REVIEW ARTICLE

Modifiers and mechanisms of multi-system polyglutamine neurodegenerative disorders: lessons from fly models

MOUSHAMI MALLIK and SUBHASH C. LAKHOTIA*

Cytogenetics Laboratory, Department of Zoology, Banaras Hindu University, Varanasi 221 005, India

Abstract

Polyglutamine (polyQ) diseases, resulting from a dynamic expansion of glutamine repeats in a polypeptide, are a class of genetically inherited late onset neurodegenerative disorders which, despite expression of the mutated gene widely in brain and other tissues, affect defined subpopulations of neurons in a disease-specific manner. We briefly review the different polyQ-expansion-induced neurodegenerative disorders and the advantages of modelling them in *Drosophila*. Studies using the fly models have successfully identified a variety of genetic modifiers and have helped in understanding some of the molecular events that follow expression of the abnormal polyQ proteins. Expression of the mutant polyQ proteins causes, as a consequence of intra-cellular and inter-cellular networking, mis-regulation at multiple steps like transcriptional and post-transcriptional regulations, cell signalling, protein quality control systems (protein folding and degradation networks), axonal transport machinery etc., in the sensitive neurons, resulting ultimately in their death. The diversity of genetic modifiers of polyQ toxicity identified through extensive genetic screens in fly and other models clearly reflects a complex network effect of the presence of the mutated protein. Such network effects pose a major challenge for therapeutic applications.

[Mallik M. and Lakhotia S. C. 2010 Modifiers and mechanisms of multi-system polyglutamine neurodegenerative disorders: lessons from fly models. *J. Genet*. **89**, 497–526]

Introduction

Neurodegeneration (Greek 'neuro' = nerve and Latin 'dēgenerāre' = to decline) literally means deterioration of neurons resulting in slow but irretrievable loss of neuronal activity. Human neurodegenerative disorders, sporadic or hereditary, are of heterogeneous etiology and lead to disorder-specific loss of neurons and thus to dysfunctioning of specific components of the central nervous system. Based on their phenotypic effects, they can be divided into two groups, viz., (i) conditions associated with tremors and movement disorders or ataxias, and (ii) conditions affecting cognitive functions and memory or dementias. However, these phenotypes are not mutually exclusive.

Neurodegenerative diseases involving triplet repeat expansion

In recent years, a growing number of neurodegenerative diseases have been found to be associated with a unique class of mutations which bring about expansion of unstable trinucleotide repeats in the genome. Such trinucleotide repeat disorders, also known as codon reiteration disorders, are caused by expansion of the reiteration frequency of the tandem triplet repeats in certain genes beyond the gene-specific normal and stable threshold. Such pathogenic mutations were first described in 1991 as the causative mutations in fragile X syndrome (FXS; Verkerk *et al*. 1991) and spinal and bulbar muscular atrophy (SBMA; Laspada *et al*. 1991). Currently, about 20 such disorders are known, nine of which are neurodegenerative and result from expansion of CAG repeats coding for polyglutamine (polyQ) tracts. Among these, Huntington's disease (HD) and Machado–Joseph disease (MJD) or Spinocerebellar ataxia 3 (SCA3) are prominent. Besides polyglutamine, several other amino acid repeats are also common in the human genome (Karlin and Burge 1996) but only a few of them have been found to undergo expansions that result in disease. Expansion of polyalanine repeats has been described in recent years as the causative agent in some neurodegenerative diseases (Albrecht and Mundlos 2005). Diseases associated with expansion of the glutamine codon (CAG/CTG) are primarily discussed here.

*For correspondence. E-mail: lakhotia@bhu.ac.in; sclakhotia@yahoo.co.in.

Keywords. neurodegeneration; triplet repeat expansion; Hsp; chaperones; proteasome; *hsrω*.

The following defining features (Plassart and Fontaine 1994; Paulson and Fischbeck 1996) are common amongst disorders caused by trinucleotide repeat expansions. (i) The expanded repeats show both somatic and germ line instability due to dynamic mutations, and more frequently expand rather than contract, in successive transmissions from one generation to the next (Pearson *et al*. 2005). (ii) The larger the expansion beyond the threshold, the greater is the severity of disease. This property results in the characteristic anticipation common in trinucleotide repeat disorders so that the age of onset decreases and severity of symptoms increases through successive generations in the affected family (Igarashi *et al*. 1992). (iii) Parental origin of the disease allele can often influence anticipation. For example, the triplet repeat is more likely to expand when inherited from the mother in myotonic dystrophy and with paternal transmission in the case of polyQ repeat disorders such as HD (reviewed in Lutz 2007).

The different neurological disorders caused by expansion of triplet (in rare cases tetra-nucleotide or penta-nucleotide) repeat sequences can be broadly divided into two distinct groups based on the location of expanded repeats in the affected gene (see tables 1–3). The first group is characterized by expansion of CAG repeats in the coding region of the target gene whereas in the second group, the repeat expansion occurs in the non-coding region of the affected gene (table 1). The first group is collectively referred to as polyglutamine or polyQ disorders (tables 1 and 3). The polyalanine (polyA) repeat expansion disorders, which exhibit a low degree of polymorphism with respect to the site and length of the repeat, have also been recently included in this category (Albrecht and Mundlos 2005). The second group includes non-coding trinucleotide repeat diseases, which are typically characterized by large and variable repeat expansions resulting in pleiotropic dysfunction in multiple tissues (table 2).

The second group of triplet expansion diseases can also be divided into two mechanistic categories: (i) diseases caused by expansion of non-coding repeats that interfere with transcription of the mutated gene resulting in a loss of protein function; and (ii) diseases caused by expansion of transcribed but non-translated repeats resulting in altered RNA function and metabolism (table 2).

Polyglutamine (CAG) repeat disorders

The polyglutamine diseases constitute a class of genetically distinct, late-onset, gain-of-function neurological disorders, that are caused by expansion of polyglutamine stretches, typically from a normal range of 4 to 36 residues to a pathogenic range of >36 tandem residues (see table 3) in different proteins (Gusella and MacDonald 2000; Everett and Wood 2004; Gatchel and Zoghbi 2005). In each of these diseases, the CAG repeat expansion occurs in the translated region of the respective disease genes (tables 1 and 3). The expansion is found in the first exon of the given gene in SCA2, SCA3, HD and SBMA diseases (Vonsattel *et al*. 1985; Laspada *et al*. 1991; Kawaguchi *et al*. 1994; Imbert *et al*. 1996) while in SCA1, SCA7 and DRPLA, the expanded CAG repeats are located in exons 8, 3 and 5, respectively (Orr *et al*. 1993; Koide *et al*. 1994; David *et al*. 1997). The main features of each of these diseases such as the causative disease genes, their genetic loci, functions of the protein products, etc are listed in table 3. With the exception of SBMA, all these neurodegenerative diseases are inherited in an autosomal dominant manner.

Studies on these pathogenic proteins reveal that the long polyQ domain alters protein conformation causing an enriched beta sheet structure (Bilen and Bonini 2007). This confers a novel toxic property on these proteins in neuronal cells resulting in death of selective neurons, although the diseased protein is expressed more widely in brain and other tissues (table 3).

Nature of polyQ toxicity

Isolated, expanded polyQ fragments by themselves are intrinsically and indiscriminately cytotoxic (Marsh *et al*. 2000), suggesting that the selective vulnerability of different subsets of neurons in each disease is due to other factors.

Table 1. Types of trinucleotide repeat disorders based on location of the expanded repeats.

Translated repeat disorders		Untranslated triplet repeat diseases	
Disease name	Mutation/repeat unit	Disease name	Mutation/repeat unit
SCA 1, 2, 3, 6, 7, 17	$(CAG)_n$	FRDA	$(GAA)_n$
HD	$(CAG)_n$	FRAXA	$(CGC)_n$
DRPLA	$(CAG)_n$	FRAXE	$(CCG)_n$
SBMA	$(CAG)_n$	FXTAS	$(CGG)_n$
		DM1	$(CTG)_n$
		DM2	$(CCTG)_n$
		SCA8	$(CTG)_n$
		SCA10	$(ATTCT)_n$
		SCA12	$(CAG)_n$
		HDL2	$(CTG)_n$

Table 2. Characteristic features of diseases caused by expansion of untranslated triplet repeats.

Disease name	Inheritance	Mutation/ repeat unit	Repeat location	Gene product	Putative function	Normal repeat length	Expanded repeat length	Main clinical features	References
FRDA	Autosomal recessive	$(GAA)_n$	Intron 1	Frataxin	Mitochondrial iron metabolism	6–32	200–1700	Sensory ataxia, slow saccades, hypertrophic cardiomyopathy, diabetes mellitus	Campuzano *et al.* (1996), Cossee *et al.* (1997), Harding (1981), Pandolfo (2002a), Pandolfo (2002b), Puccio *et al.* (2001)
FXS	X-linked	$(CGG)_n$	5′ UTR	FMRP	Translational regulation	6–52	55→2000	Mental retardation, macroorchidsm, connective tissue defects, hyperactivity and behavioral abnormalities	De Boulle *et al.* (1993), Eichler and Nelson (1996), Hagerman (2006), Merenstein *et al.* (1996), Verkerk *et al.* (1991)
FRAXE	X-linked	$(CCG)_n$	5′ end	FMR2	Transcription	4–39	200–900	Mental retardation	Gecz *et al.* (1996), Gu *et al.* (1996), Knight *et al.* (1993), Mulley *et al.* (1995)
FXTAS	X-linked	$(CGG)_n$	5′ UTR	FMR1 RNA	RNA-mediated	6–60	60–200	Ataxia, tremor, Parkinsonism, cognitive deficits	Hagerman and Hagerman (2004), Van Dam *et al.* (2005)
DM1	Autosomal dominant	$(CTG)_n$	3′ UTR	DM1/DMPK	RNA-mediated	5–37	50–10000	Myotonia, muscle weakness, cardiac conduction abnormalities, insulin resistance, cataracts, testicular atrophy, respiratory distress, mental retardation in congenital form	Brook *et al.* (1992), Fu *et al.* (1992), Mahadevan *et al.* (1992)
DM2	Autosomal dominant	$(CCTG)_n$	Intron 1	CNBP	RNA-mediated	< 27	75–11000	Similar to DM1, no congenital form	Liquori *et al.* (2001) Wheeler and Thornton (2007)
EPM1	Autosomal recessive	$(C)_4G(C)_4GCG$	Promoter	CSTB	RNA-mediated	2–3	45–70	severe stimulus sensitive myoclonus, generalized tonic-clonic seizures	Lalioti *et al.* (1997), Larson *et al.* (1999), Virtaneva *et al.* (1997)
SCA8	Autosomal dominant	$(CAG)_n$ and $(CTG)_n$	Region of overlap of the 3′ ends of *ATXN8* and *ATXN8OS*	SCA8 RNA	Antisense RNA to the actin organizing protein, Kelch-like protein 1	6–37	> 74	Ataxia, cerebellar dysfunction, polyneuropathy, slurred speech, nystagmus	Day *et al.* (2000), Koob *et al.* (1999), Moseley *et al.* (2006), Nemes *et al.* (2000)
SCA10	Autosomal dominant	$(ATTCT)_n$	Intron 9	-	Unknown	10–29	280–4500	Ataxia, tremor, cognitive and neuropsychiatric impairment	Matsuura *et al.* (2000)
SCA12	Autosomal dominant	$(CAG)_n$ / $(CTG)_n$	5′ region	PPP2R2B	Phosphatase regulation	9–28	55–78	Ataxia and seizures	Holmes *et al.* (2001)
HDL2	Autosomal dominant	$(CAG)_n$ / $(CTG)_n$	Alternatively spliced exon 2A	Junctophilin 3	PM/ER junction protein	6–28	40–59	Similar to HD	Holmes *et al.* (2001)

Table 3. Characteristics of the various polyglutamine (polyQ) repeat diseases.

Disease	Inheritance	Gene locus	Normal repeat length	Pathogenic repeat length	Gene name (protein product)	Putative function	Protein localization	Regions most affected	Main clinical features	References
HD	Autosomal dominant	4p16.3	6–34	36–121	*HD* (huntingtin)	Signalling, transport, transcription	Cytoplasmic	Striatum, cerebral cortex	Severe movement abnormalities, chorea, dystonia, cognitive deficits, psychiatric problems	DiFiglia *et al.* (1995), Gusella *et al.* (1993), The Huntington's disease collaborative research group (1993), Kehoe *et al.* (1999), MacDonald *et al.* (2003); Sapp *et al.* (1997)
DRPLA	Autosomal dominant	12p13.31	7–34	49–88	*DRPLA* (atrophin-1)	Transcriptional corepressor	Cytoplasmic	Cerebellum, cerebral cortex, basal ganglia, Luys body	Ataxia, seizures, choreoathetosis, dementia	Hayashi *et al.* (1998), Ikeuchi *et al.* (1995), Koide *et al.* (1994), Komure *et al.* (1995), Nagafuchi *et al.* (1994), Zhang *et al.* (2002)
SBMA	X-linked	Xq11–12	9–36	38–62	*AR* (androgen receptor)	Steroid-hormone receptor	Nuclear and cytoplasmic	Anterior horn and bulbar neurons, dorsal root ganglia	Motor weakness, swallowing difficulty, gynecomastia, hypogonadism, decreased fertility	Adachi *et al.* (2007), Fischbeck *et al.* (1999), Igarashi *et al.* (1992), Laspada *et al.* (1991)
SCA1	Autosomal dominant	6p21.3	6–44	39–82	*SCA1* (ataxin-1)	Transcription	Nuclear	Cerebellar Purkinje cells, dentate nucleus, brainstem	Ataxia, slurred speech, spasticity, cognitive impairments	Matilla-Duenas *et al.* (2007), Orr *et al.* (1993), Servadio *et al.* (1995), Yakura *et al.* (1974)
SCA2	Autosomal dominant	12q23-24.1	15–24	32–200	*SCA2* (ataxin-2)	RNA metabolism	Cytoplasmic	Cerebellar Purkinje cells , brainstem, fronto-temporal lobes	Ataxia, slow saccades, decreased reflexes, polyneuropathy, motor neuropathy, infantile variant with retinopathy	Gispert *et al.* (1993)
SCA3	Autosomal dominant	14q24.3-32	13–36	61–84	*SCA3* (ataxia-3)	De-ubiquitinating activity	Cytoplasmic	Cerebellar dentate neurons, basal ganglia, brainstem, spinal cord	Ataxia, parkinsonism, severe spasticity	Kawaguchi *et al.* (1994), Paulson *et al.* (1997a)
SCA6	Autosomal dominant	19p13	4–19	10–33	*CACNA1A* ($CACNA1_A$)	P/Q-type α_{1A} voltage-gated calcium channel subunit	Cell membrane	Cerebellar Purkinje cells, dentate nucleus, inferior olive	Ataxia, dysarthria, nystagmus, tremors	Zhuchenko *et al.* (1997)
SCA7	Autosomal dominant	3p14-21.1	4–35	37–306	*SCA7* (ataxin-7)	Transcription	Nuclear	Cerebellum, brainstem, macula, visual cortex	Ataxia, blindness, cardiac failure in infantile form	Benomar *et al.* (1995)
SCA17	Autosomal dominant	6p27	25–42	47–63	*SCA17* (tata binding protein)	Transcription	Nuclear	Cerebellum, basal ganglia	Ataxia, behavioural changes or psychosis, cognitive decline, seizures	Nakamura *et al.* (2001)

Since the genes causing these diseases have no homology with each other except for the highly polymorphic CAG tract, the distinct clinical and pathological features of the various polyQ diseases (table 3) indicate that the protein context around the pathogenic repeat plays a significant role in modulating the disorder (Orr 2001; Nozaki *et al*. 2001; La Spada and Taylor 2003; Masino *et al*. 2004; de Chiara *et al*. 2005; Gatchel and Zoghbi 2005; Thakur *et al*. 2009). For instance, phosphorylation of ataxin-1 at serine 776 and sumoylation of huntingtin protein have been found to be important determinants of toxicity (Chen *et al*. 2003; Emamian *et al*. 2003; Steffan *et al*. 2004). In spite of the divergent properties of the affected proteins, the various polyQ diseases share several features like: (i) mid-life onset; (ii) progressive neuronal cell loss; (iii) decline in motor and cognitive functions; (iv) anticipation; (v) a correlation between the number of CAG repeats and the severity and age at onset of the disease; and (vi) abnormal protein conformation(s) which result in protein aggregations in the affected cells (DiFiglia *et al*. 1997; Paulson *et al*. 1997b; Skinner *et al*. 1997; Walters and Murphy 2009).

Inclusion bodies

The various polyQ disorders generally show intracellular aggregates or inclusion bodies (IB) due to abnormal folding of the expanded polyQ proteins in the affected neurons in humans as well as in cell culture and animal models (Davies *et al*. 1997; Klement *et al*. 1998; Saudou *et al*. 1998; Warrick *et al*. 1998; Bates 2003). These aggregates develop in a polyQ length and time-dependent manner (Kim *et al*. 1999). In polyQ patients, the aggregates may localize in the cytoplasm, perinuclear and/or nuclear regions of the cell. These inclusion bodies sequester a variety of cellular proteins like molecular chaperones (Cummings *et al*. 1998; Warrick *et al*. 1999), some key transcription factors (McCampbell *et al*. 2000; Nucifora *et al*. 2001; Dunah *et al*. 2002; Li *et al*. 2002; Schaffar *et al*. 2004), proteasome subunits (Cummings *et al*. 1998; Chan *et al*. 2000; Bence *et al*. 2001) and cytoskeletal components (Meriin *et al*. 2003; Ganusova *et al*. 2006). The intrinsic toxicity of insoluble aggregates of proteins with expanded polyQ tract is thus believed to be aggravated by the functional depletion of the other normal cellular proteins because of their sequestration by the IBs (Stenoien *et al*. 1999; Chai *et al*. 2002; Iwata *et al*. 2005).

It is still debated if the IBs, which are hallmarks of polyQ pathogenesis, are causal to or a consequence of disease pathogenesis or represent a cellular protective mechanism (DiFiglia *et al*. 1997; Kim and Tanz 1998; Saudou *et al*. 1998; Warrick *et al*. 1998; Arrasate *et al*. 2004). Some studies have suggested that the IBs are merely structural markers of neurotoxicity and are not necessary for neuronal loss but have a protective role in case of HD, SCA1 and SCA7 (Watase *et al*. 2002; Yoo *et al*. 2003; Arrasate *et al*. 2004; Bowman *et al*. 2005) Further, mouse models expressing full length huntingtin or ataxin-1 proteins lacking the self-association domain failed to develop the typical aggregates, yet they showed specific neuronal cell loss characteristic of the disease (Klement *et al*. 1998; Hodgson *et al*. 1999). On the other hand, several studies in *Drosophila* polyQ disease models showed that polypeptides that bind to mutant huntingtin or mutant ataxin-3 and interfere with their aggregation reduce the polyQ toxicity (Apostol *et al*. 2003; Nagai *et al*. 2003). Recent studies from our laboratory have also demonstrated that suppression of polyQ toxicity in fly models of the disease by targeted depletion of Hsp60D or the large nuclear non-coding hsrω-n RNA is associated with inhibition of polyQ aggregate formation in eye disc cells (Arya *et al*. 2010; Mallik and Lakhotia 2009a). It is also believed that the potentially soluble and diffusible oligomeric structures of the expanded polyQ proteins may be the actual mediators of cytotoxicity (Ross and Poirier 2004; Bennett *et al*. 2005).

PolyQ diseases are examples of a growing group of neurodegenerative disorders in which protein homeostasis seems to be affected due to abnormal protein folding, aggregation and impaired degradation. However, several fundamental issues relating to the polyQ pathogenesis remain to be understood. For instance, why are neurons selectively vulnerable even though the mutant proteins are more widely expressed? Even in the populations of neurons that express the mutant protein, why do only certain subpopulations of neurons undergo degeneration while others do not? Are changes in conformation of mutant protein the primary cause of neurodegeneration or does the expanded polyQ stretch provide a loss-of-function or gain-of-function property to the protein or do such proteins get mislocalized in the cell resulting in disruption of their normal function/s? Are there other independent events, triggered by the expanded polyQ stretch, which also contribute to the polyQ phenotypes? It also remains to be understood if the currently accepted markers of neurodegeneration are the causal factors or consequences of the pathology? Notwithstanding these uncertainties, conformational changes in proteins with expanded polyQ stretches are believed to be the prime cause for the pathogenesis in view of the colocalization of molecular chaperones and proteasome components with the IBs and modulation of polyQ aggregation and toxicity by several chaperones (Muchowski and Wacker 2005; Rousseau *et al*. 2009; Nagai *et al*. 2010, also see table 5).

There are several possible ways through which altered conformations of the expanded polyQ proteins may cause degeneration of neuronal cells: (i) The mutant protein's intrinsic biological activity is altered because of the conformational change in the polyQ domain. (ii) The mutant protein shows altered interactions with its normal interacting partners and/or novel associations with other proteins. In particular, the misfolded polyQ proteins interact with normal cellular proteins that contain polyQ or glutamine-rich domains, because such domains are sufficient to recruit these normal proteins into polyQ IBs (Perez *et al*. 1998; Kazantsev *et al*. 1999). Except for the polyQ tract, the disease proteins are dissimilar and therefore, certain changes in protein interactions will be unique to the individual disease protein.

Table 4. Fly models of glutamine repeat disorders

Protein context	Transgene construct	PolyQ Repeat length	Transgene name	References
Pure polyglutamine	Transgenes with varying length of CAG trinucleotide repeats generated from various sources but without any disease protein context	48	UAS-Q48tag	Kazantsev *et al*. (2002)
		63	UAS-63Q	Kazemi-Esfarjani and Benzer (2002)
		79	GMR-Q79	Higashiyama *et al*. (2002)
		92	GMR-Q92	
		108	UAS-Q108	Marsh *et al*. (2000)
		127	UAS-127Q	Kazemi-Esfarjani and Benzer (2000)
Ataxin-1	Human SCA1 cDNA	82	UAS-SCA1 82Q	Feany and Bender (2000); Fernandez-Funez *et al*. (2000); Tsai *et al*. (2004)
Ataxin-3	NH_2-terminal 12 aa and C-terminal 43 aa	78	UAS-MJDtr-Q78	Warrick *et al*. (1998)
	NH_2-terminal 12 aa and C-terminal 43 aa	61	UAS-SCA3tr-Q61(S)	Chan *et al*. (2000)
	N-terminally truncated ataxin-3	62	UAS-SCA3trQ62-DsRed	Li *et al*. (2007)
	N-terminally truncated ataxin-3 with NES sequence from the Rev protein at the 3′ end	77	UAS-MJD-77QNES	Gunawardena *et al*. (2003)
	Full length of ataxin-3	78	UAS-SCA3-Q78	Warrick *et al*. (2005)
		84	UAS-SCA3-Q84	
	Full length of ataxin-3 with a point mutation in the ubiquitin protease domain	88	UAS-SCA3-Q88 C14A	
	Full length ataxin-3 with point mutations (S236A, S256A) in the ubiquitin interacting motif (UIM)	80	UAS-SCA3-Q80 UIM*	
	Full length ataxin-3 carrying a mutation in the VCP-Binding site	71	UAS-Atx3Q71HNHH	Boeddrich *et al*. (2006)
	NH_2-terminal deletion mutant of ataxin-3	79	UAS-ataxin-3ΔN79QC	Matsumoto *et al*. (2004)
Ataxin-7	SCA7 cDNA (amino acids 1-232) with an added nuclear localization signal	102	UAS-SCA7T-102Q	Latouche *et al*. (2007)
Huntingtin	NH_2-terminal 17 aa and an additional 125 aa from Huntingtin and different carboxy termini due to variations in the portion of the parental hsp70 vector 3′ region included prior to the stop codon	75	GMR-Huntingtin-Q75	Jackson *et al*. (1998)
		120	GMR-Huntingtin-Q120	
	Entire exon 1 of Huntingtin (amino acids 1-67)	93	UAS-Httex1p Q93	Steffan *et al*. (2001)
	cDNA encoding the entire exon 1 of Huntingtin followed by the proline rich PXXP domain; this domain is absent in the 103Q construct	97	UAS-Httex1p 97QP	Steffan *et al*. (2004)
		103	UAS-Httex1p 103Q	
	548 amino acid NH_2-terminal fragment of the human Huntingtin cDNA	128	UAS- Htt-Q128	Lee *et al*. (2004)
	NH_2-terminal fragment encoding the first 336 amino acids of the human Huntingtin cDNA	128	UAS-128QHtt[M64]	Kaltenbach *et al*. (2007)
	N-terminal part of human Huntingtin (amino acids 1-171)	138	UAS-HA-hHtt171aa-138Q	Mugat *et al*. (2008)
	NH_2-terminal Huntingtin exon 1	46	UAS-Httex1-Q46-eGFP	Zhang *et al*. (2010)
		72	UAS-Httex1-Q72-eGFP	
		103	UAS-Httex1-Q103-eGFP	
	N-terminal Huntingtin exon 1 fused to EGFP either with or without an NLS for nuclear targeting	48	UAS-Nhtt(48Q)EGFPNLS	Doumanis *et al*. (2009)
		152	UAS-Nhtt(152Q)EGFP	
Androgen receptor	Full length human AR	52	UAS-hAR(Q52)	Takeyama *et al*. (2002)
	Mutant hAR lacking the C-terminal E/F domain containing the ligand binding domains.	52	UAS-hAR(Q52 AF-1)	
	Human AR cDNA	112	UAS-ARtrQ112	Chan *et al*. (2002)

Table 5. Genetic modifiers of toxicity in fly models of polyQ disorders.

Genetic modifiers of polyQ toxicity (gene name)	Nature of mutant allele	Fly models of polyQ disease pathology examined						References
			SCAs					
		PolyQ model	SCA1	SCA3	SCA7	HD	SBMA	
Transcription factors/regulators								
Heat shock factor (*Hsf*)	LOF			En				Fujikake *et al*. (2008)
Sin3A (*Sin3A*)	OE			Su[3]				1. Fernandez-Funez *et al*. (2000), 2. Steffan *et al*. (2001), 3. Bilen and Bonini (2007), 4. Branco *et al*. (2008)
	LOF		En[1]			En[4], Su[2a]		
Taranis (*tara*)[b]	OE	NC[2]	En[1,2]	NC[2]		En[3]		1. Fernandez-Funez *et al*. (2000), 2. Ghosh and Feany (2004), 3. Branco *et al*. (2008)
	LOF		En[1]					
Engrailed (*en*)	OE					Su		Mugat *et al*. (2008)
Tramtrack (*ttk*)	LOF					Su		
Armadillo (*arm*)	LOF					Su		
Crooked legs (*crol*)[c]	LOF					En		Kaltenbach *et al*. (2007)
						Su		
Myocyte enhancer factor 2 (*Mef2*)	LOF					Su		
Nipped-A (*dTra1*)	LOF				Su[1]	En[2]		1. Latouche *et al*. (2007), 2. Zhang *et al*. (2010)
TBP-associated Factor 10 (*Taf10*)	LOF				Su[1]			
Skuld (*skd*)	LOF		En			En		Branco *et al*. (2008)
C-terminal Binding Protein (*dCtBP*)	LOF		En[1,2]			NC[2]		1. Fernandez-Funez *et al*. (2000), 2. Branco *et al*. (2008)
Debra (*dbr*)	OE			Su				Bilen and Bonini (2007)
	LOF			En				
Silencing mediator for retinoid and thyroid hormone receptors (SMRT)-related ecdysone receptor-interacting factor (SMRTER)	OE		Su					Tsai *et al*. (2004)
	LOF		En					
RNA-binding proteins								
Muscleblind (*mbl*)	OE			En				Li *et al*. (2008)
Mushroom-body expressed (*mub*)	OE		Su[1]		En[2]	En[3]		1. Fernandez-Funez *et al*. (2000), 2. Latouche *et al*. (2007), 3. Branco *et al*. (2008)
	LOF		En[3]			NC[3]		
Drosophila myeloid leukemia factor 1 (*dmlf*)	OE	Su				Su		Kazemi-Esfarjani and Benzer (2002)
Pumilio (*pum*)	OE	NC[2]	En[1]	NC[2]		En[3]		1. Fernandez-Funez *et al*. (2000), 2. Ghosh and Feany (2004), 3. Branco *et al*. (2008)
	LOF		NC[1], Su[3 d]			Su[3]		

Table 5 (*contd.*)

Genetic modifiers of polyQ toxicity (gene name)	Nature of mutant allele	Fly models of polyQ disease pathology examined						References
			SCAs					
		PolyQ model	SCA1	SCA3	SCA7	HD	SBMA	
Couch potato (*cpo*)	OE LOF		En[1] NC[1]			En[2]		1. Fernandez-Funez *et al*. (2000), 2. Branco *et al*. (2008)
Hrb87F (*hrb87F*)	LOF	En						Sengupta and Lakhotia (2006)
Hoi-polloi (*hoip*)	OE	En					En	Murata *et al*. (2008)
Histone acetyltransferases/deacetylases								
CREB Binding Protein (*nejire*)	OE LOF	Su[1] En[1,2]		En[2]	En[3]			1. Taylor *et al*. (2003), 2. Mallik and Lakhotia (2010), 3. Latouche *et al*. (2007)
Rpd3 (*Rpd3*)	LOF		En[1]		En[2 e]	NC[3], Su[4 f]		1. Fernandez-Funez *et al*. (2000), 2. Latouche *et al*. (2007), 3. Branco *et al*. (2008), 4. Pallos *et al*. (2008)
Sirtuin 2 (*Sir2*)	OE LOF	NC[2]	En[1,2] NC[1]	NC[2]		En[3] Su[4]		1. Fernandez-Funez *et al*. (2000), 2. Ghosh and Feany (2004), 3. Branco *et al*. (2008), 4. Pallos *et al*. (2008)
Histone deacetylase 6 (*HDAC6*)	OE LOF	Su		Su			Su En	Pandey *et al*. (2007)
Protein homeostasis pathways								
Ubiquitin (*Ubi63E*, *CR11700*)	OE LOF		En[1]	Su[3]		Su[2]		1. Fernandez-Funez *et al*. (2000), 2. Steffan *et al*. (2004), 3. Bilen and Bonini (2007)
Ubiquitin conjugases (*UbcD1/effete*, *dUbc-E2H*)	LOF		En[1]			En[2]		1. Fernandez-Funez *et al*. (2000), 2. Branco *et al*. (2008)
Ubiquitin activating enzyme (*Uba1*)	OE				Su			Latouche *et al*. (2007)
Ubiquitin Ligases (*CHIP*, *CG8209*, *Faf*, *UFD2a/CG11070*)	OE	NC[2]	Su[2]	Su[1,3]		Su[2]		1. Matsumoto *et al*. (2004), 2. Al-Ramahi *et al*. (2007), 3. Bilen and Bonini (2007)
SUMO (*smt3*)	LOF					Su		Steffan *et al*. 2004
SUMO-1 activating enzyme (*Uba2*)	LOF			En[1,2]		En[2]		1. Arya *et al*. (2010), 2. Chan *et al*. (2002)
Proteasome subunits (*Pros26*, *Prosβ2*)	LOF	En[1]					En[2]	1. Mallik and Lakhotia (2010), 2. Chan *et al*. (2002)
Full length ataxin-3 protein	OE		Su	Su		Su		Warrick *et al*. (2005)
Autophagy-specific genes (*Atg5, Atg6, Atg12*)	LOF			En[1]			En[2]	1. Bilen and Bonini (2007), 2. Pandey *et al*. (2007)
Fat facets (*faf*)	LOF					En		Kaltenbach *et al*. (2007)
Chaperones and co-chaperones								

Table 5 (*contd.*)

Genetic modifiers of polyQ toxicity (gene name)	Nature of mutant allele	Fly models of polyQ disease pathology examined						References
			SCAs					
		PolyQ model	SCA1	SCA3	SCA7	HD	SBMA	
HdJ1 (*DnaJ-1*)	OE LOF	Su[3,4]	Su[2,4]	Su[1,4] En[1]	Su[5]	Su[1]		1. Chan *et al*. (2000), 2. Fernandez-Funez *et al*. (2000), 3. Kazemi-Esfarjani and Benzer (2000), 4. Ghosh and Feany (2004), 5. Latouche *et al*. (2007)
Heat shock protein 70 (*Hsp70*)	OE LOF	Su[4]	Su[4]	Su[1,4] En[5]		Su[2]	Su[3]	1. Warrick *et al*. (1999), 2. Chan *et al*. (2000), 3. Chan *et al*. (2002), 4. Ghosh and Feany (2004), 5. Gong and Golic (2006)
Heat Shock Protein cognate 3 (*Hsc70-3*)	OE LOF		En[2]		Su[1]	En[2]		1. Latouche *et al*. (2007), 2. Branco *et al*. (2008)
Heat Shock Protein cognate 4 (*Hsc70-4*)	LOF	En[3]	En[3]	En[1,3]			En[2]	1. Warrick *et al*. (1999), 2. Chan *et al*. (2000), 3. Ghosh and Feany (2004)
CG6603 (*Hsc70Cb/Hsp110*)	OE LOF					Su En		Zhang *et al*. (2010)
Hsp60D (*hsp60D*)	LOF	Su		Su				Arya *et al*. (2010)
Hsp27	OE	NC[g] Su						Liao *et al*. (2008)
sHsp αβ crystalline (*CG14207*)	OE			Su				Bilen and Bonini (2007)
Tetratricopeptide repeat protein 2 (*Tpr2*)	OE LOF	Su[1]		Su[2] En[2]				1. Kazemi-Esfarjani and Benzer (2000), 2. Bilen and Bonini (2007)
Cellular detoxification pathway								
Superoxide dismutases (*Sod, Sod2*)	OE					NC		Bahadorani and Hilliker (2008)
Glutathione-S-transferase S1 (*GstS1*)	OE LOF		Su[1] En[1,2]			NC[2]		1. Fernandez-Funez *et al*. (2000), 2. Branco *et al*. (2008)
Aspartyl β-hydroxylase (*Asph*)	LOF					En		Kaltenbach *et al*. (2007)
Axonal transport								
Kinesin heavy chain (*Khc*)	LOF	En[1]		En[1]		En[1] Su[2h]		1. Gunawardena *et al*. (2003), 2. Kaltenbach *et al*. (2007)
Cytoplasmic dynein light chain 2 (*Cdlc2*)	LOF	En		En		En		
Dynein heavy chain 64C (*Dhc64C*)	LOF					En		Kaltenbach *et al*. (2007)
Signal transduction								
14-3-3ϵ (*14-3-3ϵ*)	OE LOF		En Su			En[1,2] Su[1,2]		1. Branco *et al*. (2008); 2. Kaltenbach *et al*. (2007)
14-3-3ζ (*14-3-3ζ/leonardo*)	OE					En		Kaltenbach *et al*. (2007)
Akt1 (*Akt1*)	OE LOF		En[1] Su[1]	NC[2]		Su[1,3] En[1]		1. Branco *et al*. (2008); 2. Bilen *et al*. (2006), 3. Lievens *et al*. (2008)

Table 5 (*contd.*)

Genetic modifiers of polyQ toxicity (gene name)	Nature of mutant allele	Fly models of polyQ disease pathology examined						References
			SCAs					
		PolyQ model	SCA1	SCA3	SCA7	HD	SBMA	
p53	LOF					Su		Bae *et al*. (2005)
Vibrator (*vib*)	OE		Su			En		Branco *et al*. (2008)
RhoGAP (*RhoGAPp190*)	OE		En			En		
Pi3K92E (*Pi3K92E*)	OE		En			Su		
Intersectin (*Dap160*)	OE	En						Scappini *et al*. (2007)
GTPase (*Rheb*)	OE					En		Doumanis *et al*. (2009)
Src oncogene at 42A (*Src42A*)	OE LOF					Su En		Kaltenbach *et al*. (2007)
Syntaxin1A (*Syx1A*)	LOF OE					Su En		
Inositol 1,4,5,-tris-phosphate receptor (*Itp-r83A*)	OE LOF					En Su		
Apoptosis								
P35	OE	NC[1,3]		Su[3]	NC[4]		NC[2]	1. Kazemi-Esfarjani P. and Benzer S., unpublished, 2. Jackson *et al*. (1998), 3. Ghosh and Feany (2004), 4. Bilen and Bonini (2007)
DIAP1 (*thread*)	OE LOF	NC[1] En[4]		Su[1,3] En[3]	Su[1], NC[5] En[4]	Su[2] En[2]	Su[3] En[3]	1. Ghosh and Feany (2004), 2. Latouche *et al*. (2007), 3. Branco *et al*. (2008), 4. Arya *et al*. (2010), 5. Bilen *et al*. (2006)
Drosophila Apaf-1-related-killer (*dark*)	LOF	Su[1]			NC[2]		Su[1]	1. Sang *et al*. (2005), 2. Bilen *et al*. (2006)
Death executioner Bcl-2 homologue (*debcl/Drob-1/dBorg-1/dBok*)	OE LOF				Su En			Senoo-Matsuda *et al*. (2005)
Buffy (*Buffy*)	OE				En			
VCP/p97/CDC48 (*ter94*)	OE LOF	Su[2]			Su[1]		NC[1]	1. Boeddrich *et al*. (2006), 2. Higashiyama *et al*. (2002)
Non-coding RNAs								
Heat shock RNA omega (*hsrω*)	OE LOF	En[1,2 i] Su[3]		Su[3]	Su[3]		En[2] Su[3]	1. Fernandez-Funez *et al*. (2000), 2. Sengupta and Lakhotia (2006), 3. Mallik and Lakhotia (2009a)
Bantam (*ban*)	OE LOF				Su En			Bilen *et al*. (2006)
microRNA processing								
Dicer-1 (*dcr-1*)	LOF				En			Bilen *et al*. (2006)
Dicer-2 (*dcr-2*)	LOF				NC			
R3D1 (*loqs*)	LOF				En			
Dicer-1 (*dcr-1*)	LOF				En			
PolyQ genes								

Table 5 (*contd.*)

Genetic modifiers of polyQ toxicity (gene name)	Nature of mutant allele	Fly models of polyQ disease pathology examined						References
			SCAs					
		PolyQ model	SCA1	SCA3	SCA7	HD	SBMA	
Ataxin-2 (*dAtx2*)	OE LOF	En[1]	En[1,2] Su[2]	En[1] Su[3]		NC[2]		1. Ghosh and Feany (2004), 2. Al-Ramahi *et al*. (2007), 3. Lessing and Bonini (2008)
Ataxin-3 (*hAtx3*)	OE		Su	Su		Su		Warrick *et al*. (2005)
Huntingtin (*dHtt*^{620aa}, *hHtt*^{548aa})	OE LOF					Su En		Mugat *et al*. (2008)
Translational regulators								
Dappled (*dpld*)	OE			Su				
Insulin growth factor II mRNA binding protein (*Imp*)	OE			Su				Bilen and Bonini (2007)
Orb2 (*orb2/CG5735*)	OE			Su				
Cytoskeletal organization and biogenesis								
Chickadee (*chic*)	OE	Su				Su		Burnett *et al*. (2008)
LaminC (*LamC*)	LOF					En		
Zipper (*zip*)	LOF					En		Kaltenbach *et al*. (2007)
Hu li tai shao (*hts*)	LOF					Su		
Peanut (*pnut*)	LOF					Su		
Transport proteins								
Embargoed (*emb*)	OE LOF			Su En				Bilen and Bonini (2007)
Rab5 (*Rab5*)	OE LOF					Su En		Ravikumar *et al*. (2008)
Nup44A (*Nup44A*)	OE		Su[1]			NC[2]		1. Fernandez-Funez *et al*. (2000), 2. Branco *et al*. (2008)
Nuclear pore protein 160 (*Nup160*)	LOF					Su		Doumanis *et al*. (2009)
Clathrin heavy chain (*Chc*)	LOF					Su		
Unc-76 (*Unc-76*)	LOF					En		Kaltenbach *et al*. (2007)
Porin (*porin*)	LOF					Su		
Sec61α (*CG9539*)	LOF			Su		Su		Kanuka *et al*. (2003)
Miscellaneous								
Yeast prion domain Sup35N	OE LOF			Su En				Li *et al*. (2007)
CG7231	LOF	En	En	En				Ghosh and Feany (2004)
CG1109	LOF					Su		Doumanis *et al*. (2009)
CG5537	LOF					Su		
G protein αi subunit 65A (*G-iα65A*)	OE LOF					En Su		
Short stop (*shot*)	LOF					En		
CG12455	OE LOF					En Su		Kaltenbach *et al*. (2007)
Phosphoglucose isomerase (*Pgi*)	LOF					En		

Table 5 (*contd.*)

Genetic modifiers of polyQ toxicity (gene name)	Nature of mutant allele	Fly models of polyQ disease pathology examined						References
			SCAs					
		PolyQ model	SCA1	SCA3	SCA7	HD	SBMA	
Rpt1 (*Rpt1*)	OE					En		
	LOF					Su		
M6 (*M6*)	OE					En		
	LOF					Su		
Lachesin (*Lac*)	LOF					En		
Pasilla (*ps*)	LOF		Su			NC		
Sc2 (*Sc2*)	LOF		En			NC		Branco *et al*. (2008)
CG14438	OE		Su			En		
	LOF		En			Su		
Polyalanines	OE					Su		Berger *et al*. (2006)

En, enhancing effect; LOF, loss-of-function; NC, no discernable change; OE, overexpression; Su, suppressing effect.

Numbers in superscripts in columns for fly models refer to the serial number of references listed in the last column of the given row in cases where more than one citations are listed.

[a]The opposing results seen in case of the HD model maybe due to use of different loss-of-function alleles. The EP insertion in the *EP(2)866* allele of *Sin3A*, used in references 1 and 4, is in opposite orientation with respect to the ATG at +1; in reference 2 another loss-of-function allele, $Sin3A^{08269}$ was used and the overexpressing EP allele used in reference 3 was $Sin3A^{B9-E}$.

[b]EP element in the *EP(3)3463* allele of the *taranis* gene used in all the three studies is inserted in sense orientation in an intron ~16.3 kb downstream of the first ATG, but −553 bp with respect of the second ATG. Thus while the taranis isoform 1A is disrupted, isoform 1B is overexpressed.

[c]The opposing results seen in case of the HD model may be due to the fact that different loss-of-function alleles of *crol* ($P(EPgy2)crol^{EY08953}$ and $P(PZ)crol^{04418}$, respectively) were used by Kaltenbach *et al*. (2007).

[d]The differing results seen in case of the SCA1 model maybe due to the fact that different loss-of-function alleles were used in each case; while Fernandez-Funez *et al*. (2000) used the pum^{13} allele, Branco *et al*. (2008) used the pum^{bem} allele.

[e] The EP-transposon insertion, *EP(3)3672*, was reported as a gain-of-function allele of *Rpd3* by Latouche *et al*. (2007); however, Fernandez-Funez *et al*. (2000) reported that although the *EP(3)3672* tranposon is inserted in sense orientation to *Rpd3*, this allele does not over-express *Rpd3*. It is to be further noted that the site of EP-transposon insertion in *EP(3)3672* is actually in the neighbouring *Src64B* gene (http://www.flybase.org), > 1 kb upstream of the *Rpd3* gene. Therefore, it remains possible that the enhancing effect of *EP(3)3672* on polyQ pathogenesis may actually be due to loss-of-function of the *Src64B* gene. This needs further examination.

[f]The differing results with *Rpd3* mutant alleles in case of the HD model may be due to different loss-of-function alleles used in the two studies; Branco *et al*. (2008) used $Rpd3^{04556}$, while Pallos *et al*. (2008) did not specify the loss-of-function allele used in their study.

[g]Though over-expression of *hsp27* attenuates mild toxicity caused by a short polyQ (UAS-41Q), it fails to alleviate the severe toxicity caused by a long polyQ (UAS-127Q) tract.

[h]The opposing results obtained with the same *Khc* mutant allele (Khc^{8}) in case of the HD model by Gunawardena *et al*. (2003) and Kaltenbach *et al*. (2007) may be because different polyQ expanded Huntingtin transgenes were used in each case.

[i]The $hsr\omega^{05241}$ allele was described by Fernandez-Funez *et al*. (2000) as a loss-of-function allele but as described by Sengupta and Lakhotia (2006), this is actually an overexpression allele of the gene.

All these may result in wider alterations in expression of genes, including those that are critical for functioning of specific neurons, so that the grossly disrupted protein homeostasis triggers the affected neuron's death. However, since a variety of cellular pathways (see section on Molecular mechanisms) are affected, the pathogenic mechanisms are indeed likely to be more complex.

Modelling human polyglutamine diseases in *Drosophila*

With a view to understand the molecular and cellular pathophysiology of polyQ-induced neurodegeneration and to discover potential and novel drug targets for therapeutics, several neurodegenerative diseases, including Alzeihmer's, Parkinson's, HD, SCA3, SCA1, SBMA and others, have been modelled in different animal systems. Human neurodegenerative diseases were initially modelled in mice (Ikeda *et al*. 1996; Lin *et al*. 1999). However, expensive maintenance and the longer time required for genetic manipulations remain the major limitations of mouse models (Reiter and Bier 2002). Therefore, these diseases have also been modelled in simpler organisms like yeast, *Caenorhabditis*, *Drosophila* etc. (Krobitsch and Lindquist 2000; Satyal *et al*. 2000; Coughlan and Brodsky 2003; Voisine and Hart 2004; Celotto and Palladino 2005; Marsh *et al*. 2009). In this context, *Drosophila* has proved to be an excellent model organism for gene function studies in relation to human diseases due to the relative ease of genetic manipulation and large-scale genetic screening (Bier 2005; Bilen and Bonini 2005; Brumby and Richardson 2005; Restifo 2005). The relative simplicity of the fly genome compared to the complex and intricate human genomic organization, the lack of many redundant genes in flies and the availability of a number of versatile genetic manipulation techniques that are impossible or impractical in mammalian models, have encouraged genetic analysis of many human diseases in fly models (Bier 2005; Bilen and Bonini 2005; Brumby and Richardson 2005; Restifo 2005). Notwithstanding the genome simplicity, many genes and pathways that were originally studied in flies have subsequently been identified in mammals. Over 50% of fly genes exhibit apparent homology to human genes, with conservation of molecular mechanisms and fundamental aspects of cell biology including regulation of gene expression, neuronal connectivity, cell signalling and cell death (Adams *et al*. 2000; Rubin *et al*. 2000). Not only basic cell biology, but also higher-order events such as organ structure and function are conserved. For instance, the fly brain is estimated to have more than 300,000 neurons and, as in mammals, the brain is organized into areas with specialized functions such as learning, olfaction, memory and vision (Hartenstein *et al*. 2008). Approximately 75% of known human disease genes have at least one homolog in *Drosophila* (Reiter *et al*. 2001; Chien *et al*. 2002). Both the normal and aberrant functions of these genes can be conveniently studied by generating mutations in the *Drosophila* homolog or by introduring the human disease gene in the fly genome and analysing the resulting cellular phenotypes. Keeping these unique advantages in view, several *Drosophila* transgenic lines (see table 4 for a list of fly models of polyQ diseases) expressing either pure polyQ tracts with some protein context (Kazemi-Esfarjani and Benzer 2000, 2002; Marsh *et al*. 2000) or full-length or truncated disease causing proteins with expanded polyQ (Jackson *et al*. 1998; Warrick *et al*. 1998, 2005; Fernandez-Funez *et al*. 2000; Steffan *et al*. 2001, 2004) have been established during the past decade (reviewed in Muqit and Feany 2002; Bilen and Bonini 2005; Sang and Jackson 2005; Marsh and Thompson 2006). The GAL4/UAS system (Brand and Perrimon 1993) provides a simple but very efficient means of spatially and temporally targeted gene expression in *Drosophila* (figure 1) and has been most commonly used to express the polyQ transgenes in the target tissue. In addition to the *GMR–GAL4* driver (Hay *et al*. 1994), which restricts expression of the polyQ transgenes to the developing eye (figure 1), a pan-neuronal *elav-GAL4* driver (Lin and Goodman 1994) has also been used. The GAL4/UAS system has been successfully used to demonstrate that, as in mammals, the neuronal cells are more sensitive to the toxic effects of the expanded polyQ proteins than the epithelial cells in flies (Warrick *et al*. 1998). Most screens for identification of modulators of the neurodegenerative phenotypes in flies expressing the polyQ transgenes have used loss-of-function or gain-of-function mutant alleles of fly homologs of the mammalian/human genes although in some studies other transgenes or chemical modifiers have also been used.

The fly model offers two relatively simple tests for neurodegeneration, viz., (i) assay of structural and functional organization of photoreceptor neurons in the eye and (ii) motor function assay though climbing ability (Jackson *et al*. 1998; Marsh and Thompson 2004). The fly's eye is completely dispensable for survival and fertility of the laboratory strains, and is tolerant of genetic disruption of basic biological processes, thus facilitating genetic studies of neurodegenerative disorders (figure 2). Besides the overall morphology of the adult eye (figure 2, A&D), the organization of ommatidial arrays in eyes of flies (figure 2, B&E) can also be easily examined by a novel and efficient nail-polish imprint technique (Arya and Lakhotia 2006). Degeneration of the photoreceptor neurons (figure 2, C&F) can be directly visualized in adult fly's eyes by the corneal neutralization or pseudopupil technique (Franceschini and Kirschfeld 1971). Functionality of the visual system can also be assessed by simple phototaxis assay (Quinn *et al*. 1974). In addition, since the signalling cascades that turn the undifferentiated eye imaginal cells of mid-stage larvae into the highly stereotypic pattern of ommatidial arrays in adult flies is fairly well understood (Dickson *et al*. 1992; Wolff and Ready 1993; Morante *et al*. 2007; Kumar 2009), the changes that accompany induced neurodegeneration

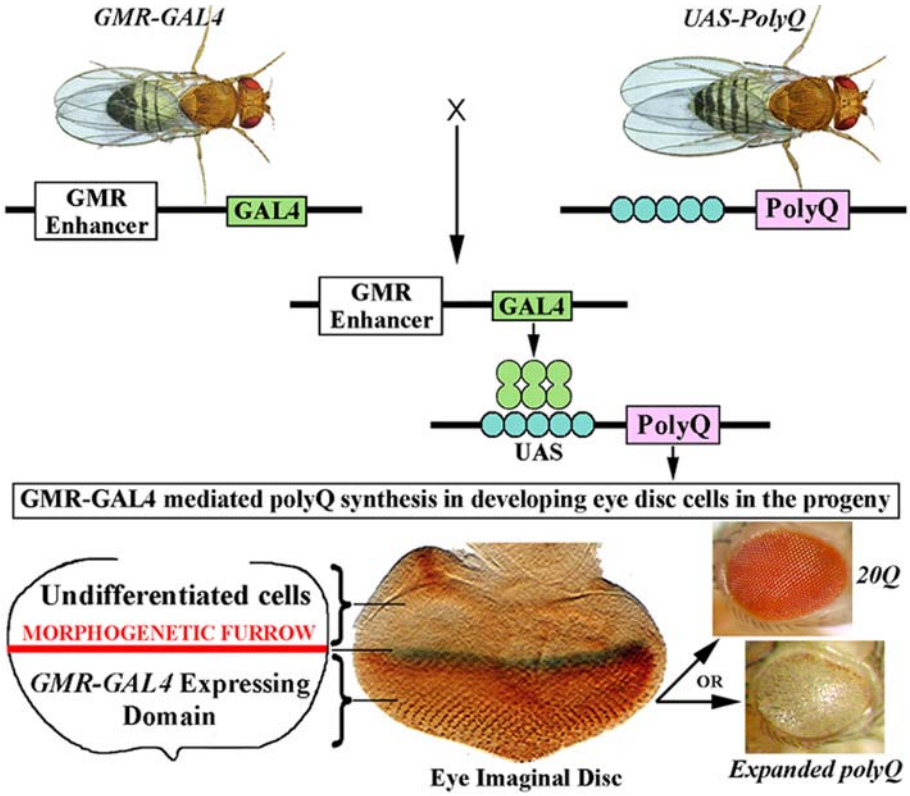

Figure 1. The binary UAS–GAL4 system (Brand and Perrimon 1993) is used for targeted expression of the polyQ protein in developing eyes of *Drosophila*. In this system, the *polyQ* responder gene is placed downstream of the yeast upstream activating sequence (*UAS*) element. In absence of the yeast GAL4 transcription factor, the *UAS-polyQ* transgene remains silent in the parental *UAS-polyQ* responder line. The *GMR-GAL4* driver is widely used to direct expression of the UAS-carrying transgene in developing eyes since the *GMR* promoter is active in eye disc cells behind the morphogenetic furrow (lower part of the figure). To activate transcription of the *UAS-polyQ* transgene, the responder flies (*UAS-polyQ*) are mated with flies carrying the *GMR-GAL4* driver. The resulting F_1 progeny larvae express the polyQ responder gene, non-pathogenic (20Q) or pathogenic (expanded polyQ) depending upon the transgene construct, in all eye disc cells behind the morphogenetic furrow. The resulting phenotype of adult eyes provides a convenient end point for assaying the neurodegeneration (see figure 2).

in the developing eyes can be followed stepwise with impressive specificity.

Global unbiased *in vivo* genetic interaction screens using a variety of gene mutations and conditional expression systems (Brand and Perrimon 1993; Chou and Perrimon 1996; Rorth 1996; Morin *et al*. 2001; Adams and Sekelsky 2002; Johnston 2002; Kuttenkeuler and Boutros 2004; Evans *et al*. 2009; http://www.flybase.org), have helped in identifying the diverse range of molecules and mechanisms involved in the neurotoxicity in these debilitating disorders. The various modifiers of polyQ toxicity identified through fly models are listed in table 5. Analyses of the modulatory action of the genetic modifiers identified in fly and other models have revealed that the proteins with expanded polyQ stretches impinge upon several different pathways like transcriptional regulation, protein quality control, axonal transport, signal transduction, apoptosis etc. (table 5; figure 3). However, since several of the identified modifiers (see table 5) do not appear to be directly linked to a defined pathway, it is obvious that other network effects also exist. The major pathways (figure 3) are discussed in the following in light of the information gained from the fly and other models.

Molecular mechanisms leading to cellular dysfunction following expression of abnormal polyQ proteins

Transcriptional dysfunction in polyQ diseases

Accumulating evidence from genetic screens and other experimental studies show that transcriptional dysregulation (see table 5) plays a key role in polyglutamine disease pathology (Helmlinger *et al*. 2006). Many transcription factors (TFs) contain polyQ or glutamine-rich domains, and the polyQ tracts themselves serve as transcriptional activators

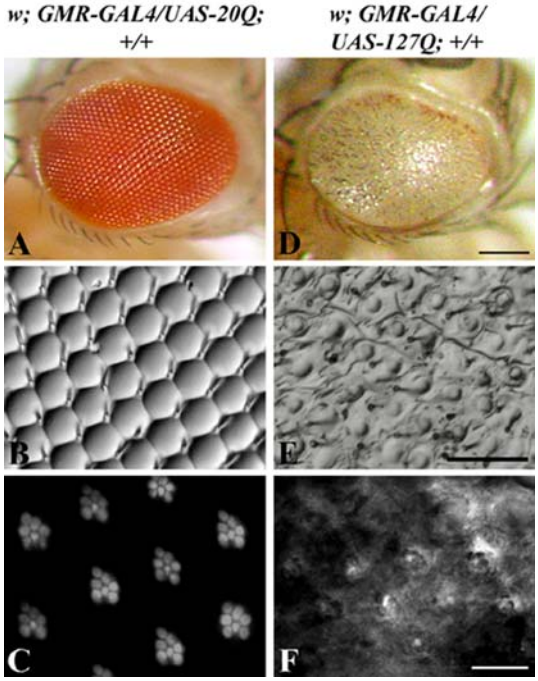

Figure 2. The retinal degeneration caused by *GMR-GAL4* driven targeted expression of the expanded polyQ protein can be easily monitored by external morphology of adult eyes (A, D), or nail-polish imprints of the eye surface (Arya and Lakhotia 2006) (B, E) or the pseudopupil (corneal neutralization, Franceschini and Kirschfeld 1971) image of the rhabdomeres in each ommatidium (C, F). The pseudopupil image reveals the precisely ordered arrangement of seven of the eight neuronal rhabdomeres in each ommatidial unit (C) Eye-specific expression of a transgene with 20Q (nonpathogenic) construct has no effect on eye morphology (A–C) while *GMR-GAL4* directed expression of the expanded pathogenic polyQ transgene results in characteristic damage as assayed by any of the three methods (D–F). Scale bars for A, D in D; B, E in E and for C, F in F = 20 μm.

(Gerber *et al*. 1994). CAG repeat expansions within two transcription factors, TATA binding protein (TBP) and androgen receptor (AR) are the causative pathogenic mutations in SCA17 and SBMA, respectively (table 3). In addition, huntingtin may function as a transcriptional corepressor by interacting with complexes that contain nuclear co-repressor proteins; likewise ataxin-1, ataxin-3 and atrophin-1 have also been implicated as transcriptional regulators (reviewed in Margolis and Ross 2001; Everett and Wood 2004; Tsai *et al*. 2004; Orr and Zoghbi 2007). The SCA7 disease protein, ataxin-7, was shown to be a component of the STAGA/TFTC histone acetyltransferase complex (Helmlinger *et al*. 2004; McMahon *et al*. 2005; Palhan *et al*. 2005). Alterations in gene expression also occur through effects on RNA processing and stability. In a genetic screen using a *Drosophila* model of SCA1 (Fernandez-Funez *et al*. 2000), several of the identified modifiers were actually found to be RNA binding and processing proteins (table 5). Deficiency of the RNA binding hnRNP Hrb87F has been shown to aggravate polyQ toxicity in a *Drosophila* model of the disease (Sengupta and Lakhotia 2006; Mallik and Lakhotia 2010). Overexpression of the non-coding hsrω RNA which forms dynamic structures called omega speckles that sequester various unengaged hnRNPs and related RNA processing proteins (Lakhotia *et al*. 1999; Prasanth *et al*. 2000), has been shown to aggravate polyQ-induced neurodegeneration while RNAi-mediated depletion of these transcripts nearly completely suppressed the polyQ toxicity in fly models expressing mutant SCA1 or SCA3 or huntingtin or a quasipure polyQ tract (Sengupta and Lakhotia 2006; Mallik and Lakhotia 2009a, also see table 5). Even in the absence of a direct interaction between the polyQ IBs and the hsrω transcripts or the hnRNPs associated with it, overabundance of the hsrω transcripts enhanced the degeneration by limiting the available pool of hnRNPs which thus compromises normal cellular functions of several other downstream proteins (see figure 3). On the other hand release of hnRNPs from omega speckles following depletion of the hsrω transcripts suppressed polyQ pathogenesis by making more of the hnRNPs available in the active pool (Mallik and Lakhotia 2009a, 2010). Likewise, CGG repeat-induced neurodegeneration in a *Drosophila* model of FXTAS was suppressed by overexpression of the hnRNPs, Hrb87F and Hrb98DE (Sofola *et al*. 2007). It remains to be seen if the suppressive effect observed upon direct overexpression of these hnRNPs extends to the polyQ diseases also. Levels of the mRNAs for proteins involved in neuronal signal transduction and calcium homeostasis are preferentially decreased in both SCA1 and HD mouse models (Lin *et al*. 2000; Vig *et al*. 2001; Panov *et al*. 2002; Strand *et al*. 2007; Lim *et al*. 2008; Runne *et al*. 2008). In a HD model, transcripts encoding neurotransmitters, neurotrophic factors like brain-derived neurotrophic factor (BDNF) and cell-adhesion proteins were also decreased, whereas mRNAs encoding heat shock proteins, proteasome and other stress-related proteins were increased (Hughes and Olson 2001; Sawa 2001).

Proteins with expanded polyQ stretches accumulate in nucleus and interact with a number of nuclear proteins including transcription factors, transcription cofactors (coactivators and corepressors) and splicing factors (reviewed in Okazawa 2003; Sugars and Rubinsztein 2003). For instance, ataxin-2 interacts with ataxin-2 binding protein 1 (A2BP1), which has been implicated in splicing (Shibata *et al*. 2000). Mutant ataxin-1 aggregates sequester the transcriptional corepressor, SMRTER (silencing mediator for retinoid and thyroid hormone receptors (SMRT)-related ecdysone receptor interacting factor), and accordingly, the SCA1-mediated eye degeneration was enhanced by a P-insertion mutation in the gene encoding the corepressor (Tsai *et al*. 2004; Table 5). Some of these interactions are sensitive to amino acid residues flanking the polyQ-tract. In several polyQ-containing proteins, the polyglutamine region is adjacent to

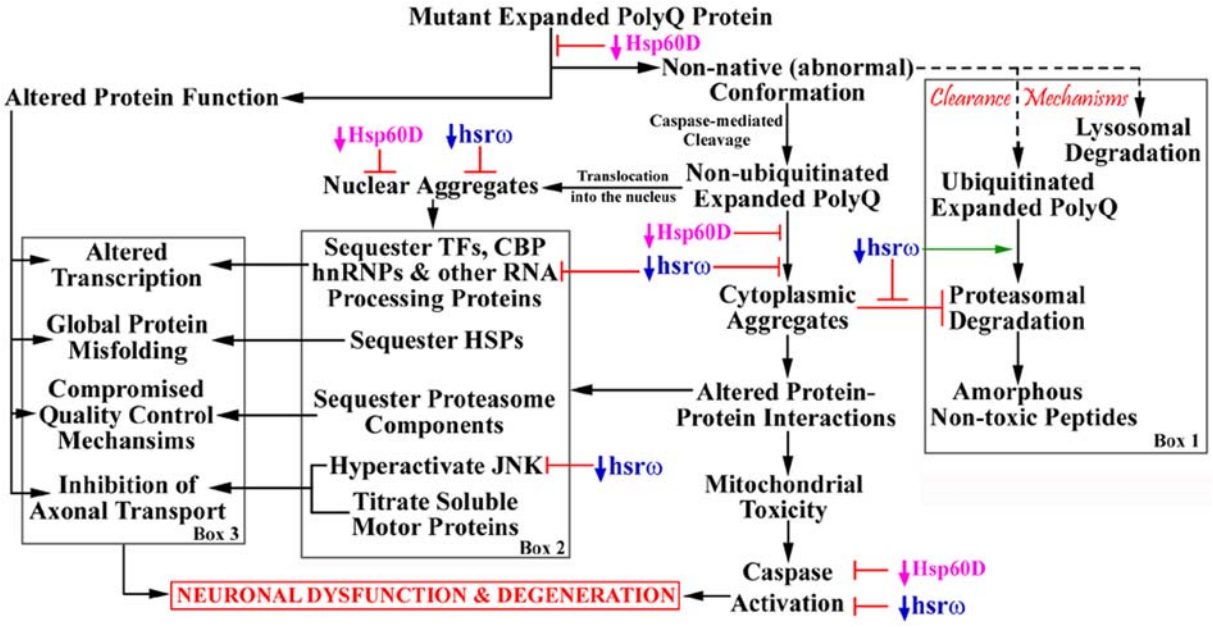

Figure 3. Mutant expanded polyQ proteins affect cell homeostasis in multiple ways. The mutant polypeptides with expanded polyQ stretches assume non-native conformation, some of which can be degraded through the lysosomal or ubiquitin-proteasome clearance paths (Box 1). However, majority of the expanded non-ubiquitinated polyQ proteins get cleaved by caspases and become toxic. Their cytoplasmic and/or nuclear aggregates sequester and thus compromise transcriptional and RNA processing machinery, chaperoning system, proteasomal components, soluble motor proteins or hyperactivate JNK (Box 2). The mutant polyQ proteins may also directly interact with other normal regulatory proteins in cells and, together with the perturbations shown in Box 2, have global consequences on transcription, protein folding, quality control mechanisms and axonal transport in the sensitive neuronal cells (Box 3), resulting in neuronal dysfunction and cell death, and thus culminating in neurodegeneration. Based on recent studies in our laboratory (Arya and Lakhotia 2008; Arya *et al*. 2010; Mallik and Lakhotia 2009a, 2009b, 2010) the multiple steps at which reduced cellular levels of the Hsp60D protein or the non-coding hsrω transcripts inhibit polyQ toxicity are also shown. A downwardly pointed arrow indicates RNAi-mediated reduction in levels of the Hsp60D protein (red) or hsrω transcripts (blue); green horizontal arrow indicates a facilitatory function while a horizontal line with a vertical bar at the end indicates an inhibitory action. It is significant that although RNAi for the Hsp60D protein or the non-coding hsrω transcripts seem to act at several steps in common, the actual mechanism is different in each case (see text for details).

a polyproline tract; in huntingtin, the polyproline region interacts with SH3-domain and WW-domain containing proteins (Faber *et al*. 1998; Sittler *et al*. 1998). It is still not definitely understood whether the functional disturbances of nuclear factors are because of their interactions with soluble polyglutamine proteins or sequestration in insoluble complexes (Schaffar *et al*. 2004). Either route may result in inappropriate or reduced activity at specific promoters or in chromatin modification by histone acetyltransferases and other enzymes.

Nuclear entry of the expanded mutant polyQ proteins appears to be critical for pathogenesis (Yang *et al*. 2002) in several diseases. For instance, SCA1 mice carrying a mutation in the nuclear localization sequence do not develop the disease (Klement *et al*. 1998). N-terminal fragments of mutant ataxin-7 have been shown to accumulate in the nucleus in an age-dependent manner (Yvert *et al*. 2001). In some cases, TFs are mislocalized or sequestered in the inclusions. TBP localizes to the IBs in human SCA3 disease brain, TAFII130 to inclusions in DRPLA and HD, and CBP to inclusions in SCA1, SCA3, HD and SBMA (Perez *et al*. 1998; McCampbell *et al*. 2000; Shimohata *et al*. 2000b; Nucifora *et al*. 2001; Stenoien *et al*. 2002). In HD patient brains, N-CoR is mislocalized and mSin3A is present in nuclear inclusions (Boutell *et al*. 1999; Steffan *et al*. 2000). Interactions with polyQ proteins are known to inhibit functions of some TFs. Mutant huntingtin represses TAFII130 promoters while expanded polyQ repeats in ataxin-3, huntingtin and atrophin-1 repress CBP-dependent gene transcription in cell models (Shimohata *et al*. 2000a; Nucifora *et al*. 2001; Jiang *et al*. 2003). Reduction of soluble CBP by sequestration (McCampbell *et al*. 2000; Nucifora *et al*. 2001) or increased turnover (Jiang *et al*. 2003) is coincident with a state of general hypoacetylation of histones, a condition that is restored by increased expression of CBP (Nucifora *et al*. 2001; Taylor *et al*. 2003; also see table 5) or treatment with HDAC inhibitors in transgenic mouse models of SBMA (Minamiyama *et al*. 2004) and HD (Ferrante *et al*. 2003; Hockly *et al*. 2003) and in fly models of polyQ diseases (Steffan *et al*. 2000, 2001; Taylor *et al*. 2003). Further, treatment with VEGF, a neurotrophic factor that is transcriptionally regulated by CBP, was found to reduce cell death in motor neuron culture model of SBMA (Sopher *et al*.

2004). Studies in our laboratory (Mallik and Lakhotia 2010) also have shown that altered hsrω transcript levels modulate polyQ toxicity (see table 5) by reciprocally affecting cellular levels of CBP via its interaction with the hnRNPs like Hrb87F and Hrb57A. Alterations in CBP expression and its metabolism, which in turn disrupt normal transcriptional regulation, thus appear to represent an important common factor for pathogenesis following expanded polyQ protein expression (Rouaux *et al*. 2004).

Failure of protein quality control mechanisms

Cells must ensure that nascent polypeptides fold correctly and must also deal with refolding of proteins damaged by physiological stress or mutations. HSPs and other molecular chaperones facilitate proper folding of polypeptides and thus maintain proteins in appropriate soluble conformation (Hendrick and Hartl 1993). If the native conformation of a protein is not achieved, either the refolding efforts by molecular chaperones continue or the protein is targeted for degradation (Hartl and Hayer-Hartl 2002). Abnormally folded proteins tend to aggregate. When the concentration of misfolded proteins exceeds cellular folding and degradative capacity, such proteins can form insoluble, intracellular aggregates, reminiscent of those seen in the polyQ disorders. For many damaged or misfolded proteins, the principal route for protein destruction is the ubiquitin-proteasome pathway (UPP) which together with the molecular chaperones carry out the major protein quality control functions in cells (Hartl and Hayer-Hartl 2002; Berke and Paulson 2003).

As evident from table 5, a variety of molecular chapernones and other protein quality control mechnisms have been found to modify the polyQ toxicity in fly models. Molecular chaperones localize to polyQ aggregates in patient tissues and in cellular and animal models (Paulson *et al*. 1997b; Cummings *et al*. 1998), suggesting that protein aggregates result from protein misfolding. Overexpression of chaperones like Hsp70, Hsc70 family members or Hsp40 has been demonstrated to suppress polyQ-mediated neuronal degeneration and cell death in *Drosophila* models (table 5), although in some studies this was not found to be accompanied by suppression of aggregation (Cummings *et al*. 1998; Warrick *et al*. 1999; Kazemi-Esfarjani and Benzer 2000; Muchowski *et al*. 2000). The sequestration of chaperones into aggregates most likely decreases the soluble pool of functioning chaperones, thereby lowering the overall protein folding capacity of the cell. This in turn may result in an environment that favours further misfolding and aggregation rather than refolding and degradation. Overexpression of chaperone proteins in fly models alters the biochemical nature of aggregates, rendering them detergent soluble, though visible inclusions may still remain (Chan *et al*. 2000). These findings support the hypothesis that polyQ proteins do in fact compromise the folding capacity of cells, resulting in accumulation of toxic oligomeric species (Satyal *et al*. 2000; Sherman and Goldberg 2001; Wyttenbach 2004; Matilla-Duenas *et al*. 2007). Genetic screens in *C. elegans* and yeast also point to a role for chaperones in buffering the toxicity of expanded polyQ proteins (Willingham *et al*. 2003; Nollen *et al*. 2004). Transgenic overexpression of Hsp70 chaperones yields only marginal benefit in polyQ mouse models, suggesting that reduced chaperone activity may not fully explain the pathology seen in polyQ disorders (Hay *et al*. 2004).

Using fly models expressing either a quasi pure polyQ tract (127Q) or the pathogenic SCA3 protein, Arya *et al*. (2010) identified Hsp60D, a member of the *Drosophila* Hsp60 family of chaperones, as a novel modifier of polyQ pathogenesis. Unlike several other chaperone proteins that reduce the polyQ toxicity when overexpressed, reduction in the cellular levels of Hsp60D in the polyQ expressing developing eye cells was found to improve the eye morphology along with concomitant reduction in the number of IBs and the associated expression of Hsp70. Further, Hsp60D-RNAi was also found to suppress the organismal lethality caused by pan-neuronal expression of the pathogenic polyQ proteins. Hsp60D thus appears to be essential for folding of the mutant polyQ polypeptides into pathological aggregates such that this protein's depletion following Hsp60D-RNAi does not allow formation of the toxic aggregates. Suppression of the polyQ phenotypes following depletion of Hsp60D was largely independent of functional proteasomal and SUMO activities but appeared to require the *Drosophila* inhibitor of apoptosis protein 1 (DIAP1).

Amongst the low molecular weight Hsps, neuronal overexpression of *hsp27* but not *hsp26* in fly models was found to attenuate cellular polyglutamine toxicity and suppress increased levels of reactive oxygen species caused by huntingtin (Hsieh *et al*. 2005; Liao *et al*. 2008). During the early disease stage of the MJD neuroblastoma cellular model, reduction of Hsp27 synthesis mitigated the ability of neuronal cells to cope with cytotoxicity induced by mutant ataxin-3, triggering the cell death process during the disease progress (Chang *et al*. 2005). However, the subsequent increase in Hsp27 levels associated with the disease progression does not provide any protection against the mutant ataxin-3-induced cytotoxic effects (Chang *et al*. 2005). Liao *et al*. (2008) further demonstrated that overexpression of *hsp27* exerts its neuroprotective effects on mutant proteins with short polyQ stretches not through its chaperone function, but instead by preventing the *hid*-induced apoptotic pathway. Overexpression of the small heat shock protein crystalline, a weak/moderate suppressor of truncated ataxin-3-induced cytotoxicity, robustly suppressed anatomical and functional defects following expression of full length ataxin-3 (Bilen and Bonini 2007).

The Hsp90 protein family is one of the most versatile molecular chaperones with a very diverse clientele including other chaperones, steroid hormone receptors, cytoskeletal components and signal transducers (Pearl and Prodromou 2006), because of which it also plays important roles in

evolvability and canalization (McManus *et al*. 2006). However, despite the wide-range actions of the Hsp90 family proteins, relatively few studies have examined interactions of Hsp90 and the mutant polyQ proteins. Most of such studies have not used direct alteration of quantitative or qualitative expression of Hsp90 gene/protein; instead they have examined effects of chemical inhibitors of Hsp90 on the polyQ phenotypes (reviewed in Waza *et al*. 2006). In a mouse model of SBMA, inhibition of Hsp90 through 17-allylamino-17-demethoxygeldanamycin (17-AAG) resulted in degradation of the mutated androgen receptor and thus ameliorated the neurodegenerative phenotype (see Waza *et al*. 2006). It will indeed be interesting to examine effects of targetted misexpression of wild type or mutant Hsp90 in the different fly models of polyQ disorders.

There is evidence that UPP function declines with age, paralleling the typically late onset of polyQ disease symptoms (Goto *et al*. 2001). The IBs in polyQ disorders are ubiquitinated and they sequester proteasome components, e.g., the 20S proteasome relocates to aggregates in SCA1 (Cummings *et al*. 1998), SCA3 (Chai *et al*. 1999) and SCA7 (Yvert *et al*. 2001; Zander *et al*. 2001) disease tissue. Eukaryotic proteasomes cannot digest polyQ chains which must be released for digestion by cellular peptidases (Venkatraman *et al*. 2004). The presence of long undegradable expanded polyQ sequences in the cell's proteasomal machinery has been shown to promote early disease onset (Venkatraman *et al*. 2004). In cell-based proteasome reporter assays, expression of pathogenic polyQ proteins caused impairment of the UPP (Bence *et al*. 2001; Jana *et al*. 2001). A specific 19S proteasome subunit was depleted in brain regions affected by neurodegeneration in SCA7 (Matilla *et al*. 2001). Using a fly model of SBMA, Chan *et al*. (2002) demonstrated that the endogenous proteasome activity was involved in clearance of the pathogenic polyQ aggregates (table 5). Conversely, in case of SCA3, overexpression of wild-type ataxin-3 which has ubiquitin-protease activity, suppressed polyQ-mediated neurodegeneration (Warrick *et al*. 2005; table 5). *In vivo* impairment of the cellular proteasomal degradation machinery using reporter transgenes has also been demonstrated in fly models expressing a quasi pure polyQ tract or the mutant SCA3 protein (Mallik and Lakhotia 2010). Further, one of the multiple mechanisms responsible for the aggravation of polyQ pathogenesis following increased expression of the *hsrω* gene in fly models could be the fact that overabundance of these transcripts itself causes proteasomal dysfunction in the cell; interestingly, reduction in hsrω transcripts improved proteasomal activity and this was associated with alleviation of polyQ toxicity (Mallik and Lakhotia 2010). The proteasome inhibitor lactacystin increased accumulation of toxic undegraded proteins, indicating that proteasomal processing of ubiquitinated substrates is a clearance mechanism which counterbalances the aggregate formation (Chai *et al*. 1999; Wyttenbach *et al*. 2000). In a mutant huntingtin expressing cell culture system, inhibition of the UPP increased huntingtin induced apoptotic cell death (Saudou *et al*. 1998). Expression of the expanded SCA1 allele in a transgenic mouse model lacking the E6-AP ubiquitin ligase accelerated disease progression while diminishing formation of IBs (Cummings *et al*. 1999; Park *et al*. 2005). However, some other studies have suggested that the UPP may not have a significant role in polyQ toxicity. For example, Bowman *et al*. (2005) did not find any adverse effect of inhibition of proteasome activity in the degenerating retina of SCA7 mice. Likewise, Bilen and Bonini (2005) also reported that limiting proteasome activity by expressing a dominant temperature-sensitive mutant proteasome subunit had no enhancing effect on SCA3 toxicity (table 5). However, Arya *et al*. (2010) found that expression of the dominant temperature-sensitive mutant proteasome did aggravate the SCA3 phenotype.

It is likely that the above noted divergent findings (Bilen and Bonini 2005; Bowman *et al*. 2005) about the relation between the protein quality control mechanisms and the polyQ toxicity may be due to different model systems or to other factors that need further examination.

Overexpression of the C-terminal Hsp70-interacting protein (CHIP), both a co-chaperone and a ubiquitin ligase which serves as the molecular link between chaperones and the UPP, rescued mutant polyQ-induced phenotypes in several *in vitro* and non-mammalian animal models (Miller *et al*. 2005; Williams *et al*. 2009). In a SCA3 mouse model, depletion of CHIP accelerated the disease phenotype in a dose-dependent manner (Miller *et al*. 2005). However CHIP was found to increase ubiquitinylation of ataxin-1, which reduced its solubility and promoted its aggregation (Choi *et al*. 2007).

Autophagy is another major degradation pathway for various intracytosolic, aggregate-prone, disease-causing proteins associated with the neurodegenerative disorders. Inclusions of N-terminal truncated huntingtin have been shown to directly enhance autophagy (Ravikumar *et al*. 2004). In HD flies, rapamycin, in addition to inducing autophagy, has been demonstrated to protect cells against neurodegeneration by decreasing synthesis of aggregation prone polyQ expanded huntingtin (Ravikumar *et al*. 2004). Expression of pathogenic ataxin-3 was found to induce autophagy (Bilen and Bonini 2007). Further, limiting the activity of autophagy genes in the presence of the pathogenic SCA3 or the polyQ expanded AR protein was found to enhance retinal degeneration (Bilen and Bonini 2007; Pandey *et al*. 2007; also see table 5).

Taken together, it appears that choking of the protein quality control mechanisms in the sensitive neurons by the expanded polyQ proteins is a major insult that the neurons face when chronically exposed to expanded polyQ.

Axonal transport defects in polyQ diseases

Several genes that affect axonal transport have been found to modulate polyQ phenotypes in the fly (table 5) and other polyQ models indicating that this is also an important target for the toxicity. Histopathological analysis of polyQ disease

brains show widespread neuritic inclusions suggesting that perturbation of transport processes may indeed contribute to pathogenesis (DiFiglia *et al*. 1997). Dystrophic neurites, which are consistently observed in the striatum of HD mouse models and human patient brains, exhibit characteristic features of blocked axons such as prominent swellings with accumulated vesicles and organelles together with polyQ aggregates (DiFiglia *et al*. 1997). The polyQ aggregates physically block transport in narrow axons. Truncated versions of huntingtin, ataxin-3 or the androgen receptor inhibit anterograde and retrograde transport in giant squid axons, mammalian tissue culture cells and fly models of HD (Gunawardena *et al*. 2003; Szebenyi *et al*. 2003; Lee *et al*. 2004; Kaltenbach *et al*. 2007; Sinadinos *et al*. 2009). Mutant polyQ proteins interact aberrantly with transport pathway proteins and thus titrate them away from their normal transport functions (Gunawardena *et al*. 2003; Lee *et al*. 2004). The huntingtin-associated protein-1 (HAP1) has been shown to interact with the prodomain of BDNF. However, this interaction was reduced in the presence of polyQ expanded huntingtin resulting in reduced release and transport of BDNF in HD mice (Wu *et al*. 2010). Expression of the expanded SCA7 allele in a transgenic mouse model has been shown to downregulate mRNA expressions of the vesicular transport proteins synaptobrevin 1 and vesicular glutamate transporter subtype 2 (VGLUT2), and upregulate mRNA levels of proteins that regulate neurotransmitter release and synaptic plasticity such as GluR2 and Rab3-interacting molecule 2 (RIM2α causing dysregulated glutamatergic transmission and consequent cerebellar malfunction (Chou *et al*. 2010). Chou *et al*. (2008) had previously demonstrated that mRNA expression of several proteins involved in glutamatergic signalling, including VGLUT2, GluR6, phospholipase C b4 and inositol trisphosphate receptor-1 (IP3R-1) were downregulated in the cerebellum of SCA3 transgenic mice. Abnormal distributions of the motor protein dynein and of mitochondria have been observed in dystrophic neurites containing aggregated expanded AR in a testosterone-treated motor neuron cell model of SBMA (Piccioni *et al*. 2002). Further, while the mRNA level of dynactin 1, an axon motor for retrograde transport, was significantly reduced in the SBMA mice, overexpression of dynactin 1 mitigated the polyQ expanded AR protein-induced neuronal toxicity in a cell culture model of SBMA (Katsuno *et al*. 2006). In addition, some of the disease proteins may have functions in axonal transport and these functions may be directly impaired by polyQ expansion as seen in the fly model of HD (Gunawardena *et al*. 2003; Szebenyi *et al*. 2003).

Signal transduction pathways

Several recent studies (see table 5) have implicated components of various signalling pathways in the pathophysiology of the polyQ disorders. For instance, upregulation of the anti-apoptotic kinase Akt in a fly model of HD was beneficial in a cell-type-specific manner (Lievens *et al*. 2008; Branco *et al*. 2008); however, it failed to ellicit a similar response in case of mutant ataxin-3 mediated neurotoxicity (Bilen *et al*. 2006). On the other hand, overexpression of *Akt1* enhanced and its downregulation was found to ameliorate the ataxin-1-induced degeneration in a fly model of SCA1 (Branco *et al*. 2008). Such divergent effects of *Akt1* reflect disease-specific perturbations in the affected neurons.

Posttranslational modification/s of the polyQ expanded protein substrates by signalling pathways appear to be important determinants in the development and progression of polyglutamine diseases. For instance, insulin-like growth factor-1 (IGF-1) completely inhibits mutant huntingtin induced neurotoxicity through activation of the prosurvival serine–threonine kinase Akt which phosphorylates mutant huntingtin at Ser^{421} and thus abrogates its proapoptotic activity (Humbert *et al*. 2002; Schilling *et al*. 2006). Furthermore, phosphorylation of the ADP-ribosylation factor-interacting protein arfaptin 2 at Ser^{260} by Akt decreased inclusion formation in a neuronal model of HD and thus promoted neuronal survival. Phosphorylated arfaptin 2 was also found to inhibit the mutant huntingtin-induced blockade of the proteasome, thereby facilitating protein degradation (Rangone *et al*. 2005). Akt also controls p53 levels via phosphorylation of Mdm2, the E3 ubiquitin ligase that triggers degradation of p53 (Zhou *et al*. 2001). Consistently, in a *Drosophila* HD model, deletion of p53 robustly suppressed the neurotoxicity associated with the expression of mutant huntingtin (Bae *et al*. 2005).

Binding partners of a large number of phosphoproteins, 14-3-3 proteins, participate in a variety of signal transduction pathways and regulate a number of cellular processes. While overexpression of 14-3-3ε enhanced SCA1 and mutant huntingtin induced degeneration in *Drosophila* models, reduction in its cellular levels abolished aggregate formation and suppressed the neurotoxicity (Kaltenbach *et al*. 2007; Branco *et al*. 2008). Overexpression of 14-3-3ζ also enhanced mutant huntingtin induced degeneration in the fly model. 14-3-3 binds with the Akt phosphorylated mutant ataxin-1 resulting in stabilization of the mutated ataxin-1 and the consequent neurotoxic effects (Chen *et al*. 2003). In HD, on the other hand, phosphorylation of the C-terminus of HAP1A promotes its interaction with the 14-3-3 proteins which in turn decrease the association of HAP1 with kinesin light chain. This diminishes HAP1A in neurites, suppresses neurite outgrowth and also blocks axonal transport (Rong *et al*. 2007).

Expression of expanded polyQ proteins has been reported to hyperphosphorylate JNK and c-Jun (Merienne *et al*. 2003; Morfini *et al*. 2006; Scappini *et al*. 2007), which also contribute to neuronal dysfunction and cell death in neurodegenerative disorders. Using fly models of HD and SBMA, Scappini *et al*. (2007) demonstrated that overexpression of the multi-domain scaffolding protein intersection (ITSN), which regulates endocytosis and signal transduction, increased polyQ aggregation through activation of the c-Jun-NH_2-terminal kinase (JNK)-MAPK pathway. Con-

versely, downregulation of ITSN or JNK inhibition attenuated the aggregation (Scappini *et al*. 2007). In a hippocampal neuronal cell line, mutant huntingtin was found to activate JNK (Liu 1998). Further, JNK and the transcription factor c-Jun were also activated in striatal neurons transfected with exon 1 of huntingtin (Garcia *et al*. 2004). Reduction in cellular levels of the *Drosophila* hsrω transcripts prevents activation of JNK (Mallik and Lakhotia 2009b) which may also contribute to suppression of the polyQ damage following hsrω-RNAi (Mallik and Lakhotia 2010). Using a *Drosophila* model of HD, Lievens *et al*. (2008) reported that expression of active ERK did not improve the neurodegenerative phenotypes in any cell type.

It is posible that several of these signal transduction pathways also work through their modulatory actions on apoptosis, which as is the final pathological consequence in the affected neurons.

Neuronal dysfunction and cell death

Neuronal cell loss is a characteristic defining feature of the polyQ diseases. Neuronal cell death can be apoptotic or necrotic. Apoptosis, a highly regulated cellular death pathway, is crucial to neurodegeneration in polyQ repeat diseases (reviewed in Dragunow *et al*. 1995; Friedlander 2003). Evidence for caspase activation has been observed in mutant huntingtin expressing brain and lymphoblasts (Sanchez *et al*. 1999). Expression of expanded polyQ in animal cell culture promotes apoptosis (Kouroku *et al*. 2002; Huynh *et al*. 2003). In addition to causing stress that activates the apoptotic programme, some polyQ-containing proteins themselves are caspase substrates (Wellington *et al*. 1998). Accordingly, a number of studies have shown modifiers of apoptosis to also modulate polyQ pathogenesis (see table 5). Proteolytic cleavage of huntingtin, a necessary step in the initiation of HD, increases its cellular toxicity while mutation of caspase-3 cleavage sites in huntingtin reduces toxicity, indicating that proteolysis of the disease protein by caspase-3 may contribute to HD progression and hence generate more toxic N-terminal fragments (Gafni *et al*. 2004). In the R6/2 mouse HD model, toxicity of the expanded huntingtin transgene was reduced in a caspase-1 dominant-negative background, and administration of caspase inhibitors like zVAD-fmk or minocycline also slowed the disease progression (Ona *et al*. 1999; Chen *et al*. 2000). Similar proteolytic processing of the polyQ expanded AR by caspase-3 (LaFevre-Bernt and Ellerby 2003) and of mutant ataxin-3 by caspase-1 (Berke *et al*. 2004) has been implicated in causing neurotoxicity. Inhibition of caspase activity has been shown to abrogate IB formation and prolong cell survival (Kim *et al*. 1999; Wang *et al*. 1999; Wellington and Hayden 2000). Sang *et al*. (2005) demonstrated that a loss-of-function mutation of *dark*, the fly homolog of human Apaf-1, suppressed neurodegeneration, cell death and effector caspase activity in Q108, HD and SCA1 expressing flies. Higashiyama *et al*. (2002) identified *ter94*, which encodes the *Drosophila* homolog of vasolin-containing protein (VCP)/p97 and is a member of the AAA+ class of ATPases, as a novel effector of polyQ-induced cell death. Loss-of-function *ter94* mutants were found to dominantly suppress cell death and neurodegeneration in *Drosophila* polyQ models (Higashiyama *et al*. 2002). Recently Boeddrich *et al*. (2006) found that VCP overexpression suppressed expanded polyQ-induced ataxin-3 aggregation and neurodegeneration. They further demonstrated that VCP directly binds to/associates with both the soluble as well as aggregated forms of mutant ataxin-3 through an arginine/lysine-rich VCP-binding motif (VBM). Consistently, overexpression of VCP had little effect on neurodegeneration induced by expression of either full length ataxin-3 carrying a mutated VCP-binding site or a truncated form of the polyQ expanded ataxin-3 lacking the VBM (Boeddrich *et al*. 2006). Kariya *et al*. (2005) showed that the endogenous peptide humanin, a neuroprotective factor, suppressed apoptotic cell death induced by mutant polyQs by inhibiting activation of apoptosis signal-regulating kinase 1 (ASK1). Expression of ataxin-2 with expanded repeats in PC12 and COS1 cells increased cell death compared with normal ataxin-2 and elevated the levels of activated caspase-3 (Huynh *et al*. 2003). These studies suggest that caspases play a role in the neuronal loss observed in polyQ disorders. However, results of experiments testing suppression of polyQ phenotypes following expression of anti-apoptotic proteins in the fly eye have been inconsistent. Both P35 and DIAP1 suppressed ataxin-1 and ataxin-3 phenotypes (Warrick *et al*. 1998; Ghosh and Feany 2004). Ghosh and Feany (2004) also reported that unlike overexpression of DIAP1 which has no effect on 127Q toxicity, P35 overexpression aggravated the phenotype. Both these proteins, however, have been reported to have no effect on either Q108 and htt-Q120 induced neurodegeneration in fly models (Ghosh and Feany 2004; Sang and Jackson 2005; Sang *et al*. 2005). On the other hand, two novel modifiers of polyQ toxicity, the Hsp60D protein and the non-coding hsrω transcripts, identified in our laboratory (Mallik and Lakhotia 2009a; Arya *et al*. 2010) have been shown to also modulate the caspase-mediated canonical death pathways in *Drosophila* (Arya and Lakhotia 2008; Mallik and Lakhotia 2009b). While depletion of Hsp60D may contribute to recovery from the polyQ damage by preventing caspase activation by inhibiting disassociation of DIAP1 from the DIAP1-effector caspase complexes (Arya and Lakhotia 2008), reduction in hsrω transcript levels ameliorate cell death phenotypes by augmenting cellular levels of DIAP1 via its interaction with the hnRNP Hrb57A (Mallik and Lakhotia 2009b).

Some studies also indicate that, in conjunction with apoptosis, caspase-independent neuronal death pathways may also contribute to the neurodegeneration observed in polyQ and other neurodegenerative diseases (Wyttenbach *et al*. 2002; Li *et al*. 2007).

Since various stress proteins/molecular chaperones have significant roles in regulation of apoptosis and cell survival

(Arya *et al*. 2007), their protective effects noted earlier may also be brought about through modulation of the cell death pathways.

Other diverse modifiers

In addition to the above specified pathways, a variety of other genetic modifiers have also been identified in fly models (table 5). These include mutations affecting cytoskeletal biogenesis, organization and trafficking, cell cycle regulation, vesicular transport, nuclear pore proteins, ion channels and pumps, cell adhesion molecules, and miRNA and nucleotide processing proteins. Many of these efficacious modifiers with widely divergent molecular functions mitigate polyQ-induced neurodegeneration by modulating events that finally impinge on basic processes like cellular transcription, protein homeostasis, axonal transport and cell death etc., which have, as discussed above, been implicated in the pathophysiology of these diseases. Several of these diverse interactors, however, are likely to modulate neurotoxicity through as yet unknown mechanisms. Data presented in table 5 suggest that the endogeneous activity of majority of these genes may normally help to protect against neurodegeneration and thus provide potential new therapeutic targets.

Epilogue

Drosophila has proved to be an excellent model system to study fundamental aspects of disease pathogenesis and modifier mechanisms, an approach that is difficult in human or other mammalian models owing to both logistical and ethical considerations. Genome-wide forward genetic analysis, candidate gene approaches, and microarray analysis using *Drosophila* polyQ disease models have been successfully exploited to uncover a variety of novel genetic modifiers of neurodegenerative phenotypes. While disease-associated pathological inclusions are intimately connected with protein processing, protein folding, transcriptional regulation and apoptosis in general, many clinical and pathological differences suggest that there are also other disease-specific mechanisms.

Identification of a large variety of genetic factors (see table 5), other than those involved in transcriptional regulation, protein quality control, axonal transport or apoptosis, is a clear indication that multiple steps are parallely and serially affected by the expanded polyQ proteins. In the context of complex intra-cellular and inter-cellular networking required for maintenance of homeostasis, existence of such apparently diverse modulators of polyQ toxicity is not surprising. The disease-specific varied phenotypes caused due to expression of proteins with expanded polyQ stretches in the sensitive neurons also reflect the complexity of networking in neuronal cells. The pleiotropic actions of reduced levels of the non-coding hsrω transcripts in suppressing polyQ pathology through multiple paths (Mallik and Lakhotia 2009a, 2010; figure 3) also exemplify the networking effects. In this context, it will be interesting to examine if the non-coding human sat III transcripts can also modulate polyQ pathogenesis since the hsrω and sat III transcripts seem to be functional analogues (Jolly and Lakhotia 2006).

Fly models have also been adapted for high-throughput testing of potential therapeutic compounds. Initial evidence for the efficacy of this approach came from findings that HDAC inhibitors protect against polyQ-mediated degeneration (Steffan *et al*. 2001). The use of an appropriate fly model to prescreen large numbers of compounds prior to testing in mammalian models appears a good strategy since that would not only significantly reduce the time and expense needed to check compounds for toxic side effects but would also help identify the most promising candidates to move into clinical trials. An understanding of the multi-system pathology manifest in different polyQ disorders indeed remains a major challenge for therapeutic exploitation of the information gleaned from the different model systems.

Acknowledgements

Research from our laboratory has been supported by grants from the Department of Science and Technology (DST) and Department of Biotechnology (DBT), Govt. of India, New Delhi to S. C. L. and M. M. was supported by the Shyama Prasad Mukherjee Fellowship from the Council for Scientific and Industrial Research, New Delhi. We thank the fly community for generously providing fly stocks and reagents used in our work on these debilitating disorders. We also thank the anonymous reviewer for the constructively crtitical comments on the manuscript.

References

Adachi H., Waza M., Katsuno M., Tanaka F., Doyu M. and Sobue G. 2007 Pathogenesis and molecular targeted therapy of spinal and bulbar muscular atrophy. *Neuropath. Appl. Neuro*. **33**, 135–151.

Adams M. D. and Sekelsky J. J. 2002 From sequence to phenotype: reverse genetics in *Drosophila melanogaster. Nat. Rev. Genet*. **3**, 189–198.

Adams M. D., Celniker S. E., Holt R. A., Evans C. A., Gocayne J. D., Amanatides P. G. *et al*. 2000 The genome sequence of *Drosophila melanogaster. Science* **287**, 2185–2195.

Albrecht A. and Mundlos S. 2005 The other trinucleotide repeat: polyalanine expansion disorders. *Curr. Opin. Genet. Dev*. **15**, 285–293.

Al-Ramahi I., Perez A. M., Lim J., Zhang M., Sorensen R., de Haro M. *et al*. 2007 dAtaxin-2 mediates expanded ataxin-1-induced neurodegeneration in a *Drosophila* model of SCA1. *PLoS Genet*. **3**, e234.

Apostol B. L., Kazantsev A., Raffioni S., Illes K., Pallos J., Bodai L. *et al*. 2003 A cell-based assay for aggregation inhibitors as therapeutics of polyglutamine-repeat disease and validation in *Drosophila. Proc. Natl. Acad. Sci. USA* **100**, 5950–5955.

Arrasate M., Mitra S., Schweitzer E. S., Segal M. R. and Finkbeiner S. 2004 Inclusion body formation reduces levels of mutant Huntingtin and the risk of neuronal death. *Nature* **431**, 805–810.

Arya R. and Lakhotia S. C. 2006 A simple nail polish imprint technique for examination of external morphology of *Drosophila* eyes. *Curr. Sci*. **90**, 1179–1180.

Arya R. and Lakhotia S. C. 2008 Hsp60D is essential for caspase-mediated induced apoptosis in *Drosophila melanogaster. Cell Stress Chaperones* **13**, 509–526.

Arya R., Mallik M. and Lakhotia S. C. 2007 Heat shock genes–integrating cell survival and deth. *J. Biosci.* **32**, 595–610.

Arya R., Nisha S. A. and Lakhotia S. C. 2010 Hsp60D - A novel modifier of polyglutamine-mediated neurodegeneration in *Drosophila. Ann. Neurosci*. **17**, 8–17.

Bae B. I., Xu H., Igarashi S., Fujimuro M., Agrawal N., Taya Y. *et al*. 2005 p53 mediates cellular dysfunction and behavioral abnormalities in Huntington's disease. *Neuron* **47**, 29–41.

Bahadorani S. and Hilliker A. J. 2008 Antioxidants cannot suppress the lethal phenotype of a *Drosophila melanogaster* model of Huntington's disease. *Genome* **51**, 392–395.

Bates G. 2003 Huntingtin aggregation and toxicity in Huntington's disease. *Lancet* **361**, 1642–1644.

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

Bennett E. J., Bence N. F., Jayakumar R. and Kopito R. R. 2005 Global impairment of the ubiquitin-proteasome system by nuclear or cytoplasmic protein aggregates precedes inclusion body formation. *Mol. Cell* **17**, 351–365.

Benomar A., Krols L., Stevanin G., Cancel G., LeGuern E., David G. *et al*. 1995 The gene for autosomal dominant cerebellar ataxia with pigmentary macular dystrophy maps to chromosome 3p12-p21.1. *Nat. Genet*. **10**, 84–88.

Berger Z., Davies J. E., Luo S., Pasco M. Y., Majoul I., O'Kane C. J. *et al*. 2006 Deleterious and protective properties of an aggregate-prone protein with a polyalanine expansion. *Hum. Mol. Genet*. **15**, 453–465.

Berke S. J. and Paulson H. L. 2003 Protein aggregation and the ubiquitin proteasome pathway: gaining the UPPer hand on neurodegeneration. *Curr. Opin. Genet. Dev*. **13**, 253–261.

Berke S. J., Schmied F. A., Brunt E. R., Ellerby L. M. and Paulson H. L. 2004 Caspase-mediated proteolysis of the polyglutamine disease protein Ataxin-3. *J. Neurochem*. **89**, 908–918.

Bier E. 2005 *Drosophila*, the golden bug, emerges as a tool for human genetics. *Nat. Rev. Genet*. **6**, 9–23.

Bilen J. and Bonini N. M. 2005 *Drosophila* as a model for human neurodegenerative disease. *Annu. Rev. Genet*. **39**, 153–171.

Bilen J. and Bonini N. M. 2007 Genome-wide screen for modifiers of Ataxin-3 neurodegeneration in *Drosophila. PLoS Genet*. **3**, 1950–1964.

Bilen J., Liu N., Burnett B. G., Pittman R. N. and Bonini N. M. 2006 MicroRNA pathways modulate polyglutamine-induced neurodegeneration. *Mol. Cell* **24**, 157–163.

Boeddrich A., Gaumer S., Haacke A., Tzvetkov N., Albrecht M., Evert B. O. *et al*. 2006 An arginine/lysine-rich motif is crucial for VCP/p97-mediated modulation of Ataxin-3 fibrillogenesis. *EMBO J*. **25**, 1547–1558.

Boutell J. M., Thomas P., Neal J. W., Weston V. J., Duce J., Harper P. S. *et al*. 1999 Aberrant interactions of transcriptional repressor proteins with the Huntington's disease gene product, Huntingtin. *Hum. Mol. Genet*. **8**, 1647–1655.

Bowman A. B., Yoo S. Y., Dantuma N. P. and Zoghbi H. Y. 2005 Neuronal dysfunction in a polyglutamine disease model occurs in the absence of ubiquitin-proteasome system impairment and inversely correlates with the degree of nuclear inclusion formation. *Hum. Mol. Genet*. **14**, 679–691.

Branco J., Al-Ramahi I., Ukani L., Perez A. M., Fernandez-Funez P., Rincon-Limas D. *et al*. 2008 Comparative analysis of genetic modifiers in *Drosophila* points to common and distinct mechanisms of pathogenesis among polyglutamine diseases. *Hum. Mol. Genet*. **17**, 376–390.

Brand A. H. and Perrimon N. 1993 Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* **118**, 401–415.

Brook J. D., McCurrach M. E., Harley H. G., Buckler A. J., Church D., Aburatani H. *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.

Brumby A. M. and Richardson H. E. 2005 Using *Drosophila melanogaster* to map human cancer pathways. *Nat. Rev. Cancer* **5**, 626–639.

Burnett B. G., Andrews J., Ranganathan S., Fischbeck K. H. and Di Prospero N. A. 2008 Expression of expanded polyglutamine targets profilin for degradation and alters actin dynamics. *Neurobiol. Dis*. **30**, 365–374.

Campuzano V., Montermini L., Molto M. D., Pianese L., Cossee M., Cavalcanti F. *et al*. 1996 Friedreich's ataxia: autosomal recessive disease caused by an intronic GAA triplet repeat expansion. *Science* **271**, 1423–1427.

Celotto A. M. and Palladino M. J. 2005 *Drosophila:* a "model" model system to study neurodegeneration. *Mol. Interv*. **5**, 292–303.

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.

Chai Y., Shao J., Miller V. M., Williams A. and Paulson H. L. 2002 Live-cell imaging reveals divergent intracellular dynamics of polyglutamine disease proteins and supports a sequestration model of pathogenesis. *Proc. Natl. Acad. Sci. USA* **99**, 9310–9315.

Chan H. Y., Warrick J. M., Gray-Board G. L., Paulson H. L. and Bonini N. M. 2000 Mechanisms of chaperone suppression of polyglutamine disease: selectivity, synergy and modulation of protein solubility in *Drosophila. Hum. Mol. Genet*. **9**, 2811–2820.

Chan H. Y., Warrick J. M., Andriola I., Merry D. and Bonini N. M. 2002 Genetic modulation of polyglutamine toxicity by protein conjugation pathways in *Drosophila. Hum. Mol. Genet*. **11**, 2895–2904.

Chang W. H., Cemal C. K., Hsu Y. H., Kuo C. L., Nukina N., Chang M. H. *et al*. 2005 Dynamic expression of Hsp27 in the presence of mutant Ataxin-3. *Biochem. Biophys. Res. Commun*. **336**, 258–267.

Chen H. K., Fernandez-Funez P., Acevedo S. F., Lam Y. C., Kaytor M. D., Fernandez M. H. *et al*. 2003 Interaction of Akt-phosphorylated Ataxin-1 with 14-3-3 mediates neurodegeneration in spinocerebellar ataxia type 1. *Cell* **113**, 457–468.

Chen M., Ona V. O., Li M., Ferrante R. J., Fink K. B., Zhu S. *et al*. 2000 Minocycline inhibits caspase-1 and caspase-3 expression and delays mortality in a transgenic mouse model of Huntington disease. *Nat. Med*. **6**, 797–801.

Chien S., Reiter L. T., Bier E. and Gribskov M. 2002 Homophila: human disease gene cognates in *Drosophila. Nucleic Acids Res*. **30**, 149–151.

Choi J. Y., Ryu J. H., Kim H. S., Park S. G., Bae K. H., Kang S. *et al*. 2007 Co-chaperone CHIP promotes aggregation of Ataxin-1. *Mol.Cell Neurosci*. **34**, 69–79.

Chou A. H., Yeh T. H., Quyang P., Chen Y. L., Chen S. Y. and Hand H. L 2008 Polyglutamine-expanded ataxin-3 causes cerebellar dysfunction of SCA3 transgenic mice by inducing transcriptional dysregulation. *Neurobiol. Dis.* **31**, 89–101.

Chou A. H., Chen C. Y., Chen S. Y., Chen W. J., Chen Y. L., Weng Y. S. *et al*. 2010 Polyglutamine-expanded Ataxin-7 causes cerebellar dysfunction by inducing transcriptional dysregulation. *Neurochem. Int*. **56**, 329–339.

Chou T. B. and Perrimon N. 1996 The autosomal FLP-DFS technique for generating germline mosaics in *Drosophila melanogaster. Genetics* **144**, 1673–1679.

Cossee M., Schmitt M., Campuzano V., Reutenauer L., Moutou C., Mandel J. L. *et al*. 1997 Evolution of the Friedreich's ataxia trinucleotide repeat expansion: founder effect and premutations. *Proc. Natl. Acad. Sci. USA* **94**, 7452–7457.

Coughlan C. M. and Brodsky J. L. 2003 Yeast as a model system to investigate protein conformational diseases. *Methods Mol. Biol*. **232**, 77–90.

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. *Nat. Genet*. **19**, 148–154.

Cummings C. J., Orr H. T. and Zoghbi H. Y. 1999 Progress in pathogenesis studies of spinocerebellar ataxia type 1. *Philos. Trans. R. Soc. London. ser. B*. **354**, 1079–1081.

David G., Abbas N., Stevanin G., Durr A., Yvert G., Cancel G. *et al*. 1997 Cloning of the SCA7 gene reveals a highly unstable CAG repeat expansion. *Nature Genet*. **17**, 65–70.

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.

Day J. W., Schut L. J., Moseley M. L., Durand A. C. and Ranum L. P. 2000 Spinocerebellar ataxia type 8: clinical features in a large family. *Neurology* **55**, 649–657.

De Boulle K., Verkerk A. J., Reyniers E., Vits L., Hendrickx J., Van Roy B. *et al*. 1993 A point mutation in the FMR-1 gene associated with fragile X mental retardation. *Nat. Genet*. **3**, 31–35.

de Chiara C., Menon R. P., Dal Piaz F., Calder L. and Pastore A. 2005 Polyglutamine is not all: The functional role of the AXH domain in the Ataxin-1 protein. *J. Mol. Biol*. **354**, 883–893.

Dickson B., Sprenger F. and Hafen E. 1992 Prepattern in the developing *Drosophila* eye revealed by an activated torso–sevenless chimeric receptor. *Genes. Dev*. **6**, 2327–2339.

DiFiglia M., Sapp E., Chase K., Schwarz C., Meloni A., Young C. *et al*. 1995 Huntingtin is a cytoplasmic protein associated with vesicles in human and rat brain neurons. *Neuron* **14**, 1075–1081.

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.

Doumanis J., Wada K., Kino Y., Moore A. W. and Nukina N. 2009 RNAi screening in *Drosophila* cells identifies new modifiers of mutant Huntingtin aggregation. *PLoS ONE* **4**, e7275.

Dragunow M., Faull R. L., Lawlor P., Beilharz E. J., Singleton K., Walker E. B. *et al*. 1995 In situ evidence for DNA fragmentation in Huntington's disease striatum and Alzheimer's disease temporal lobes. *Neuroreport* **6**, 1053–1057.

Dunah A. W., Jeong H., Griffin A., Kim Y. M., Standaert D. G., Hersch S. M. *et al*. 2002 Sp1 and TAFII130 transcriptional activity disrupted in early Huntington's disease. *Science* **296**, 2238–2243.

Eichler E. E. and Nelson D. L. 1996 Genetic variation and evolutionary stability of the FMR1 CGG repeat in six closed human populations. *Am. J. Med. Genet*. **64**, 220–225.

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.

Evans C. J., Olson J. M., Ngo K. T., Kim E., Lee N. E., Kuoy E. *et al*. 2009 G-TRACE: rapid Gal4-based cell lineage analysis in *Drosophila. Nat. Methods* **6**, 603–605.

Everett C. M. and Wood N. W. 2004 Trinucleotide repeats and neurodegenerative disease. *Brain* **127**, 2385–2405.

Faber P. W., Barnes G. T., Srinidhi J., Chen J., Gusella J. F. and MacDonald M. E. 1998 Huntingtin interacts with a family of WW domain proteins. *Hum. Mol. Genet*. **7**, 1463–1474.

Feany M. B. and Bender W. W. 2000 A *Drosophila* model of Parkinson's disease. *Nature* **404**, 394–398.

Fernandez-Funez P., Nino-Rosales M. L., de Gouyon B., She W. C., Luchak J. M., Martinez P. *et al*. 2000 Identification of genes that modify Ataxin-1-induced neurodegeneration. *Nature* **408**, 101–106.

Ferrante R. J., Kubilus J. K., Lee J., Ryu H., Beesen A., Zucker B. *et al*. 2003 Histone deacetylase inhibition by sodium butyrate chemotherapy ameliorates the neurodegenerative phenotype in Huntington's disease mice. *J. Neurosci*. **23**, 9418–9427.

Fischbeck K. H., Lieberman A., Bailey C. K., Abel A. and Merry D. E. 1999 Androgen receptor mutation in Kennedy's disease. *Philos. Trans. R. Soc. London. Ser. B.* **354**, 1075–1078.

Franceschini N. and Kirschfeld K. 1971 Pseudopupil phenomena in the compound eye of *Drosophila. Kybernetik* **9**, 159–182.

Friedlander R. M. 2003 Apoptosis and caspases in neurodegenerative diseases. *N. Engl. J. Med*. **348**, 1365–1375.

Fu Y. H., Pizzuti A., Fenwick Jr R. G., King J., Rajnarayan S., Dunne P. W. *et al*. 1992 An unstable triplet repeat in a gene related to myotonic muscular dystrophy. *Science* **255**, 1256–1258.

Fujikake N., Nagai Y., Popiel H. A., Okamoto Y., Yamaguchi M. and Toda T. 2008 Heat shock transcription factor 1-activating compounds suppress polyglutamine-induced neurodegeneration through induction of multiple molecular chaperones. *J. Biol. Chem*. **283**, 26188–26197.

Gafni J., Hermel E., Young J. E., Wellington C. L., Hayden M. R. and Ellerby L. M. 2004 Inhibition of calpain cleavage of Huntingtin reduces toxicity: accumulation of calpain/caspase fragments in the nucleus. *J. Biol. Chem*. **279**, 20211–20220.

Ganusova E. E., Ozolins L. N., Bhagat S., Newnam G. P., Wegrzyn R. D., Sherman M. Y. *et al*. 2006 Modulation of prion formation, aggregation, and toxicity by the actin cytoskeleton in yeast. *Mol. Cell Biol*. **26**, 617–629.

Garcia M., Charvin D. and Caboche J. 2004 Expanded Huntingtin activates the c-Jun terminal kinase/c-Jun pathway prior to aggregate formation in striatal neurons in culture. *Neuroscience* **127**, 859–870.

Gatchel J. R. and Zoghbi H. Y. 2005 Diseases of unstable repeat expansion: mechanisms and common principles. *Nat. Rev. Genet*. **6**, 743–755.

Gecz J., Gedeon A. K., Sutherland G. R. and Mulley J. C. 1996 Identification of the gene FMR2, associated with FRAXE mental retardation. *Nat. Genet*. **13**, 105–108.

Gerber H. P., Seipel K., Georgiev O., Hofferer M., Hug M., Rusconi S. *et al*. 1994 Transcriptional activation modulated by homopolymeric glutamine and proline stretches. *Science* **263**, 808–811.

Ghosh S. and Feany M. B. 2004 Comparison of pathways controlling toxicity in the eye and brain in *Drosophila* models of human neurodegenerative diseases. *Hum. Mol. Genet*. **13**, 2011–2018.

Gispert S., Twells R., Orozco G., Brice A., Weber J., Heredero L. *et al*. 1993 Chromosomal assignment of the second locus for autosomal dominant cerebellar ataxia (SCA2) to chromosome 12q23-24.1. *Nat. Genet*. **4**, 295–299.

Gong W. J. and Golic K. G. 2006 Loss of Hsp70 in *Drosophila* is pleiotropic, with effects on thermotolerance, recovery from heat shock and neurodegeneration. *Genetics* **172**, 275–286.

Goto S., Takahashi R., Kumiyama A. A., Radak Z., Hayashi T., Takenouchi M. *et al*. 2001 Implications of protein degradation in aging. *Ann. N. Y. Acad. Sci*. **928**, 54–64.

Gu Y., Shen Y., Gibbs R. A. and Nelson D. L. 1996 Identification of FMR2, a novel gene associated with the FRAXE CCG repeat and CpG island. *Nat. Genet*. **13**, 109–113.

Gunawardena S., Her L. S., Brusch R. G., Laymon R. A., Niesman I. R., Gordesky-Gold B. *et al*. 2003 Disruption of axonal transport by loss of Huntingtin or expression of pathogenic polyQ proteins in *Drosophila. Neuron* **40**, 25–40.

Gusella J. F. and MacDonald M. E. 2000 Molecular genetics: unmasking polyglutamine triggers in neurodegenerative disease. *Nat. Rev. Neurosci*. **1**, 109–115.

Gusella J. F., Macdonald M. E., Ambrose C. M. and Duyao M. P. 1993 Molecular-Genetics of Huntingtons-Disease. *Arch. Neurol*. **50**, 1157–1163.

Hagerman P. J. and Hagerman R. J. 2004 The fragile-X premutation: a maturing perspective. *Am. J. Hum. Genet*. **74**, 805–816.

Hagerman R. J. 2006 Lessons from fragile X regarding neurobiology, autism, and neurodegeneration. *J. Dev. Behav. Pediatr*. **27**, 63–74.

Harding A. E. 1981 Friedreich's ataxia: a clinical and genetic study of 90 families with an analysis of early diagnostic criteria and intrafamilial clustering of clinical features. *Brain* **104**, 589–620.

Hartenstein V., Younossi-Hartenstein A., Cardona A. and Pereanu W. 2008 Modeling the developing *Drosophila* brain: rationale, technique, and application. *J. Biosci*. **58**, 823–836.

Hartl F. U. and Hayer-Hartl M. 2002 Molecular chaperones in the cytosol: from nascent chain to folded protein. *Science* **295**, 1852–1858.

Hay B. A., Wolff T. and Rubin G. M. 1994 Expression of baculovirus P35 prevents cell death in *Drosophila. Development* **120**, 2121–2129.

Hay D. G., Sathasivam K., Tobaben S., Stahl B., Marber M., Mestril R. *et al*. 2004 Progressive decrease in chaperone protein levels in a mouse model of Huntington's disease and induction of stress proteins as a therapeutic approach. *Hum. Mol. Genet*. **13**, 1389–1405.

Hayashi Y., Kakita A., Yamada M., Egawa S., Oyanagi S., Naito H. *et al*. 1998 Hereditary dentatorubral-pallidoluysian atrophy: ubiquitinated filamentous inclusions in the cerebellar dentate nucleus neurons. *Acta Neuropathol*. **95**, 479–482.

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. *Hum. Mol. Genet*. **13**, 1257–1265.

Helmlinger D., Tora L. and Devys D. 2006 Transcriptional alterations and chromatin remodeling in polyglutamine diseases. *Trends Genet*. **22**, 562–570.

Hendrick J. P. and Hartl F. U. 1993 Molecular chaperone functions of heat-shock proteins. *Annu. Rev. Biochem*. **62**, 349–384.

Higashiyama H., Hirose F., Yamaguchi M., Inoue Y. H., Fujikake N., Matsukage A. *et al*. 2002 Identification of ter94, *Drosophila* VCP, as a modulator of polyglutamine-induced neurodegeneration. *Cell Death Differ*. **9**, 264–273.

Hockly E., Richon V. M., Woodman B., Smith D. L., Zhou X., Rosa E. *et al*. 2003 Suberoylanilide hydroxamic acid, a histone deacetylase inhibitor, ameliorates motor deficits in a mouse model of Huntington's disease. *Proc. Natl. Acad. Sci. USA* **100**, 2041–2046.

Hodgson J. G., Agopyan N., Gutekunst C. A., Leavitt B. R., LePiane F., Singaraja R. *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.

Holmes S. E., Hearn E. O., Ross C. A. and Margolis R. L. 2001 SCA12: an unusual mutation leads to an unusual spinocerebellar ataxia. *Brain Res. Bull*. **56**, 397–403.

Hsieh M., Tsai H. F. and Chang W. H. 2005 HSP27 and cell death in spinocerebellar ataxia type 3. *Cerebellum* **4**, 31–36.

Hughes R. E. and Olson J. M. 2001 Therapeutic opportunities in polyglutamine disease. *Nat. Med*. **7**, 419–423.

Humbert S., Bryson E. A., Cordelières 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. *Dev. Cell* **2**, 831–837.

Huynh D. P., Yang H. T., Vakharia H., Nguyen D. and Pulst S. M. 2003 Expansion of the polyQ repeat in Ataxin-2 alters its Golgi localization, disrupts the Golgi complex and causes cell death. *Hum. Mol. Genet*. **12**, 1485–1496.

Igarashi S., Tanno Y., Onodera O., Yamazaki M., Sato S., Ishikawa A. *et al*. 1992 Strong correlation between the number of cag repeats in androgen receptor genes and the clinical onset of features of spinal and bulbar muscular-atrophy. *Neurology* **42**, 2300–2302.

Ikeda H., Yamaguchi M., Sugai S., Aze Y., Narumiya S. and Kakizuka A. 1996 Expanded polyglutamine in the Machado-Joseph disease protein induces cell death in vitro and in vivo. *Nat. Genet*. **13**, 196–202.

Ikeuchi T., Koide R., Onodera O., Tanaka H., Oyake M., Takano H. *et al*. 1995 Dentatorubral-pallidoluysian atrophy (DRPLA): Molecular basis for wide clinical features of DRPLA. *Clin. Neurosci*. **3**, 23–27.

Imbert G., Saudou F., Yvert G., Devys D., Trottier Y., Garnier J. M. *et al*. 1996 Cloning of the gene for spinocerebellar ataxia 2 reveals a locus with high sensitivity to expanded CAG/glutamine repeats. *Nat. Genet*. **14**, 285–291.

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. *Proc. Natl. Acad. Sci. USA* **102**, 13135–13140.

Jackson G. R., Salecker I., Dong X., Yao X., Arnheim N., Faber P. W. *et al*. 1998 Polyglutamine-expanded human Huntingtin transgenes induce degeneration of *Drosophila* photoreceptor neurons. *Neuron* **21**, 633–642.

Jana N. R., Zemskov E. A., Wang G. and Nukina N. 2001 Altered proteasomal function due to the expression of polyglutamine-expanded truncated N-terminal Huntingtin induces apoptosis by caspase activation through mitochondrial cytochrome c release. *Hum. Mol. Genet*. **10**, 1049–1059.

Jiang H., Nucifora Jr F. C., Ross C. A. and DeFranco D. B. 2003 Cell death triggered by polyglutamine-expanded Huntingtin in a neuronal cell line is associated with degradation of CREB-binding protein. *Hum. Mol. Genet*. **12**, 1–12.

Johnston S. D. 2002 The art and design of genetic screens: *Drosophila melanogaster. Nat. Rev. Genet*. **3**, 176–188.

Jolly C. and Lakhotia S. C. 2006 Human sat III and *Drosophila* hsrω transcripts: a common paradigm for nuclear regulation of RNA processing in stressed cells through requestration of RNA processing factors. *Nucleic Acids Res.* **34**, 5508–5514.

Kaltenbach L. S., Romero E., Becklin R. R., Chettier R., Bell R., Phansalkar A. *et al*. 2007 Huntingtin interacting proteins are genetic modifiers of neurodegeneration. *PLoS Genet*. **3**, e82.

Kanuka H., Kurunaga E., Hiratou T., Igaki T., Nelson B., Okario H. and Miura M. 2003 Cytosol-endoplasmic reticulum interplay by Sec 61 alpha translocon in polyglutamine-mediated neurofoxicity in *Drosophila. Proc. Natl. Acad. Sci. USA* **100**, 11723–11728.

Kariya S., Hirano M., Nagai Y., Furiya Y., Fujikake N., Toda T. *et al*. 2005 Humanin attenuates apoptosis induced by DRPLA pro-

teins with expanded polyglutamine stretches. *J. Mol. Neurosci.* **25**, 165–169.

Karlin S. and Burge C. 1996 Trinucleotide repeats and long homopeptides in genes and proteins associated with nervous system disease and development. *Proc. Natl. Acad. Sci. USA* **93**, 1560–1565.

Katsuno M., Adachi H., Minamiyama M., Waza M., Tokui K., Banno H. *et al*. 2006 Reversible disruption of dynactin 1-mediated retrograde axonal transport in polyglutamine-induced motor neuron degeneration. *J. Neurosci*. **26**, 12106–12117.

Kawaguchi Y., Okamoto T., Taniwaki M., Aizawa M., Inoue M., Katayama S. *et al*. 1994 CAG expansions in a novel gene for Machado-Joseph disease at chromosome 14q32.1. *Nat. Genet*. **8**, 221–228.

Kazantsev A., Preisinger E., Dranovsky A., Goldgaber D. and Housman D. 1999 Insoluble detergent-resistant aggregates form between pathological and nonpathological lengths of polyglutamine in mammalian cells. *Proc. Natl. Acad. Sci. USA* **96**, 11404–11409.

Kazantsev A., Walker H. A., Slepko N., Bear J. E., Preisinger E., Steffan J. S. *et al*. 2002 A bivalent Huntingtin binding peptide suppresses polyglutamine aggregation and pathogenesis in *Drosophila. Nat. Genet*. **30**, 367–376.

Kazemi-Esfarjani P. and Benzer S. 2000 Genetic suppression of polyglutamine toxicity in *Drosophila. Science* **287**, 1837–1840.

Kazemi-Esfarjani P. and Benzer S. 2002 Suppression of polyglutamine toxicity by a Drosophila homolog of myeloid leukemia factor 1. *Hum. Mol. Genet*. **11**, 2657–2672.

Kehoe P., Krawczak M., Harper P. S., Owen M. J. and Jones A. L. 1999 Age of onset in Huntington disease: sex specific influence of apolipoprotein E genotype and normal CAG repeat length. *J. Med. Genet*. **36**, 108–111.

Kim M., Lee H. S., LaForet G., McIntyre C., Martin E. J., Chang P. *et al*. 1999 Mutant huntingtin expression in clonal striatal cells: dissociation of inclusion formation and neuronal survival by caspase inhibition. *J. Neurosci*. **19**, 964–973.

Kim T. W. and Tanzi R. E. 1998 Neuronal intranuclear inclusions in polyglutamine diseases: nuclear weapons or nuclear fallout? *Neuron* **21**, 657–659.

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.

Knight S. J., Flannery A. V., Hirst M. C., Campbell L., Christodoulou Z., Phelps S. R. *et al*. 1993 Trinucleotide repeat amplification and hypermethylation of a CpG island in FRAXE mental retardation. *Cell* **74**, 127–134.

Koide R., Ikeuchi T., Onodera O., Tanaka H., Igarashi S., Endo K. *et al*. 1994 Unstable expansion of CAG repeat in hereditary dentatorubral-pallidoluysian atrophy (DRPLA). *Nat. Genet*. **6**, 9–13.

Komure O., Sano A., Nishino N., Yamauchi N., Ueno S., Kondoh K. *et al*. 1995 DNA analysis in hereditary dentatorubral-pallidoluysian atrophy: correlation between CAG repeat length and phenotypic variation and the molecular basis of anticipation. *Neurology* **45**, 143–149.

Koob M. D., Moseley M. L., Schut L. J., Benzow K. A., Bird T. D., Day J. W. *et al*. 1999 An untranslated CTG expansion causes a novel form of spinocerebellar ataxia (SCA8). *Nat. Genet*. **21**, 379–384.

Kouroku Y., Fujita E., Jimbo A., Kikuchi T., Yamagata T., Momoi M. Y. *et al*. 2002 Polyglutamine aggregates stimulate ER stress signals and caspase-12 activation. *Hum. Mol. Genet*. **11**, 1505–1515.

Krobitsch S. and Lindquist S. 2000 Aggregation of huntingtin in yeast varies with the length of the polyglutamine expansion and the expression of chaperone proteins. *Proc. Natl. Acad. Sci. USA* **97**, 1589–1594.

Kumar J. P. 2009 The molecular circuitry governing retinal determination. *Biochim. Biophys. Acta* **1789**, 306–314.

Kuttenkeuler D. and Boutros M. 2004 Genome-wide RNAi as a route to gene function in *Drosophila. Brief. Funct. Genomic Proteomic* **3**, 168–176.

La Spada A. R. and Taylor J. P. 2003 Polyglutamines placed into context. *Neuron* **38**, 681–684.

LaFevre-Bernt M. A. and Ellerby L. M. 2003 Kennedy's disease. Phosphorylation of the polyglutamine-expanded form of androgen receptor regulates its cleavage by caspase-3 and enhances cell death. *J. Biol. Chem*. **278**, 34918–34924.

Lakhotia S. C., Ray P., Rajendra T. K. and Prasanth K. V. 1999 The non-coding transcripts of hsr-omega gene in *Drosophila:* do they regulate trafficking and availability of nuclear RNA-processing factors? *Curr. Sci.* **77**, 553–563.

Lalioti M. D., Scott H. S., Buresi C., Rossier C., Bottani A., Morris M. A. *et al*. 1997 Dodecamer repeat expansion in cystatin B gene in progressive myoclonus epilepsy. *Nature* **386**, 847–851.

Larson G. P., Ding S., Lafreniere R. G., Rouleau G. A. and Krontiris T. G. 1999 Instability of the EPM1 minisatellite. *Hum. Mol. Genet*. **8**, 1985–1988.

Laspada A. R., Wilson E. M., Lubahn D. B., Harding A. E. and 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. *J. Neurosci*. **27**, 2483–2492.

Lee W. C., Yoshihara M. and Littleton J. T. 2004 Cytoplasmic aggregates trap polyglutamine-containing proteins and block axonal transport in a *Drosophila* model of Huntington's disease. *Proc. Natl. Acad. Sci. USA* **101**, 3224–3229.

Lessing D. and Bonini N. M. 2008 Polyglutamine genes interact to modulate the severity and progression of neurodegeneration in *Drosophila. PLoS Biol*. **6**, e29.

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

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. *Mol. Cell. Biol*. **22**, 1277–1287.

Li Y., Yokota T., Gama V., Yoshida T., Gomez J. A., Ishikawa K. *et al*. 2007 Bax-inhibiting peptide protects cells from polyglutamine toxicity caused by Ku70 acetylation. *Cell Death Differ*. **14**, 2058–2067.

Liao P. C., Lin H. Y., Yuh C. H., Yu L. K. and Wang H. D. 2008 The effect of neuronal expression of heat shock proteins 26 and 27 on lifespan, neurodegeneration, and apoptosis in *Drosophila. Biochem. Biophys. Res. Commun*. **376**, 637–641.

Lievens J. C., Iche M., Laval M., Faivre-Sarrailh C. and Birman S. 2008 AKT-sensitive or insensitive pathways of toxicity in glial cells and neurons in *Drosophila* models of Huntington's disease. *Hum. Mol. Genet*. **17**, 882–894.

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.

Lin D. M. and Goodman C. S. 1994 Ectopic and increased expression of Fasciclin II alters motoneuron growth cone guidance. *Neuron* **13**, 507–523.

Lin X., Cummings C. J. and Zoghbi H. Y. 1999 Expanding our understanding of polyglutamine diseases through mouse models. *Neuron* **24**, 499–502.

Lin X., Antalffy B., Kang D., Orr H. T. and Zoghbi H. Y. 2000 Polyglutamine expansion down-regulates specific neuronal genes before pathologic changes in SCA1. *Nat. Neurosci*. **3**, 157–163.

Liquori C. L., Ricker K., Moseley M. L., Jacobsen J. F., Kress W., Naylor S. L. *et al*. 2001 Myotonic dystrophy type 2 caused by a CCTG expansion in intron 1 of ZNF9. *Science* **293**, 864–867.

Liu Y. F. 1998 Expression of polyglutamine-expanded Huntingtin activates the SEK1-JNK pathway and induces apoptosis in a hippocampal neuronal cell line. *J. Biol. Chem*. **273**, 28873–28877.

Lutz R. E. 2007 Trinucleotide repeat disorders. *Semin. Pediatr. Neurol*. **14**, 26–33.

MacDonald M. E., Gines S., Gusella J. F. and Wheeler V. C. 2003 Huntington's disease. *Neuromol. Med*. **4**, 7–20.

Mahadevan M., Tsilfidis C., Sabourin L., Shutler G., Amemiya C., Jansen G. *et al*. 1992 Myotonic dystrophy mutation: an unstable CTG repeat in the 3' untranslated region of the gene. *Science* **255**, 1253–1255.

Mallik M. and Lakhotia S. C. 2009a RNAi for the large noncoding hsrω transcripts suppresses polyglutamine pathogenesis in *Drosophila* models. *RNA Biol* **6**, 464–478.

Mallik M. and Lakhotia S. C. 2009b The developmentally active and stress-inducible noncoding hsrω gene is a novel regulator of apoptosis in *Drosophila. Genetics* **183**, 831–852.

Mallik M. and Lakhotia S. C. 2010 Improved activities of CREB-binding protein, heterogenous RNA binding proteins and proteasome following downregulation of noncoding hsromega transcripts help suppress polyQ pathogenesis in fly models. *Genetics* **184**, 927–945.

Margolis R. L. and Ross C. A. 2001 Expansion explosion: new clues to the pathogenesis of repeat expansion neurodegenerative diseases. *Trends Mol. Med*. **7**, 479–482.

Marsh J. L. and Thompson L. M. 2004 Can flies help humans treat neurodegenerative diseases? *BioEssays* **26**, 485–496.

Marsh J. L. and Thompson L. M. 2006 *Drosophila* in the study of neurodegenerative disease. *Neuron* **52**, 169–178.

Marsh J. L., Walker H., Theisen H., Zhu Y. Z., Fielder T., Purcell J. *et al*. 2000 Expanded polyglutamine peptides alone are intrinsically cytotoxic and cause neurodegeneration in *Drosophila. Hum. Mol. Genet*. **9**, 13–25.

Marsh J. L., Lukacsovich T. and Thompson L. M. 2009 Animal models of polyglutamine diseases and therapeutic approaches. *J. Biol. Chem*. **284**, 7431–7435.

Masino L., Nicastro G., Menon R. P., Dal Piaz F., Calder L. and Pastore A. 2004 Characterization of the structure and the amyloidogenic properties of the Josephin domain of the polyglutamine-containing protein Ataxin-3. *J. Mol. Biol*. **344**, 1021–1035.

Matilla A., Gorbea C., Einum D. D., Townsend J., Michalik A., van Broeckhoven C. *et al*. 2001 Association of Ataxin-7 with the proteasome subunit S4 of the 19S regulatory complex. *Hum. Mol. Genet*. **10**, 2821–2831.

Matilla-Duenas A., Goold R. and Giunti P. 2007 Clinical, genetic, molecular, and pathophysiological insights into spinocerebellar ataxia type 1. *Cerebellum* **7**, 106–114.

Matsumoto M., Yada M., Hatakeyama S., Ishimoto H., Tanimura T., Tsuji S. *et al*. 2004 Molecular clearance of Ataxin-3 is regulated by a mammalian E4. *EMBO J*. **23**, 659–669.

Matsuura T., Yamagata T., Burgess D. L., Rasmussen A., Grewal R. P., Watase K. *et al*. 2000 Large expansion of the ATTCT pentanucleotide repeat in spinocerebellar ataxia type 10. *Nat. Genet*. **26**, 191–194.

McCampbell A., Taylor J. P., Taye A. A., Robitschek J., Li M., Walcott J. *et al*. 2000 CREB-binding protein sequestration by expanded polyglutamine. *Hum. Mol. Genet*. **9**, 2197–2202.

McMahon S. J., Pray-Grant M. G., Schieltz D., Yates J. R. III and Grant P. A. 2005 Polyglutamine-expanded spinocerebellar ataxia-7 protein disrupts normal SAGA and SLIK histone acetyltransferase activity. *Proc. Natl. Acad. Sci. USA* **102**, 8478–8482.

McManus K., Scannell C. A., Rutherford S. and Carey C. C. 2006 Canalization and evolvability: tempering the effects of mutation in a changing environment. In *Radiation risk estimates in normal and emergency situations* (ed. A. A. Cigna and M. Durante), pp. 283–290. Springer Science and Buisness Media, The Netherlands.

Merenstein S. A., Sobesky W. E., Taylor A. K., Riddle J. E., Tran H. X. and Hagerman R. J. 1996 Molecular-clinical correlations in males with an expanded FMR1 mutation. *Am. J. Med. Genet*. **64**, 388–394.

Merienne K., Helmlinger D., Perkin G. R., Devys D. and Trottier Y. 2003 Polyglutamine expansion induces a protein-damaging stress connecting heat shock protein 70 to the JNK pathway. *J. Biol. Chem*. **278**, 16957–16967.

Meriin A. B., Zhang X., Miliaras N. B., Kazantsev A., Chernoff Y. O., McCaffery J. M. *et al*. 2003 Aggregation of expanded polyglutamine domain in yeast leads to defects in endocytosis. *Mol. Cell Biol*. **23**, 7554–7565.

Miller V. M., Nelson R. F., Gouvion C. M., Williams A., Rodriguez-Lebron E., Harper S. Q. *et al*. 2005 CHIP suppresses polyglutamine aggregation and toxicity in vitro and in vivo. *J. Neurosci*. **25**, 9152–9161.

Minamiyama M., Katsuno M., Adachi H., Waza M., Sang C., Kobayashi Y. *et al*. 2004 Sodium butyrate ameliorates phenotypic expression in a transgenic mouse model of spinal and bulbar muscular atrophy. *Hum. Mol. Genet*. **13**, 1183–1192.

Morante J., Desplan C. and Celik A. 2007 Generating patterned arrays of photoreceptors. *Curr. Opin. Genet. Dev*. **17**, 314–319.

Morfini G., Pigino G., Szebenyi G., You Y., Pollema S. and Brady S. T. 2006 JNK mediates pathogenic effects of polyglutamine-expanded androgen receptor on fast axonal transport. *Nat. Neurosci*. **9**, 907–916.

Morin X., Daneman R., Zavortink M. and Chia W. 2001 A protein trap strategy to detect GFP-tagged proteins expressed from their endogenous loci in *Drosophila. Proc. Natl. Acad. Sci. USA* **98**, 15050–15055.

Moseley M. L., Zu T., Ikeda Y., Gao W., Mosemiller A. K., Daughters R. S. *et al*. 2006 Bidirectional expression of CUG and CAG expansion transcripts and intranuclear polyglutamine inclusions in spinocerebellar ataxia type 8. *Nat. Genet*. **38**, 758–769.

Muchowski P. J. and Wacker J. L. 2005 Modulation of neurodegeneration by molecular chaperones. *Nat. Rev. Neurosci*. **6**, 11–22.

Muchowski P. J., Schaffar G., Sittler A., Wanker E. E., Hayer-Hartl M. K. and Hartl F. U. 2000 Hsp70 and hsp40 chaperones can inhibit self-assembly of polyglutamine proteins into amyloid-like fibrils. *Proc. Natl. Acad. Sci. USA* **97**, 7841–7846.

Mugat B., Parmentier M. L., Bonneaud N., Chan H. Y. and Maschat F. 2008 Protective role of Engrailed in a *Drosophila* model of Huntington's disease. *Hum. Mol. Genet*. **17**, 3601–3616.

Mulley J. C., Yu S., Loesch D. Z., Hay D. A., Donnelly A., Gedeon A. K. *et al*. 1995 FRAXE and mental retardation. *J. Med. Genet*. **32**, 162–169.

Muqit M. M. and Feany M. B. 2002 Modelling neurodegenerative diseases in *Drosophila:* a fruitful approach? *Nat. Rev. Neurosci*. **3**, 237–243.

Murata T., Suzuki E., Ito S., Sawatsubashi S., Zhao Y., Yamagata K. *et al*. 2008 RNA-binding protein hoip accelerates polyQ-induced

neurodegeneration in *Drosophila. Biosci. Biotechnol. Biochem*. **72**, 2255–2261.

Nagafuchi S., Yanagisawa H., Sato K., Shirayama T., Ohsaki E., Bundo M. *et al*. 1994 Dentatorubral and pallidoluysian atrophy expansion of an unstable CAG trinucleotide on chromosome 12p. *Nat. Genet*. **6**, 14–18.

Nagai Y., Fujikake N., Ohno K., Higashiyama H., Popiel H. A., Rahadian J. *et al*. 2003 Prevention of polyglutamine oligomerization and neurodegeneration by the peptide inhibitor QBP1 in *Drosophila. Hum. Mol. Genet*. **12**, 1253–1259.

Nagai Y., Fujikake N., Popiel H. A. and Wada K. 2010 Induction of molecular chaperones as a therapeutic strategy for the polyglutamine diseases. *Curr. Pharm. Biotechnol*. **11**, 188–197.

Nakamura K., Jeong S. Y., Uchihara T., Anno M., Nagashima K., Nagashima T. *et al*. 2001 SCA17, a novel autosomal dominant cerebellar ataxia caused by an expanded polyglutamine in TATA-binding protein. *Hum. Mol. Genet*. **10**, 1441–1448.

Nemes J. P., Benzow K. A., Moseley M. L., Ranum L. P. and Koob M. D. 2000 The SCA8 transcript is an antisense RNA to a brain-specific transcript encoding a novel actin-binding protein (KLHL1). *Hum. Mol. Genet*. **9**, 1543–1551.

Nollen E. A., Garcia S. M., van Haaften G., Kim S., Chavez A., Morimoto R. I. *et al*. 2004 Genome-wide RNA interference screen identifies previously undescribed regulators of polyglutamine aggregation. *Proc. Natl. Acad. Sci. USA* **101**, 6403–6408.

Nozaki K., Onodera O., Takano H. and Tsuji S. 2001 Amino acid sequences flanking polyglutamine stretches influence their potential for aggregate formation. *Neuroreport* **12**, 3357–3364.

Nucifora Jr F. C., Sasaki M., Peters M. F., Huang H., Cooper J. K., Yamada M. *et al*. 2001 Interference by huntingtin and atrophin-1 with cbp-mediated transcription leading to cellular toxicity. *Science* **291**, 2423–2428.

Okazawa H. 2003 Polyglutamine diseases: a transcription disorder? *Cell. Mol. Life Sci*. **60**, 1427–1439.

Ona V. O., Li M., Vonsattel J. P., Andrews L. J., Khan S. Q., Chung W. M. *et al*. 1999 Inhibition of caspase-1 slows disease progression in a mouse model of Huntington's disease. *Nature* **399**, 263–267.

Orr H. T. 2001 Beyond the Qs in the polyglutamine diseases. *Genes Dev*. **15**, 925–932.

Orr H. T. and Zoghbi H. Y. 2007 Trinucleotide repeat disorders. *Annu. Rev. Neurosci*. **30**, 575–621.

Orr H. T., Chung M. Y., Banfi S., Kwiatkowski Jr T. J., Servadio A., Beaudet A. L. *et al*. 1993 Expansion of an unstable trinucleotide CAG repeat in spinocerebellar ataxia type 1. *Nat. Genet*. **4**, 221–226.

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. *Proc. Natl. Acad. Sci. USA* **102**, 8472–8477.

Pallos J., Bodai L., Lukacsovich T., Purcell J. M., Steffan J. S., Thompson L. M. *et al*. 2008 Inhibition of specific HDACs and sirtuins suppresses pathogenesis in a *Drosophila* model of Huntington's disease. *Hum. Mol. Genet*. **17**, 3767–3775.

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.

Pandolfo M. 2002a Iron metabolism and mitochondrial abnormalities in Friedreich ataxia. *Blood Cells Mol. Dis*. **29**, 536–547; discussion 548–552.

Pandolfo M. 2002b The molecular basis of Friedreich ataxia. *Adv. Exp. Med. Biol*. **516**, 99–118.

Panov A. V., Gutekunst C. A., Leavitt B. R., Hayden M. R., Burke J. R., Strittmatter W. J. *et al*. 2002 Early mitochondrial calcium defects in Huntington's disease are a direct effect of polyglutamines. *Nat. Neurosci*. **5**, 731–736.

Park Y., Hong S., Kim S. J. and Kang S. 2005 Proteasome function is inhibited by polyglutamine-expanded Ataxin-1, the SCA1 gene product. *Mol. Cell* **19**, 23–30.

Paulson H. L. and Fischbeck K. H. 1996 Trinucleotide repeats in neurogenetic disorders. *Annu. Rev. Neurosci*. **19**, 79–107.

Paulson H. L., Das S. S., Crino P. B., Perez M. K., Patel S. C., Gotsdiner D. *et al*. 1997a Machado-Joseph disease gene product is a cytoplasmic protein widely expressed in brain. *Ann. Neurol*. **41**, 453–462.

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

Pearl L. H. and Prodromou C. 2006 Structure and mechanism of the Hsp90 molecular chaperone machinery. *Annu. Rev. Biochem*. **75**, 271–294.

Pearson C. E., Edamura K. N. and Cleary J. D. 2005 Repeat instability: Mechanisms of dynamic mutations. *Nat. Rev. Genet*. **6**, 729–742.

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.

Piccioni F., Pinton P., Simeoni S., Pozzi P., Fascio U., Vismara G. *et al*. 2002 Androgen receptor with elongated polyglutamine tract forms aggregates that alter axonal trafficking and mitochondrial distribution in motor neuronal processes. *FASEB J*. **16**, 1418–1420.

Plassart E. and Fontaine B. 1994 Genes with triplet repeats - a new class of mutations causing neurological diseases. *Biomed. Pharmacother*. **48**, 191–197.

Prasanth K. V., Rajendra T. K., Lal A. K. and Lakhotia S. C. 2000 Omega speckles - a novel class of nuclear speckles containing hnRNPs associated with noncoding hsr-omega RNA in *Drosophila. J. Cell Sci*. **113**, 3485–3497.

Puccio H., Simon D., Cossee M., Criqui-Filipe P., Tiziano F., Melki J. *et al*. 2001 Mouse models for Friedreich ataxia exhibit cardiomyopathy, sensory nerve defect and Fe-S enzyme deficiency followed by intramitochondrial iron deposits. *Nat. Genet*. **27**, 181–186.

Quinn W. Q., Harris W. A. and Benzer S. 1974 Conditioned behaviour in *Drosophila melanogaster. Proc. Natl. Acad. Sci. USA* **71**, 708–712.

Rangone H., Pardo R., Colin E., Girault J. A., Saudou F. and Humbert S. 2005 Phosphorylation of arfaptin 2 at Ser^{260} by Akt inhibits polyQ-Huntingtin induced toxicity by rescuing proteasome impairment. *J. Biol Chem*. **280**, 22021–22028.

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. *Nat. Genet*. **36**, 585–595.

Ravikumar B., Imarisio S., Sarkar S., O'Kane C. J. and Rubinsztein D. C. 2008 Rab5 modulates aggregation and toxicity of mutant huntingtin through macroautophagy in cell and fly models of Huntington disease. *J. Cell Sci*. **121**, 1649–1660.

Reiter L. T. and Bier E. 2002 Using *Drosophila melanogaster* to uncover human disease gene function and potential drug target proteins. *Expert Opin. Ther. Tar.* **6**, 387–399.

Reiter L. T., Potocki L., Chien S., Gribskov M. and Bier E. 2001 A systematic analysis of human disease-associated gene sequences in *Drosophila melanogaster. Genome Res*. **11**, 1114–1125.

Restifo L. L. 2005 Mental retardation genes in *Drosophila:* New ap-

proaches to understanding and treating developmental brain disorders. *Ment. Retard. Dev. Disabil. Res. Rev*. **11**, 286–294.

Rong J., Li S., Sheng G., Wu M., Coblitz B., Li M. *et al*. 2007 14-3-3 protein interacts with huntingtin-associated protein 1 and regulates its trafficking. *J. Biol. Chem.* **282**, 4748–4756.

Rorth P. 1996 A modular misexpression screen in *Drosophila* detecting tissue-specific phenotypes. *Proc. Natl. Acad. Sci. USA* **93**, 12418–12422.

Ross C. A. and Poirier M. A. 2004 Protein aggregation and neurodegenerative disease. *Nat. Med.* **10**, suppl. 10–17.

Rouaux C., Loeffler J. P. and Boutillier A. L. 2004 Targeting CREB-binding protein (CBP) loss of function as a therapeutic strategy in neurological disorders. *Biochem. Pharmacol*. **68**, 1157–1164.

Rousseau E., Kojima R., Hoffner G., Djian P. and Bertolotti A. 2009 Misfolding of proteins with a polyglutamine expansion is facilitated by proteasomal chaperones. *J. Biol. Chem*. **16**, 1917–1929.

Rubin G. M., Yandell M. D., Wortman J. R., Gabor Miklos G. L., Nelson C. R., Hariharan I. K. *et al*. 2000 Comparative genomics of the eukaryotes. *Science* **287**, 2204–2215.

Runne H., Regulier E., Kuhn A., Zala D., Gokce O., Perrin V. *et al*. 2008 Dysregulation of gene expression in primary neuron models of Huntington's disease shows that polyglutamine-related effects on the striatal transcriptome may not be dependent on brain circuitry. *J. Neurosci*. **28**, 9723–9731.

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.

Sang T. K. and Jackson G. R. 2005 *Drosophila* models of neurodegenerative disease. *NeuroTherapeutics* **2**, 438–446.

Sang T. K., Li C., Liu W., Rodriguez A., Abrams J. M., Zipursky S. L. *et al*. 2005 Inactivation of *Drosophila* Apaf-1 related killer suppresses formation of polyglutamine aggregates and blocks polyglutamine pathogenesis. *Hum. Mol. Genet*. **14**, 357–372.

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. *Ann. Neurol*. **42**, 604–612.

Satyal S. H., Schmidt E., Kitagawa K., Sondheimer N., Lindquist S., Kramer J. M. *et al*. 2000 Polyglutamine aggregates alter protein folding homeostasis in Caenorhabditis elegans. *Proc. Natl. Acad. Sci. USA* **97**, 5750–5755.

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.

Sawa A. 2001 Mechanisms for neuronal cell death and dysfunction in Huntington's disease: pathological cross-talk between the nucleus and the mitochondria? *J. Mol. Med.* **79**, 375–381.

Scappini E., Koh T. W., Martin N. P. and O'Bryan J. P. 2007 Intersectin enhances huntingtin aggregation and neurodegeneration through activation of c-Jun-NH2-terminal kinase. *Hum. Mol. Genet.* **16**, 1862–1871.

Schaffar G., Breuer P., Boteva R., Behrends C., Tzvetkov N., Strippel N. *et al*. 2004 Cellular toxicity of polyglutamine expansion proteins: mechanism of transcription factor deactivation. *Mol. Cell* **15**, 95–105.

Schilling B., Gafni J., Torcassi C., Cong X., Row R. H., LaFevre-Bernt M. A. *et al*. 2006 Huntingtin phosphorylation sites mapped by mass spectrometry: Modulation of cleavage and toxicity. *J. Biol. Chem.* **281**, 23686–23697.

Senoo-Matsuda N., Igaki T. and Miura M. 2005 Bax-like protein Drob-1 protects neurons from expanded polyglutamine-induced toxicity in *Drosophila. EMBO J.* **24**, 2700–2713.

Sengupta S. and Lakhotia S. C. 2006 Altered expression of the noncoding *hsrω* gene enhances poly-Q-induced neurotoxicity in *Drosophila. RNA Biol.* **3**, 28–35.

Servadio A., Koshy B., Armstrong D., Antalffy B., Orr H. T. and Zoghbi H. Y. 1995 Expression analysis of the Ataxin-1 protein in tissues from normal and spinocerebellar ataxia type 1 individuals. *Nat. Genet.* **10**, 94–98.

Sherman M. Y. and Goldberg A. L. 2001 Cellular defenses against unfolded proteins: a cell biologist thinks about neurodegenerative diseases. *Neuron* **29**, 15–32.

Shibata H., Huynh D. P. and Pulst S. M. 2000 A novel protein with RNA-binding motifs interacts with Ataxin-2. *Hum. Mol. Genet*. **9**, 1303–1313.

Shimohata T., Nakajima T., Yamada M., Uchida C., Onodera O., Naruse S. *et al*. 2000a Expanded polyglutamine stretches interact with TAFII130, interfering with CREB-dependent transcription. *Nat. Genet*. **26**, 29–36.

Shimohata T., Onodera O. and Tsuji S. 2000b Interaction of expanded polyglutamine stretches with nuclear transcription factors leads to aberrant transcriptional regulation in polyglutamine diseases. *Neuropathology* **20**, 326–333.

Sinadinos C., Burbidge-King T., Soh D., Thompson L. M., Marsh J. L. and Wyttenbach A. 2009 Live axonal transport disruption by mutant huntingtin fragments in *Drosophila* motor neuron axons. *Neurobiol. Dis*. **34**, 389–395.

Sittler A., Walter S., Wedemeyer N., Hasenbank R., Scherzinger E., Eickhoff H. *et al*. 1998 SH3GL3 associates with the Huntingtin exon 1 protein and promotes the formation of polygln-containing protein aggregates. *Mol. Cell* **2**, 427–436.

Skinner P. J., Koshy B. T., Cummings C. J., Klement I. A., Helin K., Servadio A. *et al*. 1997 Ataxin-1 with an expanded glutamine tract alters nuclear matrix-associated structures. *Nature* **389**, 971–974.

Sofola O. A., Jin P., Qin Y., Duan R., Liu H., de Haro M. *et al*. 2007 RNA-binding proteins hnRNP A2/B1 and CUGBP1 suppress fragile X CGG premutation repeat-induced neurodegeneration in a *Drosophila* model of FXTAS. *Neuron* **55**, 565–571.

Sopher B. L., Thomas Jr P. S., LaFevre-Bernt M. A., Holm I. E., Wilke S. A., Ware C. B. *et al*. 2004 Androgen receptor YAC transgenic mice recapitulate SBMA motor neuronopathy and implicate VEGF164 in the motor neuron degeneration. *Neuron* **41**, 687–699.

Steffan J. S., Kazantsev A., Spasic-Boskovic O., Greenwald M., Zhu Y. Z., Gohler H. *et al*. 2000 The Huntington's disease protein interacts with p53 and CREB-binding protein and represses transcription. *Proc. Natl. Acad. Sci. USA* **97**, 6763–6768.

Steffan J. S., Bodai L., Pallos J., Poelman M., McCampbell A., Apostol B. L. *et al*. 2001 Histone deacetylase inhibitors arrest polyglutamine-dependent neurodegeneration in *Drosophila. Nature* **413**, 739–743.

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.

Stenoien D. L., Cummings C. J., Adams H. P., Mancini M. G., Patel K., DeMartino G. N. *et al*. 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.

Stenoien D. L., Mielke M. and Mancini M. A. 2002 Intranuclear Ataxin-1 inclusions contain both fast- and slow-exchanging components. *Nat. Cell Biol*. **4**, 806–810.

Strand A. D., Baquet Z. C., Aragaki A. K., Holmans P., Yang L., Cleren C. *et al*. 2007 Expression profiling of Huntington's disease models suggests that brain-derived neurotrophic factor depletion plays a major role in striatal degeneration. *J. Neurosci.* **27**, 11758–11768.

Sugars K. L. and Rubinsztein D. C. 2003 Transcriptional abnormalities in Huntington disease. *Trends Genet.* **19**, 233–238.

Szebenyi G., Morfini G. A., Babcock A., Gould M., Selkoe K., Stenoien D. L. *et al*. 2003 Neuropathogenic forms of huntingtin and androgen receptor inhibit fast axonal transport. *Neuron* **40**, 41–52.

Takeyama K., Ito S., Yamamoto A., Tanimoto H., Furutani T., Kanuka H. *et al*. 2002 Androgen-dependent neurodegeneration by polyglutamine-expanded human androgen receptor in *Drosophila. Neuron* **35**, 855–864.

Taylor J. P., Taye A. A., Campbell C., Kazemi-Esfarjani P., Fischbeck K. H. and Min K. T. 2003 Aberrant histone acetylation, altered transcription, and retinal degeneration in a *Drosophila* model of polyglutamine disease are rescued by CREB-binding protein. *Genes Dev.* **17**, 1463–1468.

Thakur A. K., Jayaraman M., Mishra R., Thakur M., Chellgren V. M., Byeon I. J. L. *et al*. 2009 Polyglutamine disruption of the huntingtin exon 1 N terminus triggers a complex aggregation mechanism. *Nat. Struct. Mol. Biol.* **16**, 380–389.

The Huntington's disease collaborative research group 1993 A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell* **72**, 971–983.

Tsai C. C., Kao H. Y., Mitzutani A., Banayo E., Rajan H., McKeown M. *et al*. 2004 Ataxin-1, a SCA1 neurodegenerative disorder protein, is functionally linked to the silencing mediator of retinoid and thyroid hormone receptors. *Proc. Natl. Acad. Sci. USA* **101**, 4047–4052.

Van Dam D., Errijgers V., Kooy R. F., Willemsen R., Mientjes E., Oostra B. A. *et al*. 2005 Cognitive decline, neuromotor and behavioural disturbances in a mouse model for fragile-X-associated tremor/ataxia syndrome (FXTAS). *Behav. Brain Res.* **162**, 233–239.

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

Verkerk A., Pieretti M., Sutcliffe J. S., Fu Y. H., Kuhl D. P. A., Pizzuti A. *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.

Vig P. J., Subramony S. H. and McDaniel D. O. 2001 Calcium homeostasis and spinocerebellar ataxia-1 (SCA-1). *Brain Res. Bull*. **56**, 221–225.

Virtaneva K., D'Amato E., Miao J., Koskiniemi M., Norio R., Avanzini G. *et al*. 1997 Unstable minisatellite expansion causing recessively inherited myoclonus epilepsy, EPM1. *Nat. Genet*. **15**, 393–396.

Voisine C. and Hart A. C. 2004 Caenorhabditis elegans as a model system for triplet repeat diseases. *Methods Mol. Biol.* **277**, 141–160.

Vonsattel J. P., Myers R. H., Stevens T. J., Ferrante R. J., Bird E. D. and Richardson E. P. 1985 Neuropathological Classification of Huntingtons-Disease. *J. Neuropathol. Exp. Neurol*. **44**, 559–577.

Walters R. H. and Murphy R. M. 2009 Examining polyglutamine peptide length: a connection between collapsed conformations and increased aggregation. *J. Mol. Biol.* **393**, 978–992.

Wang G. H., Mitsui K., Kotliarova S., Yamashita A., Nagao Y., Tokuhiro S. *et al*. 1999 Caspase activation during apoptotic cell death induced by expanded polyglutamine in N2a cells. *Neuroreport* **10**, 2435–2438.

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. *Nat. Genet.* **23**, 425–428.

Warrick J. M., Paulson H. L., Gray-Board G. L., Bui Q. T., Fischbeck K. H., Pittman R. N. *et al*. 1998 Expanded polyglutamine protein forms nuclear inclusions and causes neural degeneration in *Drosophila. Cell* **93**, 939–949.

Warrick J. M., Morabito L. M., Bilen J., Gordesky-Gold B., Faust L. Z., Paulson H. L. *et al*. 2005 Ataxin-3 suppresses polyglutamine neurodegeneration in *Drosophila* by a ubiquitin-associated mechanism. *Mol. Cell* **18**, 37–48.

Watase K., Weeber E. J., Xu B., Antalffy B., Yuva-Paylor L., Hashimoto K. *et al*. 2002 A long CAG repeat in the mouse Sca1 locus replicates SCA1 features and reveals the impact of protein solubility on selective neurodegeneration. *Neuron* **34**, 905–919.

Waza M., Adachi H., Katsuno M., Minamiyama M., Tanaka F., Doyu M. *et al*. 2006 Modulation of Hsp90 function in neurodegenerative disorders: a molecular-targeted therapy against disease-causing protein. *J. Mol. Med.* **84**, 635–646.

Wellington C. L. and Hayden M. R. 2000 Caspases and neurodegeneration: on the cutting edge of new therapeutic approaches. *Clin. Genet.* **57**, 1–10.

Wellington C. L., Ellerby L. M., Hackam A. S., Margolis R. L., Trifiro M. A., Singaraja R. *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.

Wheeler T. M. and Thornton C. A. 2007 Myotonic dystrophy: RNA-mediated muscle disease. *Curr. Opin. Neurol.* **20**, 572–576.

Williams A. J., Knutson T. M., Colomer Gould V. F. and Paulson H. L. 2009 In vivo suppression of polyglutamine neurotoxicity by C-terminus of Hsp70-interacting protein (CHIP) supports an aggregation model of pathogenesis. *Neurobiol. Dis.* **33**, 342–353.

Willingham S., Outeiro T. F., DeVit M. J., Lindquist S. L. and Muchowski P. J. 2003 Yeast genes that enhance the toxicity of a mutant huntingtin fragment or alpha-synuclein. *Science* **302**, 1769–1772.

Wolff T. and Ready D. F. 1993 Pattern Formation in the *Drosophila* Retina. In *The development of Drosophila melanogaster* (ed. M. Bate and A. M. Arias), pp. 1277–1326. Cold Spring Harbor Laboratory Press, New York, USA.

Wu L. L., Fan Y., Li S., Li X. J. and Zhou X. F. 2010 Huntingtin-associated protein-1 interacts with pro-brain-derived neurotrophic factor and mediates its transport and release. *J. Biol. Chem*. **285**, 5614–5623.

Wyttenbach A. 2004 Role of heat shock proteins during polyglutamine neurodegeneration: mechanisms and hypothesis. *J. Mol. Neurosci*. **23**, 69–96.

Wyttenbach A., Carmichael J., Swartz J., Furlong R. A., Narain Y., Rankin J. *et al*. 2000 Effects of heat shock, heat shock protein 40 (HDJ-2), and proteasome inhibition on protein aggregation in cellular models of Huntington's disease. *Proc. Natl. Acad. Sci. USA* **97**, 2898–2903.

Wyttenbach A., Sauvageot O., Carmichael J., Diaz-Latoud C., Arrigo A. P. and Rubinsztein D. C. 2002 Heat shock protein 27 prevents cellular polyglutamine toxicity and suppresses the increase of reactive oxygen species caused by huntingtin. *Hum. Mol. Genet*. **11**, 1137–1151.

Yakura H., Wakisaka A., Fujimoto S. and Itakura K. 1974 Letter: Hereditary ataxia and HL-A. *N. Engl. J. Med.* **291**, 154–155.

Yang W., Dunlap J. R., Andrews R. B. and Wetzel R. 2002 Aggregated polyglutamine peptides delivered to nuclei are toxic to mammalian cells. *Hum. Mol. Genet.* **11**, 2905–2917.

Yoo S. Y., Pennesi M. E., Weeber E. J., Xu B., Atkinson R., Chen S. *et al*. 2003 SCA7 knockin mice model human SCA7 and reveal gradual accumulation of mutant Ataxin-7 in neurons and abnormalities in short-term plasticity. *Neuron* **37**, 383–401.

Yvert G., Lindenberg K. S., Devys D., Helmlinger D., Landwehrmeyer G. B. and Mandel J. L. 2001 SCA7 mouse models show selective stabilization of mutant Ataxin-7 and

similar cellular responses in different neuronal cell types. *Hum. Mol. Genet.* **10**, 1679–1692.

Zander C., Takahashi J., El Hachimi K. H., Fujigasaki H., Albanese V., Lebre A. S. *et al*. 2001 Similarities between spinocerebellar ataxia type 7 (SCA7) cell models and human brain: proteins recruited in inclusions and activation of caspase-3. *Hum. Mol. Genet.* **10**, 2569–2579.

Zhang S., Xu L., Lee J. and Xu T. 2002 *Drosophila* atrophin homolog functions as a transcriptional corepressor in multiple developmental processes. *Cell* **108**, 45–56.

Zhang S., Binari R., Zhou R. and Perrimon N. 2010 A genomewide RNA interference screen for modifiers of aggregates formation by mutant Huntingfin in *Drosophila Genetics* **184**, 1165–1179.

Zhou B. P., Liao Y., Xia W., Zou Y., Spohn B. and Hung M. C. 2001 HER-2/neu induces p53 ubiquitination via Akt-mediated MDM2 phosphorylation. *Nat. Cell Biol.* **3**, 973–982.

Zhuchenko O., Bailey J., Bonnen P., Ashizawa T., Stockton D. W., Amos C. *et al*. 1997 Autosomal dominant cerebellar ataxia (SCA6) associated with small polyglutamine expansions in the alpha 1A-voltage-dependent calcium channel. *Nat. Genet.* **15**, 62–69.

Received 26 December 2009, in revised form 16 April 2010; accepted 3 May 2010

Published on the Web: 26 November 2010