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Polyglutamine Neurodegeneration: Protein Misfolding Revisited

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Abstract

Polyglutamine diseases are a major cause of neurodegenerative disease worldwide. Recent studies highlight the importance of protein quality control mechanisms in regulating polyglutamine-induced toxicity. Drawing on these studies, we propose a model of disease pathogenesis that integrates current understanding of the role of protein folding in polyglutamine disease. We also incorporate new findings on other age-related neurodegenerative diseases in an effort to explain how protein aggregation and normal aging processes might be involved in polyglutamine disease pathogenesis.

Introduction

The polyglutamine (polyQ) diseases represent an important cause of heritable neurodegeneration, comprising at least nine disorders (reviewed in [1]). All nine result from the expansion of a CAG repeat in the respective disease genes, encoding an abnormally long glutamine tract in the disease proteins. This shared mutation suggests a common pathogenic mechanism, notwithstanding the fact that the pathogenic proteins are evolutionarily and functionally unrelated.

A decade ago, a clue to pathogenesis came from the discovery that expanded polyQ proteins accumulate within intraneuronal inclusions in select brain regions. This observation, coupled with *in vitro* experiments modeling polyQ protein aggregation, quickly led to the hypothesis that polyQ protein aggregation, and perhaps the inclusion itself, mediated neurodegeneration. The appeal of this hypothesis stemmed partly from the mechanistic link it suggested to other, more common age-related neurodegenerative disorders in which aggregates of specific proteins accumulate, including Alzheimer's disease (AD) and Parkinson's disease (PD).

Subsequently, inclusions have fallen out of favor as a primary pathogenic entity. Many cell-based, animal model and human autopsy studies suggest that inclusions are merely the byproduct of abnormal protein accumulation or perhaps even a cytoprotective response that sequesters abnormal proteins [2–5]. This has led to understandable confusion in the field about whether protein misfolding and aggregation are indeed central to disease pathogenesis. Recent studies using divergent approaches now confirm the importance of protein misfolding in polyQ disease pathogenesis. Here we synthesize these findings into a broader understanding of the role played by protein misfolding in these intriguing neurodegenerative disorders, and place them in the larger context of age-related neurodegeneration.

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Disclosure Statement

The authors declare no conflicts of interest.

Lots of Trouble From One Amino Acid

PolyQ diseases fall within the larger category of protein conformational brain disorders. As with most age-related neurodegenerative disorders, polyQ diseases are characterized by amyloid-like deposits of the disease protein in the central nervous system (CNS). Expanded polyQ proteins are prone to aggregate and, the longer the expansion, the more robust the aggregation. Disease severity also correlates directly with the length of the expansion, as measured both by the age of symptom onset and by the extent of CNS pathology: longer repeats lead to earlier onset and more widespread degeneration.

Despite their presumably shared disease mechanism, polyQ diseases differ clinically and neuropathologically. While many are degenerative spinocerebellar ataxias, one is primarily a motor neuron disorder (spinobulbar muscular atrophy) and another is characterized by striatal and cortical degeneration (Huntington's disease, HD). This preferential loss of specific neuronal populations occurs despite widespread expression of the disease proteins [6]. A closer look reveals that even the polyQ expansions differ somewhat among the diseases: although the commonly accepted disease threshold for glutamine repeats is 37–40 residues, a much smaller repeat causes disease in one disorder (SCA6), and the encoded proteins normally contain glutamine repeats up to 42 residues in two others (SCA3, SCA17). Clearly, then, not all expansions are the same. The host protein context influences both the biochemical properties of expanded polyQ and, importantly, the resultant disease phenotype.

Many hypotheses have been put forward to explain polyQ disease pathogenesis. These hypotheses are by no means mutually exclusive. For any given polyQ disease more than one mechanism likely contributes to neuronal dysfunction and eventual cell death. They include: i) misfolding of the disease protein resulting in altered function, ii) deleterious protein interactions engaged in by the mutant protein, iii) formation of toxic oligomeric complexes; iv) transcriptional dysregulation, v) mitochondrial dysfunction resulting in impaired bioenergetics and oxidative stress, vi) impaired axonal transport, vii) aberrant neuronal signaling including excitotoxicity, viii) cellular protein homeostasis impairment and ix) RNA toxicity [1,7–10]. Compelling data support each of these mechanisms in one or more polyQ diseases. But which among these are key, early events in polyQ neurodegeneration and which are secondary? As we discuss below, the most compelling argument can be made for a mechanism that combines the first three listed above: polyQ-induced conformational changes in the disease protein resulting in abnormal protein-protein interactions (Fig. 1).

The identity of the putative toxic species in polyQ disorders has been the subject of much research and debate. One possibility is that the polyQ disease protein itself is directly toxic, either as a misfolded monomer or homo-oligomeric complex. For example, Nagai and colleagues [11] recently showed that misfolded β -sheet-rich polyQ protein monomers and oligomers are toxic to cells, but properly folded α -helical polyQ monomers are not. The conformational change from α -helix to β -sheet correlated with cellular toxicity, which agrees with the generally accepted view that expanded polyQ is prone to adopt a β -sheet-rich conformation and assemble into amyloid-like complexes through a nucleation-seeded polymerization process. Based on calculations from *in vitro* reactions, a single abnormally folded monomer seeds the growth of larger oligomeric complexes and, ultimately, amyloid-like fibrils [12,13]. The structure adopted by expanded polyQ, however, is still unknown. One crystallographic study of polyQ protein has been published, describing the structure of polyQ peptide bound to a polyQ-specific monoclonal antibody [14]. In antibody-polyQ crystals, polyQ peptides have an extended linear shape. Surface plasmon resonance studies showed that isolated polyQ peptides and polyQ-expanded huntingtin possess similar antibody binding properties [14]. These results suggest that polyQ proteins adopt the same shape regardless of repeat length, which would not support the model of a toxic misfolded species. Structural

studies using circular dichroism [11], dynamic light scattering [15], nuclear magnetic resonance [16] and simulation methods [17] have reported a variety of possible polyQ structures, making it currently difficult to draw conclusions regarding local domain misfolding.

Most studies support the view that putative toxic species exist somewhere upstream of amyloid fibrils along this aggregation pathway. For example, in cells overexpressing polyQ fusion proteins, fluorescence resonance energy transfer (FRET) experiments demonstrate the formation of soluble oligomeric species whose appearance correlates with toxicity [18]. Furthermore, in some mouse models of disease, polyQ protein microaggregates that are resistant to denaturing detergents accumulate before, or simultaneous with, the first signs of CNS dysfunction, consistent with their being causative pathogenic agents [19,20].

Routes to Neuronal Toxicity

Is there, in fact, a single toxic polyQ species? In other words, is there a particular monomeric conformer or oligomer that, relative to other conformations or complexes generated by expanded polyQ proteins, is deleterious to neurons? Current evidence suggests that there is unlikely to be a single neurotoxic species. Rather, we propose that the toxicity of expanded polyQ proteins is a more general byproduct of the misfolded protein's propensity to engage in aberrant protein interactions, which can be deleterious whether mediated by monomeric protein, by homo-oligomeric complexes, or by more subtle disruptions in the balance of heteroprotein complexes through which the polyQ protein normally functions. The extent to which any of these occurs will vary depending on the structural and functional properties of the protein, the size of the expansion, the protein's subcellular localization, the occurrence of proteolytic events that partially liberate polyQ fragments from the surrounding polypeptide, and the integrity of protein homeostatic pathways in the cell. In the case of extremely long expansions or those diseases in which proteolysis generates polyQ fragments (e.g. HD), a relatively high concentration of misfolded, unconstrained polyQ will favor the formation of homo-oligomeric complexes. For example, the detergent-resistant microaggregates observed in the R6/2 transgenic mouse, which expresses a small fragment of mutant huntingtin, likely are homo-oligomers, though it should be added that some or all of the monomers comprising these microaggregates are ubiquitinated [20].

By contrast, in the more typical disease state in which a modest expansion resides within the full protein, a smaller fraction of the cellular pool of mutant protein will adopt an abnormal polyQ conformation. In this case, specific interactions ordinarily engaged in by the polyQ protein will be altered more subtly. It is important to emphasize that, rather than being structural proteins or self-sufficient enzymes, most polyQ-containing proteins tend to be regulatory cofactors within macromolecular assemblies, often linked to gene regulation [21]. Rather subtle changes in such assemblies could have tremendous consequences in neurons.

A recent example of this is provided by the SCA1 disease protein, ataxin-1. Lim and colleagues [22] showed that ataxin-1 exists in multiple heteroprotein complexes. PolyQ expansion in ataxin-1 promotes the formation of a complex containing RBM17, a putative RNA binding protein, which enhances neurotoxicity through an unknown gain of function. Simultaneously, polyQ expansion reduces the formation of an ataxin-1 complex containing the transcriptional repressor, Capicua, thereby exacerbating pathology through a loss of function [23]. These results suggest a model of pathogenesis in which conformational changes in mutant ataxin-1 alter the balance of macromolecular assemblies through which this protein acts. The resultant imbalance has deleterious consequences for specific neurons including, in this instance, cerebellar Purkinje cells. Importantly, this model does not require global protein misfolding, widespread perturbations in cellular protein homeostasis, or even the formation of mutant ataxin-1 homo-oligomers.

Another example of polyQ-induced alterations in heteroprotein complexes was recently described for the SCA17 disease protein, TATA-box binding protein (TBP) [24]. TBP normally forms transcriptionally incompetent homodimers, preventing unregulated TBP activity. PolyQ expansion, however, inhibits the formation of TBP homodimers while also promoting interaction with the transcription factor, TFIIB. The expanded TBP/TFIIB heterocomplexes lack normal DNA-binding capacity, resulting in reduced expression of TFIIB-dependent genes. SCA17 transgenic mice, like most animal models of polyQ disease, develop nuclear inclusions. Although these inclusions contain both TFIIB and TBP, this does not imply that inclusions are pathogenic structures. The presence of both proteins in inclusions may simply be the end-stage product of interactions initiated between the two proteins in the soluble, pre-aggregation state, as has been suggested elsewhere for expanded polyQ proteins that interact with transcription factors [25].

The above examples lead one to predict that interacting partners of polyQ proteins will often be genetic modifiers of pathogenesis. A recent study makes good on this prediction [26]. Using complementary approaches, Hughes and colleagues identified 234 proteins that interact with the amino-terminal domain of huntingtin. When a subset of 60 interactors were tested in a *Drosophila* model of HD, a remarkably high percentage, 45%, proved to be genetic modifiers. Thus, many modifiers of polyQ toxicity will likely prove to be the very proteins with which the disease protein normally interacts.

Evidence for Perturbations in Protein Homeostasis

The known functions of certain polyQ disease modifiers – which in some cases modulate the toxicity of several polyQ disease genes – argue that polyQ-induced protein misfolding and resultant stress on protein homeostatic pathways play a central role in pathogenesis. Several classes of heat shock protein (Hsp) are potent modulators of polyQ disease, including but not limited to the Hsp70, Hsp60/TriC/CCT and Hsp40 families [27–31]. Collectively these chaperones function cotranslationally on nascent polypeptide chains and posttranslationally on stress-denatured proteins.

Only recently was the chaperonin, TRiC, identified as a suppressor of polyQ aggregation and toxicity. A hetero-oligomer of 16 subunits, TRiC is organized in two stacked rings of 8 subunits each, forming a capsule with a central chamber reminiscent of the prokaryotic chaperonins, GroEL and GroES. TRiC interacts with the Hsp network by refolding substrates bound to Hsp70 and by functioning with Hsp70 in the assembly of oligomeric protein complexes [32]. TRiC was first identified as a modulator of polyQ protein aggregation in a genome-wide RNAi screen in *C. elegans* [28]. Three recent studies sought to explain the mechanism by which TRiC suppresses polyQ toxicity. Kitamura et al. [33] and Tam et al. [34] demonstrated that TRiC prevents the formation of toxic misfolded polyQ intermediates, as assayed by a decrease in biochemically detectable protein aggregates and cellular toxicity. Kitamura et al. [33] concluded that the TRiC holocomplex was required for suppression, whereas Tam et al. [34] showed suppression of polyQ toxicity simply by overexpressing the substrate binding domain of the TRiC subunit that bound polyQ substrates best. Behrends et al. [35] concluded, somewhat differently, that TRiC does not reduce oligomer formation but rather cooperates with Hsp70 and Hsp40 to promote the formation of nontoxic oligomers over toxic oligomers. Taken together, these TRiC studies confirm the importance of polyQ aggregation in toxicity and the role of protein quality control machinery in reducing that toxicity.

Recent genetic screens have expanded the range of modifiers to include additional classes of quality control proteins that maintain protein homeostasis, including ubiquitin-proteasome system components (Table 1). Some of these screens have identified still other genes that do not appear to function directly in protein quality control, including signal transduction pathway

components, cytoskeletal proteins and transcription factors (Table 1). Although these proteins have diverse cellular functions, many have been shown to alter the aggregation state of polyQ protein in animal models, such as α - and β -tubulin, and ribosomal components [28,30].

Gidalevitz et al. [36] asked a broader question: do expanded polyQ proteins cause unassociated proteins in the cell to misfold? That is, could the constant presence of a protein that is prone to misfold disrupt protein homeostasis throughout the cell? To answer this question, metastable temperature-sensitive proteins were expressed in worms with or without coexpressed polyQ protein. Co-expression of polyQ protein promoted the misfolding of temperature-sensitive proteins even at normally permissive temperatures. It is currently unknown whether this effect would manifest in cells expressing physiological levels of the implicated proteins, or whether there are similar endogenous, metastable proteins in neurons. Nevertheless, these results suggest a mechanism whereby polyQ proteins could wreak general havoc on cellular protein homeostasis.

Folding and Degradation Enzymes as Quality Control Partners

Quality control (QC) ubiquitin ligases, a special class of ubiquitin ligases that link protein refolding and protein degradation pathways, have recently emerged as modifiers of various age-related, neurodegenerative proteinopathies. This class of enzymes currently includes CHIP, parkin, and E4B, though others will likely be identified. Rather than simply ubiquitinating one or a few substrates, QC ubiquitin ligases act on a broader range of abnormally folded polypeptides. They accomplish this partly by constituting a scaffold that brings together components of protein refolding, most notably molecular chaperones, with ubiquitination machinery.

Among these, the C-terminus of Hsp70-interacting protein, CHIP, is of particular interest in proteinopathies. CHIP is known to interact with numerous neurodegenerative disease proteins, including polyQ disease proteins, and modulate their solubility and degradation [37–44]. CHIP plays an important role in modulating the toxicity of all polyQ proteins with which it is known to interact. So far, these include huntingtin [37], ataxin-1 [41,42], ataxin-3 [38], and androgen receptor [44]. Interestingly, CHIP's action on polyQ proteins is influenced by the substrate's protein context [31,37,42], although the mechanisms underlying this are not understood.

Currently other ubiquitin ligases in this group are less well-studied with respect to polyQ diseases, but some connections have been made. In cell models, parkin reduces the aggregation and toxicity of polyQ-containing protein and colocalizes to inclusions in HD mouse and human disease brain [45]. E4B appears to be important for the degradation of the SCA3 disease protein, ataxin-3, and suppresses ataxin-3 toxicity in flies [46]. Finally, some evidence suggests that E6-AP is also a QC ubiquitin ligase, as it regulates the aggregation, degradation and toxicity of expanded huntingtin and ataxin-3 in cell models [47] and the toxicity of expanded ataxin-1 in transgenic mice [48].

Lessons from other age-related amyloidopathies

Mutant polyQ proteins adopt amyloid-like conformations [11,49,50], suggesting that mechanistic insights from other amyloidopathies may shed light on polyQ disorders. In the most common CNS amyloidopathy, AD, the putative culprit is A β , a cleavage product of amyloid precursor protein [51]. Although extensive A β plaques accumulate in AD brain, soluble A β oligomers appear to be the principal neurotoxic species [51]. In the Tg2576 transgenic mouse model of AD, Lesné and colleagues [52] reported an extracellular A β dodecamer, A β *56, whose appearance in brain correlated with the onset of memory problems and which, when injected into rats, elicited similar cognitive deficits. Another group demonstrated that A β *56 levels in the brains of three other AD mouse models correlate with

deficits in specific learning and memory tasks [53]. Additional studies are needed to confirm that A β *56 is indeed the major, or only, oligomeric species underlying neurotoxicity in AD. Although these investigations suggest that there may be a single distinct neurotoxic A β species, it is important to note that A β is an aggregation-prone, wholly amyloidogenic fragment of a protein, and is therefore likely to generate pure homo-oligomers of discrete size. PolyQ proteins, in contrast, tend to be much larger and even when cleaved by intracellular proteases, the resultant polyQ fragments retain flanking sequences. Accordingly, they are less likely to aggregate into homogenous oligomers than A β . Nevertheless, polyQ proteins and their resultant aggregates have much in common with A β amyloid, and the methods used to identify specific A β oligomers might prove useful in detecting pathological polyQ species as well.

Another recent study has linked A β aggregation to aging pathways in *C. elegans* [54]. In the major aging pathway in worms, the insulin/IGF-1 receptor *daf-2* inhibits the transcription factor, *daf-16*. *Daf-2* signaling is critical for normal aging: when *daf-2* is inhibited, worms live longer. Another transcription factor, *hsf-1*, is important for extended lifespan secondary to *daf-2* mutation: inhibition of *hsf-1* blocks the lifespan extension resulting from *daf-2* inhibition. To complete the loop, *hsf-1* effects on aging are dependent on *daf-16*, the transcription factor that is inhibited by *daf-2* signaling. Importantly, this pathway regulates both the aging process and protein aggregation [54]. In worms treated with RNAi against *daf-2*, lifespan was extended and worms manifested A β toxicity at much later ages. Conversely, RNAi against either longevity gene, *daf-16* or *hsf-1*, caused worms to manifest A β toxicity more severely and at younger ages. This increased A β toxicity directly correlated with increased levels of A β trimer, but not with levels of A β monomer or fibrils. This suggests that A β trimers, or some higher order structure composed of A β trimers, are the toxic species. These results are not inconsistent with the findings from AD transgenic mice described above.

Further investigation of the mechanism by which *hsf-1* and *daf-16* regulate A β toxicity revealed that *hsf-1* is important for dissociating protein aggregates, whereas *daf-16* enhances the aggregation of small toxic oligomers into larger, nontoxic fibrils [54]. Thus, *daf-16* and *hsf-1* regulate distinct and complementary pathways that decrease levels of pathogenic A β aggregates and reduce the toxicity of aggregation-prone proteins. *Hsf-1* and *daf-16* have also been shown to reduce polyQ aggregation in worms [55]. Because *hsf-1* and *daf-16* regulate both protein aggregation pathways and aging pathways simultaneously, the failure of these systems during aging may explain why diseases of protein misfolding and aggregation manifest in an age-related manner. Importantly, lifespan extension in worms delays polyQ aggregation and toxicity [56], suggesting that the studies linking A β aggregation and aging may inform our understanding of how these processes are linked in polyQ disease as well.

Unanswered Questions and Routes to Therapy

Drawing on recent research findings, we propose a revised model of polyQ disease pathogenesis (Fig 1). In this model, misfolded polyQ protein adopts a conformation that provides an accessible, reactive polypeptide surface favoring aberrant protein interactions. The resultant protein complexes, whether they are subtly altered “normal” complexes or detergent-resistant microaggregates (including homo-oligomers), could contribute to neuronal dysfunction culminating in cell death. Although A β oligomers are likely the most important neurotoxic species in AD, recent evidence in the polyQ disease field suggests that the neurotoxic complexes formed by any given polyQ protein are more heterogenous. Therefore, therapeutic initiatives cannot afford to focus primarily on preventing the formation of disease-specific heteroprotein or oligomeric complexes, which may be relevant for only one or a few polyQ diseases. Rather, they should also include efforts to enhance neuronal protein quality control pathways and reduce intracellular levels of the mutant polyQ disease proteins through RNA interference or clearance pathways [24,44,57–59].

Because protein misfolding appears central to polyQ disease pathogenesis, increasing the expression or activity of neuronal protein refolding machinery holds therapeutic potential [57]. For example, Hsp90 inhibitors, which increase substrate degradation by the proteasome, are one class of drugs that modulate protein homeostasis. Certain Hsp90 inhibitors have already demonstrated efficacy in animal models of polyQ disease [58]. Further medicinal chemistry endeavors may discover compounds that act in diverse ways to enhance the activity of specific components of the neuronal protein quality control apparatus.

An important, still unanswered question in the study of polyQ diseases is, why is the nervous system selectively affected? As highly specialized postmitotic cells, neurons may handle misfolded proteins differently from other cell types or be particularly sensitive to the consequences of altered protein interactions. While little is known about why neurons are susceptible to polyQ toxicity and other cells are not, several recent studies have examined susceptibility differences among different types of neurons, revealing that distinct types of neurons handle the same protein differently. Brignull and colleagues [60] showed that a near-threshold-length polyQ protein aggregated in some *C. elegans* neurons but not others, demonstrating that different types of neurons have different protein quality control mechanisms and capacities. Meanwhile, Tagawa and colleagues [29] showed that expression of the neuroprotective chaperone Hsp70 increased more robustly in response to mutant huntingtin in cerebellar granule neurons (which are resistant to huntingtin toxicity) than in cortical neurons (which are sensitive to huntingtin toxicity). Although the mechanisms underlying selective CNS toxicity are far from clear, further efforts to elucidate the causes of CNS susceptibility to polyQ disease might identify promising therapeutic targets in these fatal proteinopathies.

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Glossary Box

Amyloid

name given to proteins that have adopted a non-native, β -sheet-rich structure and aggregate in tissue. Amyloid can be generated from many different proteins, but amyloid structures assumed by diverse proteins share common properties, including reactivity with thioflavin-S and Congo Red stains

Inclusion

an intracellular, typically spherical accumulation of proteins in the nucleus or cytoplasm, visible by light microscopy with antibodies directed against polyQ disease protein or ubiquitin. Often contain proteins involved in the ubiquitin-proteasome and molecular chaperone systems

Microaggregates

detergent-resistant protein aggregates, much smaller than inclusions, which may or may not be pure homo-oligomers of disease protein. Microaggregates are soluble in that they partition in the soluble fraction of brain lysates, but are not dissociable by denaturing detergent

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Figure 1. Possible cellular sequelae of expanded polyQ protein misfolding

PolyQ proteins, when expanded, are prone to misfold. HSF-1 can trigger the expression of certain chaperones, including Hsp70 and Hsp40, which help refold misfolded polyQ proteins. When misfolded polyQ monomers form oligomers, chaperones can help to dissociate them. Misfolded monomers can also form complexes with other proteins. These aberrant heteroprotein complexes may cause the affected proteins to adopt novel functions or alter their normal functions. Oligomers may also interact with cellular proteins to cause similar alterations in function, though currently there is no direct experimental evidence. Some protein quality control components, such as the TRiC chaperonin complex, may reduce the toxicity of oligomers by changing their size and conformation. Based on current evidence, we suggest misfolded, polyQ-expanded monomers and resultant oligomers display reactive, accessible polypeptide surfaces that cause neuronal dysfunction and ultimately cell death.

Genetic modifiers of polyglutamine toxicity in animal models

Genetic screening in animal models has identified multiple gene families that modulate polyQ toxicity, including genes encoding chaperones, components of the ubiquitin proteasome system (UPS), proteins implicated in RNA metabolism, and proteins of diverse signal transduction pathways. A unifying feature of many implicated modifier genes is involvement in the maintenance of cellular proteostasis. This table is a representative but incomplete listing, restricted to modifier genes that are 1) directly involved in cellular protein quality control, 2) have been identified in multiple animal models, or 3) have been shown to regulate polyQ protein aggregation.

Name	Class	Function	Effect on PolyQ Toxicity	Animal Model	References
Hsp70	Chaperone	Bind unfolded proteins. ATP hydrolysis	Suppressor	fly worm	28,30,31
Hsp60/TRiC/CCT	Chaperone	Bind unfolded proteins. ATP hydrolysis	Suppressor	worm	28
Hsp40	Chaperone	Bind unfolded proteins. co-chaperone for Hsp70	Suppressor	fly worm	28,30,31
α B-crystallin	Chaperone	Small heat-shock protein	Suppressor	fly	30
CHIP	Chaperone UPS ^a	Bind chaperones, ubiquitin ligase	Suppressor	fly mouse	31,37,44
E6-AP	Chaperone UPS ^a	Ubiquitin ligase, may functionally interact with Hsp70	Suppressor	mouse	48
Ubiquitin	UPS ^a	Targets proteins for degradation, various cellular processes	Suppressor	fly worm	28,30
Uba	UPS ^a	Ubiquitin activating enzyme	Suppressor	worm	28
Ubc-E2H	UPS ^a	Ubiquitin conjugating enzyme	Suppressor	fly	31
Usp9X/fat facets	UPS ^a	Deubiquitinating enzyme	Suppressor	fly	26,30
Proteasome core subunits	UPS ^a	Protein degradation	Suppressor	worm	28
Proteasome cap subunits	UPS ^a	Regulation of proteasome activity	Variable(depends on specific cap subunit)	fly worm	26,28
I4-3-3	Signal transduction	Binds phosphorylated proteins	Enhancer	fly	26,31
Akt	Signal transduction	Serine/threonine kinase	Variable(depends on polyQ disease)	fly	31
RhoGAP	Signal transduction	Regulates GTPases	Enhancer	fly	31
α - and β -tubulin	Cytoskeleton	Vesicle trafficking, cell structure	Suppressor	worm	28
Exportin-1	Nuclear export	Binds and transports proteins	Suppressor	fly	30
HSE-1	Transcription factor	Binds DNA, triggers expression of chaperones	Suppressor	worm	28
Ribosomal proteins	Protein synthesis	Protein synthesis, bind mRNA	Suppressor	worm	28

^a ubiquitin proteasome system