

Neurotoxic protein oligomerisation associated with polyglutamine diseases

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Abstract Polyglutamine (polyQ) diseases are associated with a CAG/polyQ expansion mutation in unrelated proteins. Upon elongation of the glutamine tract, disease proteins aggregate within cells, mainly in the central nervous system (CNS) and this aggregation process is associated with neurotoxicity. However, it remains unclear to what extent and how this aggregation causes neuronal dysfunction in the CNS. Aiming at preventing neuronal dysfunction, it will be crucial to determine the links between aggregation and cellular dysfunction, understand the folding pathway of polyQ proteins and discover the relative neurotoxicity of polyQ protein species formed along the aggregation pathway. Here, we review what is known about conformations of polyQ peptides and proteins in their monomeric state from experimental and modelling data, how conformational changes of polyQ proteins relate to their oligomerisation and morphology of aggregates and which cellular functions are impaired by oligomers, *in vitro* and *in vivo*. We also summarise the key modulatory cellular mechanisms and co-factors, which could affect the folding pathway and kinetics of polyQ aggregation. Although many studies have investigated the relationship between polyQ aggregation and toxicity, these have mainly focussed on investigating changes in the formation of the classical hallmark of polyQ diseases, *i.e.* microscopically

visible inclusion bodies. However, recent studies in which oligomeric species have been considered start to shed light on the identity of neurotoxic oligomeric species. Initial evidence suggests that conformational changes induced by polyQ expansions and their surrounding sequence lead to the formation of particular oligomeric intermediates that may differentially affect neurotoxicity.

Introduction

Polyglutamine (polyQ) diseases are a group of human disorders associated with a triplet CAG expansion mutation and can be classified together with fragile X syndrome, fragile X tremor/ataxia syndrome, Friedreich's ataxia and the polyalanine diseases (e.g. oculopharyngeal muscular dystrophy) into the group of the trinucleotide repeat expansion diseases [118]. The trinucleotide expansions in the genes associated with these disorders occur in the gene coding regions and are translated into a mature protein. Currently, there are 10 known polyQ disorders (Table 1). Despite widespread expression of the disease-associated proteins outside the central nervous system (CNS), all polyQ disorders are slowly progressive, fatal neurodegenerative diseases of the CNS causing a large spectrum of neurological and psychiatric symptoms (Table 1) with no cure available. Patterns of affected brain regions and CNS cell types (mainly neurons) vary according to the disease (Table 2). As in other chronic CNS diseases such as Alzheimer's- and Parkinson's disease (AD, PD), neuropathology is relatively specific to certain brain regions and CNS cell types and varies for each polyQ disease (for a recent review see [10], see Table 2). With the exception of the androgen receptor (AR) that is X-linked, the genes

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Table 1 Characteristics of proteins associated with polyglutamine diseases and reported clinical symptoms of the diseases

Disease	Protein	Size of wild-type protein (kDa)	Normal polyQ length	Pathological polyQ length	Clinical features
Huntington's Disease (HD)	Huntingtin (htt)	348	6–34	36–121	Psychiatric, cognitive and motor abnormalities
Kennedy's Disease/spinal and bulbar muscular atrophy (SBMA)	Androgen receptor (AR)	110	9–36	38–62	Proximal muscle and bulbar muscle weakness, atrophy and fasciculation, endocrine abnormalities, hand tremor, muscle cramps, neuropathy
Dentatorubral-pallidoluysian atrophy (DRPLA)	Atrophin-1	190	7–34	49–88	Ataxia, epilepsy, myoclonus, choreoathetosis, dementia
Spinocerebellar ataxia Type 1 (SCA1)	Ataxin 1 (atx-1)	87	6–44	39–82	Ataxia, progressive motor deterioration, dysarthria, oculomotor palsy, amyotrophy and sensory disturbance
SCA2	Ataxin 2 (atx-2)	145	15–24	32–200	Ataxia, Parkinsonian rigidity, bradykinesia, amyotrophy, sensory disturbance, mental deterioration
Machado-Joseph Disease (MJD/SCA3)	Ataxin 3 (atx-3)	42	13–36	61–84	Ataxia, dystonia, Parkinsonism, neuropathy, amyotrophy, ophthalmoplegia, nystagmus, eyelid retraction, faciolingual fasciculation, dysphagia, weight loss
SCA6	Ataxin 6 (atx-6)	280	4–19	10–33	Ataxia, dysarthria, oculomotor disorders, incontinence, peripheral neuropathy
SCA7	Ataxin 7 (atx-7)	96	4–35	37–306	Ataxia, retinal degeneration, dysphagia, dysarthria
SCA12	Regulatory subunit of protein phosphatase 2A (PPP2R2B)	55	7–28	66–78	Action tremor, hyperreflexia, dysarthria, gait ataxia, cognitive or psychiatric disorders
SCA17	TATA-binding protein (TBP)	42	25–42	47–63	Ataxia, dementia, epilepsy

associated with polyQ diseases are inherited in an autosomal dominant manner, in contrast to the majority of AD and PD sporadic cases. There is an inverse correlation between the length of the polyQ stretch and the age of onset of disease and its severity. The polyQ stretch is a strong determinant of disease and, e.g. in Huntington's Disease (HD) determines ca. 50% of the variation in age of onset of HD [180]. Disease occurs above a CAG/polyQ threshold of approximately 35–40 (that is variable to some extent, see Table 1).

The proteins involved in polyQ diseases appear unrelated, are found at different intracellular locations and some functions of these proteins are known or are beginning to emerge (Table 2). Spinal bulbar muscular atrophy (SBMA) is caused by a polyQ expansion in the AR, a nuclear hormone receptor that is responsive to G-protein coupled receptor signalling. The AR binds DNA and regulates target gene expression by modulating chromatin complexes and their regulatory proteins and functions in many different physiological processes including male reproductive functions [105]. The transcriptional activity of the AR is inversely correlated with the length of its CAG repeat. The actions of ataxin-1 (the polyQ-expanded protein associated

with spinocerebellar ataxia 1, SCA-1) also occur in the nucleus where it is involved in RNA processing and transcriptional repression. Ataxin-1 has also been suggested to modulate protein folding itself via interactions with the chaperone and ubiquitin–proteasome pathways [36, 50, 166]. Ataxin-2 has been implicated in RNA metabolism, the regulation of apoptosis and actin-polymerisation [115, 144, 181]. Ataxin-3 appears to mediate degradation of ubiquitinated proteins [43] via its activity as a polyubiquitin chain-editing enzyme [23]. The gene encoding ataxin-6 produces a subunit of a voltage-dependent calcium channel [53]. Ataxin-7 is a member of the transcription mediator complex STAGA [67] and hence is involved in the regulation of transcription. The human TATA-binding protein (TBP) contains 25–42 glutamines and upon expansion is a disease protein causing SCA17 [98]. Atrophin-1 that is associated with Dentatorubral-Pallidoluysian atrophy (DRPLA) acts as a transcriptional co-repressor [188] as suggested by a study in *Drosophila melanogaster*. A further role of atrophin-1 could be in assembling signalling complexes at the synapse by interacting with membrane-associated guanylate kinases [184]. Finally, huntingtin (htt) is a nucleocytoplasmic shuttling protein and directly involved in regulating

Table 2 Functions and normal cellular locations of polyglutamine disease-associated proteins and reported sites of cellular toxicity within the CNS

Disease	Protein	Potential functions	Predominant localisation of wild-type protein	Location of cellular toxicity in the CNS
Huntington's Disease (HD)	Huntingtin	Regulation of transcription, scaffolding protein, vesicle trafficking, axonal transport, postsynaptic and calcium signalling and bioenergetic homeostasis	Cytoplasmic	Caudate nuclei, striatum, cerebral cortex
Kennedy's Disease/spinal and bulbar muscular atrophy (SBMA)	Androgen receptor	Nuclear hormone receptor, modulates gene transcription	Cytoplasmic and nuclear	Spinal anterior horn, facial nucleus, hypoglossal nucleus, skeletal muscle
Dentatorubral-pallidoluysian atrophy (DRPLA)	Atrophin-1	Transcriptional co-repressor, assembling signalling complexes at the synapse	Cytoplasmic and nuclear	Globus pallidus, subthalamic nucleus, dentate nucleus, cerebral and cerebellar white matter
Spinocerebellar ataxia type 1 (SCA1)	Ataxin-1	RNA processing and transcriptional repression, modulates protein folding	Nuclear	Cerebellar Purkinje cells, brainstem, spinal cord, globus pallidus
SCA2	Ataxin-2	RNA metabolism, the regulation of apoptosis and actin-polymerisation	Cytoplasmic	Cerebellar Purkinje cells and granular cells, brainstem, substantia nigra, spinal cord and thalamus
Machado-Joseph Disease (MJD/SCA3)	Ataxin-3	Degradation of ubiquitinated proteins	Cytoplasmic	Cerebellum, substantia nigra, brainstem, dentate nucleus, globus pallidus, thalamus and spinal cord
SCA6	Ataxin-6	Subunit of a voltage-dependent calcium channel	Transmembrane	Cerebellar Purkinje cells, dentate nucleus, inferior olive
SCA7	Ataxin-7	Regulation of transcription	Nuclear	Cerebellar Purkinje cells, dentate nucleus, inferior olive, spinal cord, retina, brainstem
SCA12	Regulatory subunit of protein phosphatase 2A (PPP2R2β)	Brain-specific regulation of serine/threonine protein phosphatase 2A	Cytoplasmic and mitochondrial	Cerebral cortex and cerebellum
SCA17	TATA-binding protein	Regulation of transcription	Nuclear	Cerebral cortex and cerebellum

transcription, but is also found in the cytoplasm, in axons and at the synapse. As a scaffolding protein, htt mediates many protein–protein interactions and plays a role in vesicle interactions, fast axonal transport and likely signalling in the post-synaptic density [42, 57, 61]. Htt further appears to regulate calcium signalling and bioenergetic homeostasis [90].

It is possible that the polyQ expansion leads to a loss of function of some of the properties of polyQ proteins (reviewed in [25]) but there is a significantly larger amount of evidence that a gain-of-function due to the polyQ mutations causes CNS pathology [41, 155]. In particular, the observed protein aggregation, due to CAG mutations, is likely to at least partly explain the disease-associated neurotoxicity. Therefore, misfolding and aggregation as a toxic gain-of-function in these diseases is the subject critically reviewed in this article. There is a strong correlation between proteins in each disease exceeding a critical polyQ threshold and, as a consequence, abnormal intracellular accumulation of expanded polyQ proteins occurs. The aggregation of polyQ proteins has been intensively studied for over 10 years, following seminal observations that neurons from the first mouse model of HD and brains of patients affected by HD and Machado-Joseph Disease (MJD) contained intraneuronal inclusion bodies (IBs), hallmarks of all polyQ disorders [37, 41, 99, 120]. These observations led to the speculation that aggregation of the polyQ proteins may be causally related, at least in part, to CNS pathology. This has led to the emergence of a large body of literature on the elucidation of structural mechanisms leading to aggregation and whether/how polyQ aggregates cause cytotoxicity. In recent years, it has been increasingly suggested that oligomeric forms of polyQ proteins are key players in the neuropathology of polyQ diseases. Hence, here we review relevant insights that have been made in understanding the folding and aggregation of polyQ proteins, particularly focussing on the formation of oligomeric polyQ species and how oligomerisation of polyQ proteins may be linked to neurotoxicity.

Folding pathways and aggregation of polyQ proteins

Unfortunately, structural understanding of polyQ proteins has proved difficult for a number of reasons, including the inevitable handling difficulties of an aggregation-prone protein and the difficulty in synthesising a polyQ peptide (for a review see [163]) and therefore there is only limited information regarding structures of polyQ proteins in their monomeric and aggregated forms. The field has, however, been assisted by the exploration of similarities in protein assemblies observed in other neurodegenerative diseases such as AD and PD and these similarities have sometimes

led to hypothetical common mechanisms for aggregation and cellular toxicity of such amyloidogenic proteins [51]).

Conformation of polyQ peptides

With the sharp mind of a genius Max Perutz, proposed in 1994 that polyQ peptides could form “polar zippers”, which would form antiparallel β hairpins if formed intramolecularly and would induce aggregation of the peptide by forming intermolecular antiparallel β -sheets between main chain and side chain amides [124]. The proposition that polyQ proteins may aggregate followed the discovery in 1993 that the gene causing HD contained long stretches of CAG repeats and proved correct when IBs in neurons of the first mouse model were described 3 years afterwards [37]. Later, characteristic cross- β reflections from X-ray diffraction data of a polyQ peptide, D2Q15K, led to his proposal that a novel structure termed the “water-filled nanotube”, composed of a continuous β -strand running around a water-filled cylinder 31 Å in diameter, is formed [123]. However, this interpretation proved controversial and reanalysis of data collected by Perutz et al. revealed a more conventional cross- β sheet structure in which hydrogen bonds form between inter backbone amides and inter side chain amides [153]. An independent X-ray diffraction study of polyQ peptides of various lengths also suggested formation of β -sheets in slab-like structures [39]. Helical conformations have also been suggested from molecular mechanics calculations [88, 108], for example, a polyQ peptide was proposed to form a μ -helix with a cylindrical pore of 6.6 Å in diameter via hydrogen bonds with the side chain amides [108]. Other studies have indicated that polyQ peptides in aqueous solution adopt a random coil conformation [4, 31]. Fluorescence correlation spectroscopy using a G-Qn-C-K2 peptide suggested that the polyQ forms ensembles of interconvertible collapsed structures in aqueous solutions [34]. A study using a KK-C-Qn-W-KK peptide used the quenching of triplet state fluorescence of tryptophan by cysteine to conclude that polyQ is intrinsically stiff and adopts rod-like conformations and a rigid nature of a polyQ stretch was also suggested from single molecule force spectroscopy measurements [44]. Molecular simulation studies on homopolymeric constructs of polyQ at physiologically relevant temperatures indicated that they form ensembles of collapsed globules [169]. The apparent discrepancy as to whether polyQ forms rigid structures or fluctuating collapsed structures was addressed in a recent study using fluorescence resonance energy transfer (FRET) [174]. This study showed that in constructs with pairs of C- and N-terminal lysines, the collapse is not evident until the polyQ length exceeds 16 residues. Once this occurs, these collapsed conformations correlated with the propensity of

the peptide to aggregate. The study also suggested that polyQ peptides containing C- and N-terminal lysines were likely to be affected by end-to-end electrostatic repulsions.

In summary, conformational studies of the polyQ stretch can be heavily influenced by differing experimental conditions in which measurements have been taken including various solvents, different sequences and temperature. The only studies that have been performed on true homopolymeric polyQ have been molecular simulations, with all experimental investigations using charged residues at the N- and/or C-termini, which can potentially influence intrinsic polyQ conformations and interactions [183]. It has been suggested that the random coil conformation is only visible when the polyQ peptide is fully soluble with beta-sheet and beta-hairpin structures only observed when the peptide is at least partially aggregated [104]. Indeed recent modelling data suggest that high β -content of monomeric polyQ is thermodynamically unfavourable with the formation of intermolecular interfaces promoting the formation of β -sheet structures [168]. This is consistent with recent biophysical data from a K2Q23K2 peptide, which indicate that large soluble oligomers have no regular secondary structure [89].

This recent biophysical study [89] has also contributed significantly to our understanding of the kinetic pathway of polyQ peptide aggregation. Previous analysis of kinetic data led to the proposal of a classic nucleation-elongation mechanism with a significant lag time, during which the peptide is monomeric. The monomer would then shift from random coil to a thermodynamically unfavourable conformation inducing aggregation of the peptide [17, 31, 32]. However, using laser light scattering and size exclusion chromatography, Lee et al. [89] showed that during the so-called lag time, the polyQ peptide is not solely monomeric, but also consists of large soluble aggregates before further aggregation into insoluble precipitates.

All of the above studies of polyQ aggregation have been performed with polyQ peptides, partly due to the very large size of the polyQ disease-causing proteins (Table 1), but also justified by the fact that the only similarity between the disease-causing polyQ proteins appears to be their polyQ stretch. In the various polyQ diseases, the location of the polyQ stretch varies within the corresponding disease-causing protein, and both the protein context surrounding the polyQ stretch and the cellular location and function of the polyQ proteins are different (see “[Introduction](#)” and Table 1). However, it is now clear that the context of the polyQ stretch, posttranslational modifications of the protein, cleavage of the full-length protein, intracellular location and many other cellular factors are important determinants in the aggregation of the protein, as outlined in “[Factors affecting aggregation of polyQ proteins](#)” (see below).

Effects of protein context on conformation of the polyQ stretch

A variety of conformations of polyQ stretches have been reported when the polyQ stretch is embedded within a protein. Indeed simply adding an oligoproline stretch C-terminal to a polyQ peptide affects its aggregation kinetics and the conformation [16]. In the presence of the oligoproline stretch, the alpha-helical content, as measured by circular dichroism (CD), of the polyQ peptide, is abolished, whereas the N-terminal fusion of oligoproline had no effect on conformation of the polyQ peptide [16]. A polyQ stretch with glutathione-S-transferase (GST) fused to the N-terminus of the stretch showed a random coil conformation [103]. Alpha-helical conformations of polyQ stretches fused to thioredoxin and in ataxin-3, i.e., in a disease-causing protein context, have also been observed with CD [113]. The alpha-helical content of the polyQ stretch in ataxin-3 is significantly reduced when the polyQ length is increased from 27 residues to 78 residues, with a corresponding increase in random coil, highlighting the importance of polyQ length in the determination of structure [15]. However, an expanded polyQ stretch in myoglobin formed an antiparallel beta-pleated sheet structure [162]. In contrast to the studies with polyQ peptides mentioned above [89, 168], fourier transform infrared spectroscopic analysis showed an increase in β -structure upon the appearance of small oligomers of htt Exon 1 (httex1) [126], consistent with CD data showing early oligomers of ataxin-3 are β -sheet-rich [56], indicating a shift to β structure early on in the aggregation process. As shown by molecular modelling data [9], it is likely that the polyQ stretch destabilises proteins to form partially unfolded intermediates, prone to aggregation potentially via inter-glutamine interactions. A significant step forward in understanding the conformation of polyQ stretches has been achieved by the recent determination of the first crystal structure of a polyQ-containing protein in its native context, i.e. httex1 fused to the maltose-binding protein (MBP), although it should be noted that the MBP fusion may have effects on conformation [80]. The structure of httex1 consists of an amino-terminal α -helix that precedes the polyQ stretch, which is then followed by a polyproline helix that was modelled, due to inconclusive electron densities. The polyQ region (consisting of 17Q) was crystallised in multiple conformations, including α -helix, random coil and extended loop. Httex1 with a polyQ length of 17Q does not aggregate and therefore it is still unclear as to which changes occur in the conformation of the polyQ stretch to induce aggregation of the protein: it is unknown whether the β -strand conformations, known to exist in fibrils formed by mutant httex1, also exist in the monomeric state of mutant htt. However, it is quite possible that

different conformations do exist and that each conformation may form a different type of aggregate.

To date, there are limited data available on the kinetics of aggregation in an *in vivo* context. A recent FRET analysis of polyQ proteins containing a polyQ expansion within cells has been used to support a model of parallel β -sheet or a head-to-tail cylindrical β -sheet formation during aggregation, as signals were only detected when donor and acceptor fluorescent proteins were attached to the same side of the molecule [159]. As this experiment was performed with a polyQ stretch in atrophin-1, this model needs to be tested in other polyQ systems given the importance of flanking residues as discussed above. Another study using a cellular model addressed the proposal based on *in vitro* studies that polyQ aggregates consist of alternating beta-strand and beta-turns [165]. Cell models expressing httex1 engineered within the polyQ stretch to assume a proposed β -strand/ β -turn structure formed similar aggregates to cells expressing the normal sequence of httex1 polyQ, whereas addition of proline residues to presumably prevent this β -strand/ β -turn structure abrogated aggregation of the protein [125].

In summary, the conformations adopted by the polyQ stretch within a protein remain controversial and appear to be dependent on many conditions including protein context, temperature and polyQ length. Indeed recent crystallographic evidence suggests that in a protein context the polyQ stretch, at least in monomeric proteins, can adopt multiple conformations [80]. Although the conformation of the polyQ stretch in a protein context may be variable, it is generally accepted that in large insoluble aggregates, proteins containing a polyQ stretch can form a variety of amyloid conformations [114, 125], although these conformations may be different to the well-characterised β -amyloid-like fibrils formed in AD [35]. How and when the conformational transition to beta-sheet (if any) occurs is also a matter for debate and may indeed vary according to the polyQ-containing protein involved.

Morphological characterisation of the intermediates along the polyQ-aggregation pathway

A variety of techniques have been used in order to investigate morphological, structural and kinetic features of the pathway of polyQ aggregation. It is clear that *in vitro*, peptides or proteins with a polyQ expansion form fibrillar aggregates with similar properties to those formed by amyloid β (A β) peptide. However, at least *in vivo*, investigations using the techniques mentioned above have shown that the pathway from soluble monomeric polyQ protein to the formation of fibrillar aggregates is a multi-step process, outlined in Fig. 1 [164, 172]. As an additional step to the pathway *in vivo*, IBs, containing not only the

polyQ protein, but a variety of other proteins and cellular components (like aggresomes), are formed. These IBs, far from being static end-stage structures, are also highly dynamic [26, 81, 157].

Fibrillar polyQ protein aggregates have been well studied using EM and AFM (Fig. 1) and exhibit several defining features of amyloid, e.g. binding of thioflavin T, congo red birefringence, and they react with a “generic” anti-amyloid antibody [30, 147]. Fibrils are formed from short, sometimes curvilinear protofibrils [56, 126], tend to be approximately 10 nm in diameter and up to 2 μ m in length with a beaded morphology [15, 46, 56], have a high β -content [126], and self-associate eventually forming large branched structures [35, 93] (also see Fig. 1).

A range of aggregation intermediates between monomeric and fibrillar polyQ have been identified. Elegant studies by the Muchowski lab using AFM to study the morphology of prefibrillar aggregates of httex1 and polyQ peptides, has identified a progression of aggregates from spherical structures with 5–65 nm diameter, rare annular structures composed of approximately 100 nm diameter (made of smaller annular and spherical structures), amorphous structures, protofibrils and fibrils [94, 172]. The intermediates formed were SDS-insoluble and could be altered by changes in concentration and incubation time, as well as by the addition of heat shock proteins (HSPs). The ability of HSPs (HSP40 and 70) to increase fibrillisation with a concomitant decrease in spherical structures led to the proposal that it is a particular conformation of monomer that leads to the formation of fibrils (which can be partitioned by HSPs), with spherical, annular and amorphous aggregates being “off-pathway” for fibril formation. The same study showed that the polyQ stretch is buried upon spherical oligomer and annular oligomer formation, whereas the stretch appears to be exposed in fibrils. Small oligomeric structures of approximately 5–20 nm diameter have also been detected by others using EM and AFM to monitor aggregation of httex1 constructs and ataxin-3 *in vitro* [46, 56, 68, 111, 126] (Fig. 1), and these oligomers have been shown to decorate fibrils formed by a GST-httex1 construct, indicating that these small oligomers are added to the ordered fibrillar structures to increase length or branching of the fibrils [68]. Annular structures of polyQ proteins, observed by AFM ([94, 172]; Fig. 1), similar to those reported to be formed by α -synuclein [87] have also been predicted by molecular modelling studies on polyQ peptides [101]. These annular oligomers have been suggested to be cytotoxic via the formation of ion-permeable pores in the membrane [87], further discussed below.

Visualisation of the morphology of polyQ oligomers formed *in vivo* is challenging, but has been achieved by tandem affinity purification of oligomers of httex1 with different affinity tags formed in cultured cells followed by

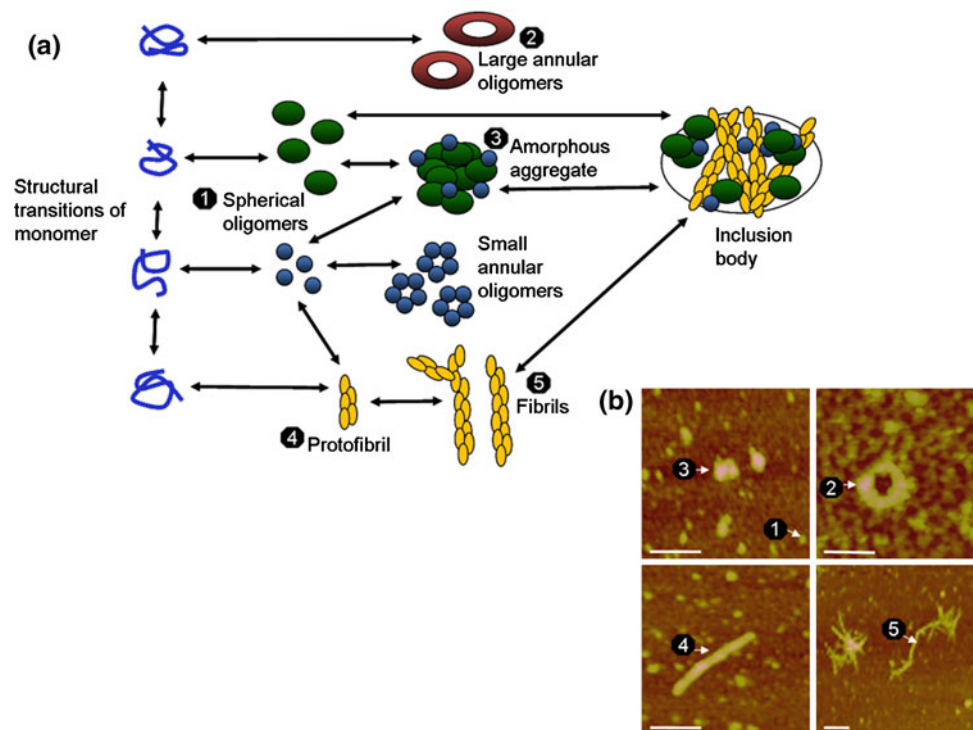


Fig. 1 Model of aggregation of polyQ proteins based on several EM and AFM studies of oligomeric forms. **a** PolyQ proteins are likely to consist of several distinct monomeric conformations (as proposed by Muchowski and colleagues [172]), one or many of which may lead to the formation of the spherical, annular or amorphous oligomeric structures, as indicated. Some of these are “on-pathway” for the formation of fibrillar structures (which could also be formed directly

from monomer), others of which are “off-pathway” for fibril formation, but may be “on-pathway” for inclusion body formation. *Numbers in circles* indicate where example AFM images of the structure are shown in **b**. **b** AFM analysis of httEx1Q53 aggregation reactions after cleavage from a GST-fusion protein. *Scale bars* 100 nm

detection by AFM and EM [111]. This revealed the formation of globular structures of 4–50 nm in diameter and the larger globules appeared to be formed from smaller structures. FCS has also been used to monitor oligomer formation in cultured cells, and shows a time-dependent increase in diffusion time and particle size [160]. This technique will be particularly useful in determining more precisely the effect of proposed polyQ-aggregation inhibitors, as demonstrated for Congo Red and QBP1 [160], which previously were usually characterised by their ability to prevent formation of SDS-insoluble material using a filter trap assay. Recently, the Seprion ligand has been used to isolate aggregated htt fragments from HD mouse models using an ELISA-based approach [143]. AFM and SDS-PAGE analysis of the aggregated protein showed that oligomeric, proto-fibrillar and fibrillar structures could be isolated and that these were similar in morphology and dimensions for two different HD mouse models and to species generated by recombinant httex1 protein. This provides useful evidence that *in vitro* similar aggregated structures can be produced to those found *in vivo*.

Factors affecting aggregation of polyQ proteins

Rather than discussing the many factors that affect polyQ aggregation in detail, which is beyond the scope of this review, we shortly summarise key factors and mechanisms that could have profound effects on the aggregation behaviour of polyQ proteins.

Postranslational modification and cleavage of polyQ proteins modulate polyQ aggregation

Recently, it has become clear that the polyQ expansion is not the only factor that determines the propensity of polyQ proteins to aggregate. Various posttranslational modifications may impact on the polyQ-aggregation process and its ensuing toxicity. For example, htt is both phosphorylated and SUMOylated at its first 17 N-terminal amino acids, which affect its aggregation properties [136, 156]. The most prevalent modification of httex1 is the acetylation and phosphorylation of threonine-3, and its phosphorylation alters httEx1 aggregation in cells and *in vivo* [2]. However,

posttranslational modifications also determine whether htt and its cleavage fragments accumulate in the nucleus or not [136]. The nuclear space appears to be an aggregation-prone environment, perhaps due to the absence of autophagic activity (suggested to degrade misfolded polyQ species, reviewed in [138]) or more directly through interactions of polyQ proteins with DNA (see below). However, htt is also phosphorylated at S421 by Akt and SGK [71, 131] and at S1181 and 1201 by Cdk5 that significantly modulate its toxic properties. In neurons activation of the IGF-1/Akt pathway and phosphorylation of S421 reduces the number of IBs found in the nucleus [71]. Ataxin-3 phosphorylation at S256 by GSK3 β has also been shown to modulate its aggregation [49]. Phosphorylation of Ataxin-1 at S776 by Akt appears critical to nuclear IB formation and S776A mutant protein does not form IBs in cultured cells [29, 47]. SUMOylation of the AR has also been shown to attenuate its aggregation [112]. It is currently unknown, how phosphorylation or indeed other modifications affect the folding/misfolding of polyQ proteins, as so far aggregation has either been recorded via IB counts within cells or measured biochemically by dot blot assays and hence effects on structural properties or oligomerisation remain to be determined.

Of course, posttranslational modifications also affect the degradation of polyQ proteins and hence their accumulation and aggregation indirectly, within a particular compartment. For example, acetylation of htt at K444 facilitates its trafficking to autophagosomes and increases its clearance via macroautophagy [77]. As many of these sites are located in the vicinity of caspase/other cleavage sites it is possible that phosphorylation also affects htt cleavage to a more aggregation-prone toxic fragment. Indeed Schilling and colleagues [150] found that mutation of S536, that is located in the proteolytic susceptibility domain of htt, to aspartic acid, inhibited calpain cleavage and reduced htt toxicity.

The cleavage of polyQ proteins such as enzymatic processing by caspases, calpains and unknown proteases is a common event during polyQ pathology, but likely also a normal physiological process for polyQ protein function [82]. Since the finding by Wellington and colleagues [179] that htt, ataxin-3, AR and atrophin-1 contain caspase cleavage sites, the *in vitro* and *in vivo* evidence for cleavage of polyQ proteins has steadily increased [173], but its role is uncertain. However, what has been clearly demonstrated is that cleavage of full-length polyQ proteins into smaller fragments modulates their intracellular localisation, aggregation and toxicity [62]. Such findings led Hayden and colleagues [63] to propose the “toxic fragment hypothesis” that has been confirmed by many cell- and animal studies. Therefore, it will be critical to explore how the cleavage and various posttranslational modifications of

polyQ proteins directly influence their conformational and oligomeric states.

Effect of cellular factors and intracellular localisation on polyQ aggregation

It is well established that a change in intracellular localisation or cell type can have a significant effect on the aggregation state of polyQ proteins. For example, the AR forms large aggregates in the cytoplasm, but AR-transport into the nucleus prevented the formation of these aggregates [12]. On the other hand, nuclear targeting of an ataxin-3 fragment increased its aggregation [122]. Targeting httex1 to the endoplasmic reticulum, the nucleus or mitochondria was found to result in profound differences in the formation of SDS-insoluble material [137]. The effect of cellular localisation and cell type on polyQ aggregation is unsurprising given the demonstrated effects on polyQ aggregation of the cytoskeleton, e.g. [110], DNA, e.g. [119], lipids, e.g. [158], glucose, e.g. [133], metals, e.g. [52, 135] and osmolytes, e.g. [72], all of which are likely to vary between cellular compartments, in addition to cleavage enzymes and enzymes involved in posttranslational modification, as discussed above. Effects of macromolecular crowding in different cell compartments could also play an important role in affecting polyQ oligomerisation. Furthermore, key regulators of polyQ aggregation such as molecular chaperones (e.g. HSPs) also vary in their presence and level of expression in particular cell types and within specific compartments [130]. An effect on aggregation of htt in relation to the presence and state of prion protein has also been reported in yeast (Rnq1) and mammalian neuronal cell lines (PrP^c) [91, 106]. Depletion of Rnq1 caused a reduction in polyQ aggregation in yeast and this effect was dependent on the prion conformation whereas in mammalian cells the depletion of PrP^c increased and its overexpression decreased Httex1 aggregation.

It is currently unclear how the oligomerisation process of a polyQ protein is affected by the combination of such factors and future studies must show the relationships between these.

Flanking sequences and domains influence polyQ aggregation

As mentioned in “Effects of protein context on conformation of the polyQ stretch” sequences flanking the polyQ stretch significantly impact on aggregation and toxicity of polyQ proteins (for a review see [146]). A striking study in yeast showed that addition of particular sequences, e.g. a FLAG tag, flanking the polyQ region of htt could convert a non-toxic protein to a toxic species and modulate the

morphology of aggregates formed [45]. The first 17 amino acids of htt directly preceding the polyQ stretch appear particularly influential, forming an amphipathic alpha-helical membrane-binding domain, disruption of which abolishes aggregation and increases toxicity, including the disruption of intracellular calcium homeostasis [7, 80, 136, 164]. In addition, the polyproline sequence following the polyQ stretch is highly influential in the aggregation and toxicity of htt [40]. Proline residues also follow the polyQ stretches in ataxin-1 and ataxin-7 and both proline and histidine residues within the polyQ stretch have been shown to mediate aggregation and toxicity of ataxin-1 [76, 151, 152]. The mutation of two residues significantly outside the polyQ stretch in the AR converted the wild-type receptor to an aggregation-prone toxic species, yet decreased the toxicity of the polyQ expanded, mutant receptor [55].

For at least some polyQ proteins (e.g. ataxin-1 and ataxin-3), regions outside the polyQ stretch are able to aggregate independently, e.g. [46], indicating that other regions can modulate and reinforce aggregation of the polyQ protein and indeed for ataxin-3, it has been reported that the formation of early ataxin-3 oligomers that can eventually lead to fibrillisation, is mediated by the Josephin domain [56]. The polyQ stretch may therefore act to destabilise surrounding domains, increasing their propensity to aggregate as has been implicated for htt [73].

What are the neurotoxic polyQ species?

The question of whether polyQ aggregation is itself neurotoxic is a highly debated issue. Many have argued that aggregation is toxic, whereas others have suggested that aggregation is simply a by-product of disease pathology and yet others imply that aggregates are protective, acting as a sink for otherwise toxic monomers. Although there have been a plethora of studies on aggregation inhibition and promotion, it is unfortunate that many previous studies testing the effects of compounds on polyQ aggregation did not investigate effects on toxicity.

Defining aggregate forms

One confounding factor in the debate on the nature of the neurotoxic polyQ species used to be the definition of the term “aggregate”. Traditionally many have used the term aggregate in the context of IBs, with measures of aggregation usually being simply a count of IBs and/or detection of SDS-insoluble material. However, now that methods to identify oligomeric species of polyQ proteins have been developed, along with the proposal of an aggregation pathway with intermediates on- and off-pathway to

fibrillisation and insoluble species, future studies should address the relationship between the specific types of aggregates and neurotoxicity. Here, the use of the new, more sensitive technologies to identify the effects of aggregation inhibitors and promoters of oligomeric intermediates, previously used to show effects on toxicity, are required. For example, a recent study using agarose gel electrophoresis to detect oligomeric species in HD mouse and cell models, showed that oligomers could be detected before any measurable behavioural phenotype and the size of the aggregates correlated with disease progression [178]. FCS has also proven to be a useful measure of alterations in aggregate size [34]. Now that different shapes and forms of polyQ species can be produced in vitro (Fig. 1) and characterised by AFM and biochemically, their neurotoxic potential can be tested. One difficulty, however, is to transfer a particular aggregate species of a sufficiently homogenous nature and quantity into cells. Recent work by Teplow and colleagues [116] in which specific oligomers of A β have been chemically stabilised via cross-linking and fractionated in pure form provides a promising step forward in this direction.

Effects of altering aggregation on toxicity

A variety of compounds have been shown to alter the IB load in cells and/or mouse models. Compounds used to promote the formation of IBs have been shown to reduce cytotoxicity in polyQ cell models [20], and in addition, cells containing an htt IB exhibited improved survival compared to cells containing diffuse mutant htt fragments [6, 145]. Strikingly, IBs have been predominantly observed in spared interneurons in the striatum of HD patient brains, with few, if any IBs found within the vulnerable medium spiny striatal neurons (MSNs) [84]. A zebra fish model also revealed that apoptotic cells killed by expression of expanded polyQ htt fragments did not contain IBs [148].

In contrast, several different methods of reducing aggregation, including addition of peptides (e.g. QBP1), expression of intrabodies, molecular chaperones and their interacting proteins and addition of oligonucleotides and a variety of small molecules have been shown to be protective in cell and animal models of polyQ diseases. However, it is worth noting that all these methods require binding of the aggregation-inhibitor to the polyQ protein and therefore have the potential to alter monomeric and oligomeric species as well as affecting aggregation per se. Methods of increasing IB formation such as increasing ubiquitination [38] have also been shown to increase toxicity. On the other hand, some studies co-expressing molecular chaperones in cell models have indicated that toxicity is modified without changing the level of IB formation [189]. Again,

one of the difficulties with all these studies is that, the method of monitoring aggregation is by IB count and/or SDS-insoluble material detection, without the effect on oligomeric species being monitored and therefore this could go some way in explaining the discrepancies, in addition to the variety of different models used. This issue is exemplified by a recent *in vivo* study of effects of aggregation, which shows that knockout of Hsp70 in the R6/2 HD mouse model significantly increased the average size and number of IBs, without affecting the total fibrillar aggregates measured biochemically, causing an increase in severity and behavioural phenotypes in this mouse model [171]. Therefore, Hsp70 may normally bind to monomeric and/or small oligomeric forms of polyQ protein that are off-pathway for fibril formation, but are toxic and lead to IB formation.

Methods which target specific aggregate structures provide great potential in investigating the most toxic species of aggregate. Towards this goal, a recent study has shown that an intrabody, which binds specifically to fibrils and increases fibrillisation of httex1, with no measurable effect on oligomers, increases toxicity of the polyQ protein [85]. A study using time-lapse live-cell fluorescence microscopy to measure aggregation of httex1 in PC12 cells was able to identify the rapid formation of “microaggregates” (<1 μm in diameter) visible by epifluorescent light microscopy, with a prolonged time required for formation of classical IBs. The detection of aggregation did not coincide with death, but detectable aggregation did correlate with an earlier cell death and the larger the final aggregate formed, the longer the cell survived [60]. Microaggregates, i.e. soluble high molecular weight complexes with a size of 700–7,000 kDa (i.e. approximately 20–200 molecules) have also been observed in a mouse model expressing polyQ-expanded ataxin-3, which are found in the soluble fraction of brain lysates, but are SDS-resistant [182]. The appearance of microaggregates in cell and mouse model systems described above, favours the “multi-nucleation” hypothesis of polyQ aggregation. Increasing the formation of microaggregates by downregulating the ubiquitin ligase, CHIP, accelerated disease progression in these mice, also indicating that the microaggregates are toxic [182]. Another study supporting the toxicity of soluble microaggregates used a yeast model expressing polyQ-expanded AR, in which a 67Q stretch formed SDS-insoluble IBs, whereas a 102Q stretch formed some irregularly shaped soluble microaggregates, but the 102Q stretch was the more toxic form [149]. In another yeast model, httex1 IBs were found to be non-toxic, whilst amorphous aggregates tended to be toxic, although this was not always the case, suggesting that morphology alone does not necessarily determine cytotoxicity [45]. It is possible that the increase in surface area of microaggregates

compared to large foci like IBs increases their toxicity, for example, by an increased ability to sequester other proteins. However, two studies show that downregulation of chaperonins causes an increase in IB formation and soluble aggregates of polyQ proteins, which is associated with an increase in toxicity but that upregulation of a chaperonin can cause the formation of multiple smaller foci, which are linked with a decrease in toxicity [83, 161].

The toxicity of polyQ proteins at the monomeric level is supported by the increased toxicity after microinjection of a monomeric thioredoxin-polyQ fusion protein with a β -sheet conformation compared to the toxicity induced by an α -helical conformation of the same protein. However, the injection of soluble oligomeric and fibrillar forms of this protein were also toxic to some extent [113]. In addition, insertion of proline residues to disrupt the monomeric conformation of the thioredoxin-polyQ protein also significantly reduced toxicity in a cell model [127]. It has also been suggested that the promotion of oligomeric species “off pathway” from fibril production can be cytoprotective. For example, the promotion of “benign” oligomers by the cooperation of chaperones (Hsp70/40) and a chaperonin (TriC) has been reported to prevent toxicity of htt [13].

In summary, it is currently unclear what the exact neurotoxic polyQ species are, but from the studies discussed above, it seems that both monomeric- and oligomeric polyQ states and assemblies confer increased cytotoxicity, compared to fibrillar structures and IBs.

Why might oligomeric polyQ species be cytotoxic?

As outlined above, there is mixed evidence regarding the toxicity of aggregation, and virtually all species of polyQ protein from monomeric polyQ, through small oligomeric and larger microscopically visible aggregate structures have been implicated in toxicity in some way. However, below we focus particularly on how oligomer formation of polyQ proteins may be toxic, although some proposed mechanisms are likely to be inseparable from toxicity caused by larger aggregates and/or IB formation.

Gain versus loss of function

As summarised in the introduction, polyQ proteins have defined roles in a normal cellular context and hence oligomerisation of polyQ proteins may lead to a loss of function (see “Introduction” for references on gain vs. loss of function during polyQ disease). Aggregation of polyQ-containing proteins into oligomers can prevent them from performing their normal cellular function either by sequestering the proteins away from their normal cellular

location (see below) or by preventing interactions with their protein network. Indeed htt protein has been shown to have a substantial protein network and has been implicated in a wide variety of cellular functions. Furthermore, mutant htt has been shown to promote fibrillogenesis of wild-type htt [24]. However, individuals that are hemizygous for htt do not show any symptoms of HD [5], indicating that a loss of function of htt is not the central cause of this disease. However, it is likely that loss of function of polyQ proteins goes some way to exacerbate the disease phenotype. A toxic gain of function is likely given that the polyQ expansion appears to be the only aspect that links the various polyQ proteins to brain pathology and that polyQ peptides are intrinsically cytotoxic [74, 102], even in organisms such as *Caenorhabditis elegans*, which have no functional homologs of the polyQ disease proteins [48].

Oligomerisation itself could cause a change in cellular localisation, which may lead to further aggregation due to abnormal interactions with cellular material. For example, a polyQ expansion in htt is known to impair its nuclear export and it can be envisaged that oligomerisation of the protein could prevent shuttling between the nucleus and cytoplasm due to an increase in size and/or a change in conformation of the protein preventing the interaction with the nuclear pore protein complex [33]. Changes in nucleocytoplasmic shuttling of ataxin-3 have also been implicated in MJD [97]. The physical accumulation of protein oligomers in unusual cellular compartments can clearly cause a whole host of problems for neurons, including synaptic dysfunction and axonal transport deficits [92, 107], that are not further discussed here.

Membrane disruption, insertion and translocation

It has been suggested for other aggregation-prone proteins, including α -synuclein, that annular oligomers can form pores in the membranes of cells, implicating membrane permeabilisation as an inducer method of toxicity for protein aggregation. Htt has been shown to associate with membranes [158]. Recently htt with a wild-type polyQ stretch has been shown to interact in vitro with lipid vesicles and lipid rafts in a HD mouse model with mutant htt interacting with lipids not recognised by the wild-type htt and having a greater affinity with lipid rafts [78]. Therefore, mutant htt could disturb membrane-dependent signalling pathways and membrane trafficking. It has been previously shown that polyQ peptides can form channels in lipid bilayers [109] and recently it was shown that an expanded polyQ stretch can cause penetration of htt into lipid bilayers [79]. The effect of membrane insertion could be due to oligomerisation, and could affect membrane protein function and/or lipid composition of the membrane, causing the membrane to be dysfunctional and possibly

forming pores, as predicted for α -synuclein. Such pores have, however, never been observed in vivo. Membrane dysfunction can be linked to many of the defects observed in polyQ disease models, including defects at the synapse and with mitochondria [65]. Indeed htt has been reported to associate with mitochondria [117]. No data are currently available for polyQ proteins other than htt in relation to membrane interactions.

In addition, fibrils formed by polyQ peptides applied extracellularly have recently been demonstrated to have the ability to cross the membrane and enter the cytoplasm [134]. This is of particular interest, as recent investigations with various other amyloid-forming proteins including tau and α -synuclein have suggested a degree of “infectivity” of these proteins due to a templated conformational change (for a review see [54]). Therefore, even if the fibrillar form of a polyQ protein is not toxic, its ability if released, e.g. due to neuronal death, to seed aggregation in another cell, could induce toxicity and perhaps propagating this “aggregation signal” throughout the CNS.

Impairment of the protein turnover machineries

Although the autophagic/lysosomal system appears to deal with the degradation of aggregation-prone molecules [187], the proteasome may be able to deal with existing polyQ aggregates, if expression of the protein in neurons is switched off [187] and there is evidence that polyQ stretches that are targeted to the UPS are at least partially degraded in cell systems [69]. These findings argue against the fact that polyQ oligomers could be trapped irreversibly in the proteasome. Although previous studies of proteasomal degradation of polyQ peptides in vitro indicate that the eukaryotic proteasome is unable to cleave within polyQ stretches [167], a recent study using mass spectrometry suggests that in the presence of a proteasome activator, PA28gamma (K188E), a fluorescein labelled polyQ peptide was able to be cleaved after each glutamine residue [128]. Despite this, oligomerisation of polyQ proteins is at least likely to increase the time required for breakdown of the protein, leading to slower degradation of the polyQ protein and also of other proteins reliant on proteasomal degradation, possibly leading to the accumulation of proteins, affecting signalling cascades (as has been suggested for anaphase-promoting complex substrates [19]) and synaptic function [176]. An increase in pure polyQ stretches being released by the proteasome would place further demand on peptidases that are able to cleave polyQ stretches, in particular puromycin-sensitive aminopeptidase [18] and could cause a further increase in aggregation and a further decline in proteasomal and peptidase activity [132]. Proteasome dysfunction has been shown to occur before the accumulation of IBs in cells [14] and therefore

oligomer formation may be the cause of the decrease in efficiency of the proteasome. Fluorescence live-cell imaging showed that the proteasome can be irreversibly sequestered within aggregates formed by overexpressed mutant httex1 or simple polyQ expansions [69] and hence as aggregates grow larger, physical “trapping” of the proteasome may also play a role in decreased efficiency of proteasomal function.

Sequestration of other cellular proteins and transcriptional impairment

The demonstration that proteins other than the polyQ disease protein itself can be found in IBs opens up the possibility that aggregation of the polyQ protein sequesters other polyQ proteins, disabling their function in some way. For example, and as discussed above, the aggregation of polyQ proteins can alter their conformation and therefore binding of other polyQ stretch-containing proteins by oligomers could cause the protein to switch to an inactive conformational state. An important class of polyQ-containing proteins are transcription factors, including CBP, TBP and Sp1. The glutamine-rich regions in these proteins are critical for the control of interactions between activator and suppressor proteins and general mediators that affect RNA polymerase II activity. Indeed several of the polyQ disease proteins have been implicated to play a role in transcription with transcriptional dysregulation being a common feature of polyQ diseases. Incorporation of these transcription factors into oligomers could severely alter the function of these factors, and direct interactions between transcription factors and polyQ proteins have been widely reported [28]. However, it is not only transcription factors, but also other cellular proteins that are sequestered and/or bind reversibly to polyQ aggregates such as molecular chaperones [185] and proteins of the UPS and autophagolysosomal system [75]. Proteins containing not only pure polyQ stretches but also Q/N-rich stretches have been reported to interact with expanded polyQ proteins, directly affecting their functions. For example, mutant htt efficiently seeds the fibrillisation of the RNA-binding protein T-cell intracellular antigen-1 (TIA-1), which is sequestered into IBs in an HD mouse model and polyQ tracts are potent inducers of amyloid formation by prion proteins, Sup35 and Rnq1, in yeast [59]. Given that the total number of Q/N-rich domains has been estimated to be 200 in the human proteome, this suggests that effects of changes in conformation and aggregation states of these proteins must somehow contribute to the pathology of polyQ diseases [66]. PolyQ aggregates have been shown to contain other proteins involved in neurodegeneration such as alpha-synuclein (involved in PD) [27], which causes a translocation of alpha-synuclein away from the synapse. Htt has

also been associated with intracellular tangles and inclusions found in AD and Pick’s Disease, especially associated with cytoskeletal filaments [154], providing evidence that an abnormal interaction with neurofilaments could contribute to HD pathology.

An even more general mechanism of polyQ-induced toxicity has been proposed, based on alterations in protein homeostasis (for a review see [8]). The expression of a polyQ stretch in several diverse temperature-sensitive mutations in *C. elegans* caused the temperature-sensitive mutation to exhibit their phenotypes at the permissive conditions, showing that the function of structurally and functionally unrelated proteins is affected by the expression of the aggregation-prone polyQ protein and indicating that aggregation of the polyQ protein may prevent the cell from dealing with unstable proteins that it would otherwise cope with by (re)-folding and clearance mechanisms [58]. This finding would provide a mechanism for the characteristic slow progression of polyQ diseases, i.e. the cell initially manages the aggregation of polyQ protein, but the accumulation of other misfolded and damaged proteins eventually overwhelms the cell’s capabilities (for a review see [65]).

Most studies have addressed the issue of sequestration of normal cellular proteins by co-immunostaining of IBs in vitro or in vivo, rather than investigating the sequestration of cellular proteins to polyQ oligomers. The relevance of sequestration of cellular proteins for polyQ disease is difficult to assess as most of the published studies did not quantify the degree of abnormal sequestration into polyQ aggregates.

ROS production

Another proposed mechanism of toxicity due to expanded polyQ proteins is the production of reactive oxygen species (ROS). Htt and ataxin-3 have both been shown to bind redox-active metals and the effect of metals in increasing aggregation of full-length polyQ proteins has also been reported [52, 135]. Our observations show that copper increases the fibrillisation of recombinant httex1 in vitro as measured by AFM and increases httex1 IB formation within cells [64]. It is therefore possible that oligomerisation of polyQ proteins creates an environment in which redox-active metals can react with molecular oxygen to form ROS, as suggested for other amyloidogenic proteins [3]. Alteration of ROS-producing systems (e.g. by mitochondria) by polyQ oligomers is another way by which oligomerisation could modulate the cellular redox-homeostasis. Both wildtype and mutant htt and htt fragments have indeed been localised to the outer mitochondrial membrane and appear to induce several mitochondrial dysfunctions (reviewed in [129]). Additionally, polyQ-expanded

htttx1 appears to disrupt the mitochondrial fusion–fission machinery [175] and to recruit a number of glycolytic enzymes such as GAPDH, enolase and fructose bis-phosphate into aggregates [177]. It is currently unclear whether and how polyQ oligomerisation disrupts the cellular redox-homeostasis.

PolyQ aggregation and the evolution of brain- and peripheral polyQ pathology

As mentioned above polyQ diseases are primarily disorders of the CNS, despite the polyQ-containing proteins being expressed in the CNS and the periphery at similar levels. PolyQ aggregation in the CNS during polyQ pathology is widespread and although immunohistochemical analysis of IBs in DRPLA, SCA3, SCA-7 and HD postmortem brain shows an overlap with the neuropathology that occurs in these disorders, it appears that polyQ aggregation is more widespread [11, 41, 70]. The brain-region-specific- and intracellular localisation of aggregated polyQ protein varies with the degree of polyQ pathology and disease progression. For example in HD, DiFiglia and colleagues [41] showed that N-terminal htt forms neuronal intranuclear inclusion bodies (NIIs) that are highly heterogeneous in composition containing a mixture of several forms of filaments/fibrils and granules/oligomers, something that is also observed in brains of MJD patients [186]. Interestingly, it has been recently shown that oligomers of cortical neurons observed by EM analysis in the human HD brain were similar in lateral dimension to oligomers observed by AFM using recombinant htttx1 ([94], see Fig. 1). Sapp et al. [140] reported that NIIs are more frequent in the cortex than in the striatum and that in earlier stages of disease accumulation of N-terminal mutant htt also occurs in the cytoplasm. Furthermore, NIIs are found in the allocortex in addition to the neostriatum and neocortex and NII frequency in the neocortex is highest in juvenile patients [96]. The frequency of the normally large ubiquitinated nuclear and cytoplasmic aggregates of mutant htt is 1–7% of cortical, striatal and thalamic neurons and neurophil of patients with adult onset of symptoms and up to 21% of cortical neurons in individuals with juvenile onset ([170] and refs therein) and therefore much lower compared to some HD rodent models (see below). However, systematic correlative biochemical studies on polyQ aggregation that also consider polyQ precursors such as oligomeric structures in the HD brain and indeed in other polyQ diseases are missing. Therefore, it is currently unclear how polyQ oligomerisation relates to the complex CNS pathology in HD and polyQ diseases and as noted in a recent review on HD neuropathology, increased effort should go into analysing human diseased brains, rather than deducing too

many aspects of polyQ pathology from animal models [170].

Numerous reports identified significant abnormalities in the peripheral tissues of HD patients including altered glucose homeostasis and (sub)-cellular alterations in fibroblasts, lymphocytes and erythrocytes, but the evidence of polyQ aggregation in such tissues is rare or non-existent (reviewed in [141]). This is in contrast to some of the mouse models of polyQ disorders that have been extensively analysed in relation to polyQ aggregation. The most studied HD rodent model is the R6/2 line expressing htttx1 with a CAG expansion of 145 [99]. In the R6/2 line, IBs are first detected in the cortex and striatum in week 3–5 after birth, before onset of symptoms co-incident with brain weight loss [142]. Later a massive production of IBs occurs in many CNS regions, and currently novel approaches for more sensitive biochemical aggregate detection are being used to understand the role of polyQ oligomerisation and pathology in R6/2 and other mouse models [86, 143, 178]. Such studies must now be extended to human CNS and peripheral tissues of polyQ pathologies to learn more about the role of oligomerisation during the evolution of pathology. For a report on peripheral events and the wide spectrum of the clinically heterogeneous group of SCAs the reader is referred to the paper by Manto [100].

Non-CNS polyQ IBs in the R6/2 HD mouse model appear to occur later, but indeed in many peripheral tissues such as skeletal muscle, heart, liver, adrenal glands, pancreas, kidney, and this correlates with a progressive decrease in size of these organs [142]. Such abnormalities could be related to the polyQ-aggregation process, but other cellular dysfunctions (e.g. altered transcription and endo/exocytosis, see above) unrelated to toxic polyQ oligomerisation may also be important. IBs in peripheral tissue have also been reported in SBMA [95] and in an SBMA mouse model [1]. The factors that determine polyQ cell-specific aggregation are unclear and likely include a multitude of parameters (see “[Effect of cellular factors and intracellular localisation on polyQ aggregation](#)”). One obvious important reason for aggregation pathology in the CNS is that the post-mitotic nature of neurons (or muscle in the periphery) prevents a reduction of aggregate load by cell division, whereas in cycling cells the aggregated material would be distributed between daughter cells. Cycling cells also have the opportunity to redistribute aggregates from the nucleus into the cytoplasm during cell division when the nuclear membrane disintegrates hence potentially reducing toxicity via autophagic/lysosomal degradation that only occurs in the cytoplasm (see “[Effect of cellular factors and intracellular localisation on polyQ aggregation](#)”). However, this reasoning would not explain neuronal subtype-specific toxicity and the sometimes observed polyQ aggregation and altered cellular function in

glial cells (e.g. astrocytes or microglia) that are increasingly believed to contribute to polyQ pathology via non-cell autonomous mechanisms [21, 22, 121, 139].

Conclusion

The current evidence indicates that a conformational change induced by the polyQ expansion, also determined by the protein sequence surrounding the polyQ stretch, significantly impacts on aggregation and toxicity. It is likely that several different conformations of monomeric polyQ protein exist and lead to particular oligomeric intermediates. It is becoming clear that although IBs are clear pathological hallmarks of the polyQ diseases, earlier intermediates are the likely cause of neuronal dysfunction. If this were the case then accumulation into larger aggregates and IBs may well be a cell-protective mechanism, at least in the short term. However, it is difficult to imagine how the presence of such a large intracellular structure, would not at some point lead to neurotoxicity, whether it be due to its large physical size disrupting cellular transport and other processes, its ability to sequester other essential cellular proteins and impair the protein turnover machinery or a combination of these factors.

As structurally defined polyQ peptides and proteins can now be produced *in vitro* and the polyQ-aggregation behaviour manipulated *in vivo*, correlative studies can be performed. This will determine the relative neurotoxic impact of different polyQ species. However, *in vivo* studies investigating the causal relationships between polyQ aggregation and toxicity remain technically challenging and the effects of oligomers on toxicity remain unclear. As no reports in the literature have been found of polyQ oligomers being present in normal individuals, it would seem prudent to focus efforts on preventing the early stages of aggregation. Thus, it is important to invest time in obtaining more detail on the structures of monomeric and oligomeric polyQ protein species produced *in vivo* and to make further attempts to model these species in order to evaluate their respective toxicities. Such studies will assist in the design of effective modifiers of early aggregation, such as intrabodies, in the hope of producing treatments to prevent and/or delay the devastating symptoms of the polyQ diseases.

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