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Phil. Trans. R. Soc. Lond. B 1999 **354**, 1057-1060
doi: 10.1098/rstb.1999.0458

THE ROYAL SOCIETY

A genetic model for human polyglutamine-repeat disease in *Drosophila melanogaster*

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To apply genetics to the problem of human polyglutamine-repeat disease, we recreated polyglutamine-repeat disease in *Drosophila melanogaster*. To do this, we expressed forms of the human gene encoding spinocerebellar ataxia type 3, also called Machado–Joseph disease (SCA-3/MJD). This gene is responsible for the most common form of human ataxia worldwide. Expression of a normal form of the MJD protein with 27 polyglutamines (MJDtr-Q27) had no phenotype. However, expression of a form of the protein with an expanded run of 78 glutamines (MJDtr-Q78) caused late onset progressive degeneration. In addition, the MJDtr-Q78 formed abnormal protein aggregates, or nuclear inclusions (NIs), whereas the control protein was cytoplasmic. These data indicate that the mechanisms of human polyglutamine-repeat disease are conserved to *Drosophila*. We are currently using this model to address potential mechanisms by which the mutant disease protein causes neural degeneration, as well as to define genes that can prevent polyglutamine-induced degeneration. By applying the power of *Drosophila* genetics to the problem of human polyglutamine-induced neural degeneration, we hope to identify ways to prevent and treat these diseases in humans.

Keywords: *Drosophila melanogaster*; degeneration; polyglutamine-repeat disease; Machado–Joseph disease; nuclear inclusions

1. INTRODUCTION

We are interested in pioneering new approaches to understand the mechanisms, and ultimately prevent, human neurodegenerative disease. To do this, we are bringing to bear the power of genetics by developing models for human brain degenerative disease in the simple system *Drosophila melanogaster* (Warrick *et al*. 1998). The polyglutamine-repeat diseases are a class of human disease that results from the expansion of a polyglutamine run within the open reading frame of the disease protein. The expanded polyglutamine run is thought to confer a dominant toxic effect on the otherwise unrelated proteins, leading to neuronal dysfunction and eventual cell loss (reviewed in Paulson & Fischbeck 1996). In order to address mechanisms by which the polyglutamine-repeat disease proteins induce neural dysfunction, as well as to identify genes that can ameliorate the effects of expanded polyglutamine proteins, we asked whether it was possible to recreate human polyglutamine-repeat disease in the fruit fly *Drosophila melanogaster*.

Drosophila has a complex nervous system, organized into neural centres much like the human brain. In addition, *Drosophila* displays complex behaviours, such as learning and memory. Genes between humans and flies are highly conserved, including entire gene pathways, such as the tyrosine kinase–ras–raf signalling pathways. *Drosophila*, however, has the advantages of a rapid reproductive cycle (ten days from egg to adult), as well as ease of growing large numbers. Thus, if it were possible to recreate polyglutamine disease in *Drosophila*, it would ultimately prove feasible to use the fly to elucidate mechanisms by which polyglutamine proteins cause neural loss, as well as to define genes or compounds that can ameliorate the effects of these proteins. A critical question is, if a phenotype is seen in *Drosophila*, to what extent does it recapitulate features of human polyglutamine-repeat disease?

2. EXPRESSION OF A HUMAN DISEASE GENE IN THE FLY

We initiated our studies with the polyglutamine-repeat disease gene (*MJDI*) responsible for the most common dominantly inherited ataxia, spinocerebellar ataxia type 3, also called Machado–Joseph disease (SCA-3/MJD). Since mouse transgenic models for Huntington's disease and SCA-3/MJD had shown that truncated versions of the respective disease proteins were more potent than full-length forms of the proteins (Ikeda *et al*. 1996; Mangiarini *et al*. 1996), we used a truncated version of MJD. The truncated gene is comprised of the C-terminal part of the MJD protein, which includes about 55 amino acids of the MJD protein, plus the polyglutamine stretch. The polyglutamine stretch included 27 glutamines for the non-disease protein (MJDtr-Q27) and 78 glutamines for the expanded polyglutamine form (MJDtr-Q78). The genes were expressed using a two-component system of targeted gene expression, the GAL4-UAS system (Brand & Perrimon 1993), such that transgene expression could be directed specifically to the nervous system as well as to other tissues as desired.

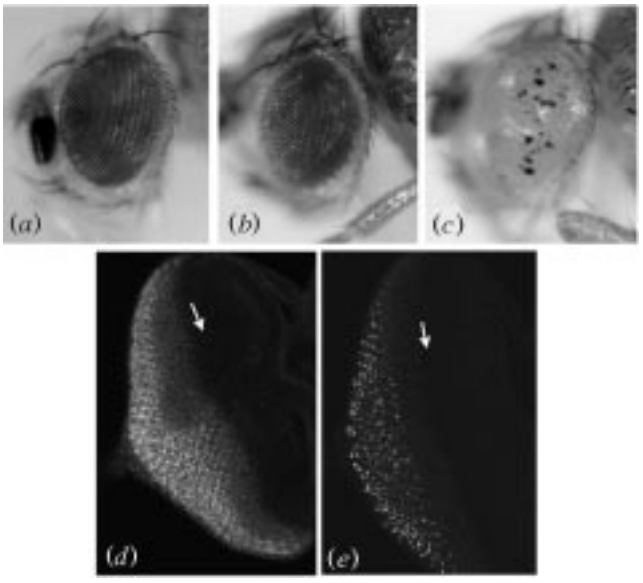

Figure 1. Expanded polyglutamine protein causes degeneration of the *Drosophila* eye. (*a*) Eye of a normal fly, showing highly ordered precise neurocrystalline lattice, and red pigmentation. (*b*) Eye of a fly expressing the control protein, MJDtr-Q27. The eye morphology is identical to that of a normal fly. Fly of genotype *w*; *gmr-GAL4/UAS-MJDtr-Q27*. (*c*) Eye of a fly expressing the expanded polyglutamine protein, MJDtr-Q78. The eye shows severe degeneration compared to the normal eye. Externally, pigmentation is lost (seen here as a light coloured eye), internally (see Warrick *et al*. 1998) the eye shows complete loss of the photoreceptor neurons. (*d*) Developing eye disc of a larva expressing the MJDtr-Q27 protein. The protein is being expressed from the start of differentiation at the furrow (arrow), and shows a diffuse cytoplasmic expression pattern. Larva of genotype *w*; *gmr-GAL4/UAS-MJDtr-Q27*. (*e*) Developing eye disc of a larva expressing the MJDtr-Q78 protein. The protein is being expressed from the start of differentiation at the furrow (arrow). The protein shows eventual concentration into brightly fluorescing nuclear inclusions. Older cells are to the left. Larva of genotype *w*; *gmr-GAL4/UAS-MJDtr-Q78*.

3. HUMAN POLYGLUTAMINE PROTEIN CAUSES LATE ONSET, PROGRESSIVE DEGENERATION IN *DROSOPHILA*

Our initial studies directed transgene expression to the eye using the *glass multiple reporter* (*gmr*) promoter. This promoter drives expression in all cells of the developing eye, including photoreceptor neurons and pigment cells. Expression of the MJDtr-Q27 protein had no effect on the eye when compared to normal eye morphology. However, expression of the MJDtr-Q78 protein caused progressive degeneration. When MJDtr-Q78 was strongly expressed, the eye failed to show pigmentation, indicating loss of pigment cells, and collapsed, indicating loss of photoreceptor neurons (figure 1). Weaker expression resulted in an eye that was only mildly disrupted; however, the eyes underwent degeneration during adult life. In this instance, degeneration was progressive, although of developmental onset. Analysis of the brain phenotype indicated that the effect occurred late in development: areas of the brain dependent upon photoreceptor neuron innervation were present, indicating that the photoreceptor neurons survived for quite a while prior to degenerating. Therefore, these studies indicate that expression of the MJDtr-Q78 protein in flies resulted in late onset, progressive cellular degeneration. This phenotype is similar to that seen in humans, and suggests that the fundamental mechanisms of human polyglutamine-repeat disease are conserved in *Drosophila*. Our findings have subsequently been confirmed by others (Jackson *et al*. 1998).

4. POLYGLUTAMINE PROTEIN FORMS NUCLEAR INCLUSIONS IN *DROSOPHILA*

Recently it has been noted that in the human disease tissue, as well as in transgenic mouse models and cells in culture, expanded polyglutamine proteins undergoes abnormal aggregation to form nuclear inclusions (Davies *et al*. 1997; DiFiglia *et al*. 1997; Paulson *et al*. 1997; Skinner *et al*. 1997). Thus we were interested in determining whether expression of such a protein in flies would also lead to nuclear inclusion formation. We found striking evidence of NI formation. The protein, when initially expressed, was cytoplasmic, but with time accumulated in the nuclei of cells, forming brightly fluorescing inclusions (figure 1).

We took these studies one step further and addressed various aspects of inclusion formation, such as whether inclusions were formed in all tissues to which transgene expression was directed. Indeed, all cells expressing the mutant protein showed evidence of inclusion formation—despite the fact that the expanded polyglutamine protein was not lethal to all cells. In fact, whereas expression of the expanded polyglutamine protein was deleterious to neurons, pigment cells and muscle cells, it was not at all deleterious to dividing epithelial cells, despite the fact that NIs formed in these cells. These studies indicate that, although inclusions may be a part of the degenerative process, the mere presence of a NI does not guarantee degeneration. Additional recent evidence confirms our finding that NIs may not be necessary to disease pathogenesis (Klement *et al*. 1998; Saudou *et al*. 1998).

5. MECHANISMS OF POLYGLUTAMINE-MEDIATED NEURODEGENERATION ARE CONSERVED IN *DROSOPHILA*

We have shown that expression of an expanded polyglutamine protein shows features of polyglutamine-repeat disease that are seen in humans (table 1; figure 2; Warrick *et al*. 1998). First, expression of the protein causes a late onset, progressive degeneration. Even when the protein is strongly expressed, degeneration is not immediate but of late developmental onset, reminiscent of juvenile cases of these diseases with particularly long CAG repeats (Paulson & Fischbeck 1996). We also showed that the degeneration was of a progressive nature. Second, the protein shows abnormal aggregation, forming NIs in the tissues in which the protein is expressed. Irrespective of whether NIs are causal to the disease, their formation has been noted for many different polyglutamine disease proteins. Hence, with respect to this characteristic feature as well, the *Drosophila* model shows conservation. Taken together, these data indicate that at least some key features of polyglutamine-repeat disease are conserved

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Table 1. *Comparison of some of the features of polyglutamine-repeat disease between* Drosophila *and humans*[a]

(*a*) Features of human polyglutamine repeat disease conserved in *Drosophila*
late-onset of the phenotype
progressive degeneration
abnormal protein aggregation in form of nuclear inclusions
(*b*) Features of human polyglutamine-repeat disease not yet addressed in *Drosophila*
selective neural sensitivity of degeneration
instability of the CAG repeat from one generation to the next (known as anticipation)

[a] For a summary of the characteristic features of human polyglutamine-repeat disease, see Paulson & Fischbeck (1996).

between humans and flies. This suggests that we should be able to use *Drosophila* to address mechanisms by which the disease proteins function, as well as to define genes or compounds that can ameliorate the phenotype.

6. DEFINING BIOLOGICAL MECHANISMS OF POLYGLUTAMINE-INDUCED DEGENERATION

Given that we have developed a fly model, we were interested in addressing aspects of polyglutamine-repeat disease that might contribute to the biological actions of the mutant protein. We had already determined that expression of a NI *per se* is not sufficient to induce degeneration, since expression of the protein in dividing epithelial cells caused no effect. We were also interested in what other proteins might be present in the NI, and whether they might contribute to the phenotype.

A number of proteins have been tested for presence in NIs, but were not found to be present (Paulson *et al*. 1997). However, initial evidence suggested the possibility that expanded polyglutamine protein may sequester other proteins with polyglutamine repeats. A mechanism of cellular degeneration, therefore, could be depletion of other essential proteins containing polyglutamine by recruitment into aggregates. To address this, we asked whether a normal eye developmental protein that contained a polyglutamine run, *eyes absent* (*eya*), would be sequestered into the NI. To do this, we co-labelled developing eye discs for Eya protein and for NI. These data showed that indeed Eya protein was recruited and concentrated in the NI (Perez *et al*. 1998). Previous studies have shown that the Eya protein is critical to eye cells; loss of *eya* function early in eye development leads to loss of all cells of the eye (Bonini *et al*. 1993). Hence, it is possible that reduction of *eya* activity may in part lead to neuronal dysfunction. We are now in a position to generate flies that overexpress *eya*, or have reduced *eya* levels, to address whether altered *eya* activity contributes to the degenerative phenotype.

A number of studies have also suggested that altered proteolytic pathways may occur in polyglutamine-repeat diseases (Davies *et al*. 1997; Paulson *et al*. 1997; Cummings *et al*. 1998). This is suggested by the fact that the NI are typically ubiquitinated, suggesting the protein is targeted for proteolysis. In addition, studies have found that proteosomes are localized to the NI in vertebrates, as well as chaperone proteins, which aid in protein folding (Cummings *et al*. 1998; H. Paulson, unpublished observations). Hence, to address a potential role of proteolysis and chaperone activity, we asked whether heat shock protein 70 (hsp70), the major chaperone protein in *Drosophila* induced upon stress, was induced with expression of expanded polyglutamine protein. These studies indicated that hsp70 is indeed upregulated, and in fact localizes to the NI. These studies indicate that in flies, as in vertebrate cells, expanded polyglutamine protein is recognized as abnormal, inducing a stress response. Thus, we are now in a position to address whether manipulating the levels of various chaperone proteins in flies can modify the phenotype induced by the expanded polyglutamine protein. Such studies will allow us to determine whether altered chaperone activity in a living organism can have an effect on the neurodegeneration.

7. SCREENING FOR GENES THAT CAN PREVENT POLYGLUTAMINE-MEDIATED DEGENERATION

In addition to testing candidate suppressor proteins suggested by vertebrate work, it is possible in flies to define new genes that can suppress polyglutamine-induced neurodegeneration. To do this, one can screen for genes that, when reduced in dosage, will modify the phenotype induced by the expanded polyglutamine protein. Therefore, one can perform screens for dominant modifiers of the phenotype. To test this, we first addressed whether expression of P35, a potent insect viral anti-cell death gene, would have any effect on the degenerative phenotype in flies. We found that concomitant expression of P35 did partially ameliorate the phenotype. These studies indicate that anti-cell death genes may be of some benefit to these diseases. Although the effect was mild in flies, a mild effect in flies could translate in humans into a significant effect on brain function.

We are also performing genetic screens to define new genes. To do this, we are mating male flies containing various mutations to females bearing the expanded polyglutamine protein. In the progeny, we then screen for flies that have a modified phenotype—in this case, show a suppressed phenotype of the expanded polyglutamine protein. By this method, we can define mutations that dominantly modify the phenotype. The screen relies on restoration of pigmentation to the flies; subsequent studies reveal the degree to which the photoreceptor neurons are also restored. This screen is currently ongoing in our laboratory. Initial studies indicate that the screen indeed allows identification of new genes that modify degeneration induced by expanded polyglutamine-protein.

8. CONCLUSIONS

We have shown that the mechanisms of human polyglutamine-repeat disease are conserved in *Drosophila*. This indicates that *Drosophila* can provide a powerful model system in which to address biological mechanisms of human polyglutamine-repeat disease, allowing direct tests of specific genes for their ability to modify neural

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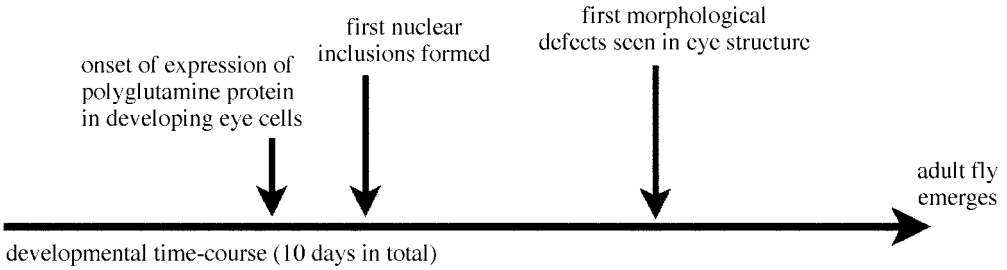

Figure 2. Time-course of events of polyglutamine-mediated neurodegeneration in *Drosophila*. The bottom line represents a developmental time line of *Drosophila*, from egg to the adult fly. Expression of the polyglutamine protein is initiated during the third-instar larval stage, about four days after egg is laid. About 12 h later, the first NIs are formed by the expanded polyglutamine protein. Several days later (about three days) the first morphological defects due to expression of the expanded polyglutamine protein are seen. The adult fly emerges at ten days: the adult eye undergoes further progressive degeneration, as well. For additional details, see Warrick *et al*. (1998).

degeneration, as well as to define new genes involved in the onset and progression of neuronal dysfunction. The extent to which aspects of human polyglutamine-repeat disease are conserved in flies is remarkable, showing characteristic late onset, progressive degeneration as noted in humans, as well as abnormal protein aggregation in the form of NIs (table 1; figure 2). Although clearly not all aspects of polyglutamine-repeat disease can be conserved between humans and flies, our *Drosophila* model will contribute to understanding mechanisms of human polyglutamine-repeat disease, by allowing rapid genetics to define conserved aspects. We have already shown that expression of the disease proteins is not lethal to all cells, and that *in vivo* in *Drosophila*, other polyglutamine-repeat proteins are sequestered into the abnormal protein aggregates. Our initial studies indeed indicate that it will prove possible to identify genes that can ameliorate the effect of these disease genes: we have already shown that P35 has effects, and have also defined some new suppressor mutations. Our efforts now are directed toward using this fly model to define additional mechanisms that contribute to polyglutamine disease progression, and to define genes that can slow or prevent altogether the effect of these disease proteins.

The author receives funding for this research through grants from the Alzheimer's Association (RG2-96-005), the David and Lucile Packard Foundation, the National Institutes of Health (EY11259), the John Merck Fund, and the Huntington's Disease Society of America Coalition for the Cure. I thank Henry Paulson for comments on the manuscript.

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