

## REVIEW ARTICLE

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

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### 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.

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### 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 (Karlín 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.

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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) <sub>n</sub>   | FRDA                                 | (GAA) <sub>n</sub>   |
| HD                          | (CAG) <sub>n</sub>   | FRAXA                                | (CGC) <sub>n</sub>   |
| DRPLA                       | (CAG) <sub>n</sub>   | FRAXE                                | (CCG) <sub>n</sub>   |
| SBMA                        | (CAG) <sub>n</sub>   | FXTAS                                | (CGG) <sub>n</sub>   |
|                             |                      | DM1                                  | (CTG) <sub>n</sub>   |
|                             |                      | DM2                                  | (CCTG) <sub>n</sub>  |
|                             |                      | SCA8                                 | (CTG) <sub>n</sub>   |
|                             |                      | SCA10                                | (ATTCT) <sub>n</sub> |
|                             |                      | SCA12                                | (CAG) <sub>n</sub>   |
|                             |                      | HDL2                                 | (CTG) <sub>n</sub>   |

**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        |                        | Main clinical features                                                                                                                                                      | References                                                                                                                                   |
|--------------|---------------------|-------------------------------------------|-------------------------------------------------------|----------------|---------------------------------------------------------------------|---------------|------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------|
|              |                     |                                           |                                                       |                |                                                                     | repeat length | Expanded repeat length |                                                                                                                                                                             |                                                                                                                                              |
| FRDA         | Autosomal recessive | (GAA) <sub>n</sub>                        | Intron 1                                              | Frataxin       | Mitochondrial iron metabolism                                       | 6–32          | 200–1700               | Sensory ataxia, slow saccades, hypertrophic cardiomyopathy, diabetes mellitus                                                                                               | Campuzano <i>et al.</i> (1996), Cossee <i>et al.</i> (1997), Harding (1981), Pandolfo (2002a), Pandolfo (2002b), Puccio <i>et al.</i> (2001) |
| FXS          | X-linked            | (CGG) <sub>n</sub>                        | 5' UTR                                                | FMRP           | Translational regulation                                            | 6–52          | 55→2000                | Mental retardation, macroorchidism, connective tissue defects, hyperactivity and behavioral abnormalities                                                                   | De Boulle <i>et al.</i> (1993), Eichler and Nelson (1996), Hagerman (2006), Merenstein <i>et al.</i> (1996), Verkerk <i>et al.</i> (1991)    |
| FRAXE        | X-linked            | (CCG) <sub>n</sub>                        | 5' end                                                | FMR2           | Transcription                                                       | 4–39          | 200–900                | Mental retardation                                                                                                                                                          | Gez <i>et al.</i> (1996), Gu <i>et al.</i> (1996), Knight <i>et al.</i> (1993), Mulley <i>et al.</i> (1995)                                  |
| FXTAS        | X-linked            | (CGG) <sub>n</sub>                        | 5' UTR                                                | FMR1 RNA       | RNA-mediated                                                        | 6–60          | 60–200                 | Ataxia, tremor, Parkinsonism, cognitive deficits                                                                                                                            | Hagerman and Hagerman (2004), Van Dam <i>et al.</i> (2005)                                                                                   |
| DM1          | Autosomal dominant  | (CTG) <sub>n</sub>                        | 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 <i>et al.</i> (1992), Fu <i>et al.</i> (1992), Mahadevan <i>et al.</i> (1992)                                                          |
| DM2          | Autosomal dominant  | (CCTG) <sub>n</sub>                       | Intron 1                                              | CNBP           | RNA-mediated                                                        | < 27          | 75–11000               | Similar to DM1, no congenital form                                                                                                                                          | Liquori <i>et al.</i> (2001) Wheeler and Thornton (2007)                                                                                     |
| EPM1         | Autosomal recessive | (C) <sub>4</sub> G(C) <sub>4</sub> GCG    | Promoter                                              | CSTB           | RNA-mediated                                                        | 2–3           | 45–70                  | severe stimulus sensitive myoclonus, generalized tonic-clonic seizures                                                                                                      | Lalioi <i>et al.</i> (1997), Larson <i>et al.</i> (1999), Virtaneva <i>et al.</i> (1997)                                                     |
| SCA8         | Autosomal dominant  | (CAG) <sub>n</sub> and (CTG) <sub>n</sub> | 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 <i>et al.</i> (2000), Koob <i>et al.</i> (1999), Moseley <i>et al.</i> (2006), Nemes <i>et al.</i> (2000)                                |
| SCA10        | Autosomal dominant  | (ATTCT) <sub>n</sub>                      | Intron 9                                              | -              | Unknown                                                             | 10–29         | 280–4500               | Ataxia, tremor, cognitive and neuropsychiatric impairment                                                                                                                   | Matsuura <i>et al.</i> (2000)                                                                                                                |
| SCA12        | Autosomal dominant  | (CAG) <sub>n</sub> / (CTG) <sub>n</sub>   | 5' region                                             | PPP2R2B        | Phosphatase regulation                                              | 9–28          | 55–78                  | Ataxia and seizures                                                                                                                                                         | Holmes <i>et al.</i> (2001)                                                                                                                  |
| HDL2         | Autosomal dominant  | (CAG) <sub>n</sub> / (CTG) <sub>n</sub>   | Alternatively spliced exon 2A                         | Junctophilin 3 | PM/ER junction protein                                              | 6–28          | 40–59                  | Similar to HD                                                                                                                                                               | Holmes <i>et al.</i> (2001)                                                                                                                  |

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

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

gates, 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 hsr $\omega$ -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 <i>et al.</i> (2002)                                                           |
|                                                                                                |                                                                                                                                                                                                                 | 63                   | UAS-63Q                        | Kazemi-Esfarjani and Benzer (2002)                                                       |
|                                                                                                |                                                                                                                                                                                                                 | 79                   | GMR-Q79                        | Higashiyama <i>et al.</i> (2002)                                                         |
|                                                                                                |                                                                                                                                                                                                                 | 92                   | GMR-Q92                        | Marsh <i>et al.</i> (2000)                                                               |
|                                                                                                |                                                                                                                                                                                                                 | 108                  | UAS-Q108                       | Marsh <i>et al.</i> (2000)                                                               |
|                                                                                                |                                                                                                                                                                                                                 | 127                  | UAS-127Q                       | Kazemi-Esfarjani and Benzer (2000)                                                       |
| Ataxin-1                                                                                       | Human SCA1 cDNA                                                                                                                                                                                                 | 82                   | UAS-SCA1 82Q                   | Feany and Bender (2000); Fernandez-Funez <i>et al.</i> (2000); Tsai <i>et al.</i> (2004) |
| Ataxin-3                                                                                       | NH <sub>2</sub> -terminal 12 aa and C-terminal 43 aa                                                                                                                                                            | 78                   | UAS-MJDr-Q78                   | Warrick <i>et al.</i> (1998)                                                             |
|                                                                                                | NH <sub>2</sub> -terminal 12 aa and C-terminal 43 aa                                                                                                                                                            | 61                   | UAS-SCA3tr-Q61(S)              | Chan <i>et al.</i> (2000)                                                                |
|                                                                                                | N-terminally truncated ataxin-3                                                                                                                                                                                 | 62                   | UAS-SCA3trQ62-DsRed            | Li <i>et al.</i> (2007)                                                                  |
|                                                                                                | N-terminally truncated ataxin-3 with NES sequence from the Rev protein at the 3' end                                                                                                                            | 77                   | UAS-MJD-77QNES                 | Gunawardena <i>et al.</i> (2003)                                                         |
|                                                                                                | Full length of ataxin-3                                                                                                                                                                                         | 78                   | UAS-SCA3-Q78                   | Warrick <i>et al.</i> (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 <i>et al.</i> (2006)                                                           |
| NH <sub>2</sub> -terminal deletion mutant of ataxin-3                                          | 79                                                                                                                                                                                                              | UAS-ataxin-3ΔN79QC   | Matsumoto <i>et al.</i> (2004) |                                                                                          |
| Ataxin-7                                                                                       | SCA7 cDNA (amino acids 1-232) with an added nuclear localization signal                                                                                                                                         | 102                  | UAS-SCA7T-102Q                 | Latouche <i>et al.</i> (2007)                                                            |
| Huntingtin                                                                                     | NH <sub>2</sub> -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 <i>et al.</i> (1998)                                                             |
|                                                                                                |                                                                                                                                                                                                                 | 120                  | GMR-Huntingtin-Q120            |                                                                                          |
|                                                                                                | Entire exon 1 of Huntingtin (amino acids 1-67)                                                                                                                                                                  | 93                   | UAS-Httex1p Q93                | Steffan <i>et al.</i> (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 <i>et al.</i> (2004)                                                             |
|                                                                                                |                                                                                                                                                                                                                 | 103                  | UAS-Httex1p 103Q               |                                                                                          |
|                                                                                                | 548 amino acid NH <sub>2</sub> -terminal fragment of the human Huntingtin cDNA                                                                                                                                  | 128                  | UAS- Htt-Q128                  | Lee <i>et al.</i> (2004)                                                                 |
|                                                                                                | NH <sub>2</sub> -terminal fragment encoding the first 336 amino acids of the human Huntingtin cDNA                                                                                                              | 128                  | UAS-128QHtt[M64]               | Kaltenbach <i>et al.</i> (2007)                                                          |
|                                                                                                | N-terminal part of human Huntingtin (amino acids 1-171)                                                                                                                                                         | 138                  | UAS-HA-hHtt171aa-138Q          | Mugat <i>et al.</i> (2008)                                                               |
|                                                                                                | NH <sub>2</sub> -terminal Huntingtin exon 1                                                                                                                                                                     | 46                   | UAS-Httex1-Q46-eGFP            | Zhang <i>et al.</i> (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 <i>et al.</i> (2009)  |                                                                                          |
|                                                                                                | 152                                                                                                                                                                                                             | UAS-Nhtt(152Q)EGFP   |                                |                                                                                          |
| Androgen receptor                                                                              | Full length human AR                                                                                                                                                                                            | 52                   | UAS-hAR(Q52)                   | Takeyama <i>et al.</i> (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 <i>et al.</i> (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                                                                                                                           |
|----------------------------------------------------------------------------------------------------------------------------|-------------------------|------------------------------------------------|---------------------------------------|-----------------|-----------------|------------------------------------|------|--------------------------------------------------------------------------------------------------------------------------------------|
|                                                                                                                            |                         | PolyQ model                                    | SCAs                                  |                 |                 | HD                                 | SBMA |                                                                                                                                      |
|                                                                                                                            |                         |                                                | SCA1                                  | SCA3            | SCA7            |                                    |      |                                                                                                                                      |
| <b>Transcription factors/regulators</b>                                                                                    |                         |                                                |                                       |                 |                 |                                    |      |                                                                                                                                      |
| Heat shock factor ( <i>Hsf</i> )                                                                                           | LOF                     |                                                |                                       | En              |                 |                                    |      | Fujikake <i>et al.</i> (2008)                                                                                                        |
| Sin3A ( <i>Sin3A</i> )                                                                                                     | OE                      |                                                |                                       | Su <sup>3</sup> |                 |                                    |      | 1. Fernandez-Funez <i>et al.</i> (2000), 2. Steffan <i>et al.</i> (2001), 3. Bilen and Bonini (2007), 4. Branco <i>et al.</i> (2008) |
|                                                                                                                            | LOF                     |                                                | En <sup>1</sup>                       |                 |                 | En <sup>4</sup> , Su <sup>2a</sup> |      |                                                                                                                                      |
| Taranis ( <i>tara</i> ) <sup>b</sup>                                                                                       | OE                      | NC <sup>2</sup>                                | En <sup>1,2</sup>                     | NC <sup>2</sup> |                 | En <sup>3</sup>                    |      | 1. Fernandez-Funez <i>et al.</i> (2000), 2. Ghosh and Feany (2004), 3. Branco <i>et al.</i> (2008)                                   |
|                                                                                                                            | LOF                     |                                                | En <sup>1</sup>                       |                 |                 |                                    |      |                                                                                                                                      |
| Engrailed ( <i>en</i> )                                                                                                    | OE                      |                                                |                                       |                 |                 | Su                                 |      | Mugat <i>et al.</i> (2008)                                                                                                           |
| Tramtrack ( <i>ttk</i> )                                                                                                   | LOF                     |                                                |                                       |                 |                 | Su                                 |      |                                                                                                                                      |
| Armadillo ( <i>arm</i> )                                                                                                   | LOF                     |                                                |                                       |                 |                 | Su                                 |      | Kaltenbach <i>et al.</i> (2007)                                                                                                      |
| Crooked legs ( <i>crol</i> ) <sup>c</sup>                                                                                  | LOF                     |                                                |                                       |                 |                 | En<br>Su                           |      |                                                                                                                                      |
| Myocyte enhancer factor 2 ( <i>Mef2</i> )                                                                                  | LOF                     |                                                |                                       |                 |                 | Su                                 |      |                                                                                                                                      |
| Nipped-A ( <i>dTral</i> )                                                                                                  | LOF                     |                                                |                                       |                 | Su <sup>1</sup> | En <sup>2</sup>                    |      | 1. Latouche <i>et al.</i> (2007), 2. Zhang <i>et al.</i> (2010)                                                                      |
| TBP-associated Factor 10 ( <i>Taf10</i> )                                                                                  | LOF                     |                                                |                                       |                 | Su <sup>1</sup> |                                    |      |                                                                                                                                      |
| Skuld ( <i>skd</i> )                                                                                                       | LOF                     |                                                | En                                    |                 |                 | En                                 |      | Branco <i>et al.</i> (2008)                                                                                                          |
| C-terminal Binding Protein ( <i>dCtBP</i> )                                                                                | LOF                     |                                                | En <sup>1,2</sup>                     |                 |                 | NC <sup>2</sup>                    |      | 1. Fernandez-Funez <i>et al.</i> (2000), 2. Branco <i>et al.</i> (2008)                                                              |
| Debra ( <i>dbr</i> )                                                                                                       | 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 <i>et al.</i> (2004)                                                                                                            |
|                                                                                                                            | LOF                     |                                                | En                                    |                 |                 |                                    |      |                                                                                                                                      |
| <b>RNA-binding proteins</b>                                                                                                |                         |                                                |                                       |                 |                 |                                    |      |                                                                                                                                      |
| Muscleblind ( <i>mb1</i> )                                                                                                 | OE                      |                                                |                                       | En              |                 |                                    |      | Li <i>et al.</i> (2008)                                                                                                              |
| Mushroom-body expressed ( <i>mub</i> )                                                                                     | OE                      |                                                | Su <sup>1</sup>                       |                 | En <sup>2</sup> | En <sup>3</sup>                    |      | 1. Fernandez-Funez <i>et al.</i> (2000), 2. Latouche <i>et al.</i> (2007), 3. Branco <i>et al.</i> (2008)                            |
|                                                                                                                            | LOF                     |                                                | En <sup>3</sup>                       |                 |                 | NC <sup>3</sup>                    |      |                                                                                                                                      |
| Drosophila myeloid leukemia factor 1 ( <i>dmlf</i> )                                                                       | OE                      | Su                                             |                                       |                 |                 | Su                                 |      | Kazemi-Esfarjani and Benzer (2002)                                                                                                   |
| Pumilio ( <i>pum</i> )                                                                                                     | OE                      | NC <sup>2</sup>                                | En <sup>1</sup>                       | NC <sup>2</sup> |                 | En <sup>3</sup>                    |      | 1. Fernandez-Funez <i>et al.</i> (2000), 2. Ghosh and Feany (2004), 3. Branco <i>et al.</i> (2008)                                   |
|                                                                                                                            | LOF                     |                                                | NC <sup>1</sup> ,<br>Su <sup>3d</sup> |                 |                 | Su <sup>3</sup>                    |      |                                                                                                                                      |

Table 5 (contd.)

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



Table 5 (contd.)

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

Table 5 (contd.)

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

Table 5 (contd.)

| Genetic modifiers of polyQ toxicity (gene name)                               | Nature of mutant allele | Fly models of polyQ disease pathology examined |                                      |                                    |      |                 |      | References                                                                                 |
|-------------------------------------------------------------------------------|-------------------------|------------------------------------------------|--------------------------------------|------------------------------------|------|-----------------|------|--------------------------------------------------------------------------------------------|
|                                                                               |                         | PolyQ model                                    | SCAs                                 |                                    |      | HD              | SBMA |                                                                                            |
|                                                                               |                         |                                                | SCA1                                 | SCA3                               | SCA7 |                 |      |                                                                                            |
| Ataxin-2 ( <i>dAtx2</i> )                                                     | OE<br>LOF               | En <sup>1</sup>                                | En <sup>1,2</sup><br>Su <sup>2</sup> | En <sup>1</sup><br>Su <sup>3</sup> |      | NC <sup>2</sup> |      | 1. Ghosh and Feany (2004), 2. Al-Ramahi <i>et al.</i> (2007), 3. Lessing and Bonini (2008) |
| Ataxin-3 ( <i>hAtx3</i> )                                                     | OE                      |                                                | Su                                   | Su                                 |      | Su              |      | Warrick <i>et al.</i> (2005)                                                               |
| Huntingtin ( <i>dHtt</i> <sup>620aa</sup> ,<br><i>hHtt</i> <sup>548aa</sup> ) | OE<br>LOF               |                                                |                                      |                                    |      | Su<br>En        |      | Mugat <i>et al.</i> (2008)                                                                 |
| <b>Translational regulators</b>                                               |                         |                                                |                                      |                                    |      |                 |      |                                                                                            |
| Dappled ( <i>dpld</i> )                                                       | OE                      |                                                |                                      | Su                                 |      |                 |      |                                                                                            |
| Insulin growth factor II mRNA binding protein ( <i>Imp</i> )                  | OE                      |                                                |                                      | Su                                 |      |                 |      | Bilen and Bonini (2007)                                                                    |
| Orb2 ( <i>orb2/CG5735</i> )                                                   | OE                      |                                                |                                      | Su                                 |      |                 |      |                                                                                            |
| <b>Cytoskeletal organization and biogenesis</b>                               |                         |                                                |                                      |                                    |      |                 |      |                                                                                            |
| Chickadee ( <i>chic</i> )                                                     | OE                      | Su                                             |                                      |                                    |      | Su              |      | Burnett <i>et al.</i> (2008)                                                               |
| LaminC ( <i>LamC</i> )                                                        | LOF                     |                                                |                                      |                                    |      | En              |      |                                                                                            |
| Zipper ( <i>zip</i> )                                                         | LOF                     |                                                |                                      |                                    |      | En              |      |                                                                                            |
| Hu li tai shao ( <i>hts</i> )                                                 | LOF                     |                                                |                                      |                                    |      | Su              |      | Kaltenbach <i>et al.</i> (2007)                                                            |
| Peanut ( <i>pnut</i> )                                                        | LOF                     |                                                |                                      |                                    |      | Su              |      |                                                                                            |
| <b>Transport proteins</b>                                                     |                         |                                                |                                      |                                    |      |                 |      |                                                                                            |
| Embargoed ( <i>emb</i> )                                                      | OE<br>LOF               |                                                |                                      | Su<br>En                           |      |                 |      | Bilen and Bonini (2007)                                                                    |
| Rab5 ( <i>Rab5</i> )                                                          | OE<br>LOF               |                                                |                                      |                                    |      | Su<br>En        |      | Ravikumar <i>et al.</i> (2008)                                                             |
| Nup44A ( <i>Nup44A</i> )                                                      | OE                      |                                                | Su <sup>1</sup>                      |                                    |      | NC <sup>2</sup> |      | 1. Fernandez-Funez <i>et al.</i> (2000), 2. Branco <i>et al.</i> (2008)                    |
| Nuclear pore protein 160 ( <i>Nup160</i> )                                    | LOF                     |                                                |                                      |                                    |      | Su              |      | Doumanis <i>et al.</i> (2009)                                                              |
| Clathrin heavy chain ( <i>Chc</i> )                                           | LOF                     |                                                |                                      |                                    |      | Su              |      |                                                                                            |
| Unc-76 ( <i>Unc-76</i> )                                                      | LOF                     |                                                |                                      |                                    |      | En              |      | Kaltenbach <i>et al.</i> (2007)                                                            |
| Porin ( <i>porin</i> )                                                        | LOF                     |                                                |                                      |                                    |      | Su              |      |                                                                                            |
| Sec61α ( <i>CG9539</i> )                                                      | LOF                     |                                                |                                      | Su                                 |      | Su              |      | Kanuka <i>et al.</i> (2003)                                                                |
| <b>Miscellaneous</b>                                                          |                         |                                                |                                      |                                    |      |                 |      |                                                                                            |
| Yeast prion domain Sup35N                                                     | OE<br>LOF               |                                                |                                      | Su<br>En                           |      |                 |      | Li <i>et al.</i> (2007)                                                                    |
| CG7231                                                                        | LOF                     | En                                             | En                                   | En                                 |      |                 |      | Ghosh and Feany (2004)                                                                     |
| CG1109                                                                        | LOF                     |                                                |                                      |                                    |      | Su              |      |                                                                                            |
| CG5537                                                                        | LOF                     |                                                |                                      |                                    |      | Su              |      | Doumanis <i>et al.</i> (2009)                                                              |
| G protein αi subunit 65A ( <i>G-ia65A</i> )                                   | OE<br>LOF               |                                                |                                      |                                    |      | En<br>Su        |      |                                                                                            |
| Short stop ( <i>shot</i> )                                                    | LOF                     |                                                |                                      |                                    |      | En              |      |                                                                                            |
| CG12455                                                                       | OE<br>LOF               |                                                |                                      |                                    |      | En<br>Su        |      | Kaltenbach <i>et al.</i> (2007)                                                            |
| Phosphoglucose isomerase ( <i>Pgi</i> )                                       | LOF                     |                                                |                                      |                                    |      | En              |      |                                                                                            |

Table 5 (contd.)

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

<sup>a</sup>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*<sup>08269</sup> was used and the overexpressing EP allele used in reference 3 was *Sin3A*<sup>B9-E</sup>.

<sup>b</sup>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.

<sup>c</sup>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*<sup>EY08953</sup> and *P(PZ)crol*<sup>04418</sup>, respectively) were used by Kaltenbach *et al.* (2007).

<sup>d</sup>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*<sup>13</sup> allele, Branco *et al.* (2008) used the *pum*<sup>bem</sup> allele.

<sup>e</sup>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* transposon is inserted in sense orientation to *Rpd3*, this allele does not overexpress *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.

<sup>f</sup>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*<sup>04556</sup>, while Pallos *et al.* (2008) did not specify the loss-of-function allele used in their study.

<sup>g</sup>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.

<sup>h</sup>The opposing results obtained with the same *Khc* mutant allele (*Khc*<sup>8</sup>) 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.

<sup>i</sup>The *hsra*<sup>05241</sup> 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.

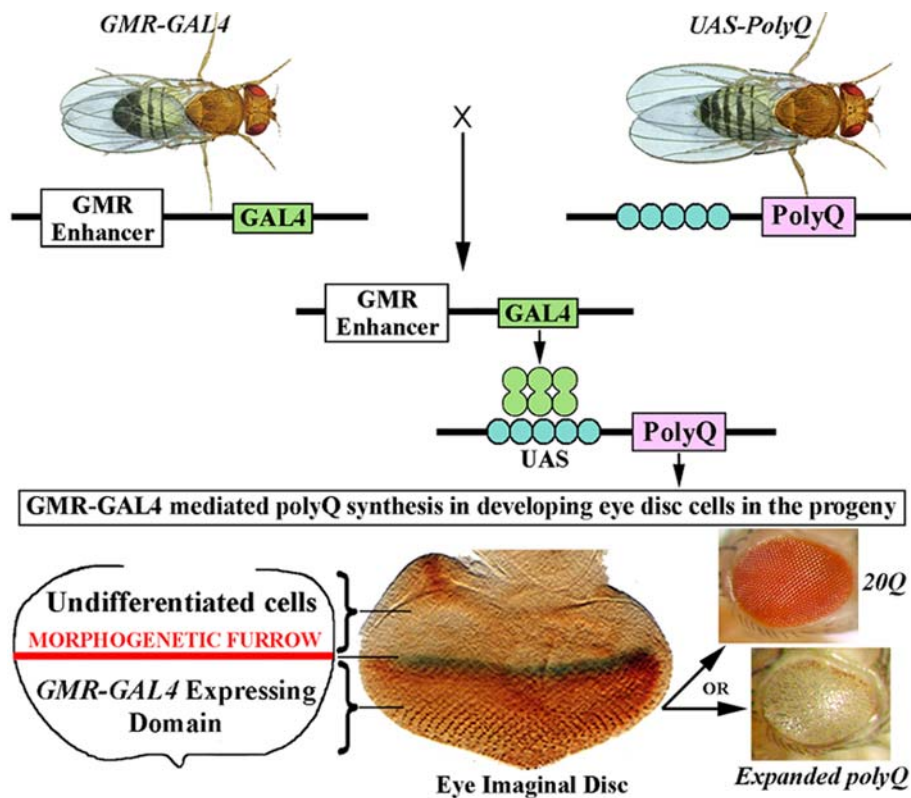
All these may result in wider alterations in expression of genes, including those that are critical for functioning of specific neurons, so that the grossly disrupted protein homeostasis triggers the affected neuron's death. However, since a variety of cellular pathways (see section on Molecular mechanisms) 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 Alzheimer'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 introducing 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 through 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<sub>1</sub> 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.

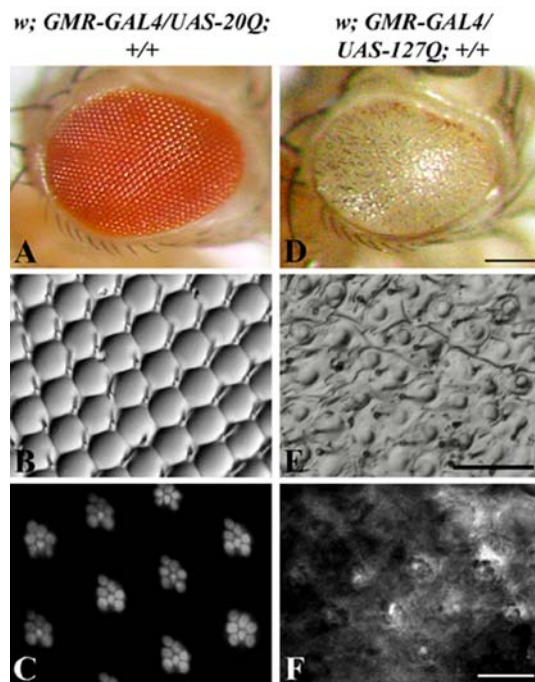
Global unbiased *in vivo* genetic interaction screens using a variety of gene mutations and conditional expression systems (Brand and Perrimon 1993; Chou and Perrimon 1996; Rorth 1996; Morin *et al.* 2001; Adams and Sekelsky 2002; Johnston 2002; Kuttenukeuler and Boutros 2004; Evans *et al.* 2009; <http://www.flybase.org>), 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

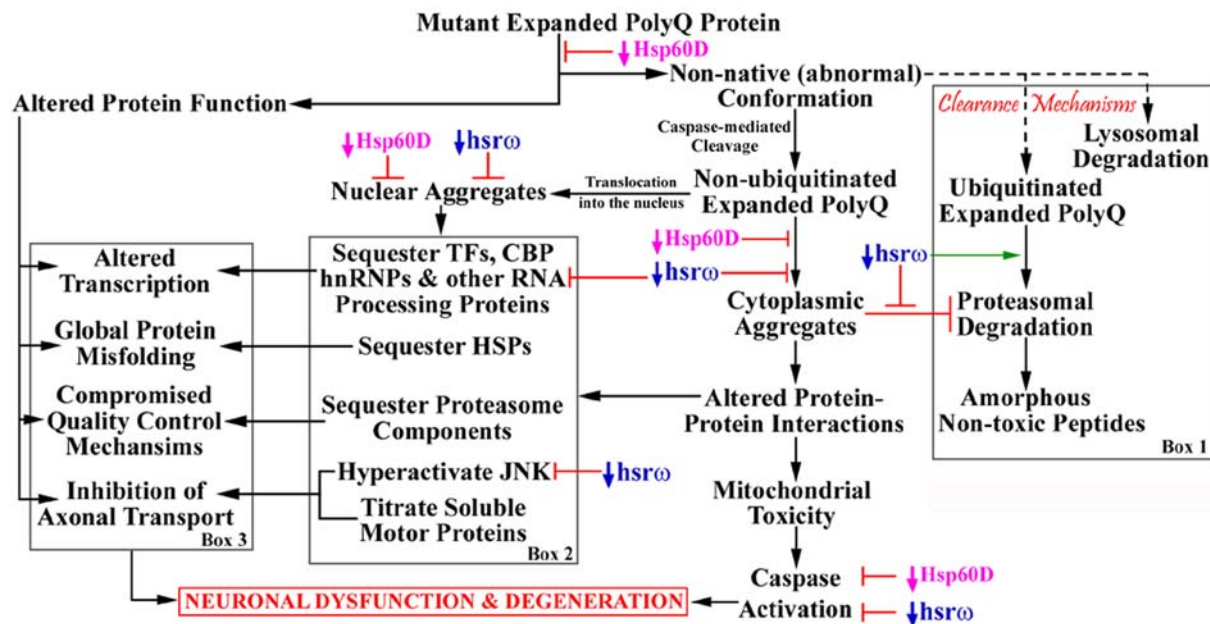


**Figure 2.** The retinal degeneration caused by *GMR-GAL4* driven targeted expression of the expanded polyQ protein can be easily monitored by external morphology of adult eyes (A, D), or nail-polish imprints of the eye surface (Arya and Lakhotia 2006) (B, E) or the pseudopupil (corneal neutralization, 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  $\mu$ m.

(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 hsr $\omega$  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 hsr $\omega$  transcripts or the hnRNPs associated with it, overabundance of the hsr $\omega$  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 hsr $\omega$  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 hsr̄ transcripts inhibit polyQ toxicity are also shown. A downwardly pointed arrow indicates RNAi-mediated reduction in levels of the Hsp60D protein (red) or hsr̄ 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 hsr̄ 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; McCamp-

bell *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 Lakhota 2010) also have shown that altered *hsr $\omega$*  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 chaperones and other protein quality control mechanisms 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 targeted 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 *hsw* gene in fly models could be the fact that overabundance of these transcripts itself causes proteasomal dysfunction in the cell; interestingly, reduction in *hsw* 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 hunt-

ingtin 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 ubiquitylation 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; Sinadinov *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 (RIM2 $\alpha$  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 elicit 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<sup>421</sup> 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<sup>260</sup> 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 14-3-3 $\epsilon$  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 14-3-3 $\zeta$  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<sub>2</sub>-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* hsr $\omega$  transcripts prevents activation of JNK (Mallik and Lakhotia 2009b) which may also contribute to suppression of the polyQ damage following hsr $\omega$ -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 possible 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 hsr $\omega$  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 hsr $\omega$  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 (Wytenbach *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 endogenous 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 parallelly 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 hsr $\omega$  transcripts in suppressing polyQ pathology through multiple paths (Mallik and Lakhotia 2009a, 2010; figure 3) also exemplify the networking effects. In this con-

text, it will be interesting to examine if the non-coding human sat III transcripts can also modulate polyQ pathogenesis since the hsr $\omega$  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.

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