CHAPTER

48

Cellular and Molecular Basis of Neurodegeneration in the CAG–Polyglutamine Repeat Diseases

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OUTLINE

INTRODUCTION TO THE CAG–POLYGLUTAMINE REPEAT DISEASES

CAG repeat expansions are responsible for nine inherited neurodegenerative disorders

The CAG–polyglutamine (PolyQ) repeat diseases are a group of inherited neurodegenerative disorders that are caused by the expansion of a CAG triplet repeat, resulting in a protein with an abnormally extended polyglutamine (polyQ) tract. The repeat expansions in these diseases occur in gene coding regions and lead to mature protein with an abnormal structure and function. To date, nine polyQ disorders have been identified, including Huntington's disease (HD), spinal and bulbar muscular atrophy (SBMA), dentatorubral—pallidoluysian atrophy (DRPLA) and six forms of spinocerebellar ataxia (SCA1,

TABLE 48-1 Genetic Features of the CAG–Polyglutamine Repeat Diseases

Disease	Causative gene	Normal CAG repeat length	Pathological CAG repeat length	Inheritance pattern
HD	Huntingtin	6–34	36–121	AD
SBMA	Androgen receptor	9–36	38–62	XL
SCA1	Ataxin-1	6–44	39–82	AD
SCA2	Ataxin-2	15–24	32–200	AD
SCA3	Ataxin-3	13–36	61–84	AD
SCA6	Calcium channel subunit alpha-1a	4–19	10–33	AD
SCA7	Ataxin-7	4–35	37–306	AD
SCA17	TATA-binding protein	25–42	47–63	AD
DRPLA	Atrophin-1	7–34	49–88	AD

HD = Huntington's disease; SBMA = Spinal and bulbar muscular atrophy; SCA = Spinocerebellar ataxia; DRPLA = Dentatorubral-pallidoluysian atrophy; XL = X-linked; AD = autosomal dominant.

2, 3, 6, 7 and 17) (Table 48-1). Since the first identification of the genetic basis of the polyQ diseases (La Spada et al., 1991), intensive research has been carried out to understand the molecular basis of these disorders. All polyQ diseases display an inverse correlation between the number of CAG repeats and the age of onset of disease, resulting in a more severe disease phenotype as the disease is transmitted from generation to generation due to increasing expansion length of the CAG mutant allele—a phenomenon known as "anticipation". The proteins involved in different polyQ diseases are expressed throughout most cells of the CNS, but differ in their function and location within the cell. Furthermore, different brain regions and neuronal cell subtypes are affected in each disease. All the diseases are inherited in an autosomal dominant manner, except for SBMA, which is X-linked.

Normal functions of polyglutamine disease proteins

The normal functions of the polyQ disease proteins were either known at the time of their identification, or determined subsequent to their discovery. For example, HD is caused by a polyQ expansion in huntingtin (htt), a nucleocytoplasmic protein found in both axons and at the synapse. Htt engages in a variety of protein–protein interactions, and has been shown to play a role in axonal transport and in the regulation of calcium signaling. A CAG–polyQ repeat expansion in the androgen receptor (AR), a nuclear receptor, causes SBMA. AR is a member of the steroid–thyroid superfamily of nuclear receptors, and drives the expression of its target genes by binding to a consensus sequence in the promoter region; it is best known for its roles in important physiological processes in the male reproductive system. The protein associated with SCA1 is ataxin-1, which appears to be involved in RNA processing and transcriptional repression. In the case of DRPLA, work done in *Drosophila* and in cell culture suggests that atrophin-1 (the causal protein in DRPLA) is a transcriptional co-repressor. Ataxin-3 acts as a poly-ubiquitin editing enzyme and hence mediates the degradation of ubiquitinated proteins. Ataxin-7 is a member of the SPT3-TAF9-ADA-GCN5 acetyl-transferase (STAGA) co-activator complex and appears to play a crucial role in regulating transcription.

EXPANDED POLYGLUTAMINE TRACTS PROMOTE PROTEIN MISFOLDING TO DRIVE NEUROTOXICITY

Disease-length polyglutamine tracts adopt a novel, toxic conformation

After being synthesized at the ribosome, proteins fold into certain three-dimensional structures, allowing them to bring key functional groups into close proximity, and also enabling them to engage in specific protein–protein interactions. A properly folded protein can perform its assigned function(s), because it possesses a normal tertiary structure. In the polyQ repeat diseases, the proteins, translated by the causative genes, contain polyQ tracts that exceed a certain length threshold, beyond which the proteins misfold and then adopt a pathological conformation. The transition to this aberrant conformation can be documented by antibody recognition of the misfolded conformer (Trottier et al., 1995). Indeed, certain antibodies (e.g., 1C2) can detect different misfolded polyglutamine proteins, even though their primary sequence varies outside of the polyQ tract. The transition to a misfolded conformer prevents their rapid degradation by the protein turnover pathways of the cell, causing mutant polyQ-containing proteins to accumulate in the cell as abnormal structures and then aggregates that are visible at the light microscope level. Consequently, protein misfolding in the polyQ diseases is a defining pathological feature.

Polyglutamine disease proteins form aggregates visible at the light microscope level

It has long been known in the polyQ research field that expanded polyQ proteins form protein aggregates visible at the light microscope level *in vivo* and *in vitro*, so these "inclusion bodies" were considered pathogenic findings in these diseases.

Intra-neuronal inclusion bodies were observed in a transgenic HD mouse model, in brain tissues of patients of SCA3 and in brain tissues from HD patients (Davies et al., 1997; DiFiglia et al., 1997; Paulson et al., 1997). This observation, along with studies modeling polyQ aggregation, led scientists to hypothesize that these inclusion bodies were the key pathogenic entities in polyQ disease. This was further supported by similar findings in other more common neurodegenerative diseases, such as Alzheimer's disease (AD) and Parkinson's disease (PD), where aggregates of specific proteins accumulate. However, a number of studies have shown that inclusion bodies are not the main reason for pathogenesis and their formation could represent a means for the cell to neutralize the toxicity of accumulating misfolded protein conformers (Arrasate & Finkbeiner, 2005; Rub et al., 2006; Slow et al., 2005).

Polyglutamine disease proteins exist as misfolded monomers, oligomers and protofibrils

PolyQ protein monomers are β-strand monomers, which can undergo oligomerization *in vitro*. But the structure of the monomers remains enigmatic. Studies carried out to elucidate the structure of monomers have suggested that they could be cylindrical or intramolecular β-sheet structures. Since all studies to date have used chemically synthesized polyQ molecules, it is impossible to predict which structures predominate in the tissues of affected patients. As noted above, in many neurodegenerative diseases including polyQ diseases, oligomers of disease proteins have been proposed to be pathogenic structures. In order to understand the structures of these oligomers, various biophysical approaches have been taken, and some investigators have proposed that the structures consist of parallel and anti-parallel β-sheets and head-to-tail cylindrical β-sheets. An anti-parallel β-sheet structure, supported by intermolecular hydrogen bonds, and termed a "polar zipper", was proposed by Max Perutz and colleagues (Perutz et al., 1994). Subsequently, a cylindrical model, termed the "nanotube model" was also suggested (Perutz et al., 2002). Work from Ron Wetzel has reaffirmed the anti-parallel β-sheet structure as a likely conformation for expanded polyQ tracts, but also pointed out that polyQ tracts adopt β-extended chains with periodic turns (Thakur et al., 2002).

In order to determine how expanded polyQ monomers assemble into oligomers, a method to observe intermolecular interactions was needed. A number of groups have addressed this challenge by adapting the fluorescence resonance energy transfer (FRET) technique to study polyQ structural interactions. Using this method, soluble polyQ oligomers were detected, and it was noted that the monomers assemble into oligomers in a head-to-head or a head-to-tail conformation (Takahashi et al., 2008). *In vivo* studies have concluded that the formation of aggregates from soluble monomeric polyQ forms is a 'multi-step process' and involves many intermediates (Thakur et al., 2009). It is also appreciated now, via *in vitro* studies, that polyQ proteins can form fibrillar aggregates that share certain properties with amyloid β (Aβ) peptide, such as binding of thioflavin T, staining by Congo red, and reacting with so-called "anti-amyloid" antibodies. These fibrils are formed from protofibrils and self associate to form branched structures. A range of intermediates have been observed between soluble monomeric forms and the fibrillar polyQ aggregates (Figure 48.1), and they include spherical structures, rare annular structures, amorphous structures, fibrils and protofibrils (Wacker et al., 2004).

Protein context plays a key role in modulating the extrinsic toxicity of the polyQ sequence. Among the nine polyQ diseases, the aggregation dynamics of three polyQ disease proteins, (ataxin-1, ataxin-2, and htt) have been characterized. All three of these proteins contain a flanking sequence that can aggregate independently of the polyQ tract. The propensity of the flanking sequence to modulate aggregation and toxicity was shown by adding certain adjacent amino acids to the same length of polyQ tract and noting the effect upon aggregation rates. The flanking domain also interferes with protein–protein interactions that determine whether aggregation will occur.

What is the toxic misfolded protein species in the polyglutamine repeat diseases?

As polyQ proteins can adopt a variety of structures, a controversial question in the field is whether soluble, aggregate, monomeric, oligomeric or intermediate forms of the protein (or some combination thereof) are toxic. In an attempt to determine if polyQ-containing monomers are toxic, one group has claimed to express such forms in cultured cells and found that they were toxic to neurons (Nagai et al., 2007). But the work was done *in vitro*, using an artificial polypeptide instead of a polyQ disease protein, and one cannot be certain as to how stable the monomers are in the culture. However, in a subsequent study carried out in living cells, polyQ oligomers exhibited greater toxicity to neuronally differentiated cells than monomeric forms (Takahashi et al., 2008). A number of other groups have presented data implicating soluble forms of polyQ disease proteins in the pathogenic pathway (Li et al., 2007; Li et al., 2002). This notion—that soluble polyQ disease proteins are likely mediating their toxic effects in the context of protein complexes to which they normally belong—has gained considerable support in the field especially over the last few years, as studies of SCA1 and SBMA have demonstrated the relevance of "native" protein interactions to disease pathogenesis (Lim et al., 2008; Nedelsky et al., 2010). This emerging view offers an explanation for an important conundrum—why the different diseases display selective patterns of cell vulnerability while exhibiting widespread and overlapping patterns of expression within the neuraxis.

THE ROLE OF PROTEIN TURNOVER PATHWAYS IN POLYGLUTAMINE DISEASE PATHOGENESIS

Are polyglutamine tracts substrates for the ubiquitin-proteasome system and autophagy pathways?

Eukaryotic cells have two main systems to degrade toxic and misfolded proteins: (1) the ubiquitin-proteasome system (UPS), and (2) the autophagy–lysosomal pathway. The UPS

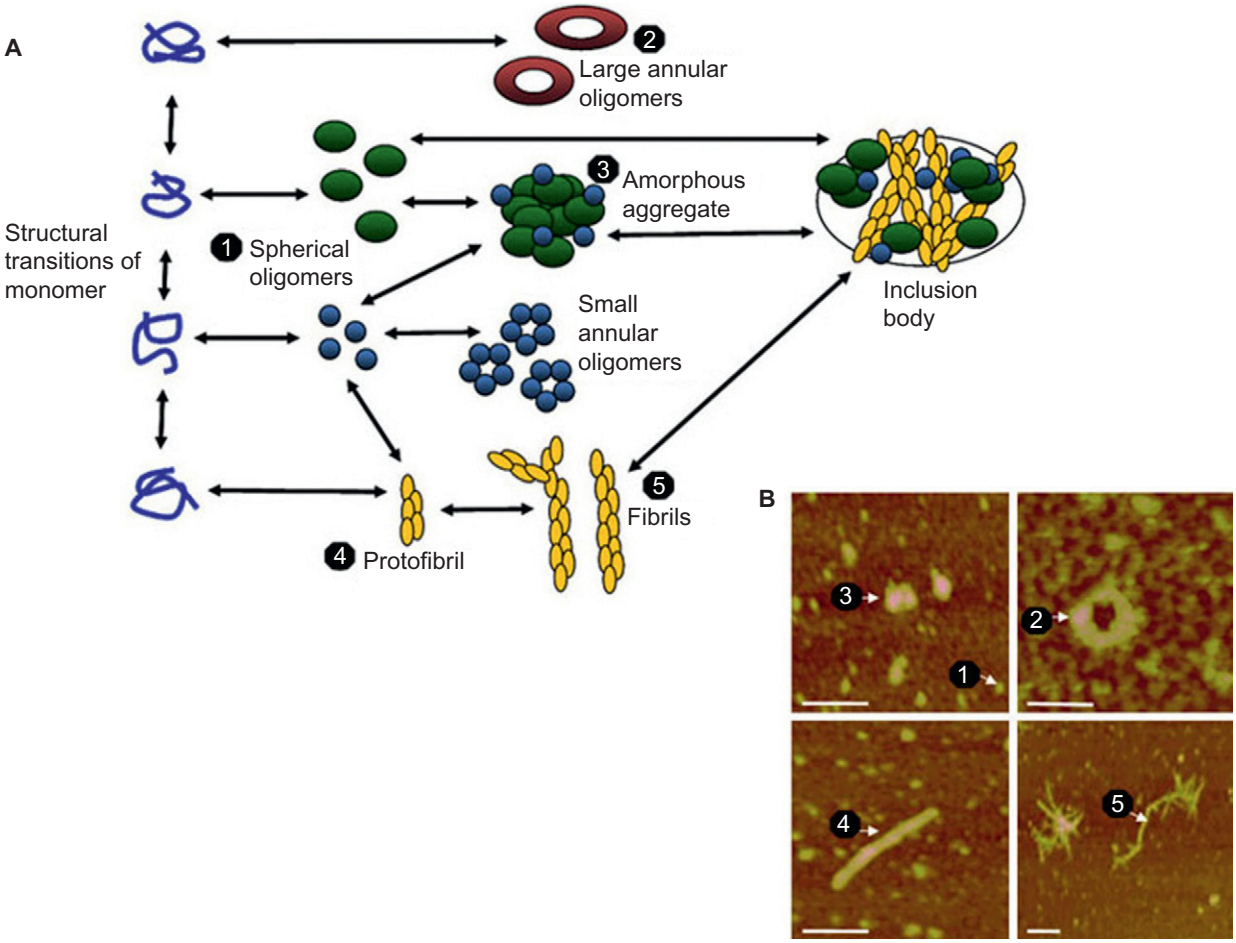

FIGURE 48.1 **Model for the formation and inter-conversion of misfolded conformers and protein aggregates in polyQ repeat disease**. (**A**) PolyQ disease proteins can adopt a variety of monomeric conformations, which convert into spherical, annular, and amorphous oligomeric forms. Certain oligomers then give rise to protofibrils, which in turn form fibrils. (**B**) Examples of these different structural conformations (enumerated to correspond to illustrated versions in panel 'a') can be documented by atomic force microscopy. From Hands & Wyttenbach, 2010; *Acta Neuropathol*, 120: 419. Used with Permission.

is the route by which the cell degrades soluble, short-lived and misfolded proteins. Such misfolded proteins are tagged by ubiquitin and then targeted to the 26S proteasome for degradation.

Early work in the polyQ field revealed evidence for inhibition of UPS function (Bence et al., 2001). Later studies then presented data suggesting that polyQ proteins cannot be degraded by the UPS (Holmberg et al., 2004; Venkatraman et al., 2004). While other groups disagreed with this conclusion, the presumed inability of the UPS to degrade polyQ disease proteins contributed to the notion that misfolded polyQ-containing proteins must be degraded by autophagy. Autophagy refers to three pathways by which cytoplasmic cargoes are delivered to the lysosome for degradation, and has thus been classified into three types: macroautophagy (often referred to as autophagy) microautophagy and chaperone-mediated autophagy. Macroautophagy, hereafter referred to as simply "autophagy," is the main pathway by which the cell degrades long-lived and misfolded proteins. Briefly, the process involves the formation of a double membrane, engulfing the macromolecules (such as misfolded proteins), organelles and other substrates, resulting in the formation of autophagosomes, which then fuse with lysosomes to form autolysosomes, where lysosomal enzymes degrade the contents and inner membrane (Figure 48.2).

Autophagy pathway involvement in polyglutamine neurodegeneration

A connection between autophagy and polyQ disease was made early on in the field, when autophagic-like vacuoles were noted in the brains of HD patients (Sapp et al., 1997). It was then shown that expression of mutant htt protein in cultured cells activates autophagy and leads to the accumulation of autophagosomes (Kegel et al., 2000). Inhibiting autophagy by the use of siRNAs against autophagy pathway genes has been found to increase the amount of polyQ aggregates (Iwata et al., 2005). This observation led investigators to test if induction of autophagy might provide neuroprotection by degrading misfolded proteins, and *in vitro* evidence for autophagic degradation of proteins with expanded polyQ tracts or polyalanine tracts was found (Kegel et al., 2000; Qin et al., 2003;

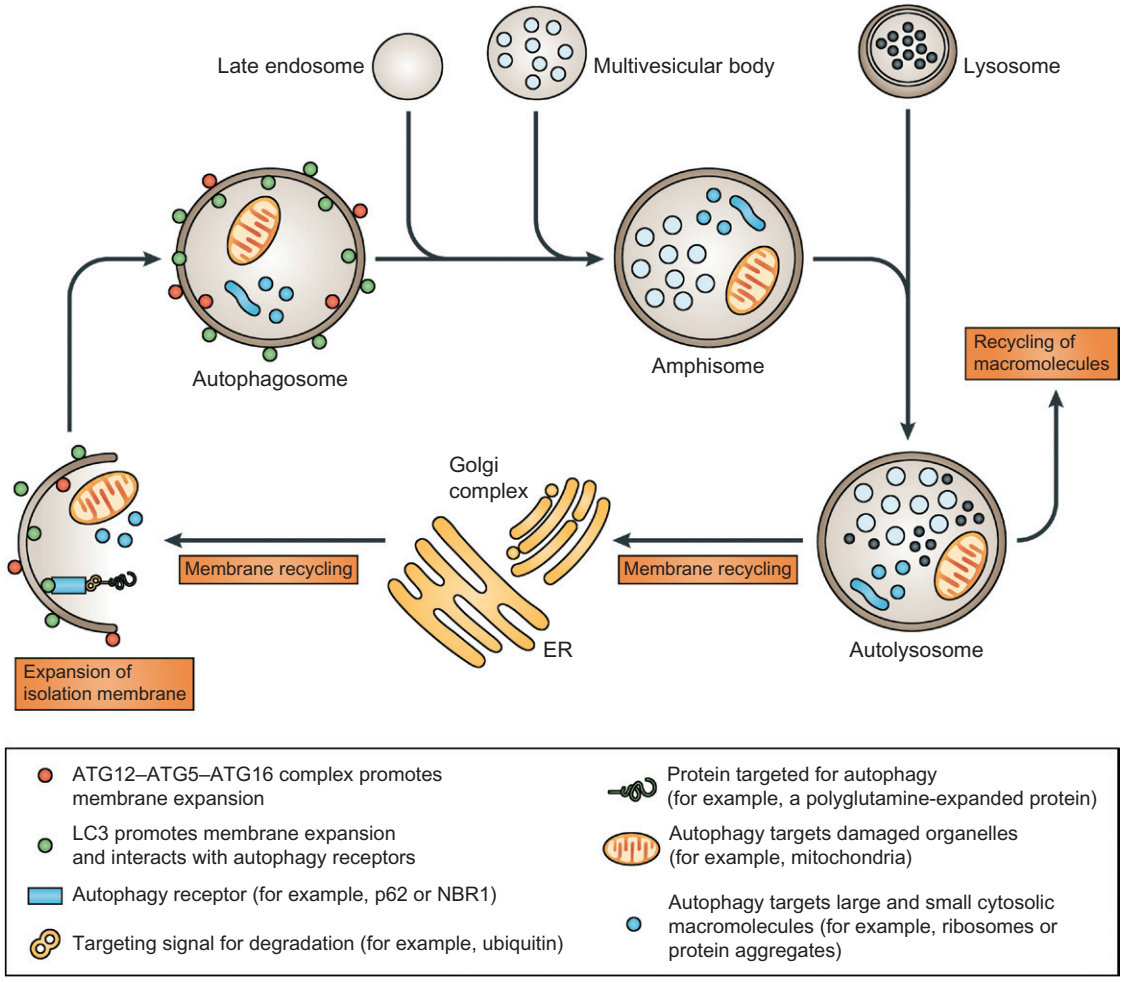

FIGURE 48.2 **The autophagy pathway in mammalian cells**. The (macro)autophagy pathway begins with the formation of an isolation membrane that engulfs proteins, macromolecules, and organelles destined for degradation. This structure then extends and fuses to form a double membrane vesicle known as an autophagosome. The formation of the vacuole is controlled by ubiquitin-like activation cascades, initiated through a multimeric complex (ATG12-ATG5-ATG16), while a second set of reactions results in conjugation of microtubule-associated protein light chain 3 (LC3) with phosphotidyl-ethanolamine. This lipidation reaction results in incorporation of LC3 into the inner and outer membrane of the autophagosome. LC3 also binds to autophagy receptors such as sequestosome 1 (SQSTM1, also known as p62). Newly formed autophagosomes go through a stepwise maturation process, first fusing with endosomes or multivesicular bodies to form amphisomes that ultimately fuse with lysosomes. Formation of the autolysosome is accompanied by a drop in pH, activating lysosomal hydrolases that then degrade the contents of the autophagosome and its inner membrane. From La Spada & Taylor, 2010; *Nat Rev Genet*, 11: 247. Used with Permission.

Ravikumar et al., 2002; Taylor et al., 2003). Subsequent work in model organisms indicated that rapamycin treatment suppresses the neurological phenotype in HD mouse and fly models by autophagy induction (Ravikumar et al., 2004). Autophagy induced by over expression of histone deacetylase 6 (HDAC6), a microtubule-associated deacetylase, can partially rescue neurodegeneration in a fly model of SBMA (Pandey et al., 2007), presumably by promoting degradation of polyQ-expanded proteins as a compensation for impaired UPS function. But if autophagy can compensate for diminished UPS function, accelerate mutant protein turnover, and yield an improved neurodegenerative phenotype, why is this protection incomplete? One possible explanation is that the autophagy pathway itself is a disease target, and thus activation of autophagy is inevitably inadequate (La Spada & Taylor, 2010). A number of studies have established that basal autophagy is critically important in post-mitotic tissues, such as neurons and myocytes, to maintain cellular health and normal function (Hara et al., 2006; Komatsu et al., 2006). Successful progression of the autophagy pathway requires that autophagosomes fuse with lysosomes. In neurons, consummation of the autophagy pathway poses special challenges, as the lysosomes are concentrated in the soma, while autophagosomes are generated in dendrites and axons, and thus must be transported to cell soma to achieve fusion with lysosomes. Consequently, neuronal autophagy is exquisitely vulnerable to defects in vesicular trafficking, as has been shown to occur in AD pathogenesis and disease progression (Boland et al., 2008; Nixon et al., 2005). Hence, accumulation of autophagic vacuoles in polyQ repeat expansion diseases and other related

neurodegenerative proteinopathies likely reflects a defect in autophagy flux, rather than autophagy induction.

Evidence for autophagy dysfunction in the polyglutamine repeat diseases

The role of autophagy in polyQ diseases has generated significant interest, especially since some workers have argued that the process can be pharmacologically manipulated to therapeutic effect (Rubinsztein et al., 2007). However, many questions remain unresolved. One important issue is whether overexpression of disease proteins could be providing an inaccurate assessment of the status of the autophagy pathway. For example, many polyQ disease studies have been performed in model systems that massively overexpress the mutant proteins, resulting in autophagy activation due to cellular stress. It is therefore preferable to examine autophagy pathway function in animal models that feature endogenous expression levels of polyQ disease proteins, ideally by using knock-in, yeast artificial chromosome (YAC) or bacterial artificial chromosome (BAC) approaches. Studies of autophagy in polyQ repeat diseases suggest that the autophagy pathway is likely perturbed, but the basis of the dysfunction remains unclear. There are at least three possible mechanisms by which this can occur: (1) Autophagy can be impaired by overwhelming the system with an excess of misfolded proteins, favoring a defective turnover of organelles and other cytoplasmic contents. (2) Autophagy pathway function can be altered due to an inappropriate interaction of the misfolded protein with a regulatory autophagy pathway factor. An example of this would be the proposed failure of cargo recognition suggested to occur in HD due to interference with p62 function (Martinez-Vicente et al., 2010). (3) Autophagy can be impaired if the disease protein is normally involved in the autophagy pathway. Although no evidence for a relationship between the normal function of polyQ proteins and the autophagy pathway has been found for most repeat diseases, in the case of HD, it appears that htt is trafficked with autophagosomes in axons and is associated with the endosomal-lysosomal system. Recently, it was shown that mutant htt impairs the activity of GTPase RAB11A in HD knock-in mouse model and results in defects in endosome recycling (Li et al., 2009a; Li et al., 2009b). Further work on the mechanistic basis of autophagy dysfunction in the different polyQ diseases will be necessary before the option of inducing autophagy as a therapy can be seriously considered.

THE IMPORTANCE OF NORMAL FUNCTION IN THE POLYGLUTAMINE REPEAT DISEASES

Interference with ataxin-7's function as a transcription regulatory protein in SCA7

In the polyQ repeat diseases, the dominant paradigm is that the glutamine tract expansion imparts toxicity to the mutant protein through a gain-of-function mechanism. However, numerous studies have also found that the polyQ tract expansion can alter the function of the protein within which it resides (La Spada & Taylor, 2010), suggesting that loss of function could also be simultaneously contributing to disease pathogenesis. One of the first disorders to be approached from the perspective of disease protein normal function is SCA7. Working independently, three groups determined that ataxin-7 is part of a transcription co-activator complex known as STAGA (Helmlinger et al., 2004; Palhan et al., 2005; Scheel et al., 2003). Importantly, one group found that polyQ-expanded ataxin-7 entered the STAGA complex and there inhibited the function of the histone acetyltransferase GCN5, resulting in transcription interference for target genes that relied upon STAGA function for their expression (Palhan et al., 2005).

Ataxin-1 protein complex associations account for SCA1 disease pathogenesis

Another more recent example of this is demonstrated by SCA1. Mutant ataxin-1 protein, in the absence of a nuclear localization signal or the ATXN1-HBP1 domain, does not cause cerebellar degeneration. This suggests that the normal function and interactions of ataxin-1 outside of the polyQ region are important for its toxic properties. More recent work has further shown that ataxin-1 exists in at least two different protein complexes, and that polyQ expansion alters the relative interactions of ataxin-1 with core components of these two complexes (Lim et al., 2008). In one case, the interaction is enhanced, and in the other, the interaction is reduced, presumably altering the function of the two complexes, either positively or negatively. The significance of the negative effect was further highlighted by a study showing that partial loss of normal ataxin-1 can cause the SCA1 phenotype, while increased expression of ataxin-1–like protein (ATXN1-L), a protein very similar to ataxin-1, can counter the loss-of-function effect, as ATXN1-L expression can rescue defects in mice lacking ataxin-1 (Crespo-Barreto et al., 2010). These studies, along with work on the AR protein in SBMA (Thomas, et al., 2006) strongly suggest that disease protein normal function is crucial for a complete understanding of polyQ disease pathogenesis.

Post-translational modifications as determinants of disease

As if the combination of gain-of-function and loss-of-function is not sufficiently challenging to comprehend polyQ disease pathogenesis, a related line of investigation has added another wrinkle to our understanding of these disorders. Indeed, it now appears that the polyQ expansion may not be necessary to cause pathology for certain polyQ repeat diseases, suggesting that the gain-of-function toxicity conferred by the polyQ tract expansion may reflect adoption of a novel conformation that can be attained through alternate pathways, in particular specific post-translational modifications.

Phosphorylation

Phosphorylation can affect protein function, localization and conformation by interfering with protein–protein interactions. A series of studies has revealed that in the case

of ataxin1, the causative gene for SCA1, phosphorylation at serine 776 (S776) is dependent upon the length of the polyQ tract, and by replacing this residue with an alanine and thereby preventing its phosphorylation, it is possible to dramatically reduce cerebellar neuropathology (Emamian et al., 2003). This finding highlights the importance of post-translational modification in the polyQ diseases and how phosphorylation can affect normal protein function. Ataxin-1 makes a protein complex with two other proteins, the transcription factor Capicua and an RNA-binding protein, RBM17. The length of polyQ tract alters the relative presence of ataxin-1 in these two complexes because polyQ-expanded ataxin-1 favors the RBM17 protein complex, rather than the Capicua protein complex. Phosphorylation status dictates the association of ataxin-1 with the two complexes, and an extended polyQ tract promotes phosphorylation at S776, which in turn favors binding with RBM17 vis-à-vis Capicua (Lim et al., 2008). Importantly, phosphomimetic amino acid substitution at S776 can produce neurotoxicity in mice in the absence of a polyQ tract expansion (Duvick et al., 2010).

In HD, the htt protein can be phosphorylated at multiple sites, especially S421, such that phosphorylation reduces the toxicity and formation of inclusion bodies (Humbert et al., 2002; Warby et al., 2005). In this case, expansion of the polyQ tract reduces the extent of this phosphorylation event. In a further study, it was shown that phosphorylation of S421 affects the recruitment of motor proteins to the endocytic vesicles, and that constitutive phosphorylation at S421 could overcome brain-derived neurotrophic factor signaling defects and thereby reduce neurotoxicity. As the htt protein undergoes protoelytic cleavage to promote toxicity, investigators have carefully scrutinized the N-terminal region of htt, cataloguing a variety of post-translational modifications within the first 17 amino acids. Using BAC transgenic mice to evaluate the importance of S13 and S16 phosphorylation, one group found that the toxicity of polyQ-expanded htt could be abolished by mimicking S13/16 phosphorylation with phosphomimetic aspartic acid substitutions (Gu et al., 2009). In an independent study, IkB kinase (IKK) was shown to mediate these serine phosphorylations, which promote the turnover of the htt protein (Thompson et al., 2009).

Acetylation

The binding of an acetyl group with lysine residues in the protein results in acetylation, a modification known to affect protein–protein and protein–DNA interactions and determine protein stability. In most cases, acetylation increases protein stability. In SCA7, acetylation at lysine 257 (K257) promotes the stability of the protein, and may be important for disease toxicity, as K257 resides within the ataxin-7 N-terminal truncation product (Mookerjee et al., 2009). In SBMA, acetylation at K632/633 regulates nuclear entry as well as the folding state of AR protein (Thomas et al., 2004). In HD, acetylation at K444 of htt protein facilitates trafficking of htt to autophagosomes and clearance of htt protein by autophagy (Jeong et al., 2009). This work indicates that acetylation can direct proteins to be degraded by autophagy, rather than stabilize them; however, to what extent this occurs, and whether it occurs in other polyQ diseases, remains unclear.

Sumoylation

Sumoylation refers to when a small ubiquitin-like modifier (SUMO) moiety is covalently linked to a lysine residue in the target protein. SUMO was observed to colocalize with neuronal inclusion bodies in the brains of patients with HD, SCA3, and DRPLA (Steffan et al., 2004). Directed studies of htt protein have implicated sumoylation in the pathogenic cascade, as sumoylation at K6 and K9 in the first 17 amino acids of the htt protein promotes mutant htt toxicity by preventing its sequestration into aggregates (Steffan et al., 2004).

RNA TOXICITY IN THE POLYGLUTAMINE REPEAT DISEASES?

PolyQ disease pathology has been extensively studied, and there are considerable data supporting proteotoxicity as the primary cause of the pathogenesis in these diseases. A number of studies, including especially work done on SCA1 and SBMA, have precluded a role for RNA toxicity in polyQ disease, as mutant RNAs do not cause any neurodegeneration in mice, when the polyQ-expanded protein does not enter the nucleus (Katsuno et al., 2002; Klement et al., 1998). However, in a *Drosophila* screen for modifiers of polyQ-expanded ataxin-3 degeneration, *muscleblind (mbl)*, a splicing regulator implicated in the RNA toxicity of CUG repeat expansion diseases, was found to modify ataxin-3 polyQ toxicity (Li et al., 2008). Although this is a provocative finding, the mechanism underlying the effect of muscleblind has remained elusive, leaving a possible role for RNA toxicity in polyQ diseases undefined.

GENE SILENCING IS A PROMISING THERAPY FOR POLYGLUTAMINE REPEAT DISEASE

RNA interference knock-down and antisense oligonucleotide knock-down: two approaches

The molecular basis of polyQ disease pathogenesis has proven to be rather complicated, as multiple cellular, neuronal and nuclear processes are deleteriously affected in these disorders. As the production of the mutant protein is the initial step in the pathogenic cascade, growing emphasis has been placed on eliminating production of the disease protein. Importantly, this approach is expected to be effective, as experimental termination of mutant protein production in inducible mouse models of HD and SCA1 results in a markedly improved phenotype (Latouche et al., 2007; Yamamoto et al., 2000; Zu et al., 2004). Consequently, therapy efforts have focused upon silencing of the mutant gene, initially by application of a RNA interference (RNAi) strategy, and more recently by developing antisense oligonucleotides (ASOs). RNAi takes advantage of the existing small RNA processing machinery of the cell to either destroy targeted mRNAs or prevent their translation, while ASOs are short oligonucleotides that bind RNA in a sequence-specific manner and then promote degradation of the target in a RNase H-dependent manner. As the polyQ diseases are dominant disorders

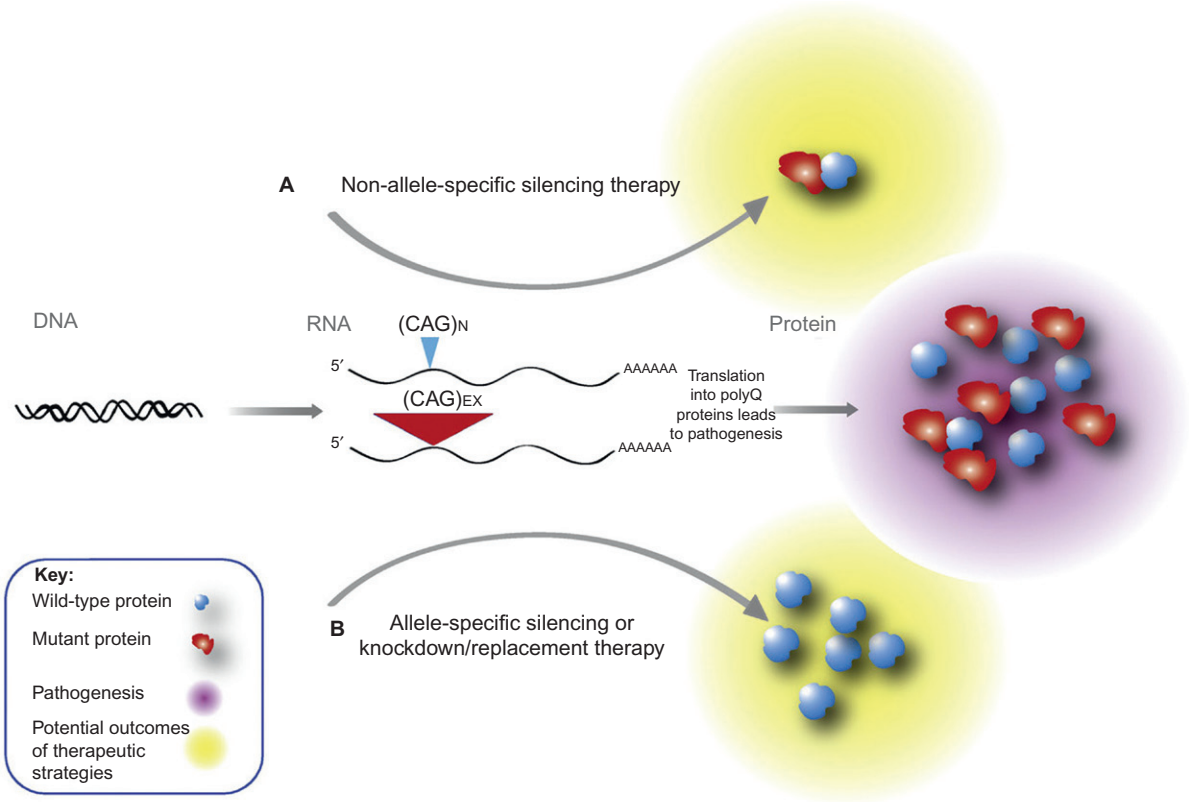

FIGURE 48.3 **Gene-silencing approaches to treat CAG – polyQ repeat disease.** Production of a mutant protein containing an expanded polyQ tract is likely the key first step in the pathogenic cascade. Gene silencing to prevent production of the disease protein can be selective (targeting only the mutant allele) or non-selective (targeting both the mutant allele and the normal allele). (**A**) Non-allele specific silencing involves indiscriminate targeting of the mRNA for the disease gene of interest. Due to the perfect nucleotide homology between the mutant and normal allele in the targeted region, levels of both mutant and normal mRNAs are reduced, yielding a decrease in the level of disease protein – but at the expense of also decreasing normal protein levels. (**B**) Allele specific silencing is typically achieved by exploiting nucleotide sequence differences between the normal and mutant alleles in the disease gene of interest. Alternatively, gene knock-down, together with gene replacement, may be pursued. In either case, the goal is to markedly reduce the levels of the mutant disease protein, while maintaining near normal levels of wild type protein. From Scholefield & Wood, 2010; *Trends Genet*, 26: 29. Used with Permission.

involving a single mutant allele, two alternative approaches have been developed: indiscriminate gene silencing and allele-specific silencing (Figure 48.3).

Indiscriminate gene silencing

In this approach, both the mutant and wild-type genes are silenced. While this approach offers the most comprehensive and straightforward algorithm for therapy development, one must consider whether reducing the normal function of the disease gene could be a problem. This is especially important because altered function of the disease protein has emerged as a factor in disease pathogenesis. Although reduced expression of the wild-type allele could pose a problem, the RNAi approach typically does not extinguish expression of the target gene; rather, the expression reduction does not exceed 60–70% of endogenous levels. One study has shown that this degree of reduction for htt is well tolerated in mice (McBride et al., 2008), suggesting that a non-allele specific approach may be a viable therapeutic option. Indeed, in 2011, a clinical trial on HD will be initiated applying this approach.

Allele-specific silencing

To achieve allele-specific silencing, the shRNA vector perfectly matches the sequence of the mutant allele, but is not completely complementary to the normal allele. The allele-specific silencing approach has been experimentally attempted in at least three polyQ diseases: HD, SCA3 and SCA7. Allele-specific silencing was achieved *in vivo* when a shRNA directed against the mutant ataxin-3 allele showed no effect on the wild-type allele (Alves et al., 2008). Recently, this silencing was also shown for SCA7, with a shRNA containing a mismatch that prevented silencing of the normal allele, but hit the mutant allele, resulting in reduced aggregates *in vitro* (Scholefield et al., 2009). In the case of HD, it may be possible to achieve specific silencing by targeting a triple deletion known as the 'delta2642' polymorphism (Zhang et al., 2009). Another allele-specific

INHIBITION OF TRANSGLUTAMINASE AS A POTENTIAL THERAPY IN POLYGLUTAMINE REPEAT DISEASES

George J. Siegel

Huntington's disease is one model for exploring the roles of the polyQ expansions of huntingtin protein (mhtt) in pathogenesis of disease and pharmacologic intervention. As pointed out in Chapter 48, mhtt leads to formation of insoluble aggregates but can also generate amyloid fibrils as well as oligomeric and protofibrillar structures containing glutamine tracts. These small, soluble species may actually be more toxic and the large insoluble aggregates may in fact be protective (see also (Mastroberardino et al., 2010; Arrasate et al., 2004)). In considering therapy, it must also be kept in mind that while the polyQ fragment may be necessary for toxicity, it may not be sufficient for all of its toxic effects and that more than one pathogenetic mechanism may be involved (see Box 49).

Therapeutic strategies addressing huntingtin gene regulation are discussed in Chapter 48 and histone deacetylase inhibitors in Box 27. The demonstration that polyQ expansions inhibit fast axoplasmic transport through a JNK-activated mechanism undoubtedly opens another fruitful arena of research into potential therapies (Morfini et al., 2009). This essay deals with the background for pharmacologic modification of transglutaminase. The polyQ stretches are substrates for crosslinking transaminations catalyzed by transglutaminases, of which there are four isoforms, the main one in brain being TG2. TG, in a strictly Ca^{2+}-dependent reaction, catalyzes formation of isopeptide bonds between the carboxamide groups of a glutamine residue on one polypeptide with, usually, the epsilon amino group of a lysine residue on another polypeptide (Jeitner et al., 2001). In postmortem HD brain, the total TG activity levels of TG2 protein and TG2-mRNA are increased together with the nuclear aggregates of mhtt. Also, elevations of intracellular Ca^{2+} and the presence of γ-L-glutamyl-L-lysine isopeptides in the mhtt aggregates are consistent features in HD brain. Moreover, CSF from HD patients contains elevated quantities of γ -glutamyl isopeptides. These facts are all consistent with upregulation of TG2, increased Ca^{2+} activation of TG2 and increased formation of γ-glutamyl-lysine cross-linkages in HD brain. Elaboration of inflammatory factors and glutamate excitotoxicity with attendant large increases in intracellular Ca^{2+}, which occur in HD brain, may lead to the upregulation of TG2. There is also evidence that mhtt enhances Ca^{2+} release from endoplasmic reticulum, another potential source for activation of TG2 (Jeitner et al., 2001; Jeitner et al., 2009). Thus, increased TG2 activity may result in toxicity in HD through unregulated transglutamination cross-linking of the polyQ with other polypeptides.

To elucidate the role of TG2 in HD, Mastroberardino et al., (2002) generated a transgenic HD mouse model (R6/1) that was also null or heterozygous for TG2. The deletion of TG2 led to significant reductions in the losses of body weight and brain weight, with increases in life span and improvement in motor behavior of the mhtt animals. TG2 ablation also led to large reductions in overall neuronal cell death and reductions in brain isopeptides but also, paradoxically, to an increased number of neuronal intranuclear insoluble aggregates. This suggested that TG2 catalyzed-crosslinking leads to toxicity but not to the formation of the insoluble aggregates and that the non-covalently formed aggregates are probably protective. A direct proof of the toxicity of diffuse polyQ expansions and the protective role of the insoluble aggregates of mhtt was demonstrated in cultured rat striatal neurons by Arrasate et al. (2004).

McConoughey et al. (2010), through a variety of experiments with transgenic mouse, cellular and *Drosophila* models expressing mhtt, showed that (1) TG2 acts in the nucleus to repress transcription factors for key metabolic enzymes; (2) TG2 activity impedes restoration of energy homeostasis after metabolic stresses (such as poisoning with 3-nitrophenol (3-NP), deprivation of glucose or oxygen); (3) TG2 inhibition or genetic ablation in mhtt-expressing cells increases mRNA for PGC-1a and *cyto c,* prolongs survival, and normalizes expression of metabolic genes and clusters of other genes; and (4) induces resistance to 3-NP. These experiments are described and referenced in detail (McConoughey et al., 2010). Transcription factors controlling the majority of nuclear-encoded mitochondrial proteins (such as SP-1, Nrf-1 and CREB) have glutamine-rich activator domains and are candidates for TG2-catalyzed cross-linking.

Therefore, investigators have explored the potential of specific TG2 inhibitors. Groups of TG2 inhibitors include siRNA, various small amine-bearing compounds and peptide-based inhibitors. Cystamine, the most widely studied, produces beneficial effects in transgenic mice. However, it also has effects independent of TG2 inhibition, including caspase-3 inhibition and increases in glutathione. However, cystamine is converted to cysteamine, which inhibits transcription of TG . High throughput screening is used to select rationally designed inhibitors that show beneficial effects in model systems (Morfini et al., 2009). One such inhibitor found through peptide screening is termed ZDON (gln-val-pro-leu). It has a higher affinity for TG2 than for TG1 or TG3 and in micromolar concentrations selectively inhibits TG2 in intact striatal neurons in Q7 and Q111 lines *in vitro*, while not inhibiting caspase-3 or raising glutathione levels. ZDON is considered a promising initial tool for developing therapy for HD (McConoughey et al., 2010).

References

Arrasate, M., Mitra, S., Schweitzer, E. S., Segal, M. R., & Finkbeiner, S. (2004, Oct 14). Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death. *Nature*, *431*(7010), 805–810.

Jeitner, T. M., Bogdanov, M. B., Matson, W. R., Daikhin, Y., Yudkoff, M., Folk, J. E., et al. (2001, Dec). N(epsilon)-(gamma-L-glutamyl)-L-lysine (GGEL) is increased in cerebrospinal fluid of patients with Huntington's disease. *Journal of Neurochemistry*, *79*(5), 1109–1112.

Jeitner, T. M., Muma, N. A., Battaile, K. P., & Cooper, A. J. (2009, Jul 1). Transglutaminase activation in neurodegenerative diseases. *Future Neurology*, *4*(4), 449–467.

Mastroberardino, P. G., & Piacentini, M. (2010, Nov). Type 2 transglutaminase in Huntington's disease: A double-edged

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sword with clinical potential. *Journal of Internal Medicine*, *268*(5), 419–431.

Mastroberardino, P. G., Iannicola, C., Nardacci, R., Bernassola, F., De Laurenzi, V., Melino, G., et al. (2002, Sep). "Tissue" transglutaminase ablation reduces neuronal death and prolongs survival in a mouse model of Huntington's disease. *Cell Death and Differentiation*, *9*(9), 873–880.

McConoughey, S. J., Basso, M., Niatsetskaya, Z. V., Sleiman, S. F., Smirnova, N. A., Langley, B. C., et al. (2010, Sep). Inhibition of transglutaminase 2 mitigates transcriptional dysregulation in models of Huntington disease. *EMBO Molecular Medicine*, *2*(9), 349–370.

Morfini, G. A., You, Y. M., Pollema, S. L., Kaminska, A., Liu, K., Yoshioka, K., et al. (2009, Jul). Pathogenic huntingtin inhibits fast axonal transport by activating JNK3 and phosphorylating kinesin. *Nature Neuroscience*, *12*(7), 864–871.

silencing approach is to knock-down both mutant and normal alleles, and then replace the normal gene (Kubodera et al., 2005). Hence, these studies suggest that selective gene silencing could be a viable therapeutic option in the polyQ disorders, and challenges the field to design more elegant RNA-targeting approaches, as the ideal treatment regimen should permit expression of the normal allele to be retained.

References

Alves, S., Nascimento-Ferreira, I., Auregan, G., Hassig, R., Dufour, N., Brouillet, E., et al. (2008). Allele-specific RNA silencing of mutant ataxin-3 mediates neuroprotection in a rat model of Machado-Joseph disease. *PLoS One*, *3*, e3341.

Arrasate, M., & Finkbeiner, S. (2005). Automated microscope system for determining factors that predict neuronal fate. *Proceedings of the National Academy of Sciences of the United States of America*, *102*, 3840–3845.

Bence, N. F., Sampat, R. M., & Kopito, R. R. (2001). Impairment of the ubiquitin-proteasome system by protein aggregation. *Science*, *292*, 1552–1555.

Boland, B., Kumar, A., Lee, S., Platt, F. M., Wegiel, J., Yu, W. H., et al. (2008). Autophagy induction and autophagosome clearance in neurons: Relationship to autophagic pathology in Alzheimer's disease. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *28*, 6926–6937.

Crespo-Barreto, J., Fryer, J. D., Shaw, C. A., Orr, H. T., & Zoghbi, H. Y. (2010). Partial loss of ataxin-1 function contributes to transcriptional dysregulation in spinocerebellar ataxia type 1 pathogenesis. *PLoS Genetics*, *6*, e1001021.

Davies, S. W., Turmaine, M., Cozens, B. A., DiFiglia, M., Sharp, A. H., Ross, C. A., et al. (1997). Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. *Cell*, *90*, 537–548.

DiFiglia, M., Sapp, E., Chase, K. O., Davies, S. W., Bates, G. P., Vonsattel, J. P., et al. (1997). Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. *Science*, *277*, 1990–1993.

Duvick, L., Barnes, J., Ebner, B., Agrawal, S., Andresen, M., Lim, J., et al. (2010). SCA1-like disease in mice expressing wild-type ataxin-1 with a serine to aspartic acid replacement at residue 776. *Neuron*, *67*, 929–935.

Emamian, E. S., Kaytor, M. D., Duvick, L. A., Zu, T., Tousey, S. K., Zoghbi, H. Y., et al. (2003). Serine 776 of ataxin-1 is critical for polyglutamine-induced disease in SCA1 transgenic mice. *Neuron*, *38*, 375–387.

Gu, X., Greiner, E. R., Mishra, R., Kodali, R., Osmand, A., Finkbeiner, S., et al. (2009). Serines 13 and 16 are critical determinants of full-length human mutant huntingtin induced disease pathogenesis in HD mice. *Neuron*, *64*, 828–840.

Hara, T., Nakamura, K., Matsui, M., Yamamoto, A., Nakahara, Y., Suzuki-Migishima, R., et al. (2006). Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. *Nature*, *441*, 885–889.

Helmlinger, D., Hardy, S., Sasorith, S., Klein, F., Robert, F., Weber, C., et al. (2004). Ataxin-7 is a subunit of GCN5 histone acetyltransferase-containing complexes. *Human Molecular Genetics*, *13*, 1257–1265.

Holmberg, C. I., Staniszewski, K. E., Mensah, K. N., Matouschek, A., & Morimoto, R. I. (2004). Inefficient degradation of truncated polyglutamine proteins by the proteasome. *The EMBO Journal*, *23*, 4307–4318.

Humbert, S., Bryson, E. A., Cordelieres, F. P., Connors, N. C., Datta, S. R., Finkbeiner, S., et al. (2002). The IGF-1/Akt pathway is neuroprotective in Huntington's disease and involves Huntingtin phosphorylation by Akt. *Developmental Cell*, *2*, 831–837.

Iwata, A., Christianson, J. C., Bucci, M., Ellerby, L. M., Nukina, N., Forno, L. S., et al. (2005). Increased susceptibility of cytoplasmic over nuclear polyglutamine aggregates to autophagic degradation. *Proceedings of the National Academy of Sciences of the United States of America*, *102*, 13135–13140.

Jeong, H., Then, F., Melia, T. J., Jr., Mazzulli, J. R., Cui, L., Savas, J. N., et al. (2009). Acetylation targets mutant huntingtin to autophagosomes for degradation. *Cell*, *137*, 60–72.

Katsuno, M., Adachi, H., Kume, A., Li, M., Nakagomi, Y., Niwa, H., et al. (2002). Testosterone reduction prevents phenotypic expression in a transgenic mouse model of spinal and bulbar muscular atrophy. *Neuron*, *35*, 843–854.

Kegel, K. B., Kim, M., Sapp, E., McIntyre, C., Castano, J. G., Aronin, N., et al. (2000). Huntingtin expression stimulates endosomal-lysosomal activity, endosome tubulation, and autophagy. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *20*, 7268–7278.

Klement, I. A., Skinner, P. J., Kaytor, M. D., Yi, H., Hersch, S. M., Clark, H. B., et al. (1998). Ataxin-1 nuclear localization and aggregation: Role in polyglutamine- induced disease in SCA1 transgenic mice. *Cell*, *95*, 41–53.

Komatsu, M., Waguri, S., Chiba, T., Murata, S., Iwata, J., Tanida, I., et al. (2006). Loss of autophagy in the central nervous system causes neurodegeneration in mice. *Nature*, *441*, 880–884.

Kubodera, T., Yokota, T., Ishikawa, K., & Mizusawa, H. (2005). New RNAi strategy for selective suppression of a mutant allele in polyglutamine disease. *Oligonucleotides*, *15*, 298–302.

La Spada, A. R., & Taylor, J. P. (2010). Repeat expansion disease: Progress and puzzles in disease pathogenesis. *Nature Reviews. Genetics*, *11*, 247–258.

La Spada, A. R., Wilson, E. M., Lubahn, D. B., Harding, A. E., & Fischbeck, K. H. (1991). Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. *Nature*, *352*, 77–79.

Latouche, M., Lasbleiz, C., Martin, E., Monnier, V., Debeir, T., Mouatt-Prigent, A., et al. (2007). A conditional pan-neuronal Drosophila model of spinocerebellar ataxia 7 with a reversible adult phenotype suitable for identifying modifier genes. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *27*, 2483–2492.

Li, L. B., Yu, Z., Teng, X., & Bonini, N. M. (2008). RNA toxicity is a component of ataxin-3 degeneration in Drosophila. *Nature*, *453*, 1107–1111.

Li, M., Chevalier-Larsen, E. S., Merry, D. E., & Diamond, M. I. (2007). Soluble androgen receptor oligomers underlie pathology in a mouse model of spinobulbar muscular atrophy. *The Journal of Biological Chemistry*, *282*, 3157–3164.

Li, S. H., Cheng, A. L., Zhou, H., Lam, S., Rao, M., Li, H., et al. (2002). Interaction of Huntington disease protein with transcriptional activator Sp1. *Molecular and Cellular Biology*, *22*, 1277–1287.

Li, X., Sapp, E., Chase, K., Comer-Tierney, L. A., Masso, N., Alexander, J., et al. (2009). Disruption of Rab11 activity in a knock-in mouse model of Huntington's disease. *Neurobiology of Disease*, *36*, 374–383.

Li, X., Standley, C., Sapp, E., Valencia, A., Qin, Z. H., Kegel, K. B., et al. (2009). Mutant huntingtin impairs vesicle formation from recycling endosomes by interfering with Rab11 activity. *Molecular and Cellular Biology*, *29*, 6106–6116.

Lim, J., Crespo-Barreto, J., Jafar-Nejad, P., Bowman, A. B., Richman, R., Hill, D. E., et al. (2008). Opposing effects of polyglutamine expansion on native protein complexes contribute to SCA1. *Nature*, *452*, 713–718.

Martinez-Vicente, M., Talloczy, Z., Wong, E., Tang, G., Koga, H., Kaushik, S., et al. (2010). Cargo recognition failure is responsible for inefficient autophagy in Huntington's disease. *Nature Neuroscience*, *13*, 567–576.

McBride, J. L., Boudreau, R. L., Harper, S. Q., Staber, P. D., Monteys, A. M., Martins, I., et al. (2008). Artificial miRNAs mitigate shRNA-mediated toxicity in the brain: Implications for the therapeutic development of RNAi. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 5868–5873.

Mookerjee, S., Papanikolaou, T., Guyenet, S. J., Sampath, V., Lin, A., Vitelli, C., et al. (2009). Posttranslational modification of ataxin-7 at lysine 257 prevents autophagy-mediated turnover of an N-terminal caspase-7 cleavage fragment. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *29*, 15134–15144.

Nagai, Y., Inui, T., Popiel, H. A., Fujikake, N., Hasegawa, K., Urade, Y., et al. (2007). A toxic monomeric conformer of the polyglutamine protein. *Nature Structural & Molecular Biology*, *14*, 332–340.

Nedelsky, N. B., Pennuto, M., Smith, R. B., Palazzolo, I., Moore, J., Nie, Z., et al. (2010). Native functions of the androgen receptor are essential to pathogenesis in a Drosophila model of spinobulbar muscular atrophy. *Neuron*, *67*, 936–952.

Nixon, R. A., Wegiel, J., Kumar, A., Yu, W. H., Peterhoff, C., Cataldo, A., et al. (2005). Extensive involvement of autophagy in Alzheimer disease: An immuno-electron microscopy study. *Journal of Neuropathology and Experimental Neurology*, *64*, 113–122.

Palhan, V. B., Chen, S., Peng, G. H., Tjernberg, A., Gamper, A. M., Fan, Y., et al. (2005). Polyglutamine-expanded ataxin-7 inhibits STAGA histone acetyltransferase activity to produce retinal degeneration. *Proceedings of the National Academy of Sciences of the United States of America*, *102*, 8472–8477.

Pandey, U. B., Nie, Z., Batlevi, Y., McCray, B. A., Ritson, G. P., Nedelsky, N. B., et al. (2007). HDAC6 rescues neurodegeneration and provides an essential link between autophagy and the UPS. *Nature*, *447*, 859–863.

Paulson, H. L., Perez, M. K., Trottier, Y., Trojanowski, J. Q., Subramony, S. H., Das, S. S., et al. (1997). Intranuclear inclusions of expanded polyglutamine protein in spinocerebellar ataxia type 3. *Neuron*, *19*, 333–344.

Perutz, M. F., Finch, J. T., Berriman, J., & Lesk, A. (2002). Amyloid fibers are water-filled nanotubes. *Proceedings of the National Academy of Sciences of the United States of America*, *99*, 5591–5595.

Perutz, M. F., Johnson, T., Suzuki, M., & Finch, J. T. (1994). Glutamine repeats as polar zippers: Their possible role in inherited neurodegenerative diseases. *Proceedings of the National Academy of Sciences of the United States of America*, *91*, 5355–5358.

Qin, Z. H., Wang, Y., Kegel, K. B., Kazantsev, A., Apostol, B. L., Thompson, L. M., et al. (2003). Autophagy regulates the processing of amino terminal huntingtin fragments. *Human Molecular Genetics*, *12*, 3231–3244.

Ravikumar, B., Duden, R., & Rubinsztein, D. C. (2002). Aggregate-prone proteins with polyglutamine and polyalanine expansions are degraded by autophagy. *Human Molecular Genetics*, *11*, 1107–1117.

Ravikumar, B., Vacher, C., Berger, Z., Davies, J. E., Luo, S., Oroz, L. G., et al. (2004). Inhibition of mTOR induces autophagy and reduces toxicity of polyglutamine expansions in fly and mouse models of Huntington disease. *Nature Genetics*, *36*, 585–595.

Rub, U., de Vos, R. A., Brunt, E. R., Sebesteny, T., Schols, L., Auburger, G., et al. (2006). Spinocerebellar ataxia type 3 (SCA3): Thalamic neurodegeneration occurs independently from thalamic ataxin-3 immunopositive neuronal intranuclear inclusions. *Brain Pathology (Zurich, Switzerland)*, *16*, 218–227.

Rubinsztein, D. C., Gestwicki, J. E., Murphy, L. O., & Klionsky, D. J. (2007). Potential therapeutic applications of autophagy. *Nature Reviews. Drug Discovery*, *6*, 304–312.

Sapp, E., Schwarz, C., Chase, K., Bhide, P. G., Young, A. B., Penney, J., et al. (1997). Huntingtin localization in brains of normal and Huntington's disease patients. *Annals of Neurology*, *42*, 604–612.

Scheel, H., Tomiuk, S., & Hofmann, K. (2003). Elucidation of ataxin-3 and ataxin-7 function by integrative bioinformatics. *Human Molecular Genetics*, *12*, 2845–2852.

Scholefield, J., Greenberg, L. J., Weinberg, M. S., Arbuthnot, P. B., Abdelgany, A., & Wood, M. J. (2009). Design of RNAi hairpins for mutation-specific silencing of ataxin-7 and correction of a SCA7 phenotype. *PLoS One*, *4*, e7232.

Slow, E. J., Graham, R. K., Osmand, A. P., Devon, R. S., Lu, G., Deng, Y., et al. (2005). Absence of behavioral abnormalities and neurodegeneration *in vivo* despite widespread neuronal huntingtin inclusions. *Proceedings of the National Academy of Sciences of the United States of America*, *102*, 11402–11407.

Steffan, J. S., Agrawal, N., Pallos, J., Rockabrand, E., Trotman, L. C., Slepko, N., et al. (2004). SUMO modification of Huntingtin and Huntington's disease pathology. *Science*, *304*, 100–104.

Takahashi, T., Kikuchi, S., Katada, S., Nagai, Y., Nishizawa, M., & Onodera, O. (2008). Soluble polyglutamine oligomers formed prior to inclusion body formation are cytotoxic. *Human Molecular Genetics*, *17*, 345–356.

Taylor, J. P., Tanaka, F., Robitschek, J., Sandoval, C. M., Taye, A., Markovic-Plese, S., et al. (2003). Aggresomes protect cells by enhancing the degradation of toxic polyglutamine-containing protein. *Human Molecular Genetics*, *12*, 749–757.

Thakur, A. K., Jayaraman, M., Mishra, R., Thakur, M., Chellgren, V. M., Byeon, I. J., et al. (2009). Polyglutamine disruption of the huntingtin exon 1 N terminus triggers a complex aggregation mechanism. Nature Structural &. *Molecular Biology*, *16*, 380–389.

Thakur, A. K., & Wetzel, R. (2002). Mutational analysis of the structural organization of polyglutamine aggregates. *Proceedings of the National Academy of Sciences of the United States of America*, *99*, 17014–17019.

Thomas, M., Dadgar, N., Aphale, A., Harrell, J. M., Kunkel, R., Pratt, W. B., et al. (2004). Androgen receptor acetylation site mutations

cause trafficking defects, misfolding, and aggregation similar to expanded glutamine tracts. *Journal of Biological Chemistry*, *279*, 8389–8395.

Thomas, P. S., Jr., Fraley, G. S., Damien, V., Woodke, L. B., Zapata, F., Sopher, B. L., et al. (2006). Loss of endogenous androgen receptor protein accelerates motor neuron degeneration and accentuates androgen insensitivity in a mouse model of X-linked spinal and bulbar muscular atrophy. *Human Molecular Genetics*, *15*, 2225–2238.

Thompson, L. M., Aiken, C. T., Kaltenbach, L. S., Agrawal, N., Illes, K., Khoshnan, A., et al. (2009). IKK phosphorylates Huntingtin and targets it for degradation by the proteasome and lysosome. *The Journal of Cell Biology*, *187*, 1083–1099.

Trottier, Y., Lutz, Y., Stevanin, G., Imbert, G., Devys, D., Cancel, G., et al. (1995). Polyglutamine expansion as a pathological epitope in Huntington's disease and four dominant cerebellar ataxias. *Nature*, *378*, 403–406.

Venkatraman, P., Wetzel, R., Tanaka, M., Nukina, N., & Goldberg, A. L. (2004). Eukaryotic proteasomes cannot digest polyglutamine sequences and release them during degradation of polyglutamine-containing proteins. *Molecular Cell*, *14*, 95–104.

Wacker, J. L., Zareie, M. H., Fong, H., Sarikaya, M., & Muchowski, P. J. (2004). Hsp70 and Hsp40 attenuate formation of spherical and annular polyglutamine oligomers by partitioning monomer. Nature Structural &. *Molecular Biology*, *11*, 1215–1222.

Warby, S. C., Chan, E. Y., Metzler, M., Gan, L., Singaraja, R. R., Crocker, S. F., et al. (2005). Huntingtin phosphorylation on serine 421 is significantly reduced in the striatum and by polyglutamine expansion *in vivo*. *Human Molecular Genetics*, *14*, 1569–1577.

Yamamoto, A., Lucas, J. J., & Hen, R. (2000). Reversal of neuropathology and motor dysfunction in a conditional model of Huntington's disease. *Cell*, *101*, 57–66.

Zhang, Y., Engelman, J., & Friedlander, R. M. (2009). Allele-specific silencing of mutant Huntington's disease gene. *Journal of Neurochemistry*, *108*, 82–90.

Zu, T., Duvick, L. A., Kaytor, M. D., Berlinger, M. S., Zoghbi, H. Y., Clark, H. B., et al. (2004). Recovery from polyglutamine-induced neurodegeneration in conditional SCA1 transgenic mice. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *24*, 8853–8861.