Human Molecular Genetics, 2000, Vol. 9, No. 6 Review 909–916

Fourteen and counting: unraveling trinucleotide repeat diseases

Christopher J. Cummings[1] and Huda Y. Zoghbi[1,2,+]

[1]Program in Cell and Molecular Biology, Departments of Pediatrics, Molecular and Human Genetics, Baylor College of Medicine and [2]Howard Hughes Medical Institute, Houston, TX 77030, USA

Received 4 February 2000; Accepted 8 February 2000

The pathological expansion of unstable trinucleotide repeats currently is known to cause 14 neurological diseases. Over the past several years, researchers have concentrated on the challenging task of identifying the mechanism by which the expanded trinucleotide repeat leads to abnormal cellular function. As a consequence, the trinucleotide repeat field has grown dramatically since the initial discovery of dynamic mutations less than a decade ago. Trinucleotide repeat expansions may prove to cause pathology through a variety of mechanisms including interference with DNA structure, transcription, RNA–protein interaction and altered protein conformations/interactions. The goal of this review is to provide a brief description of the genes harboring expanded repeats, coupled with new insights into the molecular pathways most likely to be disrupted by these expansions. Data from studies of patient material, cell culture and animal models demonstrate the complexity of the pathogenic mechanisms in each of the diseases.

INTRODUCTION

Trinucleotide repeats were once thought to be commonplace iterations in the genome, but the 1990s taught us differently. These apparently 'benign' stretches of DNA can sometimes expand and cause disease. Several defining features are shared amongst disorders caused by trinucleotide repeat expansions. First, the mutant repeats show both somatic and germline instability and, more frequently, they expand rather than contract in successive transmissions. Secondly, an earlier age of onset and increasing severity of phenotype in subsequent generations (anticipation) generally are correlated with larger repeat length. Finally, the parental origin of the disease allele can often influence anticipation, with paternal transmissions carrying a greater risk of expansion for many of these disorders.

Trinucleotide repeat diseases can be categorized into two subclasses based on the relative location of the trinucleotide repeat to a gene. The first subclass, presently accounting for six diseases, has its repeats in non-coding sequences (Table 1), whereas the second subclass is characterized by exonic $(CAG)_n$ repeats that code for polyglutamine tracts. The latter group is referred to collectively as 'polyglutamine diseases' (Table 2). This review summarizes the most recent developments in understanding the molecular mechanisms at work in these two subclasses of diseases.

NON-CODING TRINUCLEOTIDE REPEAT DISORDERS

The non-coding trinucleotide repeat diseases typically are characterized by large and variable repeat expansions that result in multiple tissue dysfunction or degeneration. Phenotypic manifestations within a disease are variable, perhaps owing to a pronounced degree of somatic heterogeneity. The larger mutations often are transmitted from a small pool of clinically silent intermediate size expansions, termed premutations. Despite these similarities, the trinucleotide repeat sequences vary in this subclass (CGG, GCC, GAA, CTG and CAG), and it is clear that the particular trinucleotide sequence, as well as its location with respect to a gene, are important defining factors in dictating the unique mechanism of pathogenesis for each disease. The pathogenic mechanism will also vary from disease to disease, depending on the consequences of the lost function of the respective proteins or, in some cases, acquired function of a toxic transcript. As each of these diseases may have unique pathophysiologies they will be discussed individually in the following sections.

Fragile X syndrome (FRAXA)

Fragile X syndrome is characterized primarily by mental retardation, macroorchidism, some dysmorphic features and hyperactivity. The disease is caused by expansion of a polymorphic $(CGG)_n$ repeat in the 5′-untranslated region (UTR) of the fragile X mental retardation gene (*FMR1*) (1,2). Expansion of the CGG repeat beyond 230 trinucleotides results in its hypermethylation together with a CpG island within the *FMR1* promoter region (3). This hypermethylation recruits transcriptional silencing machinery to the *FMR1* gene, followed by reduced *FMR1* transcription and loss of gene product (FMRP) (4–7). The mutation in fragile X syndrome is therefore a loss of the normal function of FMRP. With two K homology domains

+To whom correspondence should be addressed at: Baylor College of Medicine, Howard Hughes Medical Institute, One Baylor Plaza, Room T807, Houston, TX 77030-3498, USA. Tel: +1 713 798 6558; Fax: +1 713 798 8728; Email: hzoghbi@bcm.tmc.edu

Table 1. Diseases caused by expansion of non-coding trinucleotide repeats

Disease	Gene /Locus	Protein	Schematic representation (not to scale)	Proposed Mechanism of Disease
Fragile X syndrome	FMR1 (FRAXA) Xq27.3	FMR-1 protein (FMRP)		Loss of function: Loss of FMR1: abnormal RNA metabolism
Fragile XE syndrome	FMR2 (FRAXE) Xq28	FMR-2 protein		Loss of function: Loss of FMR2: disruption of neuronal gene regulation?
Friedreich ataxia	X25 9q13-21.1	Frataxin		Loss of function: Reduced frataxin in mitochondria causing altered iron homeostasis and mitochondrial dysfunction
Myotonic Dystrophy	DMPK 19q13	Myotonic dystrophy protein kinase (DMPK)		Loss and/or gain of function: Reduced DMPK: disruption in kinase activity Cis-effects: silencing in the DM region Dominant effects on RNA processing (CUG-binding proteins)
Spinocerebellar ataxia type 8	SCA8 13q21	None		Loss of function? Abnormal RNA (antisense) regulation?
Spinocerebellar ataxia type 12	SCA12 5q31-33	PP2A-PR55β		Loss of function? Disruption in phosphatase activity?

Upper and lower repeat sizes represent the disease and normal allele sizes, respectively (disease repeats given for FRAXA, FRAXE, X25 and DMPK are the full mutation).

Table 2. Diseases caused by expanded glutamine tracts

Disease	Gene	Locus	Protein	CAG repeat size	
				Normal	Disease
Spinobulbar muscular atrophy (Kennedy disease)	*AR*	Xq13–21	Androgen receptor (AR)	9–36	38–62
Huntington's disease	*HD*	4p16.3	Huntingtin	6–35	36–121
Dentatorubral-pallidoluysian atrophy (Haw–River syndrome)	*DRPLA*	12p13.31	Atrophin-1	6–35	49–88
Spinocerebellar ataxia type 1	*SCA1*	6p23	Ataxin-1	6–44[a]	39–82
Spinocerebellar ataxia type 2	*SCA2*	12q24.1	Ataxin-2	15–31	36–63
Spinocerebellar ataxia type 3 (Machado–Joseph disease)	*SCA3* (*MJD1*)	14q32.1	Ataxin-3	12–40	55–84
Spinocerebellar ataxia type 6	*SCA6*	19p13	α_{1A}-voltage-dependent calcium channel subunit	4–18	21–33
Spinocerebellar ataxia type 7	*SCA7*	13p12–13	Ataxin-7	4–35	37–306

[a]Alleles with 21 or more repeats are interrupted by 1–3 CAT units; disease alleles contain pure CAG tracts.

and an RGG box, FMRP is an RNA-binding protein that shuttles between the nucleus and the cytoplasm (3). In the cytoplasm, FMRP forms messenger ribonucleoprotein (mRNP) complexes and associates with ribosomes. *FMR1* has highest expression in the brain. In neurons, FMRP's association with the translational machinery in the dendritic spines suggests that it may play a role in modulating the localization and/or translation of specific target mRNAs. Therefore, mental retardation in FRAXA may result from improper protein translation during synaptic development or maintenance. The other phenotypes associated with fragile X syndrome may stem from the potential pleiotropic effects caused by abnormal RNA regulation. FMRP and two related proteins, FXR1P and FXR2P, show significant homology (60%) including conservation of the RNA-binding domains (8,9). These proteins are capable of interacting as homodimers and heterodimers and can be co-immunoprecipitated as a complex, suggesting a functional role as a ribonucleoprotein complex (10,11). Nucleolin and NUFIP (nuclear FMRP-interacting protein) are two other FMRP-associated proteins recently identified (12,13). The physiological significance of these new interactors is not yet known, but they may function as part of the FMRP–RNP complex that regulates specific RNA metabolism.

Fragile XE MR (FRAXE)

FRAXE patients have mild mental retardation and variable behavior abnormalities. FRAXE is caused by an expansion of a polymorphic $(GCC)_n$ repeat in the promoter region of the *FMR2* gene (14,15). As with FRAXA, the expanded repeats are hypermethylated, leading to transcriptional silencing of *FMR2* and subsequent loss of gene product (FMR2). Two other proteins, AF4 and LAF-4, share motifs with FMR2, and all three proteins exhibit nuclear localization, DNA-binding capacity and transcription transactivation potential (15). The putative role of FMR2 as a transcriptional activator together with its high expression levels in the hippocampus and the amygdala suggest that the cognitive and behavioral deficits in FRAXE are due to alterations in neuronal gene regulation (16–18).

Friedreich ataxia (FRDA)

Friedreich ataxia is autosomal recessive and is therefore the only triplet repeat disorder that does not show anticipation. FRDA is characterized by ataxia, dysarthria, diminished reflexes, cardiomyopathy and diabetes. Degeneration is evident in the spinal cord, dorsal root ganglia and several peripheral sensory systems. FRDA is caused by a large intronic GAA repeat expansion in the *X25* gene (or *frataxin*) which leads to reduced *X25* expression (19,20). The expanded AT-rich sequence most probably causes transcriptional interference via a self-association of the GAA/TTC tract, which stabilizes the DNA in a triplex structure (21). Reduced *X25* mRNA decreases frataxin levels, suggesting that FRDA results from a partial loss of frataxin function (20,22–25). Disruption of the yeast *X25* homolog (YFH1) causes abnormal accumulation of mitochondrial iron, loss of mtDNA, respiratory dysfunction, multiple iron–sulfur-dependent enzyme deficiencies and increased sensitivity to oxidative stress (22,26–29). These findings, together with highly conserved mitochondrial targeting signal sequences and mitochondrial membrane localization in yeast and human, suggest that frataxin is involved in iron homeostasis and respiratory function (22,26–29). In FRDA samples, studies have shown increased iron content, deficient activities of proteins involved in iron–sulfur homeostasis and hypersensitivity to iron and H_2O_2 stress (30–32). Therefore, frataxin insufficiency may result in abnormal iron–sulfur homeostasis and, in turn, mitochondrial dysfunction, free radical production, oxidative stress and cellular degeneration.

Myotonic dystrophy (DM)

DM is a multisystem disorder with highly variable phenotypes and anticipation. Myotonia, muscle weakness and progressive muscle wasting characterize adult-onset DM. Developmental abnormalities, mental handicap, hypotonia and respiratory distress are often evident in the more severe congenital myotonic dystrophy (CDM). DM is caused by an expanded CTG trinucleotide repeat tract in the 3′-UTR of the protein kinase gene, *DMPK* (33–36). The pathophysiology of DM is complex and may involve a combination of molecular defects that have varying consequences for different tissues during development. The CTG repeat may alter DMPK levels indirectly by interfering with *DMPK* transcription, RNA processing and/or translation. The result would be abnormal phosphorylation of downstream substrates. Studies from a mouse model lacking DMPK, however, argue that loss of *DMPK* function is not sufficient to cause the phenotypic spectrum of DM (37,38). The identification of two other genes flanking *DMPK* raised the possibility that CTG expansion may have *cis*-chromatin effects that could alter neighboring gene expression (39). The CTG repeat is located within the promoter of a downstream homeobox gene, termed DM locus-associated homeodomain protein (DMAHP), and immediately upstream of *DMPK* is gene 59 (also known as *DMWD*). Loss of function of either or both of these proteins could contribute to some of the features in DM. Another proposed mechanism of pathogenesis is based on a gain of function or *trans*-dominant effect of the CTG-expanded transcript, which could interfere with the normal processing and/or metabolism of various RNAs. Altered metabolism of tissue-specific RNAs could account for the various symptoms seen in DM patients. The expanded CUG repeat may sequester RNA-binding proteins, such as the CUG-binding protein (CUG-BP or hNab50), thereby interfering with their normal role in regulating RNA processing (40–45).

Spinocerebellar ataxia type 8 (SCA8)

SCA8 is a progressive ataxia with cerebellar atrophy, decreased vibration sense and brisk reflexes. Correlating with the phenotype, *SCA8* is expressed primarily in the brain, and disease is caused by an expanded CTG repeat in its 3′-terminal exon (46–48). Interestingly, SCA8 may have a CTG repeat range that is pathogenic (~110–250 repeats), with shorter and larger repeats not resulting in disease (49). Also uniquely amongst triplet repeat disorders, the *SCA8* transcript does not code for a protein and may be an endogenous antisense RNA that regulates the expression of another gene(s). A second mRNA, transcribed in an orientation opposite to that of the *SCA8* transcript, contains a common region of overlap. This 'sense' transcript encodes a protein that shares homology with the *Drosophila* KELCH gene (*KLHL1*) (50). Although the

normal function of KLHL1 in the brain is not understood, it has a conserved Kelch actin-binding domain and POZ/BTB protein–protein domains.

Spinocerebellar ataxia type 12 (SCA12)

SCA12 is a rare disease caused by a non-coding CAG trinucleotide repeat expansion in the 5′-UTR of the *PPP2R2B* gene (or *PP2A-PR55β*) (51). *PPP2R2B* encodes a brain-specific regulatory subunit of protein phosphatase 2A (PP2A). Although the CAG repeat is located near conserved promoter elements and transcriptional start sites, repeat-mediated transcriptional interference of *PPP2R2B* has not yet been shown. Future expression and function analysis of PP2A-PR55β will provide essential clues toward understanding the pathophysiology of SCA12.

POLYGLUTAMINE DISEASES

In comparison with the non-coding disorders, polyglutamine diseases have repeat expansions that are much smaller in size and variation. Although the mutant proteins do not share any homology outside the polyglutamine tract, the polyglutamine diseases have several similar features and probably share common mechanisms of pathogenesis. All eight known polyglutamine disorders are characterized by progressive neuronal dysfunction that typically begins in mid-life and results in severe neurodegeneration. Despite the ubiquitous expression of all eight genes, only a certain subset of neurons is vulnerable to degeneration. Studies of animal models and tissue culture systems have clearly demonstrated that these diseases are caused by a gain-of-function mechanism and that the expanded polyglutamine tracts are at the core of pathogenesis (52,53). Although expanded glutamine tracts in isolation are extremely toxic to cells, the selective neuronal loss particular to each disease occurs only when the full-length protein harboring an expanded glutamine tract is expressed. This idea is well supported by several recent studies of mice that were engineered to express either full-length *HD* or *DRPLA* with an expanded number of CAG repeats, and indicates that protein context helps to mediate the selective neuronal vulnerability (54–59).

Two early models were proposed to explain how long polyglutamine tracts might be toxic to neurons. Green (60) proposed that the extended polyglutamine tract is a transglutaminase substrate that can become cross-linked via isopeptide bonds. This event may enhance polyglutamine-mediated aggregation and toxicity in affected neurons (61–63). Perutz *et al.* (64) suggested that the expanded polyglutamine repeats organize into polar zippers and then aggregate. The idea that the glutamine expansion destabilizes the native conformations of the mutant proteins is extended by numerous studies of cell culture systems, animal models and patient tissues that show mutant polyglutamine protein aggregates (52,53,65). Ubiquitin-positive protein aggregates, primarily found in the nucleus, have now been reported for seven of these diseases, the exception being SCA6 where aggregates are cytoplasmic and ubiquitin negative (53,66,67). Additionally, protein aggregates with similar structure to nuclear inclusions (NIs) have been reported in the neuropil of Huntington's disease (HD) brains and transgenic mice (68,69). Occurring in dendrites and dendritic spines, neuropil aggregates are far more common in HD brains than NIs, and their appearance is well correlated with neurological findings in patients and *HD* mice (68,69).

Initially, the discovery of the NI was exciting as it suggested a common pathogenic mechanism for all polyglutamine disorders; namely, aggregates cause aberrant protein–protein interactions that affect either nuclear architecture and function and/or the function of interacting proteins. Recent findings, however, strongly indicate that NIs are neither necessary nor sufficient to initiate polyglutamine-mediated disease. First, the regional distribution of the NIs in tissue from spinal and bulbar muscular atrophy (SBMA), HD, SCA2 and SCA7 patients does not always correspond to the selective pathology (67,68,70–74). In animal models for HD, NIs were either: (i) detected in large quantities in cells that show no signs of degeneration; or (ii) detected with a very low frequency (<1%) in the striatum where the neuronal loss was prominent (54,55,75,76). Moreover, *SCA1* transgenic mice expressing mutant ataxin-1 lacking the self-association region—which is essential for ataxin-1 dimerization—develop ataxia and Purkinje cell pathology but without apparent ataxin-1 aggregation (77).

Protein misfolding and the ubiquitin–proteasome pathway

Despite evidence that indicates that NIs are dissociated from polyglutamine-induced toxicity, it is striking that expanded protein aggregates are found in all polyglutamine diseases. Even though the NIs are not the initial cause of disease, unraveling the process by which they originate may provide insight into the pathogenesis of polyglutamine disorders. A model would be that the expansion of the glutamine tract results in a misfolded protein that gains a toxic function. Expanded polyglutamine proteins may adopt energetically stable structures, which resist unfolding and therefore impede clearance by the proteasome. For example, polyglutamine-expanded ataxin-1 (92Q) is considerably more resistant to ubiquitin-mediated degradation *in vitro* compared with wild-type ataxin-1 (2Q) (78). Misfolded and potentially with extended half-lives, these proteins may interact with other proteins in an aberrant fashion and thereby trigger selective degeneration. If the capacity of the intracellular system handling these proteins is exceeded, then the NIs may develop and a cellular response to the extended presence of the mutant protein may eventuate. This concept is supported by the observation that proteasome inhibition promotes aggregation of mutant ataxin-1 and ataxin-3 in cell culture (78,79) and, in patient tissue, animal models and tissue culture systems, the proteasome and several molecular chaperones redistribute to the sites of protein aggregation (79–84). By overexpressing a dominant-negative ubiquitin-conjugating enzyme (UBC) and presumably altering the ubiquitination and clearance of mutant huntingtin in cell culture, Saudou *et al.* (85) were able to inhibit NI formation while accelerating cell death. Similarly, when crossing the *SCA1* transgenic mice with mice that lack *Ube3A*, an E3 ubiquitin–protein ligase, we discovered that Purkinje cells from the *SCA1/Ube3a* mice had significantly fewer NIs than *SCA1* littermates but markedly worse SCA1 pathology (Fig. 1A–D) (78). Together, these data suggest that impaired proteasomal degradation of mutant polyglutamine proteins may contribute to pathogenesis but, more importantly,

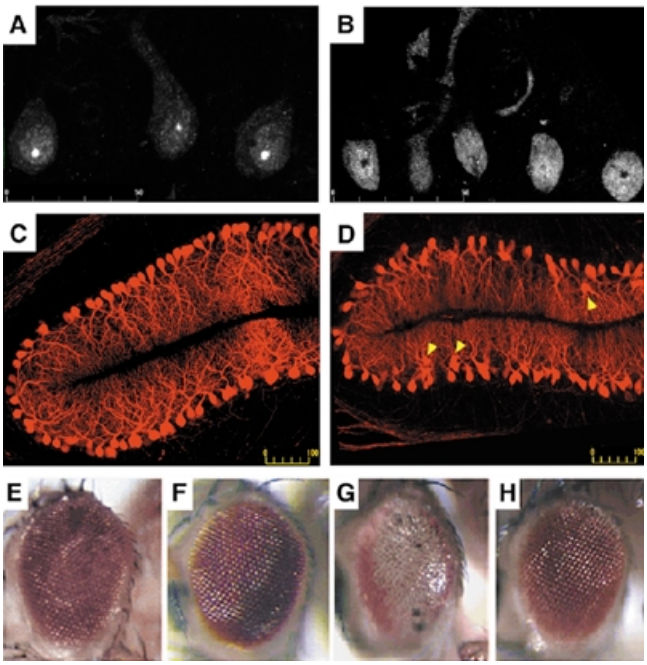

Figure 1. (A–D) *SCA1* mice lacking expression of *Ube3a*, E3–ubiquitin ligase, have a more diffuse nuclear distribution of ataxin-1 yet the cerebellar pathology is markedly worse compared with that of *SCA1* littermates. (**A**) Purkinje cells from *SCA1* mice have a sparse nuclear distribution of ataxin-1 with a concentration of the protein to the NIs. In contrast, ataxin-1 immunoreactivity is much more diffuse throughout the nucleus in Purkinje cells in the *SCA1* animals lacking *Ube3a* (**B**). A cerebellar section from an *SCA1* mouse in (**C**) shows early alterations in Purkinje cell morphology, including thinning of the dendritic arborization. A cerebellar section from an *SCA1*/*Ube3a* mouse in (**D**), however, has extensive loss of Purkinje cell dendritic arborization, a shrinkage of the molecular layer, disorganization of the Purkinje cell layer and numerous heterotopic Purkinje cells (arrowheads). The scale bar is in micrometers. Reproduced with permission from *Neuron*, Cell Press (78). (E–H) The molecular chaperone Hsp70 suppresses polyglutamine-induced neurodegeneration *in vivo* (fly eyes). (**E**) Control fly eye. (**F**) Flies expressing Hsp70 have grossly normal eye structure. (**G**) Flies expressing the expanded polyglutamine protein (MJD-Q78) have degenerate eyes that lack pigment and show severe loss of retinal structure. (**H**) Co-expression of Hsp70 ameliorates the degenerative effects of MJDtr-Q78 and the eye appears normal externally. Reproduced with permission from *Nature Genetics* (88).

the mutant proteins may be more toxic if not properly ubiquitinated, turned-over or possibly sequestered to an NI.

That molecular chaperones redistribute to NIs suggests that a chaperone stress response is being mounted to cope potentially with the toxic presence of misfolded polyglutamine proteins. Since molecular chaperones are known to be instrumental in proper protein folding, ubiquitin-dependent degradation and suppression of protein aggregation, we might infer that the cellular levels of a chaperone could directly mediate the aggregation and/or turnover of a mutant polyglutamine protein. Support for this inference comes from the fact that molecular chaperones, when overexpressed in cell culture, can decrease both the size and frequency of nuclear aggregates (80,81,84) and this decrease can be correlated with a decrease in toxicity (81). Will the chaperone-mediated benefits seen in cell culture occur, without any adverse effects, in patients? Recent data from studies of *Drosophila* models of polyglutamine disease are encouraging (86,87). In two independent studies, neurons expressing peptides with expanded glutamine repeats degenerated and showed numerous nuclear aggregates. Warrick *et al.* (88) further showed that a dominant-negative form of a fly Hsp70 enhances degeneration in the flies expressing an MJD peptide (78Q). More exciting was the observation that overexpression of Hsp70 suppresses polyglutamine-induced neurodegeneration in this animal model (Fig. 1E–H). Importantly, this suppression occurred without any effect on NI formation, arguing further against a correlation between polyglutamine-induced toxicity and NI formation.

Nuclear localization and gene expression

For most of the polyglutamine diseases, the protein aggregates are found predominantly in the nucleus, which is interesting given the varying subcellular localization of the soluble forms of these proteins. Is nuclear localization of the mutant proteins a critical event in polyglutamine disease and if so how do the mutant proteins affect nuclear function? The importance of nuclear localization of mutant ataxin-1 is evident from studies of *SCA1* transgenic mice that overexpress expanded ataxin-1 (82Q) with a mutated nuclear localization signal (NLS) (77). These mice had abundant levels of cytoplasmic mutant ataxin-1, but they never developed ataxia or pathology. Similarly, in cell culture models of HD, the addition of a nuclear export signal to mutant huntingtin drives it to the cytoplasm, and thereby suppresses aggregation and cell death; the opposite effect is seen with the addition of an NLS (85,89).

Once inside the nucleus, several recent reports suggest that mutant polyglutamine proteins may affect gene expression. For example, N-terminal huntingtin interacts in a repeat-dependent manner with the nuclear receptor co-repressor (N-CoR), which is known to repress transcription from ligand-activated receptors (90). Subcellular localization studies demonstrated that N-CoR and other associated co-repressors are redistributed by mutant huntingtin, suggesting that altered transcriptional regulation may be involved in HD. In cell culture, overexpression of full-length ataxin-1 and ataxin-7 causes these proteins to localize to the nucleus and form nuclear matrix-associated aggregates that may affect the promyelocytic oncogenic domains (PODs; also thought to be important in transcriptional regulation) (91,92). Full-length mutant AR accumulates in both cytoplasmic and nuclear matrix-associated aggregates when overexpressed in tissue culture cells, and these aggregates caused redistribution of the steroid receptor coactivator (SRC1), suggesting that SRC1-mediated transcription pathways might be affected in SBMA (84). NIs are known to sequester other polyglutamine-containing proteins, such as the TATA-binding protein (TBP) (93). Interestingly, expansion of the CAG repeat in the TBP gene may also be responsible for neurodegenerative disease (94). Analogously to the gain-of-function mechanisms in DM, but at the protein level, mutant polyglutamine repeat proteins may sequester poly(Q)-binding proteins important in transcriptional regulation. Waragai *et al.* (95) demonstrated that a polyglutamine tract-binding domain protein (PQBP-1) interacts with the glutamine tract in transcription factors and polyglutamine repeat disease proteins. This interaction is strengthened in a length-dependent manner and may affect cell survival *in vitro*. Also *in vivo*, several neuronal genes involved in calcium homeostasis and signal transduction are down-regulated specifically by the expression of expanded ataxin-1

in SCA1 transgenic mice. It is noteworthy that these alterations required nuclear localization of mutant ataxin-1 and occurred prior to any detectable pathology or visible NIs (96). Together, these results suggest that regulation of gene expression may be an important and early molecular player in the pathogenesis of polyglutamine disease.

Cleavage, caspase activity and apoptosis

Numerous studies indicate that many of the polyglutamine proteins must first be proteolytically cleaved in order to translocate to the nucleus and exert their toxic effect (53). The search for proteases that mediate this processing event has revealed that huntingtin, AR, atrophin-1 and ataxin-3 are all substrates for one or more caspases (cysteine proteases involved in apoptosis) (97,98). Sanchez *et al.* (99) proposed that aggregates of polyglutamine repeat proteins can sequester one or more procaspases, which become activated and set off the apoptotic pathway. These authors found activated caspase-8 uniquely in affected HD brain extracts, but not in controls. Additionally, preventing caspase-8-mediated apoptosis hindered the polyglutamine-induced cell death in cell culture. Caspase-1 is also activated in HD brains and transgenic mice (100). When Ona *et al*. (100) inhibited its activity in *HD* mice, endogenous huntingtin cleavage was reduced and the progression of several of the pathological features in this animal model was slowed. These results raise the possibility that caspase activity has a role in polyglutamine disease; however, the steps involved in this pathway are not yet clear. Is one or more caspase activity responsible for liberating the toxic polyglutamine peptides? Does the apoptotic pathway play a primary role in neuronal dysfunction, or is it a secondary event that occurs in dysfunctional neurons that can no longer tolerate the toxic effects of the mutant protein? Future studies will need to address the underlying pathogenic mechanisms involved in caspase activation and reconcile their relationship to the selective pattern of degeneration seen in each disorder.

CONCLUSION

The discovery of each new triplet repeat disorder brings tremendous clinical benefits, offering better classification of the diseases and facilitating early diagnosis and genetic counseling. Studies to date have made it clear that the pathological mechanisms in trinucleotide repeat disorders are complex, probably involving more than one mechanism of pathogenesis. The importance of animal models in dissecting each of these mechanisms is becoming increasingly apparent especially with the emerging insights gained from the application of fly and yeast genetics and from genetically engineered mice. Future clinical benefits will also come from advances in gene chip technologies and the human genome initiative, which together could accelerate the pace of identifying new therapeutic strategies to modulate the iron abnormalities in FRDA, or the putative alterations in gene/RNA regulation in FRAXA, FRAXE, DM and the polyglutamine disorders. Understanding the mutations in polyglutamine diseases in terms of their effect on protein–protein interactions and turnover, together with studies aimed at enhancing proper folding and degradation, may be invaluable tools required to slow or prevent these diseases. In fact, it may be possible to adopt these tools to identify therapies for other neurodegenerative 'proteinopathies' such as Alzheimer's disease, amyotrophic lateral sclerosis, prion diseases and Parkinson's disease. Despite the enormous amount of headway made in just the last few years, a sea of questions remains to be explored. Perhaps the most puzzling is a teleological one—why have these repeats expanded in the first place and what biological role do they have in evolution?

REFERENCES

1. Fu, Y.-H., Kuhl, D.P.A., Pizutti, A., Pieretti, M., Sutcliffe, J.S., Richards, S., Verkerk, A.J.M.H., Holden, J.J.A., Fenwick Jr, R.G., Warren, S.T. *et al.* (1991) Variation of the CGG repeat at the fragile X site results in genetic instability: resolution of the Sherman paradox. *Cell*, **67**, 1047–1058.
2. Verkerk, A.J.M.H., Pieretti, M., Sutcliffe, J.S., Fu, Y.-H., Kuhl, D.P.A., Pizutti, A., Reiner, O., Richards, S., Victoria, M.F., Zhang, R. *et al.* (1991) Identification of a gene (*FMR-1*) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. *Cell*, **65**, 905–914.
3. Eichler, E.E. and Nelson, D.L. (1998) The FRAXA fragile site and fragile X syndrome. In Rubinsztein, D.C. and Hayden, M.R. (eds), *Analysis of Triplet Repeat Disorders*. BIOS Scientific, Oxford, UK, pp. 13–39.
4. Eberhart, D.E. and Warren, S.T. (1996) Nuclease sensitivity of permeabilized cells confirms altered chromatin formation at the fragile X locus. *Somat. Cell Mol. Genet.*, **22**, 435–441.
5. Drouin, R., Angers, M., Dallaire, N., Rose, T.M., Khandjian, W. and Rousseau, F. (1997) Structural and functional characterization of the human FMR1 promoter reveals similarities with the hnRNP-A2 promoter region. *Hum. Mol. Genet.*, **6**, 2051–2060.
6. Schwemmle, S., de Graaff, E., Deissler, H., Glaser, D., Wohrle, D., Kennerknecht, I., Just, W., Oostra, B.A., Dorfler, W., Vogel, W. *et al.* (1997) Characterization of FMR1 promoter elements by *in vivo*-footprinting analysis. *Am. J. Hum. Genet.*, **60**, 1354–1362.
7. Coffee, B., Zhang, F., Warren, S.T. and Reines, D. (1999) Acetylated histones are associated with FMR1 in normal but not fragile X-syndrome cells. *Nature Genet.*, **22**, 98–101.
8. Siomi, M.C., Siomi, H., Sauer, W.H., Srinivasan, S., Nussbaum, R.L. and Dreyfuss, G. (1995) *FXR1*, an autosomal homolog of the fragile X mental retardation gene. *EMBO J.*, **14**, 2401–2408.
9. Kirkpatrick, L.L., McIlwain, K.A. and Nelson, D.L. (1999) Alternative splicing in the murine and human *FXR1* genes. *Genomics*, **59**, 193–202.
10. Zhang, Y., O'Connor, J.P., Siomi, M.C., Srinivasan, S., Dutra, A., Nussbaum, R.L. and Dreyfuss, G. (1995) The fragile X mental retardation syndrome protein interacts with novel homologs FXR1 and FXR2. *EMBO J.*, **14**, 5358–5366.
11. Tamanini, F., Willemsen, R., van Unen, L., Bontekoe, C., Galjaard, H., Oostra, B.A. and Hoogeveen, A.T. (1997) Differential expression of FMR1, FXR1 and FXR2 proteins in human brain and testis. *Hum. Mol. Genet.*, **6**, 1315–1322.
12. Bardoni, B., Schenck, A. and Mandel, J.L. (1999) A novel RNA-binding nuclear protein that interacts with the fragile X mental retardation (FMR1) protein. *Hum. Mol. Genet.*, **8**, 2557–2566.
13. Ceman, S., Brown, V. and Warren, S.T. (1999) Isolation of an FMRP-associated messenger ribonucleoprotein particle and identification of nucleolin and the fragile X-related proteins as components of the complex. *Mol. Cell. Biol.*, **19**, 7925–7932.
14. Knight, S.J., Flannery, A.V., Hirst, M.C., Campbell, L., Christodoulou, Z., Phelps, S.R., Pointon, J., Middleton-Price, H.R., Barnicoat, A., Pembrey, M.E. *et al.* (1993) Trinucleotide repeat amplification and hypermethylation of a CpG island in FRAXE mental retardation. *Cell*, **74**, 127–134.
15. Skinner, J.A., Foss, G.S., Miller, W.J. and Davies, K.E. (1998) Molecular studies of the fragile FRAXE and FRAXF. In Rubinsztein, D.C. and Hayden, M.R. (eds), *Analysis of Triplet Repeat Disorders*. BIOS Scientific, Oxford, UK, pp. 51–58.
16. Chakrabarti, L., Knight, S.J., Flannery, A.V. and Davies, K.E. (1996) A candidate gene for mild mental handicap at the FRAXE fragile site. *Hum. Mol. Genet.*, **5**, 275–282.
17. Gecz, J., Oostra, B.A., Hockey, A., Carbonell, P., Turner, G., Haan, E.A., Sutherland, G.R. and Mulley, J.C. (1997) FMR2 expression in families with FRAXE mental retardation. *Hum. Mol. Genet.*, **6**, 435–441.
18. Chakrabarti, L., Bristulf, J., Foss, G.S. and Davies, K.E. (1998) Expression of the murine homologue of FMR2 in mouse brain and during development. *Hum. Mol. Genet.*, **7**, 441–448.
19. Campuzano, V., Montermini, L., Molto, M.D., Pianese, L., Cossee, M., Cavalcanti, F., Monros, E., Rodius, F., Duclos, F., Monticelli, A. *et al.* (1996) Friedreich's ataxia: autosomal recessive disease caused by an intronic GAA triplet repeat expansion. *Science*, **271**, 1423–1427.

20. Cossee, M., Campuzano, V., Koutnikova, H., Fischbeck, K., Mandel, J.L., Koenig, M., Bidichandani, S.I., Patel, P.I., Molte, M.D., Canizares, J. *et al.* (1997) Frataxin fracas. *Nature Genet.*, **15**, 337–338.
21. Sakamoto, N., Chastain, P.D., Parniewski, P., Ohshima, K., Pandolfo, M., Griffith, J.D. and Wells, R.D. (1999) Sticky DNA: self-association properties of long GAA·TTC repeats in R·R·Y triplex structures from Friedreich's ataxia. *Mol. Cell*, **3**, 465–475.
22. Campuzano, V., Montermini, L., Lutz, Y., Cova, L., Hindelang, C., Jiralerspong, S., Trottier, Y., Kish, S.J., Faucheux, B., Trouillas, P. *et al.* (1997) Frataxin is reduced in Friedreich ataxia patients and is associated with mitochondrial membranes. *Hum. Mol. Genet.*, **6**, 1771–1780.
23. Bartolo, C., Mendell, J.R. and Prior, T.W. (1998) Identification of a missense mutation in a Friedreich's ataxia patient: implications for diagnosis and carrier studies. *Am. J. Med. Genet.*, **79**, 396–399.
24. Bidichandani, S., Ashizawa, T. and Patel, P.I. (1998) The GAA triplet-repeat expansion in Friedreich ataxia interferes with transcription and may be associated with an unusual DNA structure. *Am. J. Hum. Genet.*, **62**, 111–121.
25. Ohshima, K., Montermini, L., Wells, R.D. and Pandolfo, M. (1998) Inhibitory effects of expanded GAA·TTC triplet repeats from intron I of the Friedreich ataxia gene on transcription and replication *in vivo*. *J. Biol. Chem.*, **273**, 14588–14595.
26. Babcock, M., de Silva, D., Oaks, R., Davis-Kaplan, S., Jiralerspong, S., Montermini, L., Pandolfo, M. and Kaplan, J. (1997) Regulation of mitochondrial iron accumulation by Yfh1p, a putative homolog of frataxin. *Science*, **276**, 1709–1712.
27. Foury, F. and Cazzalini, O. (1997) Deletion of the yeast homologue of the human gene associated with Friedreich's ataxia elicits iron accumulation in mitochondria. *FEBS Lett.*, **411**, 373–377.
28. Koutnikova, H., Campuzano, V., Foury, F., Dolle, P., Cazzalini, O. and Koenig, M. (1997) Studies of human, mouse and yeast homologues indicate a mitochondrial function for frataxin. *Nature Genet.*, **16**, 345–351.
29. Wilson, R.B. and Roof, D.M. (1997) Respiratory deficiency due to loss of mitochondrial DNA in yeast lacking the frataxin homologue. *Nature Genet.*, **16**, 352–357.
30. Lamarche, J.B., Cote, M. and Lemieux, B. (1980) The cardiomyopathy of Friedreich's ataxia morphological observations in 3 cases. *Can. J. Neurol. Sci.*, **7**, 389–396.
31. Rotig, A., de Lonlay, P., Chretien, D., Foury, F., Koenig, M., Sidi, D., Munnich, A. and Rustin, P. (1997) Aconitase and mitochondrial iron–sulphur protein deficiency in Friedreich ataxia. *Nature Genet.*, **17**, 215.
32. Wong, A., Yang, J., Cavadini, P., Gellera, C., Lonnerdal, B., Taroni, F. and Cortopassi, G. (1999) The Friedreich's ataxia mutation confers cellular sensitivity to oxidant stress which is rescued by chelators of iron and calcium and inhibitors of apoptosis. *Hum. Mol. Genet.*, **8**, 425–430.
33. Brook, J.D., McCurrach, M.E., Harley, H.G., Buckler, A.J., Church, D., Aburatani, H., Hunter, K., Stanton, V.P., Thirion, J.P., Hudson, T. *et al.* (1992) Molecular basis of myotonic dystrophy: expansion of a trinucleotide (CTG) repeat at the 3′ end of a transcript encoding a protein kinase family member. *Cell*, **68**, 799–808.
34. Buxton, J., Shelbourne, P., Davies, J., Jones, C., Van Tongeren, T., Aslanidis, C., de Jong, P., Jansen, G., Anvret, M., Riley, B. *et al.* (1992) Detection of an unstable fragment of DNA specific to individuals with myotonic dystrophy. *Nature*, **355**, 547–548.
35. Fu, Y.-H., Pizutti, A., Fenwick, R.G., King, J.J., Rajnarayan, S., Dunne, P.W., Dubel, J., Nasser, G.A., Ashizawa, T., De Jong, P. *et al.* (1992) An unstable triplet repeat in a gene related to myotonic muscular dystrophy. *Science*, **255**, 1256–1259.
36. Mahadevan, M., Tsilfidis, C., Sabourin, L., Shutler, G., Amemiya, C., Jansen, G., Neville, C., Narang, M., Barcelo, J., O'Hoy, K. *et al.* (1992) Myotonic dystrophy mutation: an unstable CTG repeat in the 3′ untranslated region of the gene. *Science*, **255**, 1253–1255.
37. Jansen, G., Groenen, P.J., Bachner, D., Jap, P.H., Coerwinkel, M., Oerlemans, F., van den Broek, W., Gohlsch, B., Pette, D., Plomp, J.J. *et al.* (1996) Abnormal myotonic dystrophy protein kinase levels produce only mild myopathy in mice. *Nature Genet.*, **13**, 316–324.
38. Reddy, S., Smith, D.B., Rich, M.M., Leferovich, J.M., Reilly, P., Davis, B.M., Tran, K., Rayburn, H., Bronson, R., Cros, D. *et al.* (1996) Mice lacking the myotonic dystrophy protein kinase develop a late onset progressive myopathy. *Nature Genet.*, **13**, 325–335.
39. Korade-Mirnics, Z., Babitzke, P. and Hoffman, E. (1998) Myotonic dystrophy: molecular windows on a complex etiology. *Nucleic Acids Res.*, **26**, 1363–1368.
40. Timchenko, L.T., Miller, J.W., Timchenko, N.A., DeVore, D.R., Datar, K.V., Lin, L., Roberts, R., Caskey, C.T. and Swanson, M.S. (1996) Identification of a $(CUG)_n$ triplet repeat RNA-binding protein and its expression in myotonic dystrophy. *Nucleic Acids Res.*, **24**, 4407–4414.
41. Timchenko, L.T., Timchenko, N.A., Caskey, C.T. and Roberts, R. (1996) Novel proteins with binding specificity for DNA CTG repeats and RNA CUG repeats: implications for myotonic dystrophy. *Hum. Mol. Genet.*, **5**, 115–121.
42. Roberts, R., Timchenko, N.A., Miller, J.W., Reddy, S., Caskey, C.T., Swanson, M.S. and Timchenko, L.T. (1997) Altered phosphorylation and intracellular distribution of a $(CUG)_n$ triplet repeat RNA-binding protein in patients with myotonic dystrophy and in myotonin protein kinase knockout mice. *Proc. Natl Acad. Sci. USA*, **94**, 13221–13226.
43. Philips, A.V., Timchenko, L.T. and Cooper, T.A. (1998) Disruption of splicing regulated by a CUG-binding protein in myotonic dystrophy. *Science*, **280**, 737–741.
44. Amack, J.D., Paguio, A.P. and Mahadevan, M.S. (1999) *Cis* and *trans* effects of the myotonic dystrophy (DM) mutation in a cell culture model. *Hum. Mol. Genet.*, **8**, 1975–1984.
45. Lu, X., Timchenko, N.A. and Timchenko, L.T. (1999) Cardiac elav-type RNA-binding protein (ETR-3) binds to RNA CUG repeats expanded in myotonic dystrophy. *Hum. Mol. Genet.*, **8**, 53–60.
46. Schalling, M., Hudson, T.J., Buetow, K.H. and Housman, D.E. (1993) Direct detection of novel expanded trinucleotide repeats in the human genome. *Nature Genet.*, **4**, 135–139.
47. Janzen, M.L., Moseley, M.L., Benzow, K.A., Day, J.W., Koob, M.D. and Ranum, L.P.W. (1999) Limited expression of SCA8 is consistent with cerebellar pathogenesis and toxic gain of function RNA model. *Am. J. Hum. Genet.* **65** (suppl.), A276.
48. Koob, M.D., Moseley, M.L., Schut, L.J., Benzow, K.A., Bird, T.D., Day, J.W. and Ranum, L.P. (1999) An untranslated CTG expansion causes a novel form of spinocerebellar ataxia (SCA8). *Nature Genet.*, **21**, 379–384.
49. Ranum, L.P.W., Moseley, M.L., Leppert, M.F., van den Engh, G., La Spada, A.R., Koob, M.D. and Day, J.W. (1999) Massive CTG expansions and deletions may reduce penetrance of spinocerebellar ataxia type 8. *Am. J. Hum. Genet.*, **65**(suppl.), A466.
50. Koob, M.D., Moseley, M.L., Benzow, K.A., Johnson, C.M., Nemes, J.P., Ranum, L.P.W. and Day, J.W. (1999) The SCA8 transcript is an antisense RNA to a brain-specific transcript encoding a novel actin-binding protein (KLHL1). *Am. J. Hum. Genet.*, **65**(suppl.), A30.
51. Holmes, S.E., O'Hearn, E.E., McInnis, M.G., Gorelick-Feldman, D.A., Kleiderlein, J.J., Callahan, C., Kwak, N.G., Ingersoll-Ashworth, R.G., Sherr, M., Sumner, A.J. *et al.* (1999) Expansion of a novel CAG trinucleotide repeat in the 5′ region of PPP2R2B is associated with SCA12. *Nature Genet.*, **23**, 391–392.
52. Lin, X., Cummings, C.J. and Zoghbi, H.Y. (1999) Expanding our understanding of polyglutamine diseases through mouse models. *Neuron*, **24**, 499–502.
53. Zoghbi, H.Y. and Orr, H.T. (1999) Polyglutamine diseases: protein cleavage and aggregation. *Curr. Opin. Neurobiol.*, **9**, 566–570.
54. Reddy, P.H., Williams, M., Charles, V., Garrett, L., Pike-Buchanan, L., Whetsell Jr, W.O., Miller, G. and Tagle, D.A. (1998) Behavioural abnormalities and selective neuronal loss in HD transgenic mice expressing mutated full-length HD cDNA. *Nature Genet.*, **20**, 198–202.
55. Hodgson, J.G., Agopyan, N., Gutekunst, C.A., Leavitt, B.R., LePiane, F., Singaraja, R., Smith, D.J., Bissada, N., McCutcheon, K., Nasir, J. *et al.* (1999) A YAC mouse model for Huntington's disease with full-length mutant huntingtin, cytoplasmic toxicity, and selective striatal neurodegeneration. *Neuron*, **23**, 181–192.
56. Sato, T., Oyake, M., Nakamura, K., Nakao, K., Fukusima, Y., Onodera, O., Igarashi, S., Takano, H., Kikugawa, K., Ishida, Y. *et al.* (1999) Transgenic mice harboring a full-length human mutant *DRPLA* gene exhibit age-dependent intergenerational and somatic instabilities of CAG repeats comparable with those in DRPLA patients. *Hum. Mol. Genet.*, **8**, 99–106.
57. Sato, T., Yamada, M., Oyake, M., Nakao, K., Nakamura, K., Katsuki, M., Takahashi, H. and Tsuji, S. (1999) Transgenic mice harboring a full-length human *DRPLA* gene with highly expanded CAG repeats exhibit severe disease phenotype. *Am. J. Hum. Genet.*, **65**(suppl.), A30.
58. Shelbourne, P.F., Killeen, N., Hevner, R.F., Johnston, H.M., Tecott, L., Lewandoski, M., Ennis, M., Ramirez, L., Li, Z., Iannicola, C. *et al.* (1999) A Huntington's disease CAG expansion at the murine *Hdh* locus is unstable and associated with behavioural abnormalities in mice. *Hum. Mol. Genet.*, **8**, 763–774.
59. Usdin, M.T., Shelbourne, P.F., Myers, R.M. and Madison, D.V. (1999) Impaired synaptic plasticity in mice carrying the Huntington's disease mutation. *Hum. Mol. Genet.*, **8**, 839–846.
60. Green, H. (1993) Human genetic diseases due to codon reiteration: relationship to an evolutionary mechanism. *Cell*, **74**, 955–956.
61. Igarashi, S., Koide, R., Shimohata, T., Yamada, M., Hayashi, Y., Takano, H., Date, H., Oyake, M., Sato, T., Sato, A. *et al.* (1998) Suppression of aggregate formation and apoptosis by transglutaminase inhibitors in cells expressing truncated DRPLA protein with an expanded polyglutamine stretch. *Nature Genet.*, **18**, 111–117.

62. Karpuj, M.V., Garren, H., Slunt, H., Price, D.L., Gusella, J., Becher, M.W. and Steinman, L. (1999) Transglutaminase aggregates huntingtin into nonamyloidogenic polymers, and its enzymatic activity increases in Huntington's disease brain nuclei. *Proc. Natl Acad. Sci. USA*, **96**, 7388–7393.
63. Lesort, M., Chun, W., Johnson, G.V. and Ferrante, R.J. (1999) Tissue transglutaminase is increased in Huntington's disease brain. *J. Neurochem.*, **73**, 2018–2027.
64. Perutz, M.F., Johnson, T., Suzuki, M. and Finch, J.T. (1994) Glutamine repeats as polar zippers: their possible role in inherited neurodegenerative diseases. *Proc. Natl Acad. Sci. USA*, **91**, 5355–5358.
65. Paulson, H.L. (1999) Protein fate in neurodegenerative proteinopathies: polyglutamine diseases join the (mis)fold. *Am. J. Hum. Genet.*, **64**, 339–345.
66. Ishikawa, K., Fujigasaki, H., Saegusa, H., Ohwada, K., Fujita, T., Iwamoto, H., Komatsuzaki, Y., Toru, S., Toriyama, H., Watanabe, M. *et al.* (1999) Abundant expression and cytoplasmic aggregations of α1A voltage-dependent calcium channel protein associated with neurodegeneration in spinocerebellar ataxia type 6. *Hum. Mol. Genet.*, **8**, 1185–1193.
67. Koyano, S., Uchihara, T., Fujigasaki, H., Nakamura, A., Yagishita, S. and Iwabuchi, K. (1999) Neuronal intranuclear inclusions in spinocerebellar ataxia type 2: triple-labeling immunofluorescent study. *Neurosci. Lett.*, **273**, 117–120.
68. Gutekunst, C.A., Li, S.H., Yi, H., Mulroy, J.S., Kuemmerle, S., Jones, R., Rye, D., Ferrante, R.J., Hersch, S.M. and Li, X.J. (1999) Nuclear and neuropil aggregates in Huntington's disease: relationship to neuropathology. *J. Neurosci.*, **19**, 2522–2534.
69. Li, H., Li, S.H., Cheng, A.L., Mangiarini, L., Bates, G.P. and Li, X.J. (1999) Ultrastructural localization and progressive formation of neuropil aggregates in Huntington's disease transgenic mice. *Hum. Mol. Genet.*, **8**, 1227–1236.
70. Holmberg, M., Duyckaerts, C., Durr, A., Cancel, G., Gourfinkel-An, I., Damier, P., Faucheux, B., Trottier, Y., Hirsch, E.C., Agid, Y. *et al.* (1998) Spinocerebellar ataxia type 7 (SCA7): a neurodegenerative disorder with neuronal intranuclear inclusions. *Hum. Mol. Genet.*, **7**, 913–918.
71. Li, M., Miwa, S., Kobayashi, Y., Merry, D.E., Yamamoto, M., Tanaka, F., Doyu, M., Hashizume, Y., Fischbeck, K.H. and Sobue, G. (1998) Nuclear inclusions of the androgen receptor protein in spinal and bulbar muscular atrophy. *Ann. Neurol.*, **44**, 249–254.
72. Li, M., Nakagomi, Y., Kobayashi, Y., Merry, D.E., Tanaka, F., Doyu, M., Mitsuma, T., Hashizume, Y., Fischbeck, K.H. and Sobue, G. (1998) Nonneural nuclear inclusions of androgen receptor protein in spinal and bulbar muscular atrophy. *Am. J. Pathol.*, **153**, 695–701.
73. Huynh, D.P., Del Bigio, M.R., Ho, D.H. and Pulst, S.M. (1999) Expression of ataxin-2 in brains from normal individuals and patients with Alzheimer's disease and spinocerebellar ataxia 2. *Ann. Neurol.*, **45**, 232–241.
74. Kuemmerle, S., Gutekunst, C.A., Klein, A.M., Li, X.J., Li, S.H., Beal, M.F., Hersch, S.M. and Ferrante, R.J. (1999) Huntington aggregates may not predict neuronal death in Huntington's disease. *Ann. Neurol.*, **46**, 842–849.
75. Davies, S.W., Turmaine, M., Cozens, B.A., DiFiglia, M., Sharp, A.H., Ross, C.A., Scherzinger, E., Wanker, E.E., Mangiarini, L. and Bates, G.P. (1997) Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. *Cell*, **90**, 537–548.
76. Sathasivam, K., Hobbs, C., Turmaine, M., Mangiarini, L., Mahal, A., Bertaux, F., Wanker, E.E., Doherty, P., Davies, S.W. and Bates, G.P. (1999) Formation of polyglutamine inclusions in non-CNS tissue. *Hum. Mol. Genet.*, **8**, 813–822.
77. Klement, I.A., Skinner, P.J., Kaytor, M.D., Yi, H., Hersch, S.M., Clark, H.B. and Zoghbi, H.Y. (1998) Ataxin-1 nuclear localization and aggregation: role in polyglutamine-induced disease in SCA1 transgenic mice. *Cell*, **95**, 41–53.
78. Cummings, C.J., Reinstein, E., Sun, Y., Antalffy, B., Jiang, Y.-h., Ciechanover, A., Orr, H.T., Beaudet, A.L. and Zoghbi, H.Y. (1999) Mutation of the E6–AP ubiquitin ligase reduces nuclear inclusion frequency while accelerating polyglutamine-induced pathology in SCA1 mice. *Neuron*, **24**, 879–892.
79. Chai, Y., Koppenhafer, S.L., Shoesmith, S.J., Perez, M.K. and Paulson, H.L. (1999) Evidence for proteasome involvement in polyglutamine disease: localization to nuclear inclusions in SCA3/MJD and suppression of polyglutamine aggregation *in vitro*. *Hum. Mol. Genet.*, **8**, 673–682.
80. Cummings, C.J., Mancini, M.A., Antalffy, B., DeFranco, D.B., Orr, H.T. and Zoghbi, H.Y. (1998) Chaperone suppression of aggregation and altered subcellular proteasome localization imply protein misfolding in SCA1. *Nature Genet.*, **19**, 148–154.
81. Chai, Y., Koppenhafer, S.L., Bonini, N.M. and Paulson, H.L. (1999) Analysis of the role of heat shock protein (Hsp) molecular chaperones in polyglutamine disease. *J. Neurosci.*, **19**, 10338–10347.
82. Davies, S.W., Turmaine, M., Cozens, B.A., Raza, A.S., Mahal, A., Mangiarini, L. and Bates, G.P. (1999) From neuronal inclusions to neurodegeneration: neuropathological investigation of a transgenic mouse model of Huntington's disease. *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, **354**, 981–989.
83. Merry, D.E., Woods, J., Walcott, J., Bish, L., Fischbeck, K.H. and Abel, A. (1999) Characterization of a transgenic model for SBMA. *Am. J Hum. Genet.*, **65**(suppl.), A30.
84. Stenoien, D.L., Cummings, C.J., Adams, H.P., Mancini, M.G., Patel, K., DeMartino, G.N., Marcelli, M., Weigel, N.L. and Mancini, M.A. (1999) Polyglutamine-expanded androgen receptors form aggregates that sequester heat shock proteins, proteasome components and SRC-1, and are suppressed by the HDJ-2 chaperone. *Hum. Mol. Genet.*, **8**, 731–741.
85. Saudou, F., Finkbeiner, S., Devys, D. and Greenberg, M.E. (1998) Huntingtin acts in the nucleus to induce apoptosis but death does not correlate with the formation of intranuclear inclusions. *Cell*, **95**, 55–66.
86. Jackson, G.R., Salecker, I., Dong, X., Yao, X., Arnheim, N., Faber, P.W., MacDonald, M.E. and Zipursky, S.L. (1998) Polyglutamine-expanded human huntingtin transgenes induce degeneration of *Drosophila* photoreceptor neurons. *Neuron*, **21**, 633–642.
87. Warrick, J.M., Paulson, H.L., Gray-Board, G.L., Bui, Q.T., Fischbeck, K.H., Pittman, R.N. and Bonini, N.M. (1998) Expanded polyglutamine protein forms nuclear inclusions and causes neural degeneration in *Drosophila*. *Cell*, **93**, 939–949.
88. Warrick, J.M., Chan, H.Y., Gray-Board, G.L., Chai, Y., Paulson, H.L. and Bonini, N.M. (1999) Suppression of polyglutamine-mediated neurodegeneration in *Drosophila* by the molecular chaperone HSP70. *Nature Genet.*, **23**, 425–428.
89. Peters, M.F., Nucifora Jr, F.C., Kushi, J., Seaman, H.C., Cooper, J.K., Herring, W.J., Dawson, V.L., Dawson, T.M. and Ross, C.A. (1999) Nuclear targeting of mutant huntingtin increases toxicity. *Mol. Cell. Neurosci.*, **14**, 121–128.
90. Boutell, J.M., Thomas, P., Neal, J.W., Weston, V.J., Duce, J., Harper, P.S. and Jones, A.L. (1999) Aberrant interactions of transcriptional repressor proteins with the Huntington's disease gene product, huntingtin. *Hum. Mol. Genet.*, **8**, 1647–1655.
91. Skinner, P.J., Koshy, B., Cummings, C., Klement, I.A., Helin, K., Servadio, A., Zoghbi, H.Y. and Orr, H.T. (1997) Ataxin-1 with extra glutamines induces alterations in nuclear matrix-associated structures. *Nature*, **389**, 971–974.
92. Kaytor, M.D., Duvick, L.A., Skinner, P.J., Koob, M.D., Ranum, L.P. and Orr, H.T. (1999) Nuclear localization of the spinocerebellar ataxia type 7 protein, ataxin-7. *Hum. Mol. Genet.*, **8**, 1657–1664.
93. Perez, M.K., Paulson, H.L., Pendse, S.J., Saionz, S.J., Bonini, N.M. and Pittman, R.N. (1998) Recruitment and the role of nuclear localization in polyglutamine-mediated aggregation. *J. Cell Biol.*, **143**, 1457–1470.
94. Koide, R., Kobayashi, S., Shimohata, T., Ikeuchi, T., Maruyama, M., Saito, M., Yamada, M., Takahashi, H. and Tsuji, S. (1999) A neurological disease caused by an expanded CAG trinucleotide repeat in the TATA-binding protein gene: a new polyglutamine disease? *Hum. Mol. Genet.*, **8**, 2047–2053.
95. Waragai, M., Lammers, C.H., Takeuchi, S., Imafuku, I., Udagawa, Y., Kanazawa, I., Kawabata, M., Mouradian, M.M. and Okazawa, H. (1999) PQBP-1, a novel polyglutamine tract-binding protein, inhibits transcription activation by Brn-2 and affects cell survival. *Hum. Mol. Genet.*, **8**, 977–987.
96. Lin, X., Antalffy, B., Kang, D., Orr, T. and Zoghbi, H.Y. (2000) Polyglutamine expansion in ataxin-1 downregulates specific neuronal genes before pathogenic changes in spinocerebellar ataxia type 1. *Nature Neurosci.*, **3**, in press.
97. Goldberg, Y.P., Nicholson, D.W., Rasper, D.M., Kalchman, M.A., Koide, H.B., Graham, R.K., Bromm, M., Kazemi-Esfarjani, P., Thornberry, N.A., Vaillancourt, J.P. *et al.* (1996) Cleavage of huntingtin by apopain, a proapoptotic cysteine protease, is modulated by the polyglutamine tract. *Nature Genet.*, **13**, 442–449.
98. Wellington, C.L., Ellerby, L.M., Hackam, A.S., Margolis, R.L., Trifiro, M.A., Singaraja, R., McCutcheon, K., Salvesen, G.S., Propp, S.S., Bromm, M. *et al.* (1998) Caspase cleavage of gene products associated with triplet expansion disorders generates truncated fragments containing the polyglutamine tract. *J. Biol. Chem.*, **273**, 9158–9167.
99. Sanchez, I., Xu, C.J., Juo, P., Kakizaka, A., Blenis, J. and Yuan, J. (1999) Caspase-8 is required for cell death induced by expanded polyglutamine repeats. *Neuron*, **22**, 623–633.
100. Ona, V.O., Li, M., Vonsattel, J.P., Andrews, L.J., Khan, S.Q., Chung, W.M., Frey, A.S., Menon, A.S., Li, X.J., Stieg, P.E. *et al.* (1999) Inhibition of caspase-1 slows disease progression in a mouse model of Huntington's disease. *Nature*, **399**, 263–267.