

# Polyglutamine diseases: emerging concepts in pathogenesis and therapy

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**Polyglutamine diseases are a family of neurodegenerative conditions that each derive from a CAG triplet repeat expansion in a specific gene. This produces a pathogenic protein that contains a critically expanded tract of glutamines. These prototypical protein misfolding disorders include Huntington disease, spinobulbar muscular atrophy, dentatorubral-pallidoluysian atrophy and several spinocerebellar ataxias. This article reviews the emerging concepts in pathogenesis and therapy. Key ideas include the role of proteolytic cleavage, the importance of conformational change in the pathogenic proteins, the role of protein aggregation and the importance of transcriptional and metabolic disturbances. The relative role of functional perturbation in a target protein induced by a polyglutamine expansion is also discussed. Therapeutic strategies include counteracting cellular perturbations and direct targeting of polyglutamine protein expression, cleavage or conformation.**

## OVERVIEW (INTRODUCTION)

The first CAG triplet repeat disease was described in 1991: a mutation in the androgen receptor (AR) gene that causes the progressive motor neuron disease spinal bulbar muscular atrophy (SBMA) (1). Eight other related diseases have now been described; all derive from a CAG codon expansion past a specific threshold (Table 1). CAG encodes glutamine, and thus affected proteins have elongated glutamine tracts (2). These prototypical disorders of protein folding are collectively termed 'polyglutamine' diseases, and include SBMA, Huntington disease (HD), several spinocerebellar ataxias (SCAs) and dentatorubral-pallidoluysian atrophy (DRPLA) (2). Each, with the exception of SCA6 (which forms cytoplasmic aggregates that stain negative for ubiquitin) (3), features the accumulation of the mutant protein in large intranuclear inclusions (4). Initially, it was proposed that these large inclusions were the proximal cause of neurodegeneration. However, in many cases, the appearance of intranuclear inclusions has been dissociated from the pathogenic process (5–8). A more nuanced interpretation that distinguishes protein aggregation (the self-association of peptides) from inclusion bodies (macromolecular structures formed by the cell) is thus warranted (9,10). The general genetic and pathogenic features of these diseases have been reviewed

extensively in the past (2,4,11,12). This review will focus on emerging areas of research, emphasizing where mechanistic knowledge might impact treatment (see Fig. 1 for pathogenesis and Fig. 2 for therapeutic strategies).

## PATHOGENESIS

### Proteolytic cleavage

Several polyglutamine diseases, such as HD, SBMA and SCA3, appear clearly linked to proteolytic cleavage that liberates toxic polyglutamine-containing fragments (10,13–20). In others, such as SCA1, no evidence has been found for proteolysis. Recently full-length mouse models of HD were created with mutations of a caspase-6 cleavage site in the huntingtin (Htt) protein. The pathogenic phenotype was attenuated in these animals (21). However, it remains possible that multiple cleavage events could produce a variety of toxic fragments in humans (22). Further work will thus be required to determine whether or not a single protease is responsible to initiate pathogenesis in HD and other polyglutamine diseases.

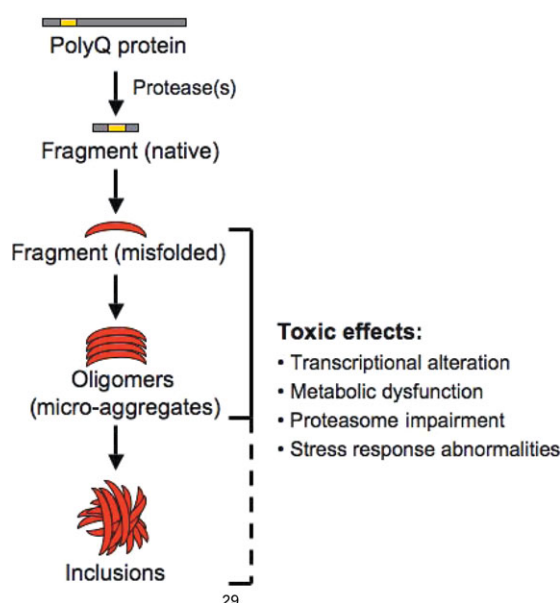
### Conformational change

An expanded polyglutamine protein is aggregation-prone *in vitro* (23), and the expanded polyglutamine tract within a

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**Table 1.** Polyglutamine diseases: emerging concepts in pathogenesis and therapy

Disease	Protein	Repeat	Normal repeat length	Pathogenic repeat length	Inclusions	Brain regions most affected
<i>Typical polyglutamine diseases (gain of function)</i>						
HD	Huntingtin	CAG	6–34	36–121	Nucleus and cytoplasm	Striatum, cerebral cortex
SBMA	Androgen receptor	CAG	9–36	38–62	Nucleus and cytoplasm	Anterior horn and bulbar neurons, dorsal root ganglia
DRPLA	Atrophin 1	CAG	7–34	49–88	Nucleus	Cerebellum, cerebral cortex, basal ganglia, Luys body
SCA1	Ataxin 1	CAG	6–39	40–82	Nucleus	Cerebellar Purkinje cells, dentate nucleus, brainstem
SCA2	Ataxin 2	CAG	15–24	32–200	Nucleus	Cerebellar Purkinje cells, brain stem, frontotemporal lobes
SCA3	Ataxin 3	CAG	13–36	61–84	Nucleus	Cerebellar dentate neurons, basal ganglia, brain stem, spinal cord
SCA7	Ataxin 7	CAG	4–35	37–306	Nucleus	Cerebellum, brain stem, macula, visual cortex
SCA17	TATA box binding protein	CAG	25–42	47–63	Nucleus	Cerebellar Purkinje cells, inferior olive
<i>Atypical polyglutamine disease (mimicked by missense mutation)</i>						
SCA6	$\alpha$ 1a voltage-dependent calcium channel subunit	CAG	4–20	20–29	Cytoplasm	Cerebellar Purkinje cells, dentate nucleus, inferior olive
<i>Atypical polyglutamine disease (reverse transcription of CTG repeats)</i>						
SCA8	Unknown	CTG	16–34	>74	Nucleus	Cerebellar Purkinje cells, granule cells, inferior olive



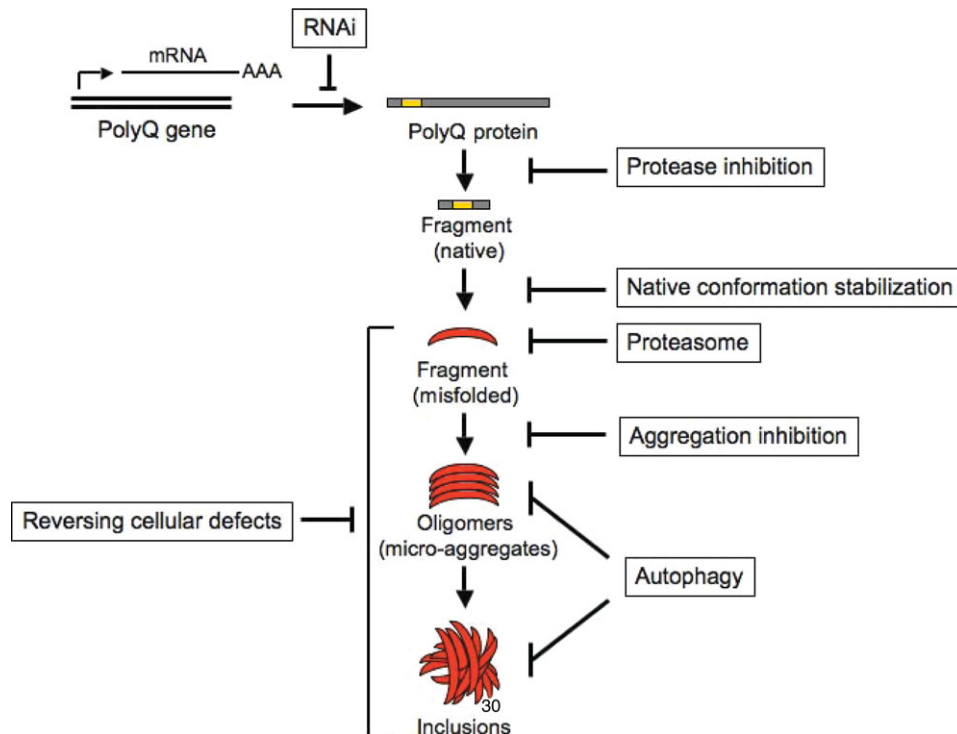
**Figure 1.** Pathogenesis of polyglutamine diseases. Many, but not all, polyglutamine diseases appear to be initiated by proteolytic cleavage to generate a toxic fragment. The expanded polyglutamine tract allows transition into a distinct conformation that may cause toxicity in several ways. The peptide may exert toxicity as a monomer or it may self-associate to form toxic oligomers. The oligomers can assemble into larger aggregated species and ultimately are deposited in macromolecular intracellular inclusions. The principal toxic effects of the aberrantly folded protein may include alterations in transcription, metabolism or impairment of the proteasome or stress response pathways.

target protein facilitates transition to a novel, toxic conformation (24,25). This aggregation-prone conformation is not an inevitable consequence of the expanded polyglutamine tract, however, and aggregation-prone conformers may form specifically in certain cell contexts (26). For example, two discernable forms of an expanded AR peptide, but not the unexpanded form, have been detected in cell extracts and appear to have distinct potential for aggregation and toxic-

ity (27). Similarly, a conformational change in expanded Htt peptide was found to precede its subsequent aggregation *in vitro* (24). Recent experiments demonstrate that two distinct conformational states result from fusion of an expanded polyglutamine tract to thioredoxin (25). In this case, an  $\alpha$ -helical state predominates initially after protein purification. After several days *in vitro*, this protein shifts to a  $\beta$ -sheet-rich conformation that is much more prone to aggregation, and which causes cellular toxicity upon microinjection into cultured cells. Intriguingly, a polyglutamine-binding peptide (QBP1) prevents the toxic conformational transition (25). Taken together, these data indicate that an expanded polyglutamine tract facilitates a crucial conformational transition, which can be influenced by numerous factors, including the protein context of the repeats and intracellular protein interactions.

### Transcription

Interactions of expanded polyglutamine proteins with specific transcription factors may perturb gene expression, and thus initiate neurodegeneration. Such interactions could involve sequestration of a target protein by polyglutamine protein monomers, or recruitment into aggregates. Many aberrant interactions between expanded polyglutamine proteins and transcriptional factors/co-factors have been described [e.g. CREB-binding protein (CBP), p300/CBP-associated factor (p/CAF), p53, Sp1, TAFII130, PQBP-1] (28). For example, CBP has been found in nuclear inclusions formed by several polyglutamine-expanded proteins including Htt (29), AR (30), ataxin-1 (31) and atrophin-1 (29) in animal disease models or human brains. This results in its depletion from normal nuclear locations and disruption of its regulation of target genes. In contrast, Sp1 directly associates with soluble Htt in a polyglutamine-dependent fashion, and this interaction represses Sp1 transcriptional activity (32,33). In its soluble form, polyglutamine expanded Htt is also reduced in its cytoplasmic interaction with the repressor element-1 transcription factor/neuron restrictive silencer factor (REST/NRSF). This



**Figure 2.** Therapeutic strategies. Therapeutic strategies fall into two categories based on whether they specifically target the polyglutamine protein (right side) or whether they counteract cellular defects induced by the toxic species (left side). In the first category, RNAi could be used to inhibit polyglutamine protein expression; protease inhibitors could be used to block generation of a toxic fragment; normal cellular interactions, peptides or small molecules could be used to help stabilize the polyglutamine protein in a non-toxic form; methods to increase degradation via activation of proteasome or autophagy pathways could reduce protein levels; inhibition of self-association could block formation of toxic aggregates. In the second category, pharmacological intervention could be used to reverse transcriptional and metabolic abnormalities.

leads to nuclear enrichment of REST/NRSF, its enhanced binding to the neuron restrictive silencer element, and transcriptional repression of the gene encoding brain-derived neurotrophic factor (BDNF) (34). Recent work also suggests that soluble mutant Htt selectively represses the transcription of PGC-1 $\alpha$ , a regulator of essential mitochondrial genes, via interfering with the CREB/TAF4-dependent transcriptional pathway (35). Likewise, a pathological interaction of expanded ataxin-1 with a transcriptional repressor capicua may produce transcriptional alterations in SCA1 (36).

Most polyglutamine interactions with transcription factors cannot yet explain cell specificity, whereas studies of ataxin-7 have revealed how transcriptional repression might possibly lead to a neuron-specific pathology. Ataxin-7 is a subunit of the TFTC/STAGA transcriptional complex (37,38) and interacts with the photoreceptor-specific transcriptional activator CRX (39). Ataxin-7 thus recruits TFC/STAGA to promoters of retina-specific genes. Polyglutamine expanded ataxin-7 suppresses the activities of both CRX and the acetyltransferase component of the TFC/STAGA complex, and thus inhibits the expression of genes vital for retinal function (38,39). Although a different study in a knock-in mouse model argued against a primary role of CRX (40), these findings still provide a possible explanation of how specific retinal degeneration might occur in SCA7. In general terms, the transcription repression model predicts that polyglutamine protein interaction with transcription factors necessary for the survival of specific groups of neurons leads to selective neuronal loss.

Most of the factors known to interact with expanded proteins such as Htt, AR and ataxin-1 are ubiquitously expressed, however, so a simple titration model cannot explain all aspects of pathology.

### Metabolism and mitochondrial dysfunction

HD patients exhibit well-described metabolic defects (41,42), characterized by weight loss despite adequate calorie intake (43). This has been linked to mitochondrial dysfunction (reviewed in 44–46). Indeed, defects in striatal glucose metabolism occur in gene carriers, years prior to the onset of motor symptoms (47,48). Htt protein might influence mitochondrial function in several ways. Recent studies have described increased mitochondrial depolarization and early calcium defects in HD patients, and in a transgenic mouse model (49,50). This could be due to a direct binding of Htt to mitochondria (50,51) or an indirect effect via transcriptional repression of PGC-1 $\alpha$ , a transcriptional co-activator that regulates mitochondrial biogenesis and respiration (35,52). It remains to be determined whether mitochondrial deficits are specific to HD (as predicted by Htt repression of PGC-1 $\alpha$ ) or whether they are a feature of polyglutamine diseases in general.

### Proteotoxic stress

The pathogenic polyglutamine length threshold that causes human disease closely matches that which predisposes

polyglutamine proteins to aggregate *in vitro* (23). Protein misfolding thus appears to play a key role in pathogenesis. Indeed, protein quality control has now been linked to several human neurodegenerative diseases (reviewed in 53–55). The brain seems uniquely susceptible to protein misfolding, as most of the major neurodegenerative diseases are associated with large intracellular inclusions. Moreover, it seems likely that protein quality control mechanisms in humans diminish with age, as most neurodegenerative protein misfolding disorders are age-dependent (56,57). Autophagy, a process whereby the cell can degrade aggregated proteins, has been implicated in resistance to polyglutamine pathology in cells, *Drosophila* and mice (58–60). Its importance has been further demonstrated by findings that loss of autophagy induces neurodegeneration in mice associated with accumulation of misfolded proteins (61,62). Likewise, proteasome malfunction has been implicated in polyglutamine pathogenesis. In cultured cells, large intracellular inclusions formed by Htt and cystic fibrosis transmembrane conductance regulatory protein are associated with proteasome impairment (63). This may indicate that proteasome blockage underlies impairment. Problems might also arise from an inability of proteasome to fully digest soluble expanded polyglutamine proteins and the generation of polyglutamine fragments (64,65). Additionally, aggregated proteins may sequester important quality control machinery (e.g. chaperones), compromising the ability of the cell to mount an appropriate stress response (66). In *Caenorhabditis elegans*, it is observed that polyglutamine aggregates can destabilize *trans*-diverse metastable proteins that contain temperature-sensitive mutations (67).

### Aggregation versus inclusion formation

Although the link between polyglutamine length, aggregation potential and toxicity is inescapable, it is crucial to discriminate the pathogenic significance of large macromolecular inclusions versus small aggregates or oligomers. Multiple studies have now dissociated large inclusions from toxicity *in vivo* (6,8) and *in vitro* (5,7). This is very consistent with the idea that such inclusions represent an end-stage of the adaptive cellular response to large quantities of misfolded protein. However, polyglutamine proteins *in vitro* clearly form small aggregates, or oligomers (23,24,68–70), and very small Htt aggregates not visible by conventional immunohistology have been detected by a polyglutamine probe in HD brain (71). Recently soluble polyglutamine oligomers were detected for the first time in a mouse model of SBMA. These oligomers were comprised of N-terminal fragments of AR. They appeared several weeks prior to symptom onset, well before any detectable inclusions, and disappeared rapidly with castration, which halts disease progression (10). Taken together, these studies are consistent with emerging reports for a variety of neurodegenerative diseases, including Alzheimer's disease and Parkinson's disease, in which the importance of protein oligomerization in pathogenesis has been emphasized (reviewed in 72,73). In summary, misfolded protein (especially small soluble oligomers) may directly interfere with critical cellular events, challenge the cell's ability to prevent more widespread misfolding, and compromise its ability to keep up with protein degradation.

### Alteration of normal protein function

Polyglutamine diseases are dominantly inherited, with an abnormal, new toxic activity of polyglutamine-expanded proteins being principally responsible for pathogenesis. Recent studies suggest that in some cases this dominant effect might in part derive from perturbation of normal polyglutamine protein function. For example, Htt inactivation in mice leads to progressive neurodegeneration (74), its function is essential for neurogenesis and postnatal development (74–77), and overexpression of wild-type Htt in transgenic mice can rescue mutant Htt toxicity (78). This may be due to the ability of Htt to promote BDNF production and transport, and its anti-apoptotic activity, which might be impaired by polyglutamine expansion (reviewed in 79). In the case of SCA1, it has now been proposed that polyglutamine expansion within ataxin-1 interferes with its modulation of the transcriptional repressor capicua in a regulatory complex (36). Duplication of an ataxin-1-like gene competes mutant ataxin-1 away from the capicua complex and suppresses SCA1 neuropathology in mice (80). These data imply that pathogenesis of SCA1 might result in part from perturbation of the normal function of ataxin-1 as a result of polyglutamine expansion. Ataxin-3 is a polyubiquitin-binding protein with ubiquitin protease activity (81,82). Patients homozygous for mutant alleles have earlier age-of-onset and more severe phenotypes than heterozygous patients. This could be explained either by loss of function or gain of toxic activity (83). Recent studies in flies demonstrate that normal ataxin-3 suppresses neurodegeneration caused by mutant ataxin-3, and this suppression depends on its ubiquitin-binding activity and protease activities (84). This implies (at least in this *Drosophila* model) that part of the neurodegenerative phenotype might derive from loss of ataxin-3 function.

It must be emphasized, however, that multiple knockout models of polyglutamine proteins have been created, and none mimics the disease phenotype (75,77,85,86). Nor, except in the atypical case of SCA6 (87), do point mutations in the target protein replicate the polyglutamine disease. In humans, loss-of-function mutations in AR cause testicular feminization, which does not feature any motor neuron disease (88). Humans with genomic deletion of genes encoding Htt (89) and ataxin-1 (90) also do not develop HD or ataxia, nor do knockout mice lacking ataxin-1 or Htt (75,77,85,86). Furthermore, mutant Htt can rescue the knockout phenotype (76,78). Finally, homozygous HD patients have similar disease severity and age-of-onset compared with heterozygote patients, effectively ruling out loss of function as the principal mechanism in this disease (91).

### Dual mechanisms

There is currently little evidence that classical polyglutamine diseases are mediated primarily by non-coding RNA abnormalities. However, recently a fascinating pathogenic mechanism for SCA8 has been described, in which reverse strand transcription of a CTG repeat produces polyglutamine peptides that can be detected in the inclusions of transgenic mouse and patient material. It remains possible that reverse strand transcription could play a more widespread role in human



disease (92), and SCA8 could thus be the first example of a new subclass of polyglutamine diseases.

## THERAPEUTIC STRATEGIES

Therapeutic strategies for polyglutamine diseases may be divided into two categories: (i) reversal of cellular defects and (ii) targeting the expression, processing or conformation of the pathogenic protein (Fig. 2).

### Reversing cellular defects

**Transcription.** Polyglutamine pathogenesis (particularly HD) has been conceived as a problem of transcriptional regulation, whereby mutant proteins disrupt the activity of key factors, many of which possess acetyltransferase activity. Indeed, HDAC inhibitors such as suberoylanilide hydroxamic acid, sodium butyrate, and phenylbutyrate, which are purported to increase gene expression, have shown efficacy in various disease models (93–98), and phenylbutyrate is in clinical trial. Although the neuroprotective effects of HDAC inhibitors are intriguing, and they may function by correcting transcriptional defects, they might also increase acetylation of other non-histone proteins [e.g. tubulin (99) and Hsp90 (100)], and upregulate levels of heat shock proteins (e.g. Hsp70) (101,102), ameliorating polyglutamine toxicity via transcription-independent mechanisms. These myriad effects are reflected in the fact that HDAC inhibitors are also effective in mouse models of other diseases, including amyotrophic lateral sclerosis (103), and immune-mediated demyelination (104). Their chronic use in humans, at least in current forms, is likely to be limited by their fairly high toxicity. Moreover, since the specific acetyltransferase targets that mediate the effects of these compounds are not known, it will be very hard to optimize them to reduce toxicity. Further studies of their molecular mechanism, and development of more selective HDAC inhibitors, might ultimately provide better therapies.

**Cellular metabolism.** Various compounds that improve energy metabolism or possess antioxidant activities have been tested and have proven effective in mouse models (e.g. creatine and coenzyme Q10) (105), a finding consistent with the link between mitochondrial defects and HD pathogenesis. Clinical trials with these drugs in HD patients, however, have not shown significant benefits (106–109). Recently, the transcriptional regulator of mitochondrial biogenesis and respiration, PGC-1 $\alpha$ , has been proposed as a target of mutant Htt (35,52). PGC-1 $\alpha$  over-expression prevents striatal atrophy in transgenic HD mice (35) and protects neuronal culture from oxidative stress-mediated death (110). Treatments that elevate PGC-1 $\alpha$  activity might thus prove beneficial.

### Targeting polyglutamine proteins

**Gene therapy.** The most straightforward approach to therapy may be to selectively reduce expression of the expanded allele. The use of small interfering RNAs to selectively knock-down gene expression has now been validated in mouse models of polyglutamine disease (111,112). Indeed, for diseases with relatively localized pathology (e.g. retina in SCA7), it is quite

feasible to consider such an approach. However, more widespread CNS pathology (e.g. cortical and striatal neurons in HD) will present a greater challenge. Long-term safety is uncertain, but ongoing trials of viral-mediated gene therapy in humans should relieve concerns. Another challenge will be to create an allele-specific sequence that only targets the mutant gene, particularly for those diseases where the normal gene is vital (e.g. HD and SCA3). Nonetheless, if such therapies are tolerated, they will clearly hold great promise.

**Proteolysis.** Several studies have implicated caspase activation in the pathogenesis of polyglutamine diseases, due to their cleavage of the polyglutamine proteins and induction of apoptosis (113–115). Indeed, the modest activity of minocycline (a putative apoptosis inhibitor) in a mouse model of HD (116) has inspired an ongoing clinical trial. The more general role of caspase activation in the neurodegenerative process is still being elucidated. However, if specific proteases are found to cleave polyglutamine proteins to generate toxic fragments, then it may be possible to create protease inhibitors that will be of benefit.

**Protein clearance.** Stimulating cellular degradation pathways that preferentially target misfolded disease proteins may be beneficial. Emerging evidence implicates autophagy as a protective mechanism in polyglutamine disease (reviewed in 117). The mTOR inhibitor rapamycin, which stimulates autophagy, has been beneficial in cell, *Drosophila* and mouse disease models (58–60) and may be a potential drug candidate. Additionally, recent high throughput chemical screens have identified compounds that selectively stimulate clearance of Htt (118) and AR proteins (119), although the underlying mechanisms are poorly understood. Activation of cellular protein clearance mechanisms might thus be a viable strategy.

**Protein aggregation.** Direct targeting of polyglutamine aggregation has been a focus of therapeutic development for several years. Pharmacological induction of molecular chaperones (e.g. Hsp70) that aid in protein refolding and degradation has been proposed (e.g. geldanamycin and geranylgeranylacetone) (120–122). However side effects associated with such strategies are likely to be limiting. Multiple attempts have been made to screen for small molecules that directly interfere with polyglutamine protein aggregation (123–128). However, despite promising data in cell-based assays and model organisms, few convincing results in mice have been described. One potential problem is that compounds designed to prevent formation of large aggregates may not stop the initial pathological misfolding of protein monomers, which will retain their capacity for pathogenesis, either as single molecules or as toxic oligomers.

**Stabilizing native conformation.** Because an expanded polyglutamine protein can exist in multiple conformations (24,25,27), it may be possible to influence the equilibrium between a toxic and non-toxic conformation by directly targeting the protein with interventions that stabilize the native conformer. (Indeed, rescue of polyglutamine toxicity by over-expression of various interacting proteins may occur by this mechanism.) This general idea was recently validated by the use of a polyglu-

tamine binding peptide (QBP1) to stabilize the native conformation of a thioredoxin/polyglutamine fusion protein. This prevents it from converting into an aggregation-prone  $\beta$ -sheet-rich confirmation (25,129,130). A similar approach has been adopted by the use of a bivalent Htt-binding peptide to suppress polyglutamine aggregation and toxicity in *Drosophila* (131), and antibody-based therapies may also be exploited in this manner (132–134). Delivery of peptides directly to the CNS remains a significant challenge and may require virus-based gene therapy. However, small molecules that have the potential to stabilize a non-toxic conformation of a polyglutamine protein (as opposed to those simply blocking aggregate formation) might ultimately achieve this goal.

Polyglutamine protein aggregation potential can be regulated by interactions with cellular binding partners (135,136). Thus, it may be possible to stabilize the mutant proteins in a less toxic form indirectly via targeting these interactions. For example, phosphorylation of ataxin-1 at Ser-776 by Akt increases its affinity for 14-3-3 protein, which increases ataxin-1 steady-state level, and likewise its toxicity (137,138). An inhibitor of the Rho-associated protein kinase, a regulator of actin dynamics, reduces Htt aggregation and toxicity (127), and genetic modifiers of actin polymerization similarly affect polyglutamine aggregation (139), presumably based on (as yet undetermined) protein interactions. Ultimately, modulation of intracellular signaling pathways that regulate such interactions could form the basis of effective treatment.

*Conflict of Interest statement.* None declared.

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