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Proteins containing expanded polyglutamine tracts and neurodegenerative disease

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Abstract

Several hereditary neurological and neuromuscular diseases are caused by an abnormal expansion of trinucleotide repeats. To date, there have been ten of these trinucleotide repeat disorders associated with an expansion of the codon CAG encoding glutamine (Q). For these polyglutamine (polyQ) diseases, there is a critical threshold length of the CAG repeat required for disease, and further expansion beyond this threshold is correlated with age of onset and symptom severity. PolyQ expansion in the translated proteins promotes their self-assembly into a variety of oligomeric and fibrillar aggregate species that accumulate into the hallmark proteinaceous inclusion bodies associated with each disease. Here, we review aggregation mechanisms of proteins with expanded polyQ-tracts, structural consequences of expanded polyQ ranging from monomers to fibrillar aggregates, the impact of protein context and post translational modifications on aggregation, and a potential role for lipids membranes in aggregation. As the pathogenic mechanisms that underlie these disorders are often classified as either a gain of toxic function or loss of normal protein function, some toxic mechanisms associated with mutant polyQ tracts will also be discussed.

Introduction

A major genetic cause of a variety of neurological and neuromuscular diseases is an expansion of trinucleotide repeats to a number much larger than what occurs in the normal, stable gene.[1–3] Diseases caused by these expansions are often termed trinucleotide repeat disorders (TRDs). In the majority of these disorders, the codon CAG, which encodes for glutamine (Q), is repeated, resulting in an expanded polyglutamine (polyQ) tract in the translated protein. This subset of TRDs is commonly referred to as CAG repeat disorders or polyQ diseases. To date, ten CAG repeat disorders have been identified (Table 1). The nature of the proteins containing an expanded polyQ domain and the associated pathologies are

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varied (Figure 1).[4, 5] That is, each mutant polyQ protein underlies a distinct neurodegenerative disease that targets different populations of neurons. These polyQ diseases include Huntington's disease (HD), the Spinocerebellar ataxias, type 1 (SCA-1), type 2 (SCA-2), type 3 (SCA-3, also known as Machado-Joseph disease), type 6 (SCA-6), type 7 (SCA-7), Dentatorubropallidoluysian atrophy (DRPLA) and Spinobulbar muscular atrophy (SBMA, also known as Kennedy's disease).[6–18] In addition CTG/CAG expansion in the JPH3 gene is associated with Huntington Disease-like 2; although, it has not formally been classified as a polyQ disorder.[19]

While the genes associated with the different polyQ diseases are structurally and functionally distinct, there are commonalities across all of these diseases. With SBMA being a notable exception due to it being X-linked, polyQ diseases are inherited in an autosomal dominant manner. PolyQ diseases are progressive, usually fatal disorders. For each disease, there is a critical threshold of polyQ expansion that must be exceeded for disease onset, and both age of onset and symptom severity are strongly correlated to increasing polyQ expansion beyond this critical threshold (Table 1). For example in HD, polyQ lengths shorter than 35 are not associated with disease, 35–39 repeats may induce disease, 40–60 repeats result in adult onset, and repeats longer than 60 elicit juvenile forms of HD.[20, 21] This correlation with polyQ length, the dominant inheritance pattern of these diseases, the late onset of symptoms, and indistinguishable clinical presentation in homozygote and heterozygote patients suggest a toxic gain of function associated with proteins containing expanded polyQ tracts. However, polyQ expansion may also inhibit the normal functions of these proteins, i.e. a toxic loss of function.[22]

Disease-related proteins containing expanded polyQ tracts, as well as synthetic polyQ peptides, aggregate into detergent-insoluble, amyloid-like fibrils.[23–28] There is also a direct correlation between the rate of fibril formation and polyQ length, with longer polyQ tracts resulting in increased rates of fibril formation.[24, 29–31] This correlation between aggregation rate and polyQ length is recapitulated in a variety of cell culture models.[32–34] Beyond the formation of fibrillar aggregates, proteins containing expanded polyQ tracts also accumulate into a variety of other aggregates, including small and large oligomers, annular structures, and amorphous aggregates.[25, 30, 35–38] Precise characterization of aggregation mechanisms and aggregate structure may prove vital in understanding how proteins with expanded polyQ tracts are toxic. Throughout the literature, the terminology used to describe these different aggregate species can be used inconsistently and ambiguously. For this reason, we present a short definitions of some of these terms for clarification of how they will be used in this review (Table 2).

One major hallmark of polyQ diseases is the accumulation of proteins with expanded polyQ tracts into proteinaceous inclusions that are microns in size. Abnormal nuclear structures were observed in HD brains over 40 years ago,[39] and the first mouse model of HD clarified that these inclusions were comprised of fibrillar aggregates of the protein huntingtin (htt) with expanded polyQ tracts.[40, 41] Similar observations have been made with the spinocerebellar ataxias.[42] While the formation of intranuclear and cytoplasmic inclusion bodies of aggregated proteins is a prominent feature of polyQ diseases,[43] controversy remains concerning their exact role in the etiology of these diseases. Several lines of

evidence support the notion that inclusion body formation, by removing soluble forms of toxic protein species, may represents a protective cellular response.[33, 44–46] These soluble forms that can be removed by the formation of large inclusions can include small oligomeric and fibrillar aggregates that are significantly smaller than the micron sized inclusions. As such, some polyQ aggregate may be benign and represent epiphenomena associated with these diseases.

Here, the underlying biochemical and biophysical aspects of the aggregation of proteins containing expanded polyQ tracts will be reviewed. We will begin by reviewing aggregation schemes associated with polyQ expansion and defining the array of aggregate species that can result. Properties of monomeric polyQ species, oligomer formation, and fibril structure will be explored in detail. Then, the influence of protein context and post-translational modifications to influence aggregation will be discussed. Finally, a brief review of potential toxic mechanisms associated with proteins containing expanded polyQ will be provided. Several other extensive reviews are available that cover other topics related to polyQ diseases such as the physical chemical and aggregation properties of polyQ,[28, 47] mouse models of polyQ diseases,[48–50] the impact of post-translational modifications on polyQ proteins,[51] mechanisms of toxicity related to polyQ,[4] RNA-mediated pathogenic mechanisms associated with polyQ diseases,[52] gene and stem cell therapeutic strategies for polyQ diseases,[53] the interaction of polyQ with other proteins,[54] and the biology of the htt protein.[55]

PolyQ-mediated protein aggregation is a complex process

A popular notion is that the aggregation of proteins with expanded polyQ tracts mediate neurodegeneration, as both protein aggregation and pathogenesis are strongly correlated with polyQ length.[26] This is consistent with the amyloid hypothesis associated with other common neurodegenerative diseases like Alzheimer's disease and Parkinson diseases that also feature the accumulation of disease-related proteins into large inclusions. The aggregation process of pure polyQ peptides and polyQ-containing proteins is a complex process involving a number of distinct aggregate species (Figure 2), making it difficult to gain consensus on the prominent aggregate species involved in toxicity.

Amyloids typically form via a nucleus-dependent growth polymerization mechanism.[56–58] With this mechanism, the rate of aggregation is dependent on a slow nucleation phase (often called the lag phase) that involves a thermodynamically unfavorable transition from a native to non-native protein conformation that initiates the formation and elongation of fibrils. This lag phase is followed by an elongation or growth phase characterized by a relatively rapid extension of fibril aggregates. The critical nucleus that forms during the lag phase can be either monomeric or multimeric in nature. Several aggregation pathways have been proposed for the formation of fibrils of polyQ-containing proteins, ranging from simple to complex (Figure 2). While the specific details associated with the aggregation of distinct proteins can be varied, there are two generic, prominent aggregation schemes associated with polyQ-containing proteins. These are: 1) re-arrangement of polyQ monomers into a structure that directly nucleates fibril formation[23, 24, 58] and 2) the formation of soluble oligomeric intermediates that undergo a structural re-arrangement into a multimeric nucleus that is on

pathway to fibril formation.[30, 59–61] In the first scenario, there can be multimeric critical nuclei that can directly nucleate fibrilization without a required structural rearrangement. The nature of the oligomeric aggregates associated with the second scheme can be quite heterogeneous and dependent on the specific polyQ-containing protein.[30, 62, 63] It should be noted that oligomers that are off pathway to fibril formation may also form.

These two generic mechanisms are not necessarily mutually exclusive as both scenarios could occur simultaneously to varying extents. Longer pure polyQ peptides (longer than 26 repeats) typically aggregate into fibrils via the monomeric nucleation-elongation model.[23, 24, 58, 64, 65] Shorter pure polyQ peptides appear to aggregate into fibrils via a tetrameric critical nucleus.[58, 65] However, some studies have suggested some smaller oligomers of pure polyQ proteins may form prior to the nucleation of fibril formation.[30, 60, 66, 6767–70] The appearance of these oligomers may be due to differences in peptide preparation protocols as commonly used solvents such as hexafluoroisopropanol promote oligomer formation,[71] and there is no formal kinetic evidence of how these oligomers contribute to fibril formation. However, individual oligomers of a pure polyQ peptide with 32 repeats were tracked on a mica surface, were shown to be stable for up to 200 min (but typically shorter than 50 min), and directly transition into extended fibril species.[30] In this instance, the presence of the surface may have promoted or stabilized these oligomeric aggregates. It should be also noted that the dominant pathway to fibril formation on the mica surface appeared to not be oligomer mediated. The potential of these environmental factors, i.e., the presence of specific solvents or surfaces, to directly alter or promote different pathways toward fibril formation may provide valuable insight into the polyQ aggregation process. It also points to the importance of designing experiments to mechanistically investigate aggregation in relevant cellular environments.

Further complicating polyQ aggregation is the observation that heterogeneous mixtures of distinct oligomers and other aggregates can form in a polyQ length- and concentration-dependent manner (Figure 3A).[30] These other aggregate types can range from large amorphous protein conglomerates to annular structures of various size (Figure 3). In addition, a variety of these other aggregates species, including some small oligomers, are off pathway to fibril formation, and these on and off pathway aggregates can perhaps form simultaneously via competing processes (Figure 2).[66, 72] PolyQ length and concentration can also push aggregation toward specific species. For example, htt exon1 with 35 repeat glutamines formed large amorphous aggregates at low concentrations in vitro, but aggregated into fibrils at higher concentrations.[30] In addition, the androgen receptor has been shown to aggregate into annular aggregates with short, non-pathogenic length polyQ domains, but with increasing polyQ length fibrillar species are observed.[73]

Understanding the variety and mixture of aggregates formed by polyQ-containing proteins is important because these different species could play varying roles in an assortment of pathological mechanisms. Some aggregate species could represent toxic entities; while others may actually impart a protective effect. In HD for example, it is true that the formation of inclusions bodies on the order of several microns in size preceded behavioral deficits in mouse models;[40] however, several observations suggest that the formation of inclusions is not involved in pathology. While it is true that large scale cellular degeneration

is observed in the striatum coinciding with the presence of intracellular aggregates comprised of mutant htt, there is only moderate degeneration within the cerebral cortex despite a typically larger inclusion load.[74] Specifically within the striatum, large interneurons typically contain more inclusions compared to medium spiny neurons, yet medium spiny neurons are selectively lost.[75] With that being said, larger perinuclear inclusions of mutant htt can directly disrupt the nuclear envelope, leading to cell death.[76] The poor correlation between inclusion body formation and toxicity is also observed in cellular models of HD.[33] By tracking the fate of individual neurons using robotic live cell imaging, the formation of inclusion bodies of mutant htt correlated with cell survival compared with cells in which htt remained diffuse, suggesting an actual protective role of sequestering htt into large inclusions.[77] Similar protective effects of inclusion formation have also been observed for the accumulation of AR in SBMA.[46]

The diffuse htt population implicated in toxicity is still a complex mixture that includes a variety of nanoscale aggregate species such as oligomers and small fibrils (Figure 3), making it difficult to pinpoint toxic species. Recently, it was estimated that the large inclusions can contain ~1 billion molecules of htt exon1; whereas, smaller diffuse aggregates can contain only up to 1,000-3,000 molecules.[78] As aggregate species as small as tetramers have been observed,[65] the potential heterogeneity of possible aggregate species containing anywhere from a few to thousands of molecules can be quite complex. Recently, a variety of super-resolution fluorescent microscopic techniques have been applied to study the aggregation of fluorescently-labeled htt.[79–81] Initially, it was verified that sub-diffraction imaging could resolve morphological details of htt fibrils by direct comparison with AFM images on the same aggregates.[79] Subsequent studies defined the spatial distributions of fluorescently-labeled htt exon1 species in a PC12m model of HD with a particular focus on small aggregate species.[81] To accomplish this, methods to make these small aggregates visible in the presence of highly fluorescent inclusion bodies were developed, and small fibrillar aggregate species were detected in cells that were reminiscent of those observed *in vitro*. While some of these small aggregates formed a perinuclear shell-like arrangement, they were also observed dispersed throughout the entire cell.[81] Interestingly, this study demonstrates that small aggregate species of htt are still present in the cell after inclusion body formation. Finally, temporal studies indicated that these fibrils only appeared in cells at late aggregation stages when inclusion bodies were already present.[80] This suggests that inclusion bodies initially form from monomeric or small oligomeric htt species. An important caveat to these studies is the addition of large fluorescent tags to htt exon1 protein in these models, as fluorescent fusion partners have been shown to alter htt aggregations and toxicity in yeast.[82] Still, taken in light of the potential protective effect of inclusion body formation,[44, 77] this suggests these non-fibrillar aggregates may play a prominent role in toxicity.

Another aspect of polyQ aggregation (as well as amyloid formation in general) is the ability of specific pre-formed aggregates to seed aggregation via a prion-like mechanism.[83, 84] In the case of polyQ proteins, this can occur with seeds inducing aggregation in the same polyQ-containing protein (self-seeding)[23] or different polyQ-containing proteins (cross-seeding).[37, 85, 86] Seeding can also occur across different polyQ lengths,[23] causing proteins containing typically non-pathogenic polyQ lengths to aggregate. Fibrillar polyQ peptide

aggregates can be internalized by mammalian cells in culture and seed aggregation, even if the polyQ length expressed in the cell is below the pathogenic threshold.[87] Furthermore, aggregates isolated from several HD transgenic mouse models seed the aggregation of pure polyQ peptides *in vitro*,[88] and seeding of mutant htt aggregation is also induced by cerebrospinal fluid from transgenic rats and human HD patients.[89] Aggregates of synthetic htt exon1-like peptides grown under conditions that promote different aggregation pathways have varying abilities to seed further aggregation.[38] This observation is interesting considering that, in a Drosophila model, polyQ amyloids formed in older flies more efficiently seeded in vitro aggregation than those derived from younger flies,[90] suggesting that aggregates formed in flies vary in their biophysical properties and perhaps aggregation mechanism as a function of fly age. The ability to seed aggregation has several key implications for polyQ diseases. For example, the seeding phenomenon plays a critical role in non-cell autonomous effects associated with polyQ diseases based on a prion-like mechanism of transmission.[83, 87, 91] These prion-like effects may cause the degeneration of neural transplants in HD patients.[92]

Structural features of monomeric polyQ peptides and polyQ-containing proteins

While aggregation is a major feature of polyQ-related diseases, the biochemical and structural properties of polyQ-containing proteins as monomers play a fundamental role in initiating the aggregation process. In general, the functional activity of a protein is dependent on its ability to correctly fold into and maintain its native state.[57] Due to their dynamic nature, it is possible that a protein can sample a variety of conformers. Typically, there is a large kinetic barrier associated with the formation of nonnative conformations.[93–96] However, there are intrinsically disordered proteins (IDPs), which lack a fixed three-dimensional structure and can range from being completely unstructured or contain unstructured regions.[97, 98] Pure polyQ tracts are archetypal IDPs due to their homopolymeric nature and polar side groups.[99] While many factors may lead to destabilization of native proteins, the expansion of the polyQ tract in CAG-disease related proteins promotes the formation of aberrant protein structures that promote aggregation into fibrils.

Many of the initial efforts to study the basic properties of polyQ were performed on pure polyQ peptides, i.e. in the absence of any other protein context. Due to polyQ's poor solubility in aqueous buffers, often charged residues, most commonly lysine, are added to the ends of pure polyQ peptides to aid in solubility. This has led to a debate concerning the potential influence of these additional residues and if this limits the applicability of results obtained using these peptides. The addition of flanking lysine residues most certainly alters the biochemical and aggregation properties of polyQ peptides as they increase solubility; however, these are often experimentally necessary additions. Furthermore, the results from such studies have provided valuable insights into polyQ biochemistry and provide a valuable baseline for comparison with polyQ tracts placed in the context of disease proteins, providing insight into the role protein context may play.

PolyQ peptides behave as a polymer in a poor solvent under aqueous conditions as polyQ peptides collapse into compact conformations that exclude water, as demonstrated by a variety of experimental techniques and computational methods.[59, 99–103104] Based on a series of circular dichroism (CD) and NMR experiments, pure polyQ monomers, both below and above the typical pathological threshold in length, are predominately disordered with little difference in secondary structure as a function of polyQ length.[23, 64, 67, 105–108] With these pure polyQ peptides, NMR studies did not detect distinct monomeric intermediates prior to aggregation into fibrils.[107] A number of computational analyses, while producing slight variations in monomeric structure between the different studies, imply that pure polyQ peptides transiently sample a variety of secondary elements, e.g. disordered structure, α-helix, β-sheet, and β-turn.[99, 102, 109–113] Some of these computational results back the experimentally observed preference of polyQ to form collapsed structures stabilized by intrapeptide H-bonding.[99, 102, 113] A clear dependence of transient structural features of monomers on polyQ length has not been unambiguously established.

An extenuating circumstance is that polyQ tracts associated with disease are incorporated into the context of larger proteins, that is, there are a variety of protein sequences directly adjacent to the polyQ tract. Flanking sequences have a profound impact on aggregation kinetics and mechanism associated with polyQ aggregation (as will be discussed later), indicating that these sequences influence structural properties associated with monomeric polyQ tracts. For example, the polyQ tract in htt is flanked on the N-terminal side by the first 17 amino acids of the protein (N17) and by a polyproline (polyP) region on its C-terminal side (Figure 1I). The addition of a polyP region to the C-terminal side of a 40-repeat polyQ stretch reduces the rate of aggregation and aggregate stability.[106] Interestingly, the addition of this polyP domain also removes α-helical content of the peptide as measured by CD spectroscopy.[106] Whereas pure polyQ peptides are intrinsically disordered, solved X-ray diffraction crystal structures of a model htt N-terminal fragment revealed a variety of clear structural elements in the polyQ tract.[114] In these structures, the N17 domain was predominately α-helical and the polyP domain formed a poly-l-proline type II (PPII) helix. Conformational variability of the polyQ domain was also recently observed in crystal structures of the carboxy-terminal region of ataxin-3.[115] There are potential caveats, however, in that these structural elements may be induced via the crystallization process or the inclusion of fusion partners to htt that can also induce secondary structure. An α-helical structure has been observed in monomeric polyQ tracts within the androgen receptor by NMR,[116] and CD spectroscopy suggests that this α-helical content increases with polyQ length.[117] Structural predictions of ataxin-1, however, suggest that its polyQ domain remains predominately intrinsically disordered.[118]

The possibility that polyQ tracts in the context of the htt proteins can obtain a variety of interchangeable, transient conformations suggests that polyQ specific antibodies may recognize a variety of different conformers of htt.[45, 119–123] The ability of polyQ-specific monoclonal antibodies specific for polyQ to alter the aggregation of a mutant htt fragment to varying degrees appears to support this notion.[124] Considerable effort has been given to determine the epitopes that different polyQ specific antibodies recognize.[32, 92–96] A variety of polyQ specific antibodies have been shown to have higher affinities for longer polyQ tracts.[119, 121, 125] Specifically, the 1C2 monoclonal antibody has a higher affinity for longer

polyQ tracts within the context of htt, SCA1, and SCA3.[125] Another antibody, MW1, preferentially binds longer polyQ tracts as well; however, this occurs via a "linear lattice" mechanism in which several antibodies can bind to a single polyQ domain as available binding epitopes increase with polyQ length.[119, 121] It was postulated that a third polyQ-specific antibody, 3B5H10, recognizes a β-hairpin structure of polyQ;[120] however, subsequent studies have revealed that 3B5H10 recognizes a similar epitope as MW1 and 1C2.[126, 127] Interestingly, the higher affinity of several polyQ antibodies for expanded polyQ tracts does not appear to be based on a unique conformational epitope associated with expansion; rather, this higher affinity is likely due to avidity effects associated with the "linear lattice" mechanism.[126, 127] In addition, all of these antibodies bind to nonpathogenic and expanded lengths of polyQ tracts.[127] The similarities in binding between polyQ specific antibodies argue against the notion that expansion of polyQ beyond a critical threshold results in a structurally unique toxic conformation.

The exact role monomeric species play in disease is a complex issue based on the ability of monomeric pure polyQ and polyQ-containing proteins to sample a variety of thermodynamically accessible transient conformations similar to a freely jointed homopolymeric chain.[99, 102, 113, 128] A subset of these conformers might lead to distinct higher order aggregation pathways. That is, at least in principle, different polyQ monomeric conformations could promote specific oligomeric or fibrillar aggregates that are mediated by distinct hydrogen-bonding networks between polyQ residues.[45, 129] Some of the monomeric forms of proteins with expanded polyQ tracts may prove toxic themselves, as a thioredoxin-polyQ fusion protein exhibits toxic properties in a meta-stable, β-sheet-rich monomeric conformation.[130] Factors modulating the transient structural properties of polyQ proteins (i.e., protein context, modifications, and environmental factors) could have downstream effects on the aggregation process.

Oligomers formed by expanded polyQ-containing proteins are diverse

With the observation that inclusion formation within cells may represent a protective mechanism against polyQ-related toxicity,[33, 44–46] there has been a recent research focus on small soluble aggregates, often termed oligomers, due to evidence pointing to their role in initiating toxic events.[30, 63, 73, 131–137] There are several difficulties associated with characterizing oligomers. First, populations of oligomers comprised of polyQ-containing proteins are often heterogenic (Figure 3A).[30, 72, 73] Second, oligomeric species are transiently populated as they are intrinsically unfavorable species and often represent on-pathway intermediates to fibril formation.[138, 139] That being noted, there is also the potential to form oligomeric species that are off-pathway to fibril formation.[132, 140]

While there have been numerous reports of oligomers comprised of polyQ containing proteins,[30, 36, 63, 73, 132, 135, 137, 140–145] the role of these structures in CAG repeat disorders has not been fully elucidated. Oligomeric species of polyQ-containing proteins can range in size from small dimers, tetramers, octamers, and dodecamers[28, 36, 78] to much larger oligomers containing upwards of tens and even hundreds of individual proteins.[30, 14678] PolyQ peptides containing the first 17 amino acids of htt (N17) form a variety of oligomers, including tetramers, octamers and larger oligomers,[36] that appear to be direct precursors to

fibril formation. That is, self-association of the N17 domain localizes expanded polyQ tracts, facilitating further aggregation into fibrils.[36, 61] Supporting these findings, species ranging from a monomer to a tetramer of the N17 domain of htt peptides were detected in the gas phase by ion mobility mass spectrometry (MS) analysis.[147] Oligomer formation is a rate-limiting step in fibrillization of AR[148] and ataxin-3.[149, 150] While this suggests that oligomers may act as an on pathway nucleation site for fibril formation,[151] off pathway oligomers may serve as a reservoir that supplies monomers to elongating fibrils.[36] As such, different oligomers may have distinct roles in the formation and eventual elongation of fibrils. Mutant htt exon1 can also form oligomers that are generic to amyloid formation in general, as they are recognized by the A11 antibody that is specific for a generic motif in amyloid derived oligomers.[142, 152] Further complicating the situation, the formation of oligomers are mediated by the exact protein fragment involved in the process. Different N-terminal fragments of htt aggregate into distinct oligomer species. For example, htt exon1 forms a distinct subset of oligomers that were not observed in aggregation of htt shortstop, a 117 amino acid fragment that is non-toxic.[63]

While many of the studies detecting oligomeric aggregates were performed with purified proteins, there is increasing evidence that oligomers exist in cellular environments, animal models, and HD patients.[137, 143, 144, 153, 15478] While most studies of htt oligomerization have been performed with N-terminal fragments, full length htt detected from HD brains or HD mouse models was monomeric;[155] however, dimers and other larger multimers of full-length htt purified from insect[156] or mammalian cells[157] have been detected. Using the Seprion ligand, oligomers were isolated from mouse brains from both R6/2 and HdhQ150 knock-in mice, and these oligomers were similar in morphology to those formed by purified htt exon1 proteins in vitro.[137] The R6/2 mouse model expresses htt-exon1 with an expanded polyQ domain and exhibit a progressive neurological phenotype that recapitulates several features of HD.[158] The HdhQ150 mouse is a knock-in model in which a short polyQ repeat in murine htt was replaced with a 150Q repeat, resulting in measurable abnormalities in the live mouse and neuropathological features consistent with HD.[159, 160] Further studies with the HdhQ150 mouse model demonstrated that a soluble oligomer pool forms within one month of age, but this oligomer pool declines with age as insoluble aggregates form.[144] Oligomers similar in size to those observed by AFM in vitro were detected in HD patient brains with EM using the immunoperoxidase method and a primary antibody to N17.[30] Htt exon1 with a non-disease length polyQ domain can form dimers in cells;[78, 157] however with an expanded polyQ domain, an invariant oligomer pool of htt oligomers is present in cells.[154] Using a number and brightness method to analyze confocal images of cells, it was determined that mutant htt forms oligomers that are 5-15 proteins in size and on pathway to inclusion formation in living cells.[161] Oligomers have also been directly linked to ER-stress.[143, 162, 163] Recently, it was reported that expanded htt exon1 expressed in PC12 cells did not remain monomeric; rather, tetramers were detected.[78] Small, diffusible aggregates larger than tetramers formed in PC12 within 6-9 h, and sedimentable aggregates were present within 12 h, making it clear that htt oligomers of various size can exist in the cellular environment.[78] Interestingly, significant nuclear DNA damage occurred when diffusible aggregates (potentially oligomers and smaller fibrils) start to accumulate, and this damage represented an early potential toxic event.[78] A novel oligomeric specie of the androgen

receptor with expanded polyQ was isolated from PC12 cells via cryo-extraction, and these oligomers were less compacted compared with late stage aggregates and correlated with toxicity.[62]

Structural analysis of polyQ fibrils

Amyloid-like fibrils formed by a number of proteins share several biochemical properties. These include a cross-β sheet quaternary structure and detergent insolubility.[164] Fibrils of polyQ-containing proteins share most of these traditional amyloid hallmarks.[165] While the polyQ length associated with disease thresholds is typically on the order of 35-40 repeat glutamines, pure polyQ peptides shorter than this can form fibrils under certain conditions.[24] Interestingly, ataxin-3 containing either non-pathogenic or pathogenic length polyQ tracts aggregate into fibrils in vitro.[149, 166–168] Furthermore, the rate of fibril formation is positively correlated to polyQ length with longer polyQ sequences aggregating faster.[23, 24, 30, 31, 64, 85, 106] Structurally, Max Perutz first postulated that the β-sheet structure of polyQ fibrils are stabilized by hydrogen bonding between side-chains, creating a polar zipper,[169] and this basic motif has been supported by structural analysis of polyQ fibrils. Using magic angle spinning solid state nuclear magnetic resonance (ssNMR), the amyloid core of htt exon 1 fibrils was confirmed to be a steric zipper structure that contains β-hairpins.[170, 171] The polyQ core of htt fibrils is also rigidly packed based on these SS-NMR studies[170, 171] and electroparamagentic resonance spectroscopy.[172]

Many other amyloid-forming proteins have the ability to form polymorphic fibrils structures,[173–176] and EM images of fibrils of various htt fragments appear different morphologically compared with fibrils of pure polyQ peptides.[25, 26, 61, 85] Despite this apparent difference in morphology, the same polyQ core structure in htt fibrils is always observed by ssNMR, despite dramatic differences in aggregation behavior and aggregation conditions.[170, 171, 177, 178] A conserved core of polyQ may underlie observations that polyQ peptides with different flanking sequences form similar extended, fibrillar aggregates on an anionic surface[179] Internally, polyQ fibril cores are assembled from differently structured monomers containing β-hairpins, providing an “intrinsic” polymorphism supported by ssNMR studies.[170]

Flanking sequences adjacent to polyQ tracts influence aggregation and toxicity

The ability of protein context to alter aggregation of polyQ tracts has been established via a wide array of systems,[5, 36, 61, 105, 110, 149, 150, 179–185] suggesting a potentially critical role of flanking sequences to polyQ structure and to potential mechanisms of aggregation. For example, α-helical structure in the polyQ domain in a fragment of the androgen receptor is promoted by a preceding leucine-rich region, inhibiting aggregation.[116] Addition of a three-helical B domain from staphylococcus aureus protein A retards aggregation, and adding more of these domains further slows aggregation.[186] The AXH domain and the nuclear localization signal in ataxin-1 are involved in inclusion formation.[187–190] The AXH domain itself has a propensity to aggregate and may be involved in initiating fibrilization.[189, 191, 192] The pathogenic threshold length associated with some polyQ diseases appears dependent on

the polarity of its flanking sequence.[193] The polyQ length threshold increases when flanked by extended high polarity or alternating low and high polarity amino acids when compared to a long sequence of low polarity amino acids.[193]

Considerable attention has been focused on the impact of flanking sequences in htt on aggregation, and both the N17 and polyP regions flanking the polyQ tract have been shown to alter aggregation (Figure 1I). The addition of a 10-residue polyP sequence to the C-terminus of a polyQ peptide impacts the underlying conformation of polyQ and aggregation rates.[61, 106, 181, 194] Strikingly, addition of a polyP domain to the N-terminus of a polyQ domain does not affect aggregation.[106] At least in vitro, the ability of C-terminal polyP domains to slow aggregation appears to be related to its influence on the transient conformational sampling of the polyQ tract.[106, 181, 194] The polyP domain typically has a PPII-like helical structure that has been observed to propagate into the polyQ tract in crystal structures.[114] However, this propagation of PPII-like helical structure into the polyQ tract is not observed in the fibril structure[171], suggesting that if this propagation does occur in monomers in solution that it is lost during the aggregation process. The addition of a polyP domain to the C-terminal side of polyQ stabilizes the protein reducing the rate of aggregation and increases the required polyQ repeat length that results in fibril formation.[106] Some computational studies support the ability of polyP to prevent polyQ to convert to a β-sheet rich conformation as a monomer.[110] The ability of polyP to influence polyQ aggregation has been extended into the cellular environment with impacts on toxicity. In yeast cells, the absence of the 38 amino acid proline-rich region affects the shape and number of polyQ inclusions and also activated toxicity in yeast cells.[182, 195] A scFv of a polyP specific antibody, MW7, promotes htt turnover in HEK293 cells and mouse models of HD, reducing htt toxicity.[196, 197] This same antibody inhibits htt aggregation in vitro and destabilized pre-formed htt fibrils,[124] perhaps by stabilize a conformation of the polyP domains that prevents the necessary conformational changes in the polyQ domain associated with fibril core structure.

The N17 flanking sequence also influences polyQ structure, aggregation mechanisms, aggregate stability, and other important biochemical/biophysical properties of htt. Specifically, N17 drives the initial interaction between htt exon 1 monomers by self-association to form oligomers.[103, 178, 179, 185, 198] N17 attached to the N-terminal side of synthetic PolyQ increases aggregation rates exponentially.[61, 195] Unlike polyP, N17's influence on polyQ aggregation is not position-dependent as it promotes polyQ aggregation when incorporated onto the C-terminal end of polyQ peptides and even when separated from the polyQ by a solubility imparting sequence like lysines.[61] It also appears that N17 preferentially shifts the aggregation mechanisms to an oligomer mediated pathway.[61, 199, 200] Interestingly, N17 can interact with two regions of htt exon1, N17 and the polyQ tract.[201] One proposed mechanism is that the aggregation process occurs via self-association of N17 from different htt peptides to form small, α-helix rich oligomers that bring the polyQ tracts into close proximity to facilitate fibril formation[36, 61, 202] Alternatively, intermolecular interactions between N17 and polyQ may serve a role in initial steps leading to aggregation.[201] Computational studies have also observed intermolecular interactions associated with N17.[200] Support for both intramolecular N17/N17 and intermolecular N17/polyQ interactions playing a role in aggregation is provided by the effect

of the addition of exogenous N17 peptides lacking polyQ tracts to aggregating model peptides.[185, 201] When N17 peptides are incubated with pure polyQ peptides, the aggregation of the polyQ peptides is enhanced.[201] When N17 peptides are incubated with htt-mimicking peptides that contain the N17 domain, aggregation is suppressed.[185] Similar mechanisms of aggregation are associated with the Josephin domain bringing polyQ tracts together to aid fibril formation in ataxin-3.[149, 150] In cellular models, N17 mediated oligomerization appears to be a rate-determining step in aggregation as htt exon 1 oligomer populations are relatively stable even when monomers are recruited into inclusion bodies.[38, 203]

At low concentrations, N17 is essentially disordered.[36, 204] However, N17 can form an amphipathic α-helix, consisting of a predominately hydrophilic face and a predominately hydrophobic face, that is observed in some aggregate structures of htt to varying extents[66, 146, 177] and plays a role in aggregation.[201] Amphipathic α-helices tend to be disordered until interacting directly with a binding partner, as when N17 domains from different monomers interact to form α-helix rich oligomers.[36] Amphipathic α-helices are also often associated with lipid membrane binding, particularly highly curved membranes, in a regulated manner.[205] N17 indeed functions as a lipid binding domain[179, 204, 206, 207] and demonstrates preferential binding to curved membranes.[208] Understanding how binding partners induce α-helical structure in N17 could be important, as the ability of N17 to form an amphipathic α-helix has been implicated in the formation of a variety of helix bundles associated with the initial phases of htt aggregation.

Since the flanking amino acids on either side of polyglutamine affect toxicity,[182] the existence of possible interactions between the two regions are possible.[209, 210] Elegant FRET based studies in cells suggest that the polyQ domain in htt acts as a flexible hinge facilitating an interaction between N17 the polyP region creating a molecular switch that regulates htt activity.[210] This action was correlated with polyQ length with a critical threshold in line with HD where the flexibility of the polyQ domain is sufficiently lost, leading to dysfunction of the switch and perhaps toxicity.[210] Interplay between the two flanking sequences in htt diseases also modulates the interaction of htt with lipids, as having both N17 and the C-terminal polyP domains flanking the polyQ tract resulted in enhanced aggregation on bilayers, destabilized membrane structure, and lead directly to membrane leakage compared with peptides that contained only one of the flanking sequences.[179] This observation was further supported by computational studies demonstrating that N17 regulates htt/lipid interactions and the presence of both N17 and polyP enhance the interaction of htt with bilayers.[211]

While it is well-appreciated that flanking sequences influence polyQ structure and aggregation, most studies with recombinantly produced htt proteins have exogenous amino acids at the N-terminus. Chemically synthesized polyQ peptides typically have flanking lysines added to one or more termini. While often necessary from a technical point of view, the addition of exogenous tags can also exert influence on the biochemical and aggregation properties of polyQ-containing proteins. In a yeast model, sequences flanking the polyQ region of huntingtin exon I heavily influenced toxicity and aggregate morphology.[182] Specifically, a non-native FLAG tag promoted toxicity. The addition of a myc-tag preceding

N17 in full htt exon 1 can shift the balance between aggregation from an oligomeric-mediated mechanism toward a monomer aggregation pathway, suggesting that the N17 promoted oligomer formation can be influenced by steric hindrances.[146] This same myc-tag can reduce the affinity of htt exon1 for lipid membranes.[212] Recently, a variety of fluorescent fusion partners were shown to differentially influence htt aggregations and toxicity in yeast.[82] Such observations make it important to remember the experimental context of aggregation studies of polyQ proteins. Recently, successful efforts to synthesize and characterize full length htt exon1 lacking any non-native amino acids have been reported,[78, 146170213] and further biochemical analysis of these proteins without exogenous tags and amino acids should provide clearer insights into polyQ structure and aggregation.

Post-translational modifications impact aggregation and toxicity

An additional factor influencing polyQ-related disease are post translational modifications that can occur within polyQ-containing proteins. For example, phosphorylation, acetylation, and SUMOylation of AR influence its structure and stability.[214–217] There are two verified phosphorylation sites within ataxin-1, one of which is located near the polyQ domain.[118] There are at least 48 distinct PTMs associated with htt that have been identified.[55] Several of these PTMs modulate toxicity of mutant htt, and much effort has been exerted to understand the role of PTMs in the disease state. PTMs have been implicated in htt localization, function, and aggregation. For example, acetylation at lysine 444 tags mutant htt for clearance via the autophagic-lysosomal pathway leading to neuroprotection in a *C.elegans* HD model,[218] and phosphorylation at S434 or S536 regulates proteolysis by caspase 3 and calpain with a reduction in associated toxicity.[219, 220] Phosphorylation at S421 in cellular models reduces accumulation of htt fragments in the nucleus,[221] and decreases NMDA-mediated excitotoxicity.[222] The ability to phosphorylate S421 is altered by polyQ expansion, which results in a smaller fraction of htt phosphorylated at this site.[223, 224] Recently, mimicking S421 phosphorylation improved behavioral dysfunction and striatal neurodegeneration in mice by increasing proteasome-dependent turnover of htt.[225] Several other PTMs have been demonstrated to function in axonal transport.[157, 220, 226, 227] Here, we will focus on PTMs that occur within the N17 domain of htt, as several of these impact polyQ structure and aggregation.[228] Several PTMs occur in this domain – i.e., phosphorylation,[229–232] acetylation,[233] ubiquitination,[234] SUMOylation,[235, 236] palmitoylation,[237] oxidation,[238] and transglutamination.[239] Additionally, the initiator methionine of N17 can be proteolytically removed, and this may occur rapidly after or during synthesis.[157, 229] Several PTMs in N17 impact htt function, translocation, aggregation, and the toxicity of mutant htt.[51, 229, 235, 240, 241]

The htt N17 domain has three 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), and phosphorylation in N17 has been linked to reduced toxicity.[227, 229, 231, 232, 240, 242, 243] The addition of phosphomimetic mutation at S13 and S16 within full length htt with 97Q expressed in a transgenic mouse model reduced visible htt inclusions within the brain and ameliorated HD–like behavioral phenotypes.[232] Phosphorylation at S13 and S16 can be triggered by ganglioside, GM1, and initiate a protective effect that restores normal motor function in transgenic mice.[230] Phosphomimetic

amino acid substitution at T3 promotes htt aggregation in cultured cells but reduced neurodegeneration in *Drosophila* models of HD.[229] Based on studies using a variety of phosophomimetic combinations of mutations at serine 13 and 16, phosphorylation at these sights was linked to a significant decrease in aggregation rates and reduced structural stability of the resulting fibrils.[177, 232] As such, phosphorylation, which is often viewed from a signaling point of view, has biophysical consequences that impact structural features of N17 and htt aggregation. For example, phosphorylation has a generic ability to destabilize α-helicity in proteins,[244] this has been specifically demonstrated for htt peptides.[177] Inhibited aggregation of htt due to phosphorylation in N17 could be partially attributed to destabilization of α-helical mediated self-association of N17 into tetramers, which appears to be an early step in fibril formation.[36]

Ubiquitination, SUMOylation, and acetylation can also occur within N17 of htt at lysine residues 6, 9, and 15. Ubiquitination of N17 tags htt for degradation by the ubiquitin proteasome system (UPS), reducing the toxicity of the mutant htt.[245] Based on cell and animal models, SUMOylation of N17 plays a role in mediating HD pathogenesis.[235, 236] SUMOylation at K6 and K9 reduced aggregation by stabilizing htt exon1 fragments in cultured cells and exacerbates toxicity in *Drosophila*.[235] A small G protein, Rhes, binds mutant htt with a higher affinity compared with wild type and acts as a SUMO E3 ligase, resulting in higher concentration of soluble levels of htt with expanded polyQ with a concomitant enhancement of toxicity.[246] Proteomic mapping by MS demonstrated that K9 is substantially acetylated in mammalian cell lysates,[233] and this residue is preferentially chemically acetylated in vitro.[247] Interestingly, acetylation of N17 reduces aggregation into fibrils while promoting the formation of large oligomeric species.[247] Acetylation of N17 also decreases htt's affinity for lipid membranes, and computational studies suggested that K9 may play a key role in the initial binding of N17 to lipids.[247]

Interaction with lipids influence polyQ aggregation and localization

PolyQ peptides, htt exon1, and ataxin-3 all bind lipid membranes in a polyQ-length dependent manner.[167, 212, 248] Both normal and mutant htt localizes to numerous subcellular compartments, i.e. endosomes, pre-synaptic, and clathrin-coated vesicles, and dendritic plasma membrane.[249] Htt also associates with acidic phospholipids[250] and localizes to brain membrane fractions.[251] In cell lines expressing expanded n-terminal htt fragments, multi-vesicular membranes, autophagosomes, and mitochondria are incorporated into inclusion bodies.[252, 253] Collectively, this suggests a strong htt/lipid interaction. As previously mentioned, the N17 domain of htt can adopt a highly conserved amphipathic α-helix, which has been shown to bind membranes.[198, 204, 206]

With the localization of htt to membranous surfaces well established, it is appropriate to note that lipids are a common modulator of amyloid formation, as studies of α-synuclein,[254, 255] islet amyloid polypeptide,[256] β-amyloid,[257–259] and polyQ[179, 212, 260, 261] all demonstrate altered aggregation in the presence of membranes when compared to bulk solution. With regards to the amyloid formation in general, several physicochemical properties of lipid membrane, i.e. fluidity, curvature, elasticity, modulus, surface charge, and degree of hydration, influence protein aggregation.[262, 263] Although lipid bilayers can directly

influence aggregation, lipid membranes can also be directly disrupted by the expanded polyQ proteins, leading to permeabilization.[179, 264] Htt aggregates can associate directly lipid membranes and induce local mechanical changes.[68, 179, 212]

High local concentrations due to subcellular localization of polyQ-containing proteins on lipid membranes may create aggregation nucleation sites or even stabilize specific conformers or aggregate species involved in toxicity that otherwise are only transiently formed in bulk solution. In this regard, the α-helical content of htt is altered in the presence of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine (POPS): POPC SUVs, based on CD spectroscopy.[198] Htt/lipid interaction is further modulated by membrane composition and polyQ length.[248, 265] For example, exogenous cholesterol protects lipid bilayers from htt induced disruption,[264] which is particularly important provided that HD patients have altered cholesterol homeostasis.[266, 267] Membrane degradation of the nuclear envelope, ER, and mitochondria has been described to the presence of mutant htt,[76, 162] and expanded polyQ can generically distort ER membranes and the nuclear envelope in cell culture.[163] Stabilized oligomers species of htt are associated with mitochondrial structural proteins, and can lead to mitochondrial fragmentation, abnormal mitochondrial dynamics, and oxidative DNA damage.[268]

Toxic Mechanisms attributed to polyQ expansion

In terms of discovering toxic mechanisms associated with expanded polyQ tracts, the impact of expressing mutant polyQ-containing proteins in cells lines, primary cultures, and animal models has been extensively pursued. Similar to other amyloid-associated diseases, expanded polyQ tracts within proteins are often associated with a toxic gain of function (Figure 4).[94, 269, 270] Many common toxic mechanisms have been hypothesized to contribute to pathology across these diseases, including nuclear DNA damage,[78, 232, 271] transcription dysregulation,[272, 273] impairing function of other proteins via sequestration within aggregates,[274275] interference with central protein quality control and clearance mechanisms,[276–278] alteration in endocytosis and microtubule-based transport,[249] disruption of cellular/subcellular membranes,[179, 264] and mitochondrial dysfunction.[279, 280] These varied toxic mechanisms may act in a synchronistic or cooperative manner to produce pathology. Alternatively, these toxic pathways could potentially act in series with one mechanism triggering the next, resulting in a cascading effect of toxicity. Unraveling how these toxic pathways are interdependent will require careful temporal analysis of these processes with a particular focus on determining the earliest events. Some of these mechanisms will be discussed in further detail below.

Aggregate species often have excessive exposure of hydrophobic residues that facilitate aberrant interactions with other proteins in the cellular milieu. For example, numerous cell regulatory proteins are co-opted into aggregate structures, effectively sequestering these proteins and disabling their functionality. Key proteins that are found to be co-localized within protein aggregates function in a variety of roles including transcription, vesicle trafficking, redox-homeostasis, and protein homeostasis. Accordingly, polyQ-expanded disease proteins can accumulate in the nucleus, and interfere with transcription factors and

regulators, leading to toxicity.[281] Oligomers of expanded polyQ-containing proteins directly interact with functional proteins containing polyQ tracts, i.e. the transcriptions-factors CBP, TBP, and Sp1, impairing their function.[135, 272] Specifically, htt interference of CBP-mediated transcription occurs in a polyQ length-dependent manner.[275, 282, 283] Nuclear inclusions of ataxin-3 can be seeded by itself or ataxin-1, and several unrelated proteins, i.e., TATA-binding protein and Eyes Absent protein, are recruited into nuclear inclusions comprised of ataxin 3.[284]

While observed in many age-related amyloid diseases, the sequestration of components of the degradation and protein quality control systems by aggregates of polyQ-containing proteins also occurs.[276–278, 285, 286] Several components of protein quality control machinery, including the proteasome and molecular chaperones, are irreversibly pulled into protein aggregates, and the presence of proteins containing expanded polyQ tracts can eventually overwhelm the cells ability to effectively maintain protein homeostasis.[152, 287] Beyond sequestration, proteins with expanded polyQ tracts can impact protein homeostasis indirectly, as a diverse set of metastable proteins that do not co-localize have been shown to lose function with expression of mutant polyQ-containing proteins.[278] Protease-mediated cleaving events produce a variety of N-terminal fragments of mutant htt which can translocate to the nucleus and interfere with transcription.[33, 55, 288, 289] Furthermore, HSF1, the major transcription factor for stress-inducible molecular chaperones, is dysregulated in HD patients and mouse models, reducing chaperone levels and suggesting an additional transcriptional mechanisms for impaired protein quality control.[290, 291] Combined, the direct and indirect impairment of protein quality control can initiate a feedback loop that exasperates the situation with age.

Disease mechanisms discussed so far are associated with a toxic gain of function associated with expanded polyQ. Most patients with polyQ-related diseases are heterozygotes, expressing one mutant allele and one wild-type allele. As a result, both wild type and mutant forms are typically present in vivo with the concentration of the fully functional wild type forms being approximately cut in half compared to the non-disease state. Deleterious effects associated with disease can also arise from a loss of function associated with expanded polyQ. For example, htt is associated with a variety of beneficial functions including vesicle trafficking,[292, 293] mediating endocytosis and endosomal trafficking,[294, 295] regulating ciliogenesis,[296–298] and regulating transcription.[283, 299] While knocking out htt results in embryonic lethality in mice,[300–302] HD-like pathology develops in mice when htt is knocked out in adulthood.[303] Heterozygous htt knockout mice also have impaired motor activity, cognitive deficits, and neurodegeneration;[301, 304] although, the phenotype is milder compared with mouse models overexpressing mutant htt fragments.[305] Endocrine associated symptoms in SBMA are potentially due to reduce AR activity,[306] and expanded polyQ disrupts the interaction between transcriptional coactivators and the amino-terminal transactivation domain of AR.[307] Evidence points to ataxin-1 being involved in transcription regulation, and loss of this function due to expanded polyQ can contribute to pathology.[308, 309] These findings support that loss of normal function of proteins containing expanded polyQ tracts may also contribute to disease pathology.

Conclusions

Due to the complexity in structure, dynamics, and aggregation properties of proteins containing expanded polyQ tracts, consensus on underlying toxic specie(s) and their mode of operation in disease has not been reached. As there are likely numerous bioactive aggregate species potentially within the range of monomers through oligomers and eventually fibrils, the precise characterization of aggregates and underlying structure and bio-reactivity will prove invaluable in understanding the underlying toxic mechanisms associates with these proteins. While much progress has been made since the mutations associated with these diseases were first discovered, unequivocally identifying precise toxic species has remained elusive. Biochemical and biophysical analyses of the aggregation of polyQ have revealed that a myriad of factors contribute to aggregation in vitro, i.e. expansion length, flanking sequences, membranes, and post-translational modifications. Aggregation reactions can be quite diverse, resulting in complex, heterogeneous mixtures of aggregates with varying stabilities. When placed in context of the environmental complexity within cells, potential factors modulating aggregation are even more numerous. As cellular dysfunction and toxicity likely lies in the balance between these various species of different polyQ-containing proteins, therapies designed to manipulate aggregation will need to critically evaluate the extent to which the mixture of monomeric/oligomeric/fibrillar aggregates are altered, as some aggregates may be toxic while others are protective. Ultimately to identify toxic entities in these polyQ diseases, novel experimental techniques and approaches capable of distinguishing the complex mixtures of aggregates in cells and correlating this information to toxic outcomes will need to be further developed and applied.

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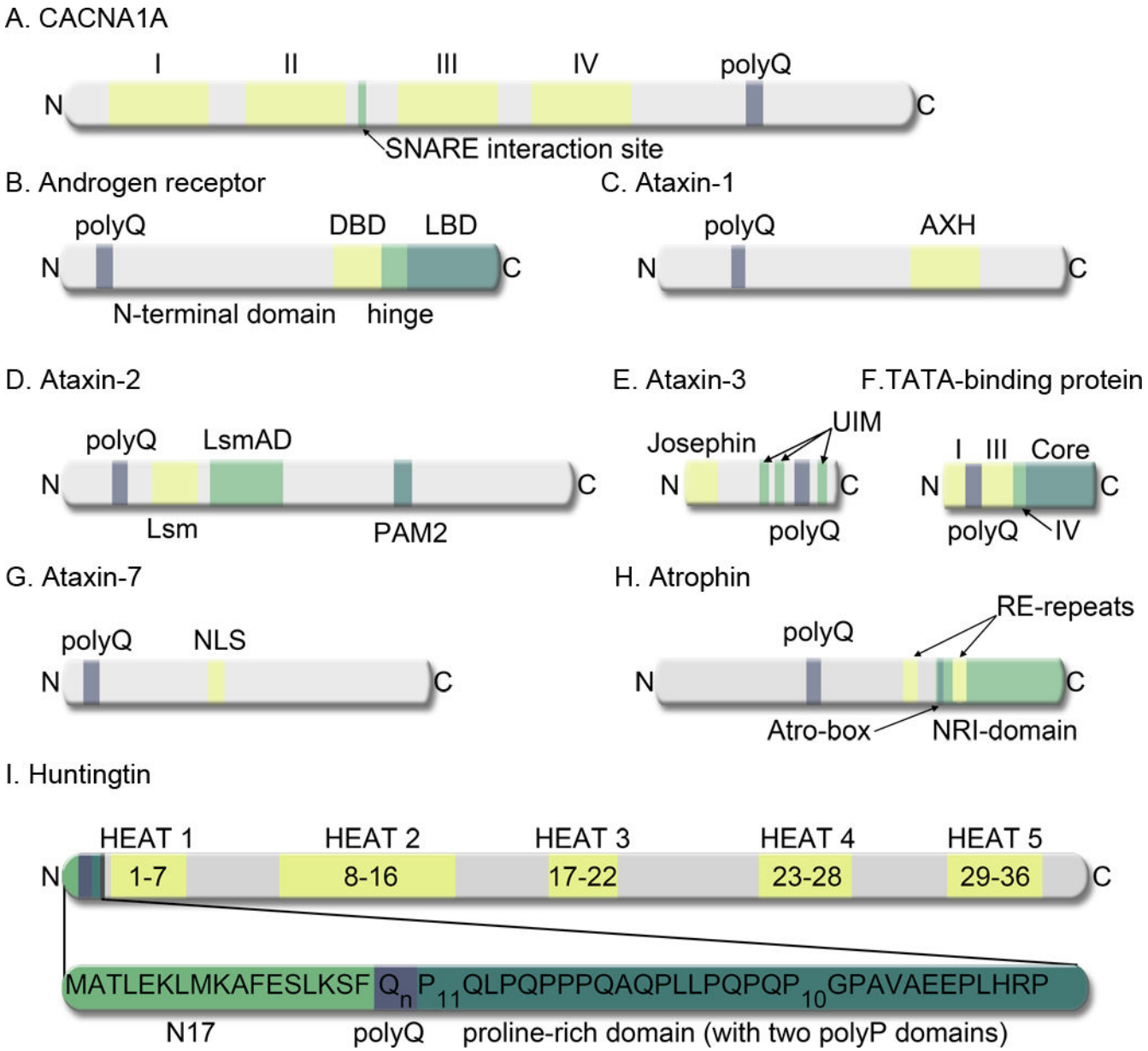

Figure 1. Schematic representation of various proteins associated with CAG-disorders

A, CACNA1A, in addition to the polyQ tract, contains four homologous domains (I–IV), each with six transmembrane segments. There is a SNARE interacting site between domains II and III. B, Androgen receptor, in addition to the polyQ tract, contains an N-terminal domain (NTD), a DNA-binding domain (DBD), a hinge region, and a ligand-binding domain (LBD). C, Ataxin-1 contains a polyQ tract and an AXH domain. D, Ataxin-2, contains the polyQ domain, a Like RNA splicing domain Sm1 and Sm2 (Lsm), a Like-Sm-associated domain (LsmAD), and a poly (A)-binding protein interacting motif 2 (PAM2). E, Ataxin-3 contains an N-terminal Josephin domain, three Ub-interacting motifs (UIM), and the polyQ domain. F, The N-terminal region of the Tata-box binding protein consists of four domains (I-IV) followed by a core region. The polyQ region is domain II. G, Ataxin-7 contains a polyQ domain and a putative nuclear localization signal (NLS). H, Atrophin-1, in addition to

the polyQ tract, contains two arginine-glutamic acid dipeptide repeats (RE repeats), a nuclear receptor interacting domain (NRI domain), and a highly conserved Atro-box domain. I, The full-length htt protein contains several HEAT repeats. The inset indicates the location of htt exon1, with the N17, polyQ, and proline-rich domains indicated.

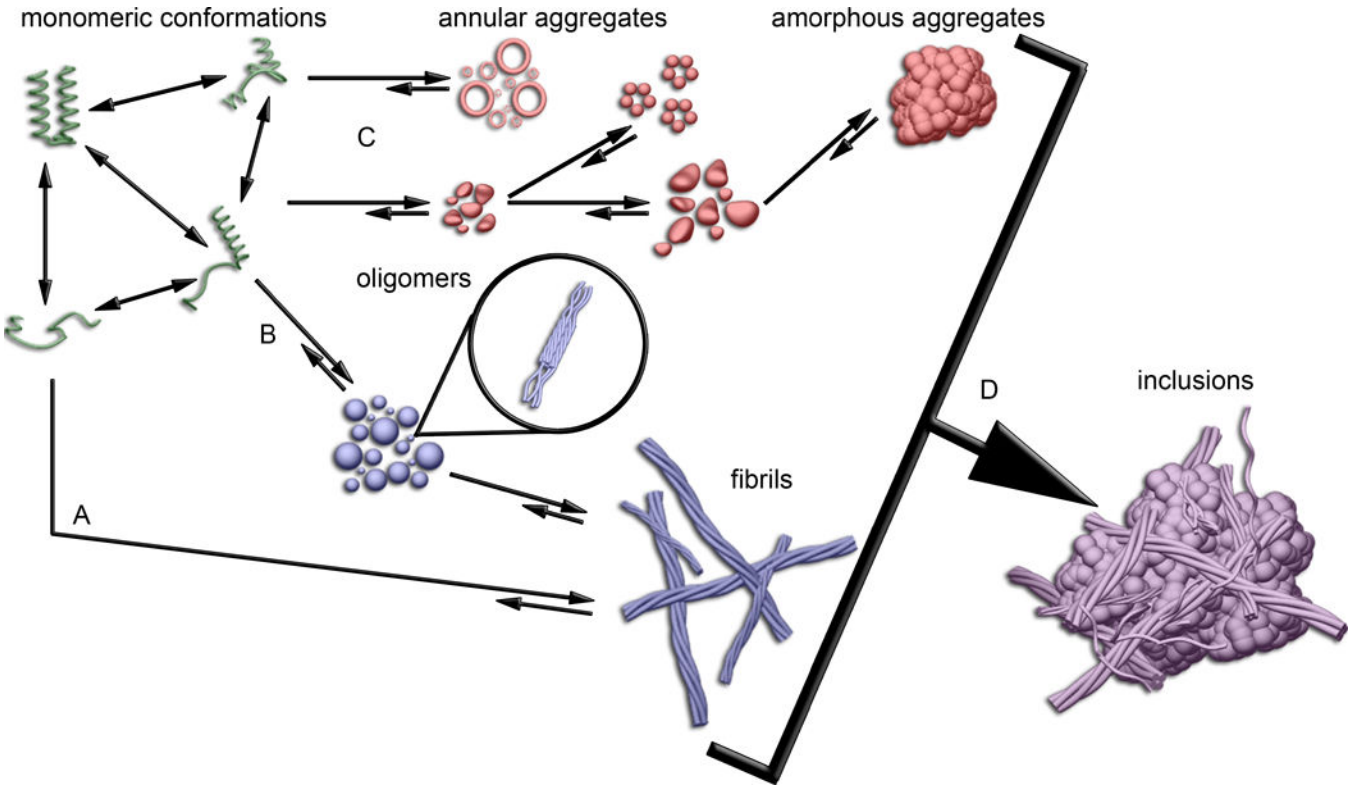

Figure 2. A schematic model for misfolding and aggregation of proteins containing expanded polyQ tracts

Monomeric proteins (shown in green) can sample a variety of distinct conformations, with the relative number and stability of these conformers potentially being polyQ length-dependent. Some of these monomeric conformation are aggregation prone and may lead to distinct aggregation pathway, some on pathway (shown in blue) to fibril formation and some off pathway (shown in red) to fibril formation. There appear to be two generic aggregation mechanisms toward fibril formation: (A) a monomeric critical nucleus that leads directly to fibrils and (B) fibril formation via oligomeric intermediates. Protein context of the polyQ domain can influence which pathway is dominant, and flanking sequences may facilitate the formation of some oligomeric intermediates. These two mechanisms are not necessarily mutually exclusive. (C) There are also several off-pathway aggregation routes that can lead to distinct oligomers of various sizes, a variety of annular aggregates, and large amorphous structures. (D) All of these higher order aggregates may accumulate together to form the large inclusions that are hallmarks of polyQ diseases.

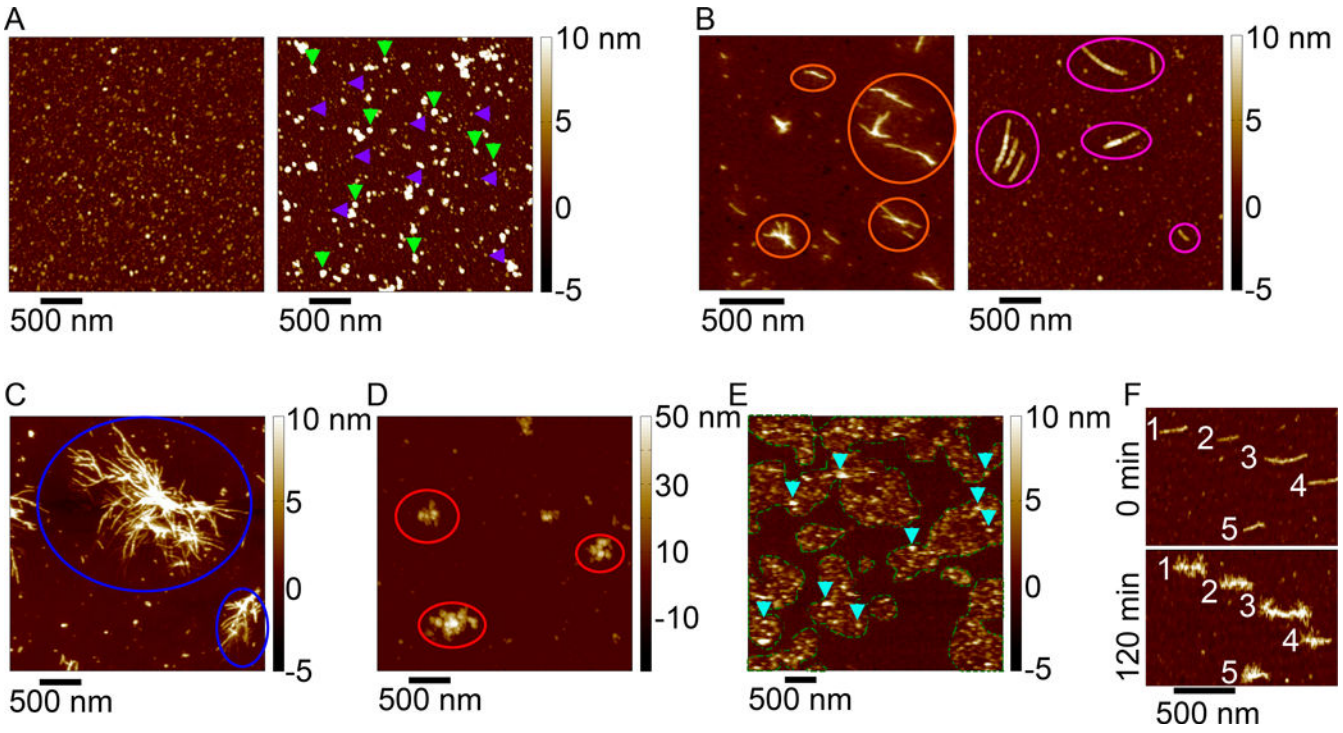

Figure 3. Atomic force microscopy images of a variety of aggregates formed by htt-exon1 proteins

(A) Htt exon1 can form a variety of globular, oligomeric species (purple arrows indicate oligomers ~5 nm in height; green arrows indicate oligomers ~10 nm in height). (B) Two morphologically distinct fibrils structures formed by exon1, (orange circles indicates thinner, smooth fibril structures; pink circles indicate thicker fibrils with a beaded morphology). (C) Blue circles indicate large bundles of htt exon1 fibrils. (D) Large, amorphous aggregates of htt exon1 are indicated by red arrows (note the color scale goes up to 50 nm). (E) When htt exon1 aggregates on a lipid bilayer, a variety of oligomeric aggregates (blue arrows) associated with regions of increased membrane roughness (outlined with the green dashes lines) are observed. (F) When adding monomeric htt exon1 to pre-formed fibrils, the monomer can accumulate around the fibrils and form a variety of branching points (numbers indicated the same fibril at 0 minutes and 120 minutes after exposure to monomeric htt exon1).

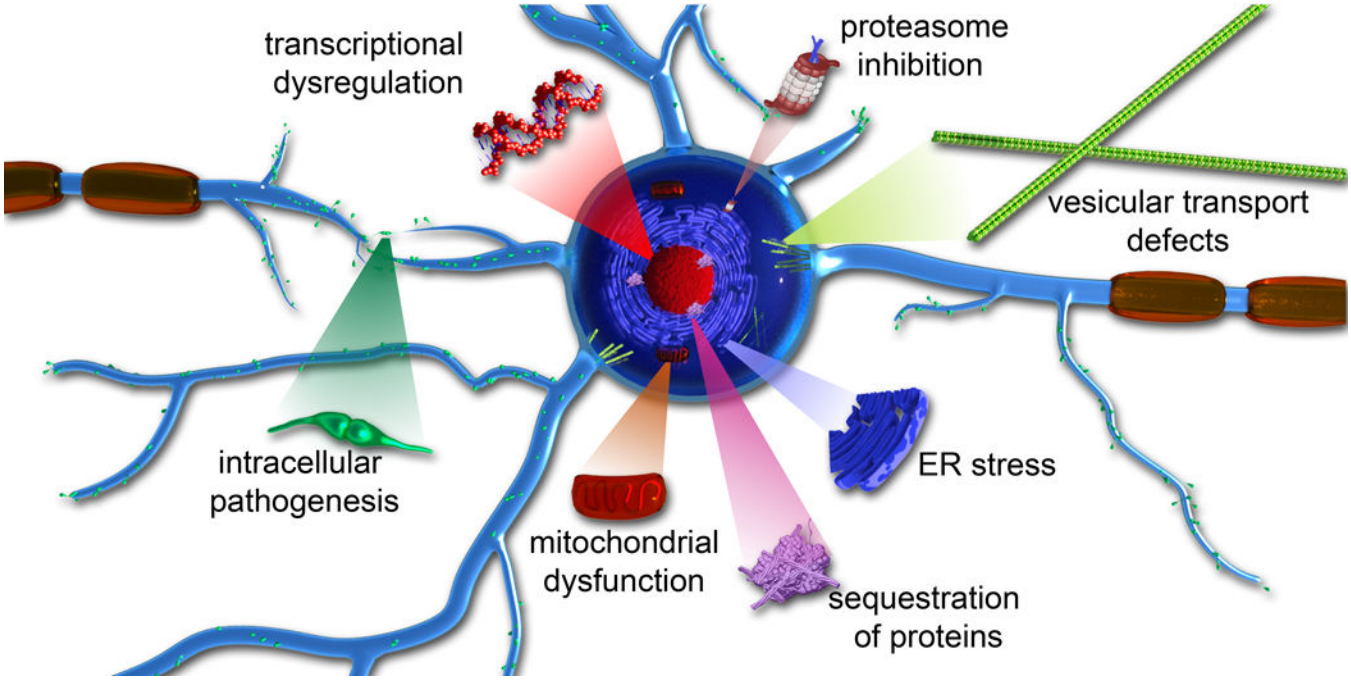

Figure 4.
Toxic gain of function pathogenic mechanisms associated with polyQ diseases.

Table 1

CAG-repeat disorders

Disease	Common Name	Protein	PolyQ*
Huntington Disease (HD)		Huntingtin	36–100
Spinobulbar Muscular Atrophy (SBMA)	Kennedy's Disease	Androgen Receptor	38–65
Dentatorubral-pallidoluysian atrophy (DRPLA)	Haw River Syndrome	Atrophin-1	49–88
Spinocerebellar Ataxia Type 1 (SCA1)		Ataxin-1	39–88
Spinocerebellar Ataxia Type 2 (SCA2)		Ataxin-2	33–77
Spinocerebellar Ataxia Type 3 (SCA3)	Machado-Joseph	Ataxin-3	55–86
Spinocerebellar Ataxia Type 6 (SCA6)		CACNA1A	21–33
Spinocerebellar Ataxia Type 7 (SCA7)		Ataxin-7	38–120
Spinocerebellar Ataxia Type 12 (SCA12)		PPP2R2B	66–78
Spinocerebellar Ataxia Type 17 (SCA17)		TATA-box Binding Protein	47–63

* Indicates the typical pathogenic polyQ range associated with each disease.

Table 2

Definitions of different aggregate species associated with polyQ aggregation

Aggregate Species	Definition
amorphous aggregate	Protein aggregates that do not have a fibrillar morphology but are larger than what is typically classified as an oligomer. Amorphous aggregates often have a granular appearance as assessed by EM or AFM.
amyloid fibril	Filamentous peptide or protein aggregates characterized by thermodynamic stability, high insolubility, and highly ordered cross-β rich structure. They are often comprised of intertwined protofilaments.
annular aggregate	Ring-shaped aggregates that are sometimes further classified as annular protofibrils or annular oligomers in the literature. Ring diameter can vary greatly. Smaller annular aggregates are hypothesized to potentially form pathogenic pores in membranes.
critical nucleus	The specie that directly initiates fibril formation. The critical nucleus may be monomeric or multimeric.
inclusion body	A large (on the order of microns) accumulation of aggregated material (including fibrils and other aggregate species) within a cell. Their large size makes them easily visible by light microscopy.
off pathway aggregate	An aggregate specie that is associated with an aggregation mechanism that does not lead to fibril formation.
oligomer	Small (on the order of 1-15 nm) protein aggregates with a globular morphology. The term oligomer has been used to describe a variety of aggregates ranging from small multimeric species containing 3-10 proteins to larger structures comprised of over 100 proteins. Oligomers are also often sub-categorized. Sub-categories include reference to their composition (i.e. tetramers, dodecamers, etc.) and being on or off pathway to fibril formation.
on pathway aggregate	An aggregate specie that is associated with an aggregation mechanism that leads directly to fibrils.
protofibril	A soluble, short filamentous aggregate that is smaller than a mature fibril and is often considered an intermediate aggregation structure.
protofilament	A single strand that is intertwined to from a mature fibril.