

## Deciphering the Enigma of Human Poly(Q) Disorders: Contribution of *Drosophila melanogaster*

M Dhruva Singh, Soram Idiyasan Chanu, Surajit Sarkar

M Dhruva Singh, Department of Biochemistry and Molecular Biology, Pennsylvania State University, University Park, PA 16802, USA  
Soram Idiyasan Chanu, Surajit Sarkar, Department of Genetics, University of Delhi South Campus, Benito Juarez Road, Dhaula Kuan, New Delhi-110 021, India

Correspondence to: Surajit Sarkar, Department of Genetics, University of Delhi South Campus, Benito Juarez Road, Dhaula Kuan, New Delhi-110 021, India.

Email: [sarkar@south.du.ac.in](mailto:sarkar@south.du.ac.in)

Telephone: +91-11-24157331 Fax: + 91-11-24112761

Received: August 27, 2015 Revised: December 6, 2015

Accepted: December 11, 2015

Published online: March 1, 2016

### ABSTRACT

Poly(Q) disorders includes neurodegenerative disorders associated with irreversible loss of specific neurons in adult brain. The causative factor has been identified as the abnormal expansion of CAG repeats in protein coding region of the gene which leads to formation of protein Inclusion Bodies (IBs) and altered cellular physiology. The molecular pathogenesis of poly(Q) disease involves protein mis-folding, aggregate formation, blockage of axonal transport, mitochondrial dysfunction and global transcriptional dysregulation. Formation of inclusion bodies is a hallmark and common characteristic feature of all poly(Q) disorders. In view of limitation attached with human genetics, *Drosophila* has emerged as an excellent system to model the human neurodegenerative disorders due to availability of flexible yet powerful genetic tools and owing to the similarity between some of the fundamental cellular processes with humans. *Drosophila* system has been extensively utilized not only to decipher the mechanistic detail of diseases pathogenesis but also to screen several genetic and chemical modifiers which could potentially help in designing novel therapeutic strategies. The *Drosophila melanogaster*, thus, expected to contribute meaningfully towards novel discoveries to design novel therapeutic strategies to combat the devastating human neurodegenerative disorders.

© 2016 ACT. All rights reserved.

**Key words:** Polyglutamine diseases; Inclusion bodies; *Drosophila*

Singh MD, Chanu SI, Sarkar S. Deciphering the Enigma of Human Poly(Q) Disorders: Contribution of *Drosophila melanogaster*. *International Journal of Neurology Research* 2016; 2(1): 216-223  
Available from: URL: <http://www.ghrnet.org/index.php/jnr/article/view/1361>

### INTRODUCTION

Polyglutamine [poly(Q)] diseases represent a group of neurodegenerative disorder which exhibit some common clinical symptoms and share comparable mechanism of molecular pathogenesis. Some of the common symptoms of poly(Q) disorders include loss of body coordination, memory, cognitive thinking and difficulty in speech<sup>[1]</sup>. There are nine of the neurological diseases: Spinal and bulbar muscular atrophy (SBMA), Huntington's disease (HD), six of the Spinocerebellar ataxias (SCA1, 2, 3, 6, 7 and 17) and Dentatorubral pallidoluysian atrophy (DRPLA) which are commonly grouped as poly(Q) diseases<sup>[2,3,4]</sup>. In general, poly(Q) disorders are late onset, progressive in nature and cause degeneration of specific population of neurons in the brain as per the characteristics of each disease type<sup>[2]</sup>. For instance, in HD basal ganglia and cortical neurons are highly affected whereas purkinje cells in cerebellum are mostly affected in case of SCA3. Most forms of the poly(Q) disorders are dominantly inherited except spinal and bulbar muscular atrophy which shows X-linked recessive inheritance pattern<sup>[5]</sup>.

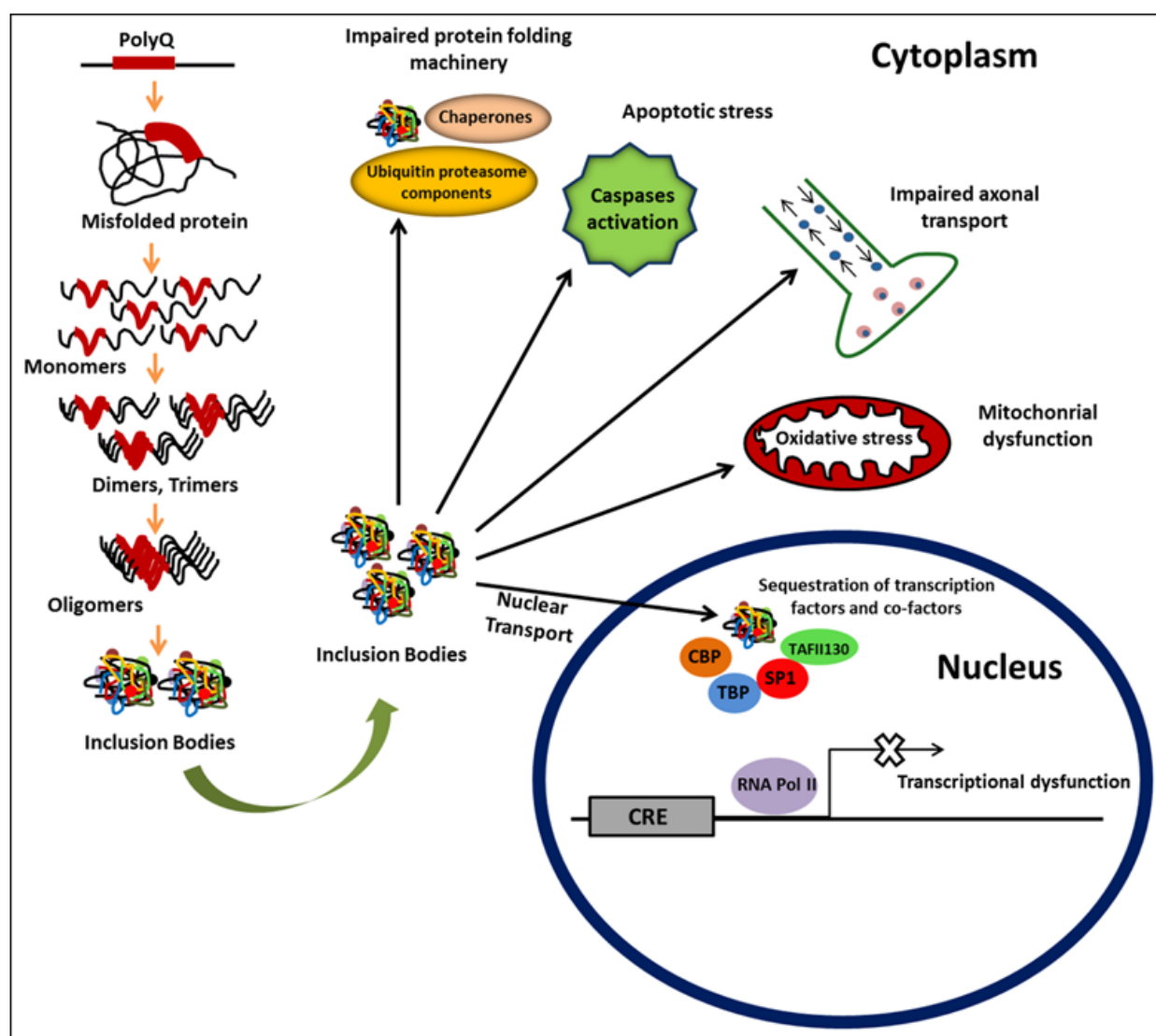
### MOLECULAR MECHANISM UNDERLYING THE PATHOGENESIS OF POLY(Q) DISORDERS

The first case of poly(Q) disease was reported by George Huntington in 1872 and named "chorea" due to dance like movement of patient suffering from this neurodegenerative condition<sup>[6]</sup>. For several years the underlying mechanism and cause of disease remain obscure but the advancement in biological sciences particularly in later part of twentieth century paved way to the understanding of predominant

basis of the onset of disease. It was found that expansion of CAG repeat in the coding sequence of androgen receptor was associated with X-linked neuromuscular disorder, commonly known as Spinal and Bulbar Muscular Atrophy (SBMA) or Kennedy's disease<sup>[7]</sup>. Subsequently, genetics of Huntington's disease (HD) was also deciphered and expansion of CAG repeat was established as the primary cause<sup>[8]</sup>. Thereafter, subsequent investigations in this area led to identification of 7 more of diseases caused by expansion triplet repeat other than CAG. For instance, fragile X syndrome (FRAXA) and Fragile X-associated tremor/ataxia syndrome (FXTAS) were identified to be associated with expansion of CGG repeats on the X chromosome<sup>[9,10]</sup>. On other hand Fragile XE mental retardation (FRAXE) is caused by expansion of GCC on X chromosome<sup>[11]</sup>. Myotonic dystrophy (DM) and Spinocerebellar ataxia type 8 (SCA8) develop due to abnormal expansion of CTG repeats on the respective genes, whereas FRDA (Friedreich's ataxia) is caused by expansion of GAA repeat in the frataxin gene located on X chromosome<sup>[12]</sup>. Interestingly, Spinocerebellar ataxia type 12 (SCA12) is also caused by the expansion of CAG repeats in the untranslated region of the gene, and therefore, it is not considered as a poly(Q) diseases<sup>[13]</sup>.

## FORMATION OF PROTEIN INCLUSION BODIES (IB) AND IMPAIRMENT OF TRANSCRIPTIONAL MACHINERY

Expansion of CAG repeats leads to major changes in cellular physiology such as formation of protein Inclusion Bodies (IBs), defects in protein folding machinery, impairment of transcriptional machinery, defect in axonal transport system, cellular apoptosis, mitochondrial defects and dysregulation of intracellular  $Ca^{2+}$  homeostasis (Figure 1). As depicted in Figure 1, expansion of CAG repeats leads to elongation of glutamine tract of the protein and alters its native conformation. The mis-folded protein interacts with other proteins comprising glutamine repeats and interrupts the molecular function(s) of the target proteins. Subsequently, such interaction results into formation of microscopic molecular aggregates commonly known as inclusion bodies<sup>[14,15]</sup>. The kinetics of the polymerization of poly(Q) proteins involve prolong lag period which facilitates formation of the nucleus of protein aggregate followed by extension period that allows rapid incorporation of monomers to



**Figure 1** Schematic representation of the molecular events leading to manifestation of poly(Q) diseases. Expansion of CAG repeats causes misfolding of target protein and subsequent formation of protein oligomers and Inclusion Bodies (IBs). Formation of inclusion bodies leads to defects in protein folding machinery and axonal transport system, mitochondrial dysfunction and cellular apoptosis. Transportation of IBs in nucleus results in sequestration of various transcription factors and impairment of transcriptional machinery. (CBP- CREB binding protein; TBP- TATA binding proteins; Sp1- Specificity protein 1; TAFII130- Transcription factor II 130).

form larger aggregates<sup>[16,17]</sup>. The propensity of the mutant proteins to form aggregate arises from their ability of self-interaction which is achieved above certain pathological threshold limit<sup>[18-20]</sup>. Mutated huntingtin protein (Htt) form  $\beta$ -pleated sheet conformations in-vitro similar to amyloid with a fibrillar structure<sup>[18]</sup>. Above the threshold limit, the poly(Q) protein are suggested to form polar zipper and associate with each other by hydrogen bonds<sup>[21]</sup>. Intriguingly, proteins with endogenous poly(Q) repeats confers instability in the conformation after acquiring additional poly(Q) in the existing stretch<sup>[19]</sup>.

In neuronal cell culture, the transfection of mutant Htt resulted in formation of IBs depending upon the length of poly(Q) repeats; with longer repeats forming more number of inclusion bodies<sup>[22,23]</sup>. Transfection studies suggest that truncated mutant Htt translocate into the nucleus rapidly and exhibit more tendencies to form aggregates than the full length of the protein<sup>[24,25]</sup>. The overexpression of both, full length and truncated form of ataxin-3 protein results in aggregate formation, cellular toxicity and neurodegeneration<sup>[26-29]</sup>. In rat striatal neurons, addition of expanded poly(Q) repeats with an inert protein such as poly(Q)-green fluorescent protein (GFP) fusion construct resulted in formation of inclusion bodies demonstrating that the toxic moiety lies in the elongated poly(Q) tract<sup>[30]</sup>. Interestingly, in all the cases of murine model of Huntington's disease, the N-terminal fragment of the mutated Htt was always found to be associated with protein aggregates. Subsequent studies have demonstrated that co-expression of the N-terminal of Htt protein along with its full length counterpart further accelerates the formation of inclusion bodies suggesting a clear role of N-terminal fragments in formation of protein aggregate<sup>[31-33]</sup>.

Interestingly, similar kinds of protein aggregates have been observed in the brain tissues of SCA1 patient and cell lines expressing mutant ataxin-1<sup>[34]</sup>. Likewise, transgenic mice expressing mutant ataxin-1 in the cerebellar region develop characteristics of ataxia phenotype with the formation of inclusion bodies<sup>[35]</sup>. In majority of poly(Q) disorders, the mis-folded proteins present in nuclear inclusion bodies are highly ubiquitinated<sup>[27,36]</sup>. Occurrence of the ubiquitinated form of mutant ataxin-3 aggregates have also been reported in neurons of SCA3 patients<sup>[37]</sup>. Similarly, ubiquitinated inclusions are also found in brain tissues of SCA2 and SCA7 patients<sup>[38,39]</sup>. In SCA17, the nuclear inclusions associated with ubiquitin and TATA binding proteins (TBP) are found in putamen and frontal cortex<sup>[40]</sup>. In contrast, inclusion bodies in SCA6 patients were not ubiquitinated and were relatively cytoplasmic<sup>[41]</sup>. In spite of that, degeneration of Purkinje cells are quite prevalence in SCA6 patients and mutant CACNA1A transfected cell lines developed perinuclear inclusions resulting in apoptosis<sup>[41]</sup>.

The poly(Q) repeats alters the function of calcium channel which could be one of the major factors driving the cellular degeneration<sup>[42]</sup>. Analysis of the brain cells of patients suffering from poly(Q) disorder revealed that in addition to the respective disease protein(s) the IBs were also comprised of several essential proteins/factors<sup>[43]</sup>. It appears that sequestration of essential proteins/ factors in IBs from their respective sites leads to altered homeostasis and cellular toxicity. The IBs gradually increase in size and grow as high molecular weight protein body and subsequently get transported into the nucleus by an unknown mechanism<sup>[43]</sup>. After reaching into the nuclear compartment it sequesters several transcription factors and results in alteration of transcriptional activity of the cell<sup>[43,44,45]</sup>.

Formation of inclusion bodies is a hallmark and common characteristic feature of all poly(Q) disorders<sup>[46,28]</sup>. Interestingly, in most of the poly(Q) diseases the flanking DNA sequence which

surround the CAG repeat domain largely contribute on the dynamics of inclusion body formation<sup>[47,48]</sup>. In Huntington's disease, first exonic sequence which flanks the CAG repeats is crucial for the formation and regulation of inclusion bodies<sup>[49]</sup>. Similarly, in SCA3, relative position of poly(Q) stretch and a highly conserved domain, Josephin determines the kinetics of inclusion body formation<sup>[47]</sup>.

Poly(Q) proteins exhibit intriguing characteristics of the ability to associate with other proteins harboring short non-pathogenic glutamine repeats by homotypic glutamate interactions<sup>[16,50]</sup>. Moreover, proteins with expanded poly(Q) repeats show tendency to accumulate in nucleus where presence of highly concentrated solutes allows it to interact with many transcription factors and cofactors<sup>[51,52]</sup>. In affected individual, the IBs grow in size with the progression of age and translocate themselves into nucleus. Inside the nucleus they bind to many more of glutamine rich transcription factors such as cAMP response element binding protein binding protein (CBP), TBP (TATA binding protein), TAFII130 and Specificity protein 1 (sp1)<sup>[53-58]</sup>. The binding and sequestration of transcription factors make a significant negative impact on the rate of cellular transcription. One of the major factors that caught great attention is CBP which is a key regulator of transcription<sup>[53-58]</sup>.

CBP (CREB binding protein) is one of the key transcription factors which interacts with several other factors and regulate transcription of crucial genes<sup>[59-61]</sup>. CBP core protein has histone acetyltransferase (HAT) activity which facilitates histone acetylation and opening of the histone chain around the DNA to make the DNA readily available for RNA polymerase<sup>[57,62]</sup>. In Huntington's disease, the mutant Htt protein sequesters CBP into the inclusion bodies<sup>[53-55]</sup>. Moreover, CBP protein has also been found to localise with the inclusion bodies in post-mortem tissues, cell culture and poly(Q) disease models<sup>[63]</sup>. The sequestration of CBP by inclusion bodies leads to hypoacetylation and undermines the transcription activity depriving cell of the survival factors and ultimately cell is destined for apoptosis. The role of defective histone acetylation on poly(Q) pathogenesis is substantially documented by the fact that inhibitors of Histone deacetylase such as Suberoylanilide hydroxamic acid (SAHA), Trichostatin A (TSA) and Sodium butyrate alleviates the neurodegeneration in animal models and cell lines<sup>[53,64]</sup>.

In addition to the compromised function of several transcription factors as discussed above, mice model of SCA1 shows reduced transcriptional efficiency of six additional neuronal genes which are involved in signal transduction and calcium homeostasis<sup>[65]</sup>. The down regulations of these genes are evident in Purkinje cells much before the detectable phenotype of SCA1<sup>[65]</sup>. In case of SCA3, several inflammatory response genes show up-regulation while expressions of several cell-surface receptor genes are repressed<sup>[66]</sup>. It appears that the loss of normal function of ataxin-3 protein together with the toxic gain of functions by mutant ataxin-3 contribute to the disease pathogenesis<sup>[66]</sup>.

## MODELLING OF HUMAN POLY(Q) DISORDERS IN *DROSOPHILA MELANOGASTER*

*Drosophila* has emerged as an excellent system to model the human neurodegenerative disorders owing to the similarity between some of the fundamental cellular processes with humans and the fact that flies and humans share many structurally and functionally related gene families. It has been found that ~75% of the human disease causing genes have functional *Drosophila* homologues showing

overall identity ranging from 40% between homologues to as much as 80-90% between conserved functional domains<sup>[67,68]</sup>. Presence of extensive yet easily analyzable form of nervous system also makes it suitable for disease modelling. Several added advantages that *Drosophila* offers over other conventional model systems such as yeast, *C. elegans* and human cell cultures include its genetic tractability due to a short generation time and life-span, ease of maintaining large population within the confines of a laboratory, high fecundity, presence of balancer chromosomes, a well worked out developmental processes and anatomy, absence of meiotic recombination in males, relatively small genome contained on four completely sequenced and annotated chromosomes and ability to perform large-scale genetic screens to identify potential modifiers of known disease phenotypes<sup>[69]</sup>. In addition, availability of a large number of various mutant lines available at several *Drosophila* stock centres also makes it a popular model organism. The most advantageous aspect of poly(Q) disease modelling in *Drosophila* lies in the easy screening of genetic and chemical modifiers which subsequently help in designing novel therapeutic strategies.

Adult flies with their sophisticated brain and nervous system organized into specialized neural centres are capable of exhibiting complex behaviours such as learning and memory, much like the human brain. Interference in such well-coordinated motor behaviours serves as a highly sensitive readout of neuronal dysfunction and subsequent genetic analysis. Moreover, fly models are extremely useful while carrying out pharmacological screens for identifying novel therapeutic drug targets as they lack a blood brain barrier that can prevent the access of the drug to the central nervous system tissues<sup>[70]</sup>. Moreover, the response towards many drugs that act within the CNS is similar to the effects observed in mammalian systems<sup>[70]</sup>. The life cycle of *Drosophila* consist of four stages: embryonic, larval, pupal and adult stage. The larval stage can be further subdivided into 3 stages namely 1st instar stage, second instar stage and third instar stage. The neurogenesis in *Drosophila* starts around 5 hours after egg laying<sup>[71,72]</sup>. The Central nervous system of *Drosophila* is composed of neurons and glial cells. The neurons are tightly associated with glial cells by a structure known as commissure<sup>[73]</sup>. In *Drosophila* various adult organs and anatomical structure develops from 10 pairs of imaginal discs and the genital disc.

One of the most valuable tools that *Drosophila* bestows for neurodegenerative disease modelling is the use of its compound eyes for easy and direct evaluation of neurodegeneration. The adult *Drosophila* eye is developed from the rudimentary organ called as eye imaginal disc<sup>[74,75]</sup>. The eyes are not indispensable for the survival and presence of regular hexagonal lattice of each ommatidium gives easily recognizable phenotype even with the naked eye<sup>[76]</sup>. Expression of pathogenic poly(Q) protein containing 108 CAG repeat in eye perfectly mimics the degeneration of neuronal tissues as found in post-mortem brain of HD patient<sup>[72]</sup>. Interestingly, the development of disease could be traced from the third instar larval eye imaginal disc. The eye disc corresponds to the posterior region of a common imaginal disc which develops into adult eye and the anterior part develops to antennal structure. The morphogenetic furrow is the area where intense cell division and neuronal differentiation takes place<sup>[74,75]</sup>.

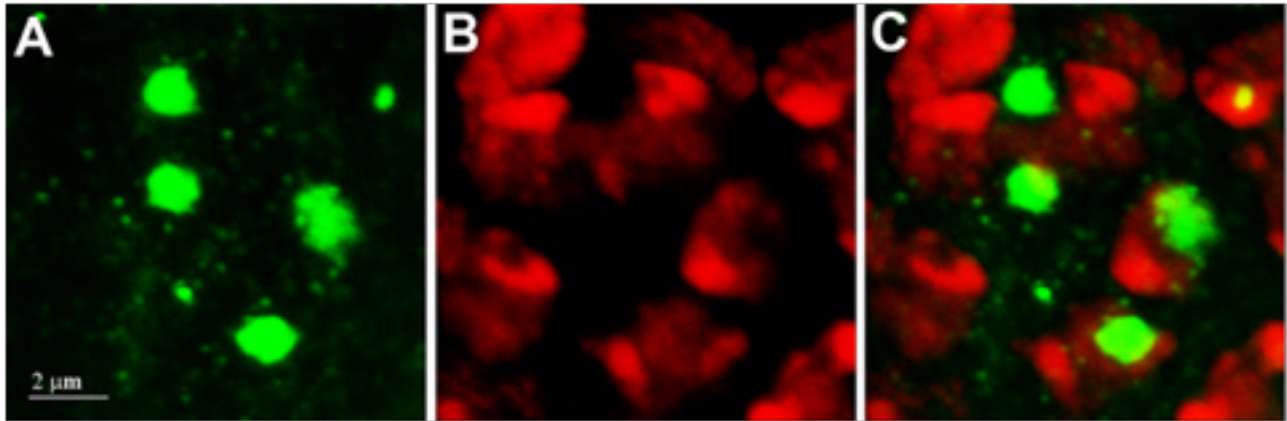
The compound eye of *Drosophila* is composed of individual unit called ommatidium. Each ommatidium is composed of different units of cells i.e. 2 primary cells, 6 secondary cells, 3 tertiary cells, 8 photoreceptors cells and 4 cone cells. The 7th photoreceptors cells lie above the 8th photoreceptor cells, and therefore, not visible at the same visual focal plane<sup>[77]</sup>. In addition to eye tissues, *Drosophila*

brain is also used as model organ in many of the neurodegenerative disorders. *Drosophila* adult brain is consists of about 10,000 neurons, organised into regions with specialized functions such as learning, olfaction, memory and vision<sup>[78,79]</sup>. The mushroom body is highly advanced information processing centre composed of about 2,500 neurons with densely packed cell bodies know as Kenyon cells<sup>[80]</sup>. Mushroom body is homologous to the hippocampus of human brain and involved in olfactory memory and learning<sup>[80]</sup>.

Several approaches have been exploited so far to study neurodegeneration in *Drosophila*. Initially, the classical forward genetic approach was utilized wherein random mutations were created to select a phenotype showing brain degeneration which was then analysed with the aim of identifying the gene responsible for the mutant phenotype<sup>[81-83]</sup>. However, such screens either remain incomplete due to ignorance of redundant loci and epigenetic effects or difficult to undertake due to non-measurable phenotypes. Additionally a classical genetic approach takes significantly longer. Therefore, alternative reverse genetics-based approaches have been undertaken to elucidate pathogenic pathways and remedial strategies of neurodegenerative disorders. Moreover, the dominant nature of most of the mutant alleles of poly(Q) disorders suggested that perhaps these diseases could be modelled in *Drosophila* by introducing the mutant allele into the fly genome and producing transgenics. A binary system was used for this purpose which benefits from the property of the yeast transcriptional activator GAL4 protein to bind to a small upstream activator sequence (UAS) of genes to drive transcription. Foreign genes can be cloned into transposable P-element vectors under the control of a UAS sequence and crossed with transgenic flies expressing the yeast Gal4 gene in a tissue-specific manner<sup>[84]</sup>. In this context it is important to note that the transgene remains silent until crossed with a desired Gal4 line. Most commonly used promoters in case of neurodegenerative disorders include pan-neuronal elav-Gal4 driver<sup>[85,86]</sup> and eye specific glass multiple reporter element (gmr) driver<sup>[87,88]</sup>. Neurodegeneration can be monitored either by measuring the loss of visible photoreceptors neurons in the eyes or by evaluating motor function by the climbing assay or by checking for the lethality of the organism. There are many advantages of using the UAS-Gal4 system for transgenic expression: first, the conditional nature of expression which prevents deleterious effects of constitutive expression of transgene; second, a single UAS transgenic line can be ectopically expressed in many different tissue types, depending upon the GAL4 line being used; third, the system is capable of yielding much higher levels of expression of the gene of interest as compared to direct promoter-fused transgenes.

Expression of mutant form of AR, ataxin-1, ataxin-3 and Htt produces neuropathological condition reminiscent of human form<sup>[28,89,90]</sup>. The severity of poly(Q) diseases was also found to be dependent upon the length of poly(Q) repeat as found in human disease conditions. Moreover the progressive nature was also established in flies as the defects are observed only in mature larvae, pupal stage or in adult stages. The earliest SCA1 models generated in *Drosophila* were SCA1Q82 and SCA1Q30 transgenic lines<sup>[89]</sup>. The overexpression of SCA1Q82 produced a strong phenotype with complete degeneration of retinal structures and neuronal cell masses. Comparatively, expression of SCA1Q30 produces milder phenotype with partial degeneration of retinal structures. Interestingly, temperature mediated increased expression of SCA1Q30 was found to enhance the degenerative phenotypes<sup>[89]</sup>. Moreover, overexpression of wild type ataxin-1 was also found to induce neurodegeneration<sup>[89]</sup>.

Targeted expression of truncated form of SCA3 containing 78 poly(Q) repeats induces neuronal degeneration in *Drosophila* eye<sup>[28]</sup>.



**Figure 2** (A) Formation of Inclusion Bodies (IBs) in eye imaginal disc of GMR-Gal4 driven UAS-SCA3trQ78(S) third instar *Drosophila* larvae as detected by HA (Green) staining. (B) DAPI staining showing positioning nucleus. (C) Merged view of image A and B.

However, expression of normal length of 27 poly(Q) repeats did not produce any degenerative phenotype<sup>[28,91]</sup>. As depicted in Figure 2, formation of inclusion bodies are equally evident in targeted *Drosophila* tissues as found in affected human brain. Occurrence of such inclusion bodies in *Drosophila* tissues was accompanied by enhanced cell death indicating implication of inclusion bodies in progression of neurodegeneration. The designing of *Drosophila* HD model was done by using truncated form of htt gene containing 1st exon with the expanded form of CAG repeats<sup>[92]</sup>. The expression of HttQ93 produces progressive loss of photoreceptors with subsequent aging<sup>[92]</sup>. The transgenic stocks containing Q0 and Q128 repeats were generated using the N-terminal fragments of Htt gene with first 588 amino acids along with the caspase cleavage site. Targeted expression of Q0 in *Drosophila* eye did not produce any morphological defect, whereas the expression of Q128 reduces the photoreceptors depolarisation and loss of synaptic transmission<sup>[93]</sup>. The aggregates were also formed along with the axons which carry the synaptic stimulus to the CNS<sup>[93]</sup>.

Modelling of human poly(Q) and other neurological disorders in model organisms provides an ultimate tool for devising novel therapeutic strategies to suppress progression or manifestation of disease phenotypes<sup>[94,95,96]</sup>. Moreover, analysis of behavioural changes and neuropathological features could be studied in greater detail as the cellular processes of disease pathogenesis are completely recapitulated in most of the model system. It also facilitates in identification of modifier genes and pathways which could be potentially utilized as drug targets. The *Drosophila* model system is one of the oldest as well as one of the widely used model system for devising the testing paradigms such as genetic screens and chemical screens of poly(Q) disorders. Several genetic and chemical modifiers of human poly(Q) disorders have been identified utilizing *Drosophila* disease models. Moreover, some of such modifiers are being investigated and potential drug targets and to design novel therapeutic approaches.

## CONCLUDING REMARKS

In brief, *Drosophila* provides a powerful genetic tool to investigate various aspects of human neurodegenerative poly(Q) disorders. However, given an exceptional model system and the varieties of tools available to study modified phenotypes, the precise mechanism which leads poly(Q) induced toxicity still stands as a debatable issue. Therefore, efforts should be given to unravel the molecular nature of the neurotoxic species arises in each disease type, and to decipher

the key neuronal functions which get altered due to accumulation of toxic protein aggregates. Moreover, parallel comprehensive studies should be planned for not only to decipher the mechanistic details of disease pathogenicity but also to perform large scale screening for the designated molecules and genetic modifiers to assess their potential as the modifier of disease phenotypes, which could be verified further for their reproducibility in higher model systems. The *Drosophila melanogaster* has, thus, proved its worth in the field of neurobiology and will continue to contribute meaningfully towards novel discoveries to combat the devastating human neurological disorders.

## ACKNOWLEDGEMENTS

Research work in laboratory is supported by grants from the Department of Biotechnology (DBT), Government of India, New Delhi; DU/DST-PURSE scheme and Delhi University R & D fund.

## CONFLICT OF INTEREST

None of the authors have a conflict of interest with regard to the work in the manuscript.

## REFERENCES

- Cummings CJ, Zoghbi HY. Trinucleotide repeats: mechanisms and pathophysiology. *Annu Rev Genomics Hum Genet* 2000; 1: 281-328.
- Everett CM, Wood NW. Trinucleotide disease and neurodegenerative disease. *Brain* 2004; 127: 2385-2405.
- Landles C, Bates GP. Huntingtin and the molecular pathogenesis of Huntington's disease. *Fourth in molecular medicine review series. EMBO Rep* 2004; 5: 958-963.
- Paulson H. Machado-Joseph disease/spinocerebellar ataxia type 3. *Handb Clin Neurol* 2012; 103: 437-449.
- Katsuno M, Adachi H, Minamiyama M, Waza M, Tokui K, Banno H, Suzuki K, Onoda Y, Tanaka F, Doyu M, Sobue G. Reversible disruption of dynactin 1-mediated retrograde axonal transport in polyglutamine-induced motor neuron degeneration. *J Neurosci* 2006; 26: 12106-12117.
- Huntington G. On Chorea. *Med Surg Rep* 1872; 26: 317-321.
- La Spada AR, Wilson EM, Lubahn DB, Harding AE, Fischbeck KH. Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. *Nature* 1991; 352: 77-79.
- Huntington's disease Collaborative Research group. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell* 1993; 72: 971-983.

9. Verkerk AJ, Pieretti M, Sutcliffe JS, Fu YH, Kuhl DPA, Pizzuti A, Reiner O, Richards S, Victoria MF, Zhang F, Eussen BE, van Ommen GB, Blonden LAJ, Riggins GJ, Chastain JL, Kunst CB, Galjaard H, Caskey CT, Nelson DL, Oostra BA, Warren ST. Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. *Cell* 1991; 65: 905-914.
10. Hagerman PJ, Hagerman RJ. The fragile-X premutation: a maturing perspective. *Am J Hum Genet* 2004; 74: 805-816.
11. Gecz J, Gedeon AK, Sutherland GR, Mulley JC. Identification of the gene FMR2, associated with FRAXE mental retardation. *Nat Genet* 1996; 13: 105-8.
12. Campuzano V, Montermini L, Moltò MD, Pianese L, Cossée M, Cavalcanti F, Monros E, Rodius F, Duclos F, Monticelli A, Zara F, Cañizares J, Koutnikova H, Bidichandani SI, Gellera C, Brice A, Trouillas P, De Michele G, Filla A, De Frutos R, Palau F, Patel PI, Di Donato S, Mandel JL, Coccozza S, Koenig M, Pandolfo M. Friedreich's ataxia: autosomal recessive disease caused by an intronic GAA triplet repeat expansion. *Science* 1996; 271: 1423-1427.
13. Cholfin JA, Sobrido MJ, Perlman S, Pulst SM, Geschwind DH. The SCA12 mutation as a rare cause of spinocerebellar ataxia. *Arch Neurol* 2001; 58: 1833-1835.
14. Rüb U, de Vos RA, Brunt ER, Sebestény T, Schöls L, Auburger G, Bohl J, Ghebremedhin E, Gierga K, Seidel K, den Dunnen W, Heinsen H, Paulson H, Deller T. Spinocerebellar ataxia type 3 (SCA3): thalamic neurodegeneration occurs independently from thalamic ataxin-3 immunopositive neuronal intranuclear inclusions. *Brain Pathol* 2006; 16: 218-227.
15. Shao J, Diamond MI. Polyglutamine diseases: emerging concepts in pathogenesis and therapy. *Hum Mol Genet* 2007; 16: R115-R123.
16. Ellisdon AM, Pearce MC, Bottomley SP. Mechanisms of ataxin-3 misfolding and fibril formation: kinetic analysis of a disease-associated polyglutamine protein. *J Mol Biol* 2007; 368: 595-605.
17. Takahashi Y, Okamoto Y, Popiel HA, Fujikake N, Toda T, Kinjo M, Nagai Y. Detection of polyglutamine protein oligomers in cells by fluorescence correlation spectroscopy. *J Biol Chem* 2007; 282: 24039-48.
18. Scherzinger E, Sittler A, Schweiger K, Heiser V, Lurz R, Hasenbank R, Bates GP, Lehrach H, Wanker EE. Self-assembly of polyglutamine-containing huntingtin fragments into amyloid-like fibrils: implications for Huntington's disease pathology. *Proc Natl Acad Sci USA* 1999; 96: 4604-4609.
19. Chen S, Berthelie V, Hamilton JB, O'Nuallain B, Wetzel R. Amyloid-like features of polyglutamine aggregates and their assembly kinetics. *Biochemistry* 2002; 41: 7391-7399.
20. Duim WC, Jiang Y, Shen K, Frydman J, Moerner WE. Super-resolution fluorescence of huntingtin reveals growth of globular species into short fibers and coexistence of distinct aggregates. *ACS Chem Biol* 2014; 9: 2767-2778.
21. Perutz MF, Johnson T, Suzuki M, Finch JT. Glutamine repeats as polar zippers: their possible role in inherited neurodegenerative diseases. *Proc Natl Acad Sci USA* 1994; 91: 5355-5358.
22. Reddy PH, Williams M, Charles V, Garrett L, Pike-Buchanan L, Whetsell WO Jr, Miller G, Tagle DA. Behavioural abnormalities and selective neuronal loss in HD transgenic mice expressing mutated full-length HD cDNA. *Nat Genet* 1998; 20: 198-202.
23. Kim M, Lee HS, LaForet G, McIntyre C, Martin EJ, Chang P, Kim TW, Williams M, Reddy PH, Tagle D, Boyce FM, Won L, Heller A, Aronin N, DiFiglia M. Mutant huntingtin expression in clonal striatal cells: dissociation of inclusion formation and neuronal survival by caspase inhibition. *J Neurosci* 1999; 19: 964-973.
24. Li SH, Li XJ. Aggregation of N-terminal huntingtin is dependent on the length of its glutamine repeats. *Hum Mol Genet* 1998; 7: 777-782.
25. Martindale D, Hackam A, Wiczorek A, Ellerby L, Wellington C, McCutcheon K, Singaraja R, Kazemi-Esfarjani P, Devon R, Kim SU, Bredesen DE, Tufaro F, Hayden MR. Length of huntingtin and its polyglutamine tract influences localization and frequency of intracellular aggregates. *Nat Genet* 1998; 18: 150-154.
26. Ikeda H, Yamaguchi M, Sugai S, Aze Y, Narumiya S, Kakizuka A. Expanded polyglutamine in the Machado-Joseph disease protein induces cell death in vitro and in vivo. *Nat Genet* 1996; 13: 196-202.
27. Paulson HL, Perez MK, Trotter Y, Trojanowski JQ, Subramony SH, Das SS, Vig P, Mandel JL, Fischbeck KH, Pittman RN. Intranuclear inclusions of expanded polyglutamine protein in spinocerebellar ataxia type 3. *Neuron* 1997; 19: 333-344.
28. Warrick JM, Paulson HL, Gray-Board GL, Bui QT, Fischbeck KH, Pittman RN, Bonini NM. Expanded polyglutamine protein forms nuclear inclusions and causes neural degeneration in *Drosophila*. *Cell* 1998; 93: 939-949.
29. Evert BO, Wüllner U, Schulz JB, Weller M, Groscurth P, Trotter Y, Brice A, Klockgether T. High level expression of expanded full-length ataxin-3 in vitro causes cell death and formation of intranuclear inclusions in neuronal cells. *Hum Mol Genet* 8: 1169-1176.
30. Moulder KL, Onodera O, Burke JR, Strittmatter WJ, Johnson EM Jr. Generation of neuronal intranuclear inclusions by polyglutamine-GFP: analysis of inclusion clearance and toxicity as a function of polyglutamine length. *J Neurosci* 1999; 19: 705-715.
31. Wellington CL, Singaraja R, Ellerby L, Savill J, Roy S, Leavitt B, Cattaneo E, Hackam A, Sharp A, Thornberry N, Nicholson DW, Bredesen DE, Hayden MR. Inhibiting caspase cleavage of huntingtin reduces toxicity and aggregate formation in neuronal and nonneuronal cells. *J Biol Chem* 2000; 275: 19831-19838.
32. Wheeler VC, Gutekunst CA, Vrbanc V, Lebel LA, Schilling G, Hersch S, Friedlander RM, Gusella JF, Vonsattel JP, Borchelt DR, MacDonald ME. Early phenotypes that presage late-onset neurodegenerative disease allow testing of modifiers in Hdh CAG knock-in mice. *Hum Mol Genet* 2002; 11: 633-640.
33. Hermel E, Gafni J, Propp SS, Leavitt BR, Wellington CL, Young JE, Hackam AS, Logvinova AV, Peel AL, Chen SF, Hook V, Singaraja R, Krajewski S, Goldsmith PC, Ellerby HM, Hayden MR, Bredesen DE, Ellerby LM. Specific caspase interactions and amplification are involved in selective neuronal vulnerability in Huntington's disease. *Cell Death Differ* 2004; 11: 424-438.
34. Skinner PJ, Koshy BT, Cummings CJ, Klement IA, Helin K, Servadio A, Zoghbi HY, Orr HT. Ataxin-1 with an expanded glutamine tract alters nuclear matrix-associated structures. *Nature* 1997; 389: 971-974.
35. Clark HB, Orr HT. Spinocerebellar ataxia type 1-modeling the pathogenesis of a polyglutamine neurodegenerative disorder in transgenic mice. *J Neuropathol Exp Neurol* 2000; 59: 265-270.
36. Yvert G, Lindenberg KS, Devys D, Helmlinger D, Landwehrmeyer GB, Mandel JL. SCA7 mouse models show selective stabilization of mutant ataxin-7 and similar cellular responses in different neuronal cell types. *Hum Mol Genet* 2001; 10: 1679-1692.
37. Evers MM, Toonen LJ, van Roon-Mom WM. Ataxin-3 protein and RNA toxicity in spinocerebellar ataxia type 3: current insights and emerging therapeutic strategies. *Mol Neurobiol* 2014; 49: 1513-1531.
38. Holmberg M, Duyckaerts C, Dürr A, Cancel G, Gourfinkel-An I, Damier P, Faucheux B, Trotter Y, Hirsch EC, Agid Y, Brice A. Spinocerebellar ataxia type 7 (SCA7): a neurodegenerative disorder with neuronal intranuclear inclusions. *Hum Mol Genet* 1998; 7: 913-918.
39. Bergink S, Severijnen LA, Wijgers N, Sugasawa K, Yousaf H, Kros JM, van Swieten J, Oostra BA, Hoeijmakers JH, Vermeulen W, Willemsen R. The DNA repair-ubiquitin-associated HR23 proteins are constituents of neuronal inclusions in specific neuro-

- degenerative disorders without hampering DNA repair. *Neurobiol Dis* 2006; 23: 708-716.
40. Nakamura K, Jeong SY, Uchihara T, Anno M, Nagashima K, Nagashima T, Ikeda S, Tsuji S, Kanazawa I. SCA17, a novel autosomal dominant cerebellar ataxia caused by an expanded polyglutamine in TATA-binding protein. *Hum Mol Genet* 2001; 10: 1441-1448.
  41. Ishikawa K, Fujigasaki H, Saegusa H, Ohwada K, Fujita T, Iwamoto H, Komatsuzaki Y, Toru S, Toriyama H, Watanabe M, Ohkoshi N, Shoji S, Kanazawa I, Tanabe T, Mizusawa H. Abundant expression and cytoplasmic aggregations of [alpha]1A voltage-dependent calcium channel protein associated with neurodegeneration in spinocerebellar ataxia type 6. *Hum Mol Genet* 1999; 8: 1185-1193.
  42. Toru S, Murakoshi T, Ishikawa K, Saegusa H, Fujigasaki H, Uchihara T, Nagayama S, Osanai M, Mizusawa H, Tanabe T. Spinocerebellar ataxia type 6 mutation alters P-type calcium channel function. *J Biol Chem* 2000; 275: 10893-10898.
  43. McCampbell A, Taylor JP, Taye AA, Robitschek J, Li M, Walcott J, Merry D, Chai Y, Paulson H, Sobue G, Fischbeck KH. CREB-binding protein sequestration by expanded polyglutamine. *Hum Mol Genet* 2000; 9: 2197-2202.
  44. Cornett J, Smith L, Friedman M, Shin JY, Li XJ, Li SH. Context-dependent dysregulation of transcription by mutant huntingtin. *J Biol Chem* 2006; 281: 36198-36204.
  45. Mohan RD, Abmayr SM, Workman JL. The expanding role for chromatin and transcription in polyglutamine disease. *Curr Opin Genet Dev* 2014; 26: 96-104.
  46. Saudou F, Finkbeiner S, Devys D, Greenberg ME. Huntingtin acts in the nucleus to induce apoptosis but death does not correlate with the formation of intracellular inclusions. *Cell* 1998; 95: 55-66.
  47. Saunders HM, Gilis D, Rooman M, Dehouck Y, Robertson AL, Bottomley SP. Flanking domain stability modulates the aggregation kinetics of a polyglutamine disease protein. *Protein Sci* 2011; 20: 1675-1681.
  48. Thakur AK, Jayaraman M, Mishra R, Thakur M, Chellgren VM, Byeon IJ, Anjum DH, Kodali R, Creamer TP, Conway JF, Gronenborn AM, Wetzel R. Polyglutamine disruption of the huntingtin exon 1 N terminus triggers a complex aggregation mechanism. *Nat Struct Mol Biol* 2009; 16: 380-389.
  49. Lakhani VV, Ding F, Nikolay Dokholyan NV. Polyglutamine induced misfolding of huntingtin exon 1 is modulated by the flanking sequences. *PLoS Comput Biol* 2010; 6: e1000772.
  50. Chen S, Bertheliev V, Yang W, Wetzel R. Polyglutamine aggregation behavior in vitro supports a recruitment mechanism of cytotoxicity. *J Mol Biol* 2001; 311: 173-182.
  51. Yamada M, Tsuji S, Takahashi H. Pathology of CAG repeat diseases. *Neuropathology* 2000; 20: 319-325.
  52. Lim J, Crespo-Barreto J, Jafar-Nejad P, Bowman AB, Richman R, Hill DE, Orr HT, Zoghbi HY. Opposing effects of polyglutamine expansion on native protein complexes contribute to SCA1. *Nature* 2008; 452: 713-718.
  53. Steffan JS, Kazantsev A, Spasic-Boskovic O, Greenwald M, Zhu YZ, Gohler H, Wanker EE, Bates GP, Housman DE, Thompson LM. The Huntingtin's disease protein interacts with p53 and CREB binding protein and represses transcription. *Proc Natl Acad Sci USA* 2000; 97: 6763-6768.
  54. Shimohata T, Nakajima T, Yamada M, Uchida C, Onodera O, Naruse S, Kimura T, Koide R, Nozaki K, Sano Y, Ishiguro H, Sakoe K, Ooshima T, Sato A, Ikeuchi T, Oyake M, Sato T, Aoyagi Y, Hozumi I, Nagatsu T, Takiyama Y, Nishizawa M, Goto J, Kanazawa I, Davidson I, Tanese N, Takahashi H, Tsuji S. Expanded polyglutamine stretches interact with TAFIII30, interfering with CREB-dependent transcription. *Nat Genet* 2000; 26: 29-36.
  55. Nucifora FC Jr, Sasaki M, Peters MF, Huang H, Cooper JK, Yamada M, Takahashi H, Tsuji S, Troncoso J, Dawson VL, Dawson TM, Ross CA. Interference by huntingtin and atrophin-1 with cbp-mediated transcription leading to cellular toxicity. *Science* 2001; 291: 2423-2428.
  56. Taylor JP, Taye AA, Campbell C, Kazemi-Esfarjani P, Fischbeck KH, Min KT. Aberrant histone acetylation, altered transcription, and retinal degeneration in a *Drosophila* model of polyglutamine disease are rescued by CREB-binding protein. *Genes Dev* 2003; 17: 1463-1468.
  57. Singh MD, Raj K, Sarkar S. *Drosophila* Myc, a novel modifier suppresses the poly(Q) toxicity by modulating the level of CREB binding protein and histone acetylation. *Neurobiol Dis* 2014; 63: 48-61.
  58. Chanu SI, Singh MD, Nisha, Sarkar S. Transcriptional up-regulation and its impact on poly(Q) disorders. *Ther Targets Neurol Dis* 2014; 1: e312.
  59. Goodman RH, Smolik S. CBP/p300 in cell growth, transformation, and development. *Genes Dev* 2000; 14: 1553-1577.
  60. Chan HM, La Thangue NB. p300/CBP proteins: HATs for transcriptional bridges and scaffolds. *J Cell Sci* 2001; 114: 2363-2373.
  61. Kumar JP, Jamal T, Doetsch A, Turner FR, Duffy JB. CREB binding protein functions during successive stages of eye development in *Drosophila*. *Genetics* 2004; 168: 877-893.
  62. Janknecht R. The versatile functions of the transcriptional coactivators p300 and CBP and their roles in disease. *Histol Histopathol* 2002; 17: 657-668.
  63. Jiang H, Nucifora FC Jr, Ross CA, DeFranco DB. Cell death triggered by polyglutamine-expanded huntingtin in a neuronal cell line is associated with degradation of CREB-binding protein. *Hum Mol Genet* 2003; 12: 1-12.
  64. Bates EA, Victor M, Jones AK, Shi Y, Hart AC. Differential contributions of *Caenorhabditis elegans* histone deacetylases to huntingtin polyglutamine toxicity. *J Neurosci* 2006; 26: 2830-2838.
  65. Lin X, Antalfy B, Kang D, Orr HT, Zoghbi HY. Polyglutamine expansion down-regulates specific neuronal genes before pathologic changes in SCA1. *Nat Neurosci* 2000; 3: 157-163.
  66. Evert BO, Vogt IR, Vieira-Saecker AM, Ozimek L, de Vos RA, Brunt ER, Klockgether T, Wüllner U. Gene expression profiling in ataxin-3 expressing cell lines reveals distinct effects of normal and mutant ataxin-3. *J Neuropathol Exp Neurol* 2003; 62: 1006-1018.
  67. Reiter LT, Potocki L, Chien S, Gribskov M, Bier E. A systematic analysis of human disease-associated gene sequences in *Drosophila melanogaster*. *Genome Res* 2001 11: 1114-1125.
  68. Marsh JL, Thompson LM. *Drosophila* in the study of neurodegenerative disease. *Neuron* 2006; 52: 169-178.
  69. Yu Z, Bonini NM. Modeling human trinucleotide repeat diseases in *Drosophila*. *Int Rev Neurobiol* 2011; 99: 191-212.
  70. Charng WL, Yamamoto S, Bellen HJ. Shared mechanisms between *Drosophila* peripheral nervous system development and human neurodegenerative diseases. *Curr Opin Neurobiol* 2014; 27: 158-64.
  71. Hartenstein V, Campos-Ortega JA. Early neurogenesis in wild-type *Drosophila melanogaster*. *Roux's Arch Dev Biol* 1984; 193: 308-325.
  72. Marsh JL, Pallos J, Thompson LM. Fly models of Huntington's disease. *Hum Mol Genet* 2003; 12: R187-R193.
  73. Therianos S, Leuzinger S, Hirth F, Goodman CS, Reichert H. Embryonic development of the *Drosophila* brain: formation of commissural and descending pathways. *Development* 1995; 121: 3849-3860.
  74. Cagan RL, Ready DF. The emergence of order in the *Drosophila* pupal retina. *Dev Biol* 1989; 136: 346-362.
  75. Tomlinson A, Ready DF. Neuronal differentiation in the *Drosophila* ommatidium. *Dev Biol* 1987; 120: 366-376.
  76. Stowers RS, Schwarz TL. A genetic method for generating *Drosophila* eyes composed exclusively of mitotic clones of a single genotype. *Genetics* 1999 152: 1631-1639.
  77. Hsiung F, Moses K. Retinal development in *Drosophila*: specify-

- ing the first neuron. *Hum Mol Genet* 2002; 11: 1207–1214.
78. Peng H, Chung P, Long F, Qu L, Jenett A, Seeds AM, Myers EW, Simpson JH. BrainAligner: 3D registration atlases of *Drosophila* brains. *Nat Methods* 2011; 8: 493-500.
  79. Hartenstein V, Spindler S, Peraanu W, Fung S. The development of the *Drosophila* larval brain. *Adv Exp Med Biol* 2008; 628: 1-31
  80. Heisenberg M. Mushroom body memoir: from maps to models. *Nat Rev Neurosci* 2003; 4: 266-275.
  81. Buchanan RL, Benzer S. Defective glia in the *Drosophila* brain degeneration mutant drop-dead. *Neuron* 1993; 10: 839-850.
  82. Kretzschmar D, Hasan G, Sharma S, Heisenberg M, Benzer S. The swiss cheese mutant causes glial hyperwrapping and brain degeneration in *Drosophila*. *J Neurosci* 1997; 17: 7425-7432.
  83. Muqit MM, Feany MB. Modelling neurodegenerative diseases in *Drosophila*: a fruitful approach? *Nat Rev Neurosci* 2002; 3: 237-243.
  84. Brand AH, Perrimon N. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* 1993; 118: 401-415.
  85. Lin DM, Goodman CS. Ectopic and increased expression of Fasciclin II alters motoneuron growth cone guidance. *Neuron* 1994; 13: 507-523.
  86. Robinow S, White K. The locus elav of *Drosophila melanogaster* is expressed in neurons at all developmental stages. *Dev Biol* 1988; 126: 294-303.
  87. Ellis MC, O'Neill EM, Rubin GM. Expression of *Drosophila* glass protein and evidence for negative regulation of its activity in non-neuronal cells by another DNA-binding protein. *Development* 1993; 119: 855-865.
  88. Hay BA, Wolff T, Rubin GM. Expression of baculovirus P35 prevents cell death in *Drosophila*. *Development* 1994; 120: 2121-2129.
  89. Fernandez-Funez P, Nino-Rosales ML, de Gouyon B, She WC, Luchak JM, Martinez P, Turiegano E, Benito J, Capovilla M, Skinner PJ, McCall A, Canal I, Orr HT, Zoghbi HY, Botas J. Identification of genes that modify ataxin-1-induced neurodegeneration. *Nature* 2000; 408: 101-106.
  90. Takeyama K, Ito S, Yamamoto A, Tanimoto H, Furutani T, Kanuka H, Miura M, Tabata T, Kato S. Androgen-dependent neurodegeneration by polyglutamine-expanded human androgen receptor in *Drosophila*. *Neuron* 2002; 35: 855-864.
  91. Bonini NM. A genetic model for human polyglutamine-repeat disease in *Drosophila melanogaster*. *Philos Trans R Soc Lond B Biol Sci* 1999; 354: 1057-1060.
  92. Steffan JS, Agrawal N, Pallos J, Rockabrand E, Trotman LC, Slepko N, Illes K, Lukacovich T, Zhu YZ, Cattaneo E, Pandolfi PP, Thompson LM, Marsh JL. SUMO modification of Huntingtin and Huntington's disease pathology. *Science* 2004; 304: 100-104.
  93. Lee WC, Yoshihara M, Littleton JT. Cytoplasmic aggregates trap polyglutamine-containing proteins and block axonal transport in a *Drosophila* model of Huntington's disease. *Proc Natl Acad Sci USA* 2004; 101: 3224-3229.
  94. Lu B, Vogel H. *Drosophila* models of neurodegenerative diseases. *Annu Rev Pathol* 2009; 4: 315-342.
  95. Debattisti V, Scorrano LD. *Melanogaster*, mitochondria and neurodegeneration: small model organism, big discoveries. *Mol Cell Neurosci* 2013; 55: 77-86.
  96. Konsolaki M. Fruitful research: drug target discovery for neurodegenerative diseases in *Drosophila*. *Expert Opin Drug Discov* 2013; 8: 1503-1513.