Smith DL, Faull RLM, Roos RAC, Howland D, Detloff PJ, Housman DE, Bates GP. Aberrant splicing of HTT generates the pathogenic exon 1 protein in Huntington disease. Proc Natl Acad Sci USA 2013; 110: 2366–70.

11. Neuwald AF, Hirano T. HEAT repeats associated with condensins, cohesins, and other complexes involved in chromosome-related functions. Genome Res 2000; 10: 1445–52.

12. DiFiglia M, Sapp E, Chase KO, Davies SW, Bates GP, Vonsattel JP, Aronin N. Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. Science 1997; 277: 1990–3.

13. Gutekunst CA, Li SH, Yi H, Mulroy JS, Kuemmerle S, Jones R, Rye D, Ferrante RJ, Hersch SM, Li XJ. Nuclear and neuropil aggregates in Huntington's disease: relationship to neuropathology. J Neurosci 1999; 19: 2522–34.

14. Atwal RS, Xia J, Pinchev D, Taylor J, Epand RM, Truant R. Huntingtin has a membrane association signal that can modulate huntingtin aggregation, nuclear entry and toxicity. Hum Mol Genet 2007; 16: 2600–15.

15. Kegel-Gleason KB. Huntingtin interactions with membrane phospholipids: strategic targets for therapeutic intervention? J Huntingt Dis 2013; 2: 239–50.

16. Leitman J, Ulrich Hartl F, Lederkremer GZ. Soluble forms of polyQ-expanded huntingtin rather than large aggregates cause endoplasmic reticulum stress. Nat Commun 2013; 4: 2753.

17. Weber JJ, Sowa AS, Binder T, Hübener J. From pathways to targets: understanding the mechanisms behind polyglutamine disease. BioMed Res Int 2014; 2014: 22.

18. Penney JB, Vonsattel JP, MacDonald ME, Gusella JF, Myers RH. CAG repeat number governs the development rate of pathology in Huntington's disease. Ann Neurol 1997; 41: 689–92.

19. Snell RG, Macmillan JC, Cheadle JP, Fenton I, Lazarou LP, Davies P, Macdonald ME, Gusella JF, Harper PS, Shaw DJ. Relationship between trinucleotide repeat expansion and phenotypic variation in Huntingtons disease. Nat Genet 1993; 4: 393–7.

20. Tobin AJ, Signer ER. Huntington's disease: the challenge for cell biologists. Trends Cell Biol 2000; 10: 531–6.

21. Roizin L, Stellar S, Willson N, Whittier J, Liu JC. Electron microscope and enzyme studies in cerebral biopsies of Huntington's chorea. Trans Am Neurol Assoc 1974; 99: 240–3.

22. Zoghbi HY, Orr HT. Glutamine repeats and neurodegeneration. Annu Rev Neurosci 2000; 23: 217–47.

23. Arrasate M, Finkbeiner S. Protein aggregates in Huntington's disease. Exp Neurol 2012; 238: 1–11.

24. Arrasate M, Mitra S, Schweitzer ES, Segal MR, Finkbeiner S. Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death. Nature 2004; 431: 805–10.

25. Miller J, Arrasate M, Brooks E, Libeu CP, Legleiter J, Hatters D, Curtis J, Cheung K, Krishnan P, Mitra S, Widjaja K, Shaby BA, Lotz GP, Newhouse Y, Mitchell EJ, Osmand A, Gray M, Thulasiramin V, Saudou F, Segal M, Yang XW, Masliah E, Thompson LM, Muchowski PJ, Weisgraber KH, Finkbeiner S. Identifying polyglutamine protein species in situ that best predict neurodegeneration. Nat Chem Biol 2011; 7: 925–34.

26. Muchowski PJ, Ning K, D'Souza-Schorey C, Fields S. Requirement of an intact microtubule cytoskeleton for aggregation and inclusion body formation by a mutant huntingtin fragment. Proc Natl Acad Sci USA 2002; 99: 727–32.

27. Saudou F, Finkbeiner S, Devys D, Greenberg ME. Huntingtin acts in the nucleus to induce apoptosis but death does not correlate with the formation of intranuclear inclusions. Cell 1998; 95: 55–66.

28. Scherzinger E, Lurz R, Turmaine M, Mangiarini L, Hollenbach B, Hasenbank R, Bates GP, Davies SW, Lehrach H, Wanker EE. Huntingtin-encoded polyglutamine expansions form amyloid-like protein aggregates in vitro and in vivo. Cell 1997; 90: 549–58.

29. Nagai Y, Inui T, Popiel HA, Fujikake N, Hasegawa K, Urade Y, Goto Y, Naiki H, Toda T. A toxic monomeric conformer of the polyglutamine protein. Nat Struct Mol Biol 2007;14: 332–40.

30. Chen S, Berthelier V, Yang W, Wetzel R. Polyglutamine aggregation behavior in vitro supports a recruitment mechanism of cytotoxicity. J Mol Biol 2001; 311: 173–82.

31. Vitalis A, Wang X, Pappu RV. Quantitative characterization of intrinsic disorder in polyglutamine: Insights from analysis based on polymer theories. Biophys J 2007; 93: 1923–37.

32. Vitalis A, Wang X, Pappu RV. Atomistic simulations of the effects of polyglutamine chain length and solvent quality on conformational equilibria and spontaneous homodimerization. J Mol Biol 2008; 384: 279–97.

33. Wang XL, Vitalis A, Wyczalkowski MA, Pappu RV. Characterizing the conformational ensemble of monomeric polyglutamine. Proteins 2006; 63: 297–311.

34. Scherzinger E, Sittler A, Schweiger K, Heiser V, Lurz R, Hasenbank R, Bates GP, Lehrach H, Wanker EE. Self-assembly of polyglutamine-containing huntingtin fragments into amyloid-like fibrils: implications for Huntington's disease pathology. Proc Natl Acad Sci USA 1999; 96: 4604–9.

35. Chen SM, Berthelier V, Hamilton JB, O'Nuallain B, Wetzel R. Amyloid-like features of polyglutamine aggregates and their assembly kinetics. Biochemistry 2002; 41: 7391–9.

36. Wetzel R. Physical chemistry of polyglutamine: intriguing tales of a monotonous sequence. J Mol Biol 2012; 421: 466–90.

37. Lunkes A, Mandel JL. A cellular model that recapitulates major pathogenic steps of Huntington's disease. Hum Mol Genet 1998; 7: 1355–61.

38. Hackam AS, Singaraja R, Wellington CL, Metzler M, McCutcheon K, Zhang TQ, Kalchman M, Hayden MR. The influence of Huntingtin protein size on nuclear localization and cellular toxicity. J Cell Biol 1998; 141: 1097–105.

39. Tanaka M, Morishima I, Akagi T, Hashikawa T, Nukina N. Intra- and intermolecular β-pleated sheet formation in glutamine-repeat inserted myoglobin as a model for polyglutamine diseases. J Biol Chem 2001; 276: 45470–5.

40. Wacker JL, Zareie MH, Fong H, Sarikaya M, Muchowski PJ. Hsp70 and Hsp40 attenuate formation of spherical and annular polyglutamine oligomers by partitioning monomer. Nat Struct Mol Biol 2004; 11: 1215–22.

41. Chen SM, Ferrone FA, Wetzel R. Huntington's disease age-of-onset linked to polyglutamine aggregation nucleation. Proc Natl Acad Sci USA 2002; 99: 11884–9.

42. Kar K, Jayaraman M, Sahoo B, Kodali R, Wetzel R. Critical nucleus size for disease-related polyglutamine aggregation is repeat-length dependent. Nat Struct Mol Biol 2011; 18: 328.

43. Walters RH, Murphy RM. Examining polyglutamine peptide length: a connection between collapsed conformations and increased aggregation. J Mol Biol 2009; 393: 978–92.

44. Walters RH, Murphy RM. aggregation kinetics of interrupted polyglutamine peptides. J Mol Biol 2011; 412: 505–19.

45. Nucifora LG, Burke KA, Feng X, Arbez N, Zhu SS, Miller J, Yang GC, Ratovitski T, Delannoy M, Muchowski PJ, Finkbeiner S, Legleiter J, Ross CA, Poirier MA. Identification of novel potentially toxic oligomers formed in vitro from mammalian-derived expanded huntingtin exon-1 protein. J Biol Chem 2012; 287: 16017–28.

46. Legleiter J, Lotz GP, Miller J, Ko J, Ng C, Williams GL, Finkbeiner S, Patterson PH, Muchowski PJ. Monoclonal antibodies recognize distinct conformational epitopes formed by polyglutamine in a mutant huntingtin fragment. J Biol Chem 2009; 284: 21647–58.

47. Bernacki JP, Murphy RM. Model discrimination and mechanistic interpretation of kinetic data in protein aggregation studies. Biophys J 2009; 96: 2871–87.

48. Nekooki-Machida Y, Kurosawab M, Nukina N, Ito K, Oda T, Tanaka M. Distinct conformations of in vitro and in vivo amyloids of huntingtin-exon1 show different cytotoxicity. Proc Natl Acad Sci USA 2009; 106: 9679–84.

49. Kim M. Beta conformation of polyglutamine track revealed by a crystal structure of Huntingtin N-terminal region with insertion of three histidine residues. Prion 2013; 7: 221–8.

50. Kim MW, Chelliah Y, Kim SW, Otwinowski Z, Bezprozvanny I. secondary structure of huntingtin amino-terminal region. Structure 2009; 17: 1205–12.

51. Bulone D, Masino L, Thomas DJ, Biagio PLS, Pastore A. The interplay between PolyQ and protein context delays aggregation by forming a reservoir of protofibrils. PLoS One 2006; 1: e111.

52. Duennwald ML, Jagadish S, Giorgini F, Muchowski PJ, Lindquist S. A network of protein interactions determines polyglutamine toxicity. Proc Natl Acad Sci USA 2006; 103: 11051–6.

53. Duennwald ML, Jagadish S, Muchowski PJ, Lindquist S. Flanking sequences profoundly alter polyglutamine toxicity in yeast. Proc Natl Acad Sci USA 2006; 103: 11045–50.

54. Ignatova Z, Gierasch LM. Extended polyglutamine tracts cause aggregation and structural perturbation of an adjacent β barrel protein. J Biol Chem 2006; 281: 12959–67.

55. La Spada AR, Taylor JP. Polyglutamines placed into context. Neuron 2003; 38: 681–4.

56. Menon RP, Pastore A. Expansion of amino acid homo-sequences in proteins: insights into the role of amino acid homo-polymers and of the protein context in aggregation. Cell Mol Life Sci 2006; 63: 1677–85.

57. Nozaki K, Onodera O, Takano H, Tsuji S. Amino acid sequences flanking polyglutamine stretches influence their potential for aggregate formation. Neuroreport 2001; 12: 3357–64.

58. Bhattacharyya A, Thakur AK, Chellgren VM, Thiagarajan G, Williams AD, Chellgren BW, Creamer TP, Wetzel R. Oligoproline effects on polyglutamine conformation and aggregation. J Mol Biol 2006; 355: 524–35.

59. Darnell G, Orgel JPRO, Pahl R, Meredith SC. Flanking polyproline sequences inhibit β-sheet structure in polyglutamine segments by inducing PPII-like helix structure. J Mol Biol 2007; 374: 688–704.

60. Qin ZH, Wang YM, Sapp E, Cuiffo B, Wanker E, Hayden MR, Kegel KB, Aronin N, DiFiglia M. Huntingtin bodies sequester vesicle-associated proteins by a polyproline-dependent interaction. J Neurosci 2004; 24: 269–81.

61. Khoshnan A, Ko J, Patterson PH. Effects of intracellular expression of anti-huntingtin antibodies of various specificities on mutant huntingtin aggregation and toxicity. Proc Natl Acad Sci USA 2002; 99: 1002–7.

62. Southwell AL, Khoshnan A, Dunn DE, Bugg CW, Lo DC, Patterson PH. Intrabodies binding the proline-rich domains of mutant huntingtin increase its turnover and reduce neurotoxicity. J Neurosci 2008; 28: 9013–20.

63. Southwell AL, Ko J, Patterson PH. Intrabody gene therapy ameliorates motor, cognitive, and neuropathological symptoms in multiple mouse models of Huntington's disease. J Neurosci 2009; 29: 13589–602.

64. Crick SL, Ruff KM, Garai K, Frieden C, Pappu RV. Unmasking the roles of N- and C-terminal flanking sequences from exon 1 of huntingtin as modulators of polyglutamine aggregation. Proc Natl Acad Sci USA 2013; 110: 20075–80.

65. Mishra R, Hoop CL, Kodali R, Sahoo B, van der Wel PCA, Wetzel R. Serine phosphorylation suppresses huntingtin amyloid accumulation by altering protein aggregation properties. J Mol Biol 2012; 424: 1–14.

66. Sivanandam VN, Jayaraman M, Hoop CL, Kodali R, Wetzel R, van der Wel PCA. The aggregation-enhancing huntingtin N-terminus is helical in amyloid fibrils. J Am Chem Soc 2011; 133: 4558–66.

67. Kelley NW, Huang X, Tam S, Spiess C, Frydman J, Pande VS. The predicted structure of the headpiece of the huntingtin protein and its implications on huntingtin aggregation. J Mol Biol 2009; 388: 919–27.

68. Williamson TE, Vitalis A, Crick SL, Pappu RV. Modulation of polyglutamine conformations and dimer formation by the N-Terminus of huntingtin. J Mol Biol 2010; 396: 1295–309.

69. Thakur AK, Jayaraman M, Mishra R, Thakur M, Chellgren VM, Byeon IJL, Anjum DH, Kodali R, Creamer TP, Conway JF, Gronenborn AM, Wetzel R. Polyglutamine disruption of the huntingtin exon 1 N-terminus triggers a complex aggregation mechanism. Nat Struct Mol Biol 2009; 16: 380–9.

70. Vitalis A, Pappu RV. Assessing the contribution of heterogeneous distributions of oligomers to aggregation mechanisms of polyglutamine peptides. Biophys Chem 2011; 159: 14–23.

71. Jayaraman M, Kodali R, Sahoo B, Thakur AK, Mayasundari A, Mishra R, Peterson CB, Wetzel R. Slow amyloid nucleation via α-helix-rich oligomeric intermediates in short polyglutamine-containing huntingtin fragments. J Mol Biol 2012; 415: 881–99.

72. Olshina MA, Angley LM, Ramdzan YM, Tang JW, Bailey MF, Hill AF, Hatters DM. Tracking mutant huntingtin aggregation kinetics in cells reveals three major populations that include an invariant oligomer pool. J Biol Chem 2010; 285: 21807–16.

73. Burke KA, Kauffman KJ, Umbaugh CS, Frey SL, Legleiter J. The interaction of polyglutamine peptides with lipid membranes is regulated by flanking sequences associated with huntingtin. J Biol Chem 2013; 288: 14993–5005.

74. Burke KA, Godbey J, Legleiter J. Assessing mutant huntingtin fragment and polyglutamine aggregation by atomic force microscopy. Methods 2011; 53: 275–84.

75. Sahoo B, Singer D, Kodali R, Zuchner T, Wetzel R. aggregation behavior of chemically synthesized, full-length huntingtin exon1. Biochemistry 2014; 53: 3897–907.

76. Mishra R, Jayaraman M, Roland BP, Landrum E, Fullam T, Kodali R, Thakur AK, Arduini I, Wetzel R. Inhibiting the nucleation of amyloid structure in a huntingtin fragment by targeting α-helix-rich oligomeric intermediates. J Mol Biol; 2012; 415: 900–17.

77. Drin G, Antonny B. Amphipathic helices and membrane curvature. FEBS Lett 2010; 584: 1840–7.

78. Brass V, Bieck E, Montserret R, Wölk B, Hellings JA, Blum HE, Penin F, Moradpour D. An amino-terminal amphipathic α-helix mediates membrane association of the hepatitis C virus nonstructural protein 5A. J Biol Chem 2002; 277: 8130–9.

79. Georgieva Elka R, Xiao S, Borbat Peter P, Freed Jack H, Eliezer D. Tau binds to lipid membrane surfaces via short amphipathic helices located in its microtubule-binding repeats. Biophys J 2014; 107: 1441–52.

80. Cui H, Lyman E, Voth GA. Mechanism of membrane curvature sensing by amphipathic helix containing proteins. Biophys J 2011; 100: 1271–9.

81. Chaibva M, Burke KA, Legleiter J. Curvature enhances binding and aggregation of huntingtin at lipid membranes. Biochemistry 2014; 53: 2355–65.

82. Gouttenoire J, Roingeard P, Penin F, Moradpour D. Amphipathic α-helix AH2 is a major determinant for the oligomerization of hepatitis C virus nonstructural protein 4B. J Virol 2010; 84: 12529–37.

83. Westerlund JA, Weisgraber KH. Discrete carboxyl-terminal segments of apolipoprotein E mediate lipoprotein association and protein oligomerization. J Biol Chem 1993; 268: 15745–50.

84. Nagarajan A, Jawahery S, Matysiak S. The effects of flanking sequences in the interaction of polyglutamine peptides with a membrane bilayer. J Phys Chem B 2014; 118: 6368–79.

85. Arndt JR, Brown RJ, Burke KA, Legleiter J, Valentine SJ. Lysine residues in the N-terminal huntingtin amphipathic α-helix play a key role in peptide aggregation. J Mass Spectrom 2015; 50: 117–26.

86. Rossetti G, Cossio P, Laio A, Carloni P. Conformations of the Huntingtin N-term in aqueous solution from atomistic simulations. FEBS Lett 2011; 585: 3086–9.

87. Dlugosz M, Trylska J. Secondary structures of native and pathogenic huntingtin N-terminal fragments. J Phys Chem B 2011; 115: 11597–608.

88. Bugg CW, Isas JM, Fischer T, Patterson PH, Langen R. Structural features and domain organization of huntingtin fibrils. J Biol Chem 2012; 287: 31739–46.

89. Burke KA, Hensal KM, Umbaugh CS, Chaibva M, Legleiter J. Huntingtin disrupts lipid bilayers in a polyQ-length dependent manner. BBA Biomembr 2013; 1828: 1953–61.

90. Hoop CL, Lin H-K, Kar K, Hou Z, Poirier MA, Wetzel R, van der Wel PCA. Polyglutamine amyloid core boundaries and flanking domain dynamics in huntingtin fragment fibrils determined by solid-state nuclear magnetic resonance. Biochemistry 2014; 53: 6653–66.

91. Jayaraman M, Mishra R, Kodali R, Thakur AK, Koharudin LMI, Gronenborn AM, Wetzel R. Kinetically competing huntingtin aggregation pathways control amyloid polymorphism and properties. Biochemistry 2012; 51: 2706–16.

92. Colby DW, Chu Y, Cassady JP, Duennwald M, Zazulak H, Webster JM, Messer A, Lindquist S, Ingram VM, Wittrup KD. Potent inhibition of huntingtin aggregation and cytotoxicity by a disulfide bond-free single-domain intracellular antibody. Proc Natl Acad Sci USA 2004; 101: 17616–21.

93. Colby DW, Garg P, Holden T, Chao G, Webster JM, Messer A, Ingram VM, Wittrup KD. Development of a human light chain variable domain (VL) intracellular antibody specific for the amino terminus of huntingtin via yeast surface display. J Mol Biol 2004; 342: 901–12.

94. Tam S, Geller R, Spiess C, Frydman J. The chaperonin TRiC controls polyglutamine aggregation and toxicity through subunit-specific interactions. Nat Cell Biol 2006; 8: 1155–62.

95. Tam S, Spiess C, Auyeung W, Joachimiak L, Chen B, Poirier MA, Frydman J. The chaperonin TRiC blocks a huntingtin sequence element that promotes the conformational switch to aggregation. Nat Struct Mol Biol 2009; 16: 1279–85.

96. McLear JA, Lebrecht D, Messer A, Wolfgang WJ. Combinational approach of intrabody with enhanced Hsp70 expression addresses multiple pathologies in a fly model of Huntington's disease. FASEB J 2008; 22: 2003–11.

97. Wolfgang WJ, Miller TW, Webster JM, Huston JS, Thompson LM, Marsh JL, Messer A. Suppression of Huntington's disease pathology in Drosophila by human single-chain Fv antibodies. Proc Natl Acad Sci USA 2005; 102: 11563–8.

98. Wacker JL, Huang S-Y, Steele AD, Aron R, Lotz GP, Nguyen Q, Giorgini F, Roberson ED, Lindquist S, Masliah E, Muchowski PJ. Loss of Hsp70 exacerbates pathogenesis but not levels of fibrillar aggregates in a mouse model of Huntington's disease. J Neurosci 2009; 29: 9104–14.

99. Lotz GP, Legleiter J, Aron R, Mitchell EJ, Huang S-Y, Ng C, Glabe C, Thompson LM, Muchowski PJ. Hsp70 and hsp40 functionally interact with soluble mutant huntingtin oligomers in a classic ATP-dependent reaction cycle. J Biol Chem 2010; 285: 38183–93.

100. Monsellier E, Redeker V, Ruiz-Arlandis G, Bousset L, Melki R. Molecular interaction between the chaperone Hsc70 and the N-terminal flank of huntingtin exon 1 modulates aggregation. J Biol Chem 2015; 290: 2560–76.

101. Li P, Huey-Tubman KE, Gao T, Li X, West AP Jr, Bennett MJ, Bjorkman PJ. The structure of a polyQ-anti-polyQ complex reveals binding according to a linear lattice model. Nat Struct Mol Biol 2007; 14: 381–7.

102. Ko J, Ou S, Patterson PH. New anti-huntingtin monoclonal antibodies: implications for huntingtin conformation and its binding proteins. Brain Res Bull 2001; 56: 319–29.

103. Trottier Y, Lutz Y, Stevanin G, Imbert G, Devys D, Cancel G, Saudou F, Weber C, David G, Tora L, Agid Y, Brice A, Mandel JL. Polyglutamine expansion as a pathological epitope in huntingtons disease and 4 dominant cerebellar ataxias. Nature 1995; 378: 403–6.

104. Bennett MJ, Huey-Tubman KE, Herr AB, West AP, Ross SA, Bjorkman PJ. A linear lattice model for polyglutamine in CAG-expansion diseases. Proc Natl Acad Sci USA 2002; 99: 11634–9.

105. Lecerf JM, Shirley TL, Zhu Q, Kazantsev A, Amersdorfer P, Housman DE, Messer A, Huston JS. Human single-chain Fv intrabodies counteract in situ huntingtin aggregation in cellular models of Huntington's disease. Proc Natl Acad Sci USA 2001; 98: 4764–9.

106. Steffan JS, Agrawal N, Pallos J, Rockabrand E, Trotman LC, Slepko N, Illes K, Lukacsovich T, Zhu YZ, Cattaneo E, Pandolfi PP, Thompson LM, Marsh JL. SUMO modification of Huntingtin and Huntington's disease pathology. Science 2004; 304: 100–4.

107. Pennuto M, Palazzolo I, Poletti A. Post-translational modifications of expanded polyglutamine proteins: impact on neurotoxicity. Hum Mol Genet 2009; 18: R40–R47.

108. Ehrnhoefer DE, Sutton L, Hayden MR. Small changes, big impact: posttranslational modifications and function of huntingtin in Huntington disease. Neuroscientist 2011; 17: 475–92.

109. Dorval V, Fraser PE. SUMO on the road to neurodegeneration. BBA Mol Cell Res 2007; 1773: 694–706.

110. Díaz Hernández M, Valera AG, Morán MA, Gómez Ramos P, Alvarez Castelao B, Castaño JG, Hernández F, Lucas JJ. Inhibition of 26S proteasome activity by huntingtin filaments but not inclusion bodies isolated from mouse and human brain. J Neurochem 2006; 98: 1585–96.

111. Borrell-Pages M, Zala D, Humbert S, Saudou F. Huntington's disease: from huntingtin function and dysfunction to therapeutic strategies. Cell Mol Life Sci 2006; 63: 2642–60.

112. O'Rourke JG, Gareau JR, Ochaba J, Song W, Rasko T, Reverter D, Lee J, Monteys AM, Pallos J, Mee L, Vashishtha M, Apostol BL, Nicholson TP, Illes K, Zhu YZ, Dasso M, Bates GP, Difiglia M, Davidson B, Wanker EE, Marsh JL, Lima CD, Steffan JS, Thompson LM. SUMO-2 and PIAS1 modulate insoluble mutant huntingtin protein accumulation. Cell Rep 2013; 4: 362–75.

113. Hannoun Z, Greenhough S, Jaffray E, Hay RT, Hay DC. Post-translational modification by SUMO. Toxicology 2010; 278: 288–93.

114. Aiken CT, Steffan JS, Guerrero CM, Khashwji H, Lukacsovich T, Simmons D, Purcell JM, Menhaji K, Zhu YZ, Green K, LaFerla F, Huang L, Thompson LM, Marsh JL. Phosphorylation of threonine 3 implications for huntingtin aggregation and neurotoxicity. J Biol Chem 2009; 284: 29427–36.

115. Di Pardo A, Maglione V, Alpaugh M, Horkey M, Atwal RS, Sassone J, Ciammola A, Steffan JS, Fouad K, Truant R,

Sipione S. Ganglioside GM1 induces phosphorylation of mutant huntingtin and restores normal motor behavior in Huntington disease mice. Proc Natl Acad Sci USA 2012; 109: 3528–33.

116. Thompson LM, Aiken CT, Kaltenbach LS, Agrawal N, Illes K, Khoshnan A, Martinez-Vincente M, Arrasate M, O'Rourke JG, Khashwji H, Lukacsovich T, Zhu YZ, Lau AL, Massey A, Hayden MR, Zeitlin SO, Finkbeiner S, Green KN, LaFerla FM, Bates G, Huang L, Patterson PH, Lo DC, Cuervo AM, Marsh JL, Steffan JS. IKK phosphorylates Huntingtin and targets it for degradation by the proteasome and lysosome. J Cell Biol 2009; 187: 1083–99.

117. Gu XF, Greiner ER, Mishra R, Kodali R, Osmand A, Finkbeiner S, Steffan JS, Thompson LM, Wetzel R, Yang XW. Serines 13 and 16 are critical determinants of full-length human mutant huntingtin induced disease pathogenesis in HD mice. Neuron 2009; 64: 828–40.

118. Jeong H, Then F, Melia TJ, Mazzulli JR, Cui L, Savas JN, Voisine C, Paganetti P, Tanese N, Hart AC, Yamamoto A, Krainc D. Acetylation targets mutant huntingtin to autophagosomes for degradation. Cell 2009; 137: 60–72.

119. Kalchman MA, Graham RK, Xia G, Koide HB, Hodgson JG, Graham KC, Goldberg YP, Gietz RD, Pickart CM, Hayden MR. Huntingtin is ubiquitinated and interacts with a specific ubiquitin-conjugating enzyme. J Biol Chem 1996; 271: 19385–94.

120. Yanai A, Huang K, Kang R, Singaraja RR, Arstikaitis P, Gan L, Orban PC, Mullard A, Cowan CM, Raymond LA, Drisdel RC, Green WN, Ravikumar B, Rubinsztein DC, El-Husseini A, Hayden MR. Palmitoylation of huntingtin by HIP14is essential for its trafficking and function. Nat Neurosci 2006; 9: 824–31.

121. Kahlem P, Green H, Djian P. Transglutaminase action imitates Huntington's disease: selective polymerization of huntingtin containing expanded polyglutamine. Mol Cell 1998; 1: 595–601.

122. Warby SC, Chan EYW, Metzler M, Gan L, Singaraja RR, Crocker SF, Robertson HA, Hayden MR. Huntingtin phosphorylation on serine 421 is significantly reduced in the striatum and by polyglutamine expansion in vivo. Hum Mol Genet 2005; 14: 1569–77.

123. Humbert S, Saudou F. Huntingtin phosphorylation and signaling pathways that regulate toxicity in Huntington's disease. Clin Neurosci Res 2003; 3: 149–55.

124. Watkin EE, Arbez N, Waldron-Roby E, O'Meally R, Ratovitski T, Cole RN, Ross CA. Phosphorylation of mutant huntingtin at serine 116 modulates neuronal toxicity. PLoS One 2014; 9: e88284.

125. Atwal RS, Desmond CR, Caron N, Maiuri T, Xia J, Sipione S, Truant R. Kinase inhibitors modulate huntingtin cell localization and toxicity. Nat Chem Biol 2011; 7: 453–60.

126. Subramaniam S, Sixt KM, Barrow R, Snyder SH. Rhes, a striatal specific protein, mediates mutant-huntingtin cytotoxicity. Science 2009; 324: 1327–30.

127. Jana NR, Dikshit P, Goswami A, Kotliarova S, Murata S, Tanaka K, Nukina N. Co-chaperone CHIP associates with expanded polyglutamine protein and promotes their degradation by proteasomes. J Biol Chem 2005; 280: 11635–40.

128. Cong X, Held JM, DeGiacomo F, Bonner A, Chen JM, Schilling B, Czerwieniec GA, Gibson BW, Ellerby LM. Mass spectrometric identification of novel lysine acetylation sites in huntingtin. Mol Cell Proteiomics 2011; 10: M111.009829.

129. Gunawardena S, Her L-S, Brusch RG, Laymon RA, Niesman IR, Gordesky-Gold B, Sintasath L, Bonini NM, Goldstein LSB. disruption of axonal transport by loss of huntingtin or expression of pathogenic polyQ proteins in Drosophila. Neuron 2003; 40: 25–40.

130. Gauthier LR, Charrin BC, Borrell-Pages M, Dompierre JP, Rangone H, Cordelieres FP, De Mey J, MacDonald ME, Lessmann V, Humbert S, Saudou F. Huntingtin controls neurotrophic support and survival of neurons by enhancing BDNF vesicular transport along microtubules. Cell 2004; 118: 127–38.

131. Lee W-CM, Yoshihara M, Littleton JT. Cytoplasmic aggregates trap polyglutamine-containing proteins and block axonal transport in a Drosophila model of Huntington's disease. Proc Natl Acad Sci USA 2004; 101: 3224–9.

132. Pal A, Severin F, Lommer B, Shevchenko A, Zerial M. Huntingtin-HAP40 complex is a novel Rab5 effector that regulates early endosome motility and is up-regulated in Huntington's disease. J Cell Biol 2006; 172: 605–18.

133. Persichetti F, Trettel F, Huang CC, Fraefel C, Timmers HTM, Gusella JF, MacDonald ME. Mutant huntingtin forms in vivo complexes with distinct context-dependent conformations of the polyglutamine segment. Neurobiol Dis 1999; 6: 364–75.

134. Zheng Z, Li A, Holmes BB, Marasa JC, Diamond MI. An N-terminal nuclear export signal regulates trafficking and aggregation of huntingtin (Htt) protein exon 1. J Biol Chem 2013; 288: 6063–71.

135. Kegel KB, Sapp E, Yoder J, Cuiffo B, Sobin L, Kim YJ, Qin ZH, Hayden MR, Aronin N, Scott DL, Isenberg F, Goldmann WH, DiFiglia M. Huntingtin associates with acidic phospholipids at the plasma membrane. J Biol Chem 2005; 280: 36464–73.

136. Suopanki J, Gotz C, Lutsch G, Schiller J, Harjes P, Herrmann A, Wanker EE. Interaction of huntingtin fragments with brain membranes – clues to early dysfunction in Huntington's disease. J Neurochem 2006; 96: 870–84.

137. Strehlow ANT, Li JZ, Myers RM. Wild-type huntingtin participates in protein trafficking between the Golgi and the extracellular space. Hum Mol Genet 2007; 16: 391–409.

138. Myre MA, Lumsden AL, Thompson MN, Wasco W, MacDonald ME, Gusella JF. Deficiency of huntingtin has pleiotropic effects in the social amoeba Dictyostelium discoideum. PLoS Genet 2011; 7: e1002052.

139. Ritch JJ, Valencia A, Alexander J, Sapp E, Gatune L, Sangrey GR, Sinha S, Scherber CM, Zeitlin S, Sadri-Vakili G, Irimia D, DiFiglia M, Kegel KB. Multiple phenotypes in Huntington disease mouse neural stem cells. Mol Cell Neurosci 2012; 50: 70–81.

140. Jacobsen JC, Gregory GC, Woda JM, Thompson MN, Coser KR, Murthy V, Kohane IS, Gusella JF, Seong IS, MacDonald ME, Shioda T, Lee J-M. HD CAG-correlated gene expression changes support a simple dominant gain of function. Hum Mol Genet 2011; 20: 2846–60.

141. Caviston JP, Holzbaur ELF. Huntingtin as an essential integrator of intracellular vesicular trafficking. Trends Cell Biol 2009; 19: 147–55.

142. Rockabrand E, Slepko N, Pantalone A, Nukala VN, Kazantsev A, Marsh JL, Sullivan PG, Steffan JS, Sensi SL, Thompson LM. The first 17 amino acids of Huntingtin modulate its sub-cellular localization, aggregation and effects on calcium homeostasis. Hum Mol Genet 2007; 16: 61–77.

143. Michalek M, Salnikov ES, Bechinger B. Structure and topology of the huntingtin 1-17 membrane anchor by a combined

solution and solid-state NMR approach. Biophys J 2013; 105: 699–710.

144. Michalek M, Salnikov ES, Werten S, Bechinger B. Membrane interactions of the amphipathic amino-terminus of huntingtin. Biochemistry 2013; 52: 847–58.

145. Caron NS, Desmond CR, Xia J, Truant R. Polyglutamine domain flexibility mediates the proximity between flanking sequences in huntingtin. Proc Natl Acad Sci USA 2013; 110: 14610–5.

146. Burke KA, Yates EA, Legleiter J. Amyloid-forming proteins alter the local mechanical properties of lipid membranes. Biochemistry 2013; 52: 808–17.

147. Xia JR, Lee DH, Taylor J, Vandelft M, Truant R. Huntingtin contains a highly conserved nuclear export signal. Hum Mol Genet 2003; 12: 1393–403.

148. McLaughlin S, Murray D. Plasma membrane phosphoinositide organization by protein electrostatics. Nature 2005; 438: 605–11.

149. Yeung T, Gilbert GE, Shi J, Silvius J, Kapus A, Grinstein S. Membrane phosphatidylserine regulates surface charge and protein localization. Science 2008; 319: 210–3.

150. Linder ME, Deschenes RJ. Palmitoylation: policing protein stability and traffic. Nat Rev Mol Cell Biol 2007; 8: 74–84.

151. Manneville J-B, Casella J-F, Ambroggio E, Gounon P, Bertherat J, Bassereau P, Cartaud J, Antonny B, Goud B. COPI coat assembly occurs on liquid-disordered domains and the associated membrane deformations are limited by membrane tension. Proc Natl Acad Sci USA 2008; 105: 16946–51.

152. Rocks O, Peyker A, Kahms M, Verveer PJ, Koerner C, Lumbierres M, Kuhlmann J, Waldmann H, Wittinghofer A, Bastiaens PIH. An acylation cycle regulates localization and activity of palmitoylated Ras isoforms. Science 2005; 307: 1746–52.

153. Lemmon MA. Membrane recognition by phospholipid-binding domains. Nat Rev Mol Cell Biol 2008; 9: 99–111.

154. McMahon HT, Gallop JL. Membrane curvature and mechanisms of dynamic cell membrane remodelling. Nature 2005; 438: 590–6.

155. Lobsiger CS, Cleveland DW. Glial cells as intrinsic components of non-cell-autonomous neurodegenerative disease. Nat Neurosci 2007; 10: 1355–60.

156. Giorgini F, Guidetti P, Nguyen QV, Bennett SC, Muchowski PJ. A genomic screen in yeast implicates kynurenine 3-monooxygenase as a therapeutic target for Huntington disease. Nat Genet 2005; 37: 526–31.

157. Yang W, Dunlap JR, Andrews RB, Wetzel R. Aggregated polyglutamine peptides delivered to nuclei are toxic to mammalian cells. Hum Mol Genet 2002; 11: 2905–17.

158. Ren P-H, Lauckner JE, Kachirskaia I, Heuser JE, Melki R, Kopito RR. Cytoplasmic penetration and persistent infection of mammalian cells by polyglutamine aggregates. Nat Cell Biol 2009; 11: 219–25.

159. Cicchetti F, Saporta S, Hauser RA, Parent M, Saint-Pierre M, Sanberg PR, Li XJ, Parker JR, Chu Y, Mufson EJ, Kordower JH, Freeman TB. Neural transplants in patients with Huntington's disease undergo disease-like neuronal degeneration. Proc Natl Acad Sci USA 2009; 106: 12483–8.