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

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Abstract

Huntington's disease (HD) is caused by a polyglutamine (polyQ) domain that is expanded beyond a critical threshold near the N-terminus of the huningtin (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 polyQ domain, such as the first seventeen amino acids on the N terminus (Nt17), influence aggregation, aggregate stability, other important biochemical properties of the protein, and ultimately it role in pathogenesis. Here, we review the impact of Nt17 on both htt aggregation mechanisms and kinetics, structural properties of Nt17 in both monomeric and aggregate forms, the potential role of post translational modifications (PTMs) that occur in Nt17 in HD, and Nt17's function as a membrane targeting domain.

Keywords

Huntington's disease; huntingtin; polyglutamine; amyloid; lipid binding; post-translational modifications

Introduction

Huntington's disease (HD), a fatal neurodegenerative disorder, is caused by an expansion of a polyglutamine (polyQ) tract near the N-terminus of the protein huntingtin (htt) (Figure 1) (1). This polyQ expansion directly leads to htt aggregation into fibrils and a variety of other aggregates (2–4). While the exact mass of htt is dependent on the size of the polyQ domain, full-length htt is approximately 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

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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). Downstream of exon 1 are a number of HEAT repeats that are 40-amino acid-long sequences that are involved in protein–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, tubulovesicles, 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 pathogenesis, 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 neurodegenerative 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 greater than 60 (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 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 post translational 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 exon1 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 models 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 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 exon1 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 assemble 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 is 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 pathway 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 (AFM) (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 AFM 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 exon1 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, these studies suggest a potentially critical role of flanking sequences to 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, alter both aggregation kinetics and conformational properties of the polyQ tract (58). By inducing 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 Huntington's disease, 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 membrane 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 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 (Figure 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 (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 NMR all point to transient helical properties of Nt17 in the monomer (67, 69, 86, 87). Separate analytical untracentrifugation (AUC) 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 AUC 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 300K and transiently exists as a straight helix (67).

Compelling evidence demonstrate that the α-helix is conserved in fibrils (66, 85, 88). Solid state NMR and FTIR 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 FTIR experiments. Additionally, ssNMR chemical shifts and precise intrapeptide NOE 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 mass spectrometry (MS) (85); however, pre-aggregated htt exon 1 inefficiently binds to lipid membranes, suggesting 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 (EPR) (88). Analysis of these EPR 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 polymorphic fibrils 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 huntingtin 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 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 the binding pocket induces helicity in the proposed random structure of Nt17, or the intrabody only recognizes helical Nt17. Intrabodies that were co-expressed with a pathogenic GFP-fused htt exon 1 (97 Q) in HEK293 mammalian cells caused a dramatic decrease in huntingtin 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.

Post translational modifications in Nt17

Nt17 also contains numerous sites that can be post-translationally modified, with some sites associated with multiple potential modifications (Figure 1D) (106–108). These post translational modification (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 (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 phosphometic 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 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 finding 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 exon1 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 (UPS), 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 is 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 *C. 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 the 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 Nt17's ability 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 micrutobule-dependent vesicle transport (141). Nt17 facilitates the trafficking of htt exon 1 to membranes associated with the 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 (PC) or a mixture of PC and phosphatidylserines (PS) 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 are 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.

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Abbreviations

AH	amphipathic α-helix
AUC	analytical untracentrifugation
AFM	atomic force microscopy
CD	circular dichroism spectropolarimetry
EPR	electron paramagnetic resonance
FTIR	Fourier transform infrared spectroscopy
Htt	huningtin protein
HD	Huntington's disease
MS	mass spectrometry
NMR	nuclear magnetic resonance spectroscopy
NOE	Nuclear Overhauser Effect
PC	phosphatidylcholines
PS	phosphatidylserines
polyQ	polyglutamine
polyP	polyproline
PTMs	post translational modifications
scFv	single chain variable fragment
ssNMR	solid state nuclear magnetic resonance spectroscopy

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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.

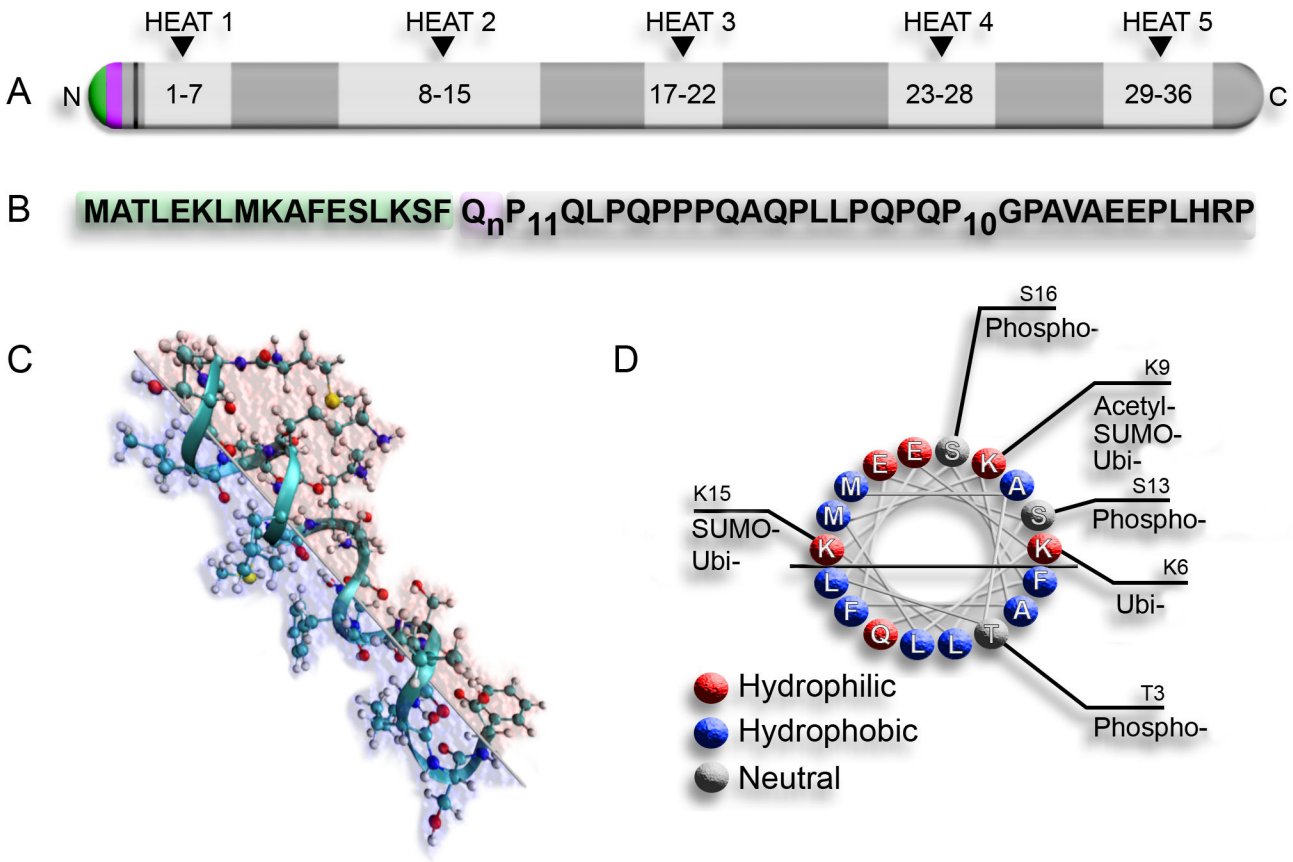

Figure 1. Schematic representation of various features of the htt protein

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), 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 post-translational modification.

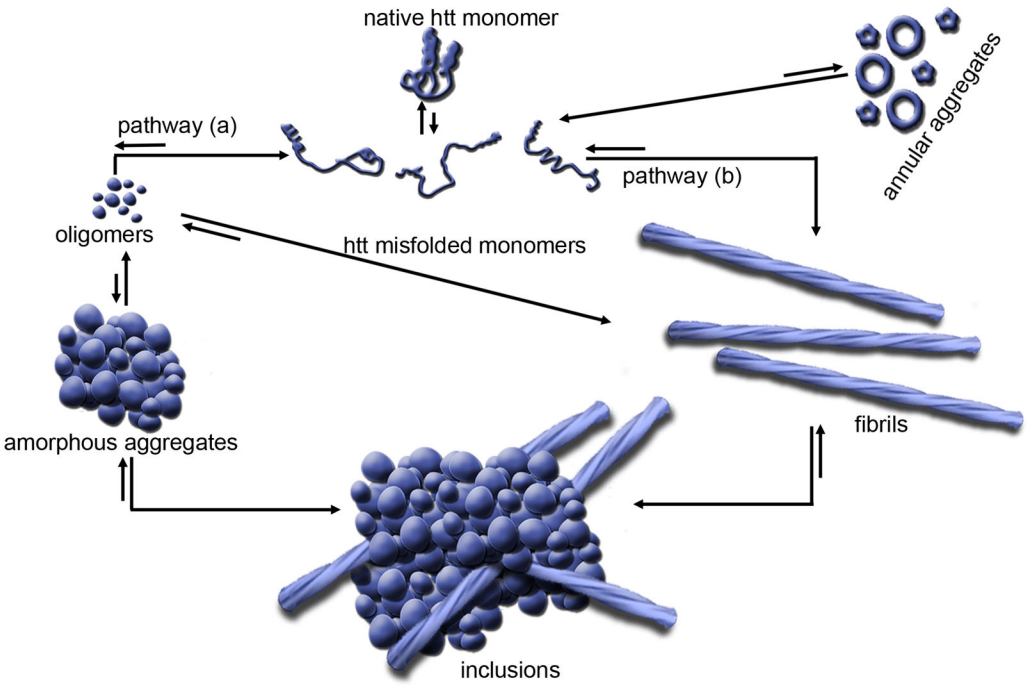

Figure 2. A schematic model for htt aggregation

A native monomer can sample a variety of distinct, misfolded monomer conformations, with the relative number and stability of these conformers potentially being polyQ length-dependent. Some misfolded monomers likely lead to aggregates, such as annular aggregates, that likely are off-pathway to fibril formation. There appears 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 HD.