Neurobiology of Disease 40 (2010) 29–39

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

Review

Neurodegenerative models in *Drosophila:* Polyglutamine disorders, Parkinson disease, and amyotrophic lateral sclerosis

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ARTICLE INFO

Article history:
Received 2 April 2010
Revised 18 May 2010
Accepted 19 May 2010
Available online 31 May 2010

Keywords:
Polyglutamine
Synuclein
Parkin
DJ-1
PINK1
LRRK2
SOD
VAPB
TDP-43
Drosophila
Neurodegeneration

ABSTRACT

Neurodegenerative diseases encompass a large group of neurological disorders. Clinical symptoms can include memory loss, cognitive impairment, loss of movement or loss of control of movement, and loss of sensation. Symptoms are typically adult onset (although severe cases can occur in adolescents) and are reflective of neuronal and glial cell loss in the central nervous system. Neurodegenerative diseases also are considered progressive, with increased severity of symptoms over time, also reflective of increased neuronal cell death. However, various neurodegenerative diseases differentially affect certain brain regions or neuronal or glial cell types. As an example, Alzheimer disease (AD) primarily affects the temporal lobe, whereas neuronal loss in Parkinson disease (PD) is largely (although not exclusively) confined to the nigrostriatal system. Neuronal loss is almost invariably accompanied by abnormal insoluble aggregates, either intra- or extracellular. Thus, neurodegenerative diseases are categorized by (a) the composite of clinical symptoms, (b) the brain regions or types of brain cells primarily affected, and (c) the types of protein aggregates found in the brain. Here we review the methods by which *Drosophila melanogaster* has been used to model aspects of polyglutamine diseases, Parkinson disease, and amyotrophic lateral sclerosis and key insights into that have been gained from these models; Alzheimer disease and the tauopathies are covered elsewhere in this special issue.

Contents

Introduction

The last 12 years have seen quite an increase in the number of laboratories using invertebrates to model human neurodegenerative diseases. The simple fruit fly *Drosophila* has been the subject of hundreds of manuscripts detailing phenotypes and defining genetic and chemical modifiers. Here we will discuss those approaches taken using mostly misexpression of human disease genes; *Drosophila* genes that cause neurodegeneration when mutated have been the subject of

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Available online on ScienceDirect (www.sciencedirect.com).

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doi:10.1016/j.nbd.2010.05.026

an exhaustive recent review (Lessing and Bonini, 2009). Models of Alzheimer disease and other tauopathies are covered elsewhere in this special issue.

Why flies?

Although fruit flies at first glance may seem far removed from human biology, fundamental cellular processes are very similar between humans and flies; these include regulation of gene expression, subcellular trafficking, synaptic transmission, synaptogenesis, and cell death. In addition, many genes and signaling pathways are conserved between human and flies, including key regulatory pathways in mammals, such as Wnt, Ras/extracellular regulated kinase (ERK), and Toll-like pathways; each of these was first identified in flies and later found to have homolog and conserved functions in mammals. Flies have simpler genetics (only 4 pairs of homologous chromosomes, as compared to 23 in humans; 12,000 genes as compared to 20,000 in humans) and simpler nervous systems (~200,000 neurons compared to ~100 billion neurons in humans); nonetheless, flies are still capable of performing complex motor behaviors, such as walking, climbing, and flying, and they can be trained using fear-conditioning paradigms to test learning and memory. *Drosophila* behavior can be useful in parsing out the effects of a gene product on neuronal function and connectivity. The relative simplicity of *Drosophila* systems and genetics makes it ideal for creating animal models of complex disorders that can individually model a subset of phenotypes associated with a disease to simplify analysis of the disease.

Another advantage of *Drosophila* as a model organism is its short reproductive and developmental cycles (10–14 days from embryo to reproductively mature adults), as well as the ease of creating genetic deletions, insertions, knockdowns, or transgenics as compared to mammals. Maintenance of stocks is simple and inexpensive, making *Drosophila* an ideal laboratory animal, as hundreds of different mutant and transgenic lines can all be kept within the confines of a single laboratory. A range of genetic manipulations have been developed in *Drosophila* that are impossible or impractical in mammals. Most of these manipulations have taken advantage of the naturally found transposable element in *Drosophila*, the P element. Although the natural P element originally consisted of inverted repeats surrounding a transposase gene to allow for hopping into endogenous chromosomes, the P element in the hands of researchers has since taken on a life of its own and has becomes the dominant tool in creating disease models in *Drosophila*. P elements insert semirandomly into the genome, although there are certain "hotspots" in chromatin integration, and preferentially insert into promoter regions, disrupting transcription of the downstream gene. The simplest application of this phenomenon has been libraries of "P element mutants," which largely represent null alleles of the affected gene. Mapping the flanking regions around the insertion is a straightforward process and allows for much more rapid mapping mutations as compared to classical chemically induced mutagens, e.g., ethyl methane sulfonate (EMS).

Taken a step further, P elements can be engineered to carry genes other than transposase while they maintain the inverted repeats, thus allowing for genomic insertion when another source of transposase is present. Fusion of a cell- or tissue-specific promoter and/or enhancer placed 5′ to the gene within the P element allows for the creation of transgenic lines that express the gene of interest in a tissue-specific manner, taking advantage of the P element's ability to insert into the host genome without the need for homologous recombination. Further, multiple P-element-derived trangenes can be used in combination with each other to allow for tissue-specific misexpression of either other transgenes or endogenous genomic sequences. The GAL4/UAS system of expression (Brand and Perrimon, 1993) uses yeast-derived GAL4, a transcription factor that binds to the upstream activating sequence (UAS) enhancer element, driving expression of the gene immediately downstream of the UAS. Thus, two transgenes are required: (1) a transgene expressing GAL4 under control of tissue-specific enhancers and (2) a transgene carrying a UAS placed just upstream of a gene of interest. There are several advantages to this binary expression system. First is the conditional nature of the expression, such that if expression of the gene is deleterious, the precursor lines can be maintained and crossed as needed to carry out experiments. Second is that a single UAS transgenic line can be ectopically expressed in many different tissue types, depending on the tissue-specific enhancer line used to express the GAL4 gene (such as line is commonly referred to as a "driver"). This is highly advantageous especially in creating models of neurodegeneration, as different tissue types or even different cell types within the same tissue may manifest different phenotypes caused by the same misexpressed gene. Third is that the GAL4/UAS system often yields much higher expression of the gene of interest than direct promoter-fused transgenes, and expression can be further augmented by adding more copies of either the driver or the UAS construct.

Together, these features of *Drosophila* make it an ideal organism to perform genetic and pharmacological screens for modifiers of neurodegeneration, once a phenotype has been established, be it morphological or behavioral. Such screens often take advantage of large collections of fly lines with mapped mutations, or mapped insertions with "empty" UAS constructs, termed "enhancer–promoter" or "EP" elements, which allow for the overexpression of an endogenous gene that is near the UAS insertion, in combination with a source of GAL4. These collections (Rorth, 1996; Bellen et al., 2004) can be obtained from the large stock centers found worldwide, such as the Bloomington *Drosophila* Stock Center maintained by Indiana University in Bloomington, Indiana. These lines and other similar collections can then be crossed to the disease model tester strain, allowing for the rapid identification of modifiers. The GAL4–UAS system can also be used to express siRNAs in cell-specific manner and thus can be used to knock down gene targets as well. Pharmacological screens can similarly be carried out in a high-throughput manner, as drugs and other chemical compounds can be readily mixed into fly food media, on which both developing larvae and adult flies feed. Such screens can yield novel genetic interactions in disease processes and help to further refine proposed and established mechanisms of pathology.

Polyglutamine diseases

Polyglutamine diseases are caused by mutations that lead to expansions of unstable CAG repeats, which are translated as glutamine (symbolized in biochemical short-hand by the letter Q) in normal functioning proteins. Huntington disease (HD) and the dominant spinocerebellar ataxias (SCA) are representative of this class of disease; these disorders show characteristic features in patients, such as (a) nuclear inclusions containing the mutant protein, (b) repeat length inversely correlated with age of onset, (c) motor impairment, and (d) age-dependent degeneration. Polyglutamine diseases are due to single-gene defects and were the first neurodegenerative models successfully created in *Drosophila* that used human transgenes. Examples of some retinal phenotypes of human disease genes are shown in Fig. 1. We created a model of HD using expression of truncated wild-type and mutant forms of huntingtin/htt (Jackson et al., 1998), and Warrick et al. (1998) reported a model of SCA 3 or Machado-Joseph disease (SCA3/MJD) expressing truncated ataxin 3 (also referred to as MJD1), also using different glutamine repeat lengths; both papers demonstrated that increased polyQ expansion led to more severe degeneration, age-dependent degeneration, and repeat length-dependent nuclear aggregation (Figs. 1B and D). These models provided a platform to demonstrate that (a) human disease genes can yield parallel neurodegenerative effects in *Drosophila*, and (b) the fly eye can serve as a model tissue to monitor neurodegeneration, serving as a readout to identify genetic modifiers of neurotoxicity. A few investigators have also shown that polyQ expression in glia can cause lethality and neurodegeneration