Cell-autonomous and non-cell autonomous protection of DNAJB6 in Huntington’s disease

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CHAPTER 3

A *D. melanogaster* model for the expression of transgenes in neurons and astrocytes

ABSTRACT

*D. melanogaster* is widely used in *in vivo* research because of the availability of flexible, well-established and -characterised genetic tools that allow the controlled expression of transgenes in a cell-specific or tissue-specific manner, through the use of binary expression systems and specific promoters. Hence, to investigate the role of HSPs expression in neurons and astrocytes in neurodegenerative diseases, we generated a *D. melanogaster* model for the expression of disease-associated polyglutamine (PolyQ) proteins and chaperones. To do so, we used the attP-site specific PhiC31 integrase system of *Drosophila* that allows the site-specific insertion of the transgenic constructs in the fly genome. We employed two different and independent binary expression systems - the GAL4-UAS and LexA-LexO systems - to drive the expression of the PolyQ protein in all neurons and the expression of chaperones in either neuronal cells or in astrocytes. We show that the two expression systems can drive the expression of the transgenes in a completely independent and non-overlapping manner and that levels of expression of the Lex-driven transgene can be controlled by the use of two different LexA trans-activators lines, LG for moderate and LhG for stronger expression. The *D. melanogaster* model generated as such is excellently suited for the investigation of cell-autonomous and non cell-autonomous effects of chaperones in PolyQ pathology and to study the potential interplay between neurons and astrocytes in these diseases.
1. Introduction

The aim of our project was to explore if Heat Shock Proteins (HSPs) - key regulators of the cellular protein quality control (PQC) system (Hartl et al., 2011) – can protect against the toxicity and neurodegeneration mediated by toxic aggregates of PolyQ proteins expressed in neurons, either when such chaperones are expressed in the same neuronal cells (cell-autonomous actions) or when these are expressed exclusively in the astrocytes (non-cell autonomous actions).

Whereas a vast amount of literature shows that specific HSPs in neurons can protect against toxic aggregates associated with neurodegenerative diseases in a cell-autonomous manner (Kakkar et al., 2014), limited to no data are available whether chaperones may also be protective in a non cell-autonomous manner, such as, for example, when they are expressed in non-neuronal cells. Interestingly, however, data from brain of patients and animal models of diseases show that several HSPs of various chaperone families, including small HSP and DNAJ, are up-regulated in astrocytes during neurodegeneration (Chapter 2, section 3.4.2). We hypothesized that such HSP-upregulation in these specific glial cells might not be merely a stress marker or a compensatory effect. Instead, we speculated that this might rather be part of a protective non-cell autonomous response of astrocytes in protein aggregation diseases and that the increased HSP levels in astrocytes might provide an additional level of neuroprotection. The proper evaluation of such a possibility requires the development of an in vivo model system allowing the cell-type specific expression of the disease-related proteins and chaperones in either neurons or astrocytes that can be regulated in a tightly controllable, inter-independent manner.

The methodological strategy that we choose was to generate an in vivo model for polyglutamine (PolyQ) disease, in which the disease-related protein is driven exclusively in neurons, where it would result in a disease-related phenotype (i.e. reduction in lifespan), caused by the aggregation of the PolyQ protein. To answer our questions, the expression system should allow the co-expression of a protective chaperone in the same neurons, to investigate cell-autonomous protective effects of the chaperone. Next, the model should give the possibility to compare such effects to what happens if the same chaperone is expressed only in astrocytes for eventual non-cell autonomous protective effects. As chaperone, we choose DNAJB6b (Hanai et al., 2003) for which our laboratory had established a comprehensive set of data on its capacity to have strong cell autonomous protective effects in PolyQ diseases, such as Huntington’s (Hageman et al., 2010; Kakkar et al., 2016).

D. melanogaster has been considered the ideal choice as in vivo model system to conduct our study. The central nervous system of D. melanogaster is well characterized in terms of neuronal structures and connections (Rein et al., 2002). Moreover, several morphologically types of glial cells in the adult Drosophila brain have been described, including astrocyte-like cells (Doherty et al., 2009) that are morphologically and molecularly similar to their mammalian counterparts. Astrocytes of Drosophila have extended, branched and ramified processes that establish structural and functional contacts with the synapse-rich regions of the fly brain (Doherty et al., 2009). The cell-specific expression of transgenes in the neuronal cells and astrocytes in Drosophila is permitted by
the use of well-characterized cell type specific promoters for such cells (Yao et al., 1993; Xiong et al., 1994; Doherty et al., 2009). Controlled and independent expression of the different transgenes is feasible by the use of the binary expression systems of Drosophila, i.e. the GAL4-UAS system (Brand and Perrimon, 1993) and the LexA-LexO system (Yagi et al., 2010). In addition, genome-site-specific insertion of the transgene (i.e. the attP-site specific PhiC31 integrase system; Bischof et al. 2007) can be used to avoid “position effects” due to aspecific insertion of the contract, allowing better comparison of the different phenotypes in the various experimental fly lines (Bischof et al. 2007). Finally, the genetics of D. melanogaster is well established and instrumental to the generation and maintenance of the fly lines (Greenspan, 2004).

In this Chapter, the generation of the D. melanogaster lines that are used for our experimental studies in chapter 4 and 5 will be described.

## 2. Materials and methods

### Vectors

UAS/LexO vectors were obtained by cloning the sequences of HttQ100-mRFP (Prof. T. Littleton Group, MIT) or V5-DNAJB6 (isoform B) or eGFP (Clontech) in the multiple cloning site of pUAS attB or pLexO attB (Prof. K. Basler Group, UZH). Driver (Promoter cell-specific expression) vectors were obtained starting from the backbone of plasmids pDPP-Gal4 attB or pDPP-LG attB or pDPP-LhG attB (Prof. K. Basler Group, UZH). DPP promoter was substituted with the sequence of promoter elav (pan-neuronal, from pElav-Casper vector, Prof. Liqun Luo, Stanford University), repo (pan-glial, from pENTRY-D-TOPO-Repo4.3 vector, Prof. C. Klämbt, University of Münster) or alrm (astrocytic, from pAlrm-Casper vector, Prof. M. Freeman, UMASS). All obtained vectors were sequenced. See table T1 for vectors list.

### Genotypes

Fig.4B: 1) active combinations w(-); UAS eGFP (D)/+; promoter Gal4/+ or w(-); UAS eGFP/ promoter LexA; +/- or w(-); promoter Gal4/+; LexO eGFP (A)/+.

Fig.5A: w(-); UAS eGFP/+; promoter Gal4/+ or w(-);promoter LexA (LG or LhG)/+; LexO eGFP/+.

Negative control: w(-);+/-;+/-.

Fig.5B: w(-); UAS eGFP(D)/+; promoter Gal4/+ or w(-);promoter LexA (LG or LhG)/+; LexO eGFP(A)/+.

Negative control: w(-);+/-;+/-.
Antibodies and reagents

Antibodies (dilutions are indicated in brackets for western blots (WB) and immunofluorescence (IF)) against huntingtin (Chemicon, MAB2166, WB 1:5000), eGFP (Clontech-Living Colours, cat.No.632375, WB 1:5000), α-tubulin (Sigma Aldrich, clone DM1A, WB 1:2000), V5 epitope tag in DNAJB6b (Thermo Fisher Scientific, cat. No.R960-25, WB 1:2000, IF 1:50), NC-82 (DSHB, WB 1:5000) were used. DAPI for nuclei staining (cat.No.D1306) was from Thermo Fisher Scientific. 20% SDS Solution (cat.No.1610418) was from BioRad. PBS components (NaCl cat.No.S9888, KCl cat.No.P9541, Na2HPO4 cat.No.255793, KH2PO4 cat.No.V000225), Tween-20 (cat.No.P2287), Triton X-100 (cat.No.T8787), Bovine Serum Albumin (cat.No.A2058, BSA), glycerol (cat.No.G5516), 3.7% Formaldehyde (cat.No.11-0705 SAJ), Tris base (cat.No.T1503) and β-mercaptoethanol (cat.No.M6250) were from Sigma Aldrich.

D. melanogaster stocks maintenance

All stocks and experimental flies were kept in polystyrene vials 25x95 mm filled with 8 ml/vial of solidified media (17 g/l Agar; 26 g/l Yeast; 54 g/l Sugar; 1.3 mg/l Nipagin). All experimental flies were maintained in a humidified and temperature controlled incubator at 25 °C on a 12 hours’ light and 12 hours’ dark cycle (Premium ICH Insect Chamber, Snijders Labs). Experimental flies, anesthetized on a CO2 pad, were selected according to their gender and phenotype by light microscope visualization.

Western Blotting D. melanogaster total head lysates preparation

30-40 D. melanogaster adults with specific phenotype, gender, age (days after pupal eclosion) and condition were collected; after freezing in liquid nitrogen and vortexing of entire flies, separated heads were collected, counted and lysed in SDS-rich buffer (SDS 1.45%, Glycerol 20%; Tris Base 0.2 M. 2.5 µl of buffer/head) using sonication (3 pulses of 50 Watt for 5 seconds). Homogenized lysate was then centrifuged at 1000 x g for 3 seconds to separate cuticle debris from supernatant. Proteins in supernatant were collected and quantified using spectrophotometry (Implant NanoPhotometer UV/Vis). Protein content was equalized. Samples, supplied with β-mercaptoethanol 5% and bromophenol blue, were boiled at 99 °C for 5 minutes. Equal amounts of volume were resolved on SDS-PAGE. Flies of the same line were collected from different vials and the entire experiment was repeated at least 2 times.

Western Blotting and Blot quantification

Following the preparation of samples, proteins were resolved by SDS-PAGE, transferred to nitrocellulose membrane and processed for Western Blotting. Primary antibodies (at concentrations mentioned above) were prepared in 3% BSA/PBS-Tween 20 0.1%, secondary antibodies at concentration 1:5000 (Invitrogen, horse peroxidase conjugated to IGG or IGM) in 5% milk/PBS-Tween 20 0.1%. For visualization membranes were incubated with Pierce ECL Western Blotting
substrate (cat. No. 32106) for 2 minutes and visualized using ChemiDoc Touch Imaging System (BioRad). Blots have been quantified using Image Lab Version 5.2.1 software (BioRad).

3. Results and Discussion

To generate the *D. melanogaster* model for comparing cell-autonomous and non-cell autonomous effects of chaperones in neurodegenerative diseases, we used the GAL4-UAS (Brand and Perrimon, 1993) and LexA-LexO (Yagi et al., 2010) expression systems of *Drosophila*. These systems have been shown to drive expression of transgenes in a completely independent and non-overlapping manner.

The expression of two different transgenes in specific cell types (i.e. neurons or astrocytes) of *D. melanogaster* brain is allowed by the use of cell-specific and well-characterized promoters (“drivers”) in the expression system. For the pan-neuronal expression of transgene, we used the promoter elav (Yao et al., 1993). To selectively express the second transgene in astrocytes, we used the promoter alrm (Doherty et al., 2009). Alternatively to alrm, and as additional control, we used the pan-glial promoter repo (Xiong et al., 1994) to express the second transgene in all glial cells.

The disease-causing PolyQ protein used in our *D. melanogaster* model of neurodegenerative disease is Huntingtin (HTT). We used a construct, hereafter referred to as HttQ100-mRFP, encoding for human PolyQ-HTT exons 1-12 (Weiss et al., 2012). As previously said, the chaperone used in our study is human DNAJB6 (short nuclear and cytosolic isoform B; Hanai et al., 2003).

3.1 Generation of the *D. melanogaster* lines

To generate the *D. melanogaster* lines for our experiments, the sequences of the above-indicated cell-specific promoters or the transgenes (for the PolyQ protein and the chaperone) were cloned into the respective GAL4-UAS and LexA-LexO plasmids, and sent to Best Gene Inc. injection service (https://www.thebestgene.com/HomePage.do), for injection and transformation of embryos of *Drosophila* (see Material and Methods and table T1).

These plasmids carry an attB site that allows the site specific insertion of the contracts in the fly genome, through the use of the FlyC31 system (Bischof et al. 2007). In this system, established by the Basler group, a library of several *D. melanogaster* lines have been generated, each line containing a specific landing platform with an attP site. Several lines of FlyC31 system are available with different positions of the attP landing site in the *D. melanogaster* genome (including chromosomes 2 and 3). The generation of the transgenic flies is based on the use of PhiC31 integrase, a serine-type recombinase that mediates the sequence-specific recombination between two largely different attachment sites, attB and attP, which share a 3 bp central region where such crossover occurs (Thorpe et al., 2000). This allows the transgenesis of the attB construct at the attP landing site.
Differently from other systems to generate transgenic flies (such as the P-element mediated transformation), the integration of the \textit{attB} vector at the \textit{attP}-site in the fly genome using the PhiC31 integrase is really efficient, irreversible and, more importantly, site-specific at the desired location (non-random integration). First, this allows to pre-select the positions of the constructs on the fly chromosomes, allowing to plan the required crosses to obtain the flies with the desired genotypes for the experiments in advance. Second, since the position of the transgenic sequence is controlled, all constructs integrated into the same locus can be directly compared (i.e. eGFP-expressing construct as control, and DNAJB6-expressing construct as experimental condition). Third, the \textit{attP} landing sites are selected to avoid insertion of the construct into life-essential endogenous fly genes or nearby enhancers and silencers sequences which can affect the transgene expression (“position effect”) in an unpredictable manner (Bischof et al. 2007).

Transgenic flies were detected by verifying the presence of the “red eyes” marker (w+) and an RFP marker that is expressed in the eyes of the adult via the 3xP3 promoter. The genetic sequences for these two markers are located at the site of insertion of the construct and are between LoxP sequences (Bischof et al. 2007). When needed (e.g. in experiments using fluorescence signals from the transgenes), the sequences for these two markers were removed by using a CRE-Recombinase line. The removal of the two markers were verified at the optical and fluorescent microscopes respectively. Importantly, the remaining transgenic sequences of the construct (e.g. the sequence for UAS-transgene of interest) are not removed by the CRE-recombinase (Bischof et al. 2007). All the lines were isogenised to remove background mutations by backcrossing each of them for 6 generations with the control stock w\textsuperscript{1118} line.

\textbf{3.2 Binary expression and crossing}

All the crossings required to generate the experimental flies described below were performed following the instructions and recommendations of the manual “Fly Pushing - The Theory and Practice of Drosophila Genetics” by Ralph J. Greenspan. For crossings, lines carrying balancer chromosomes, such as IF/CyO (for the second chromosome) or MKRS/TM6B (for the third chromosome) were used. Balancer chromosomes carry specific dominant markers which can be easily identified from the phenotype of the flies (e.g. flies carrying the marker CyO show curly wings), allowing the proper selection of desired flies for crossing or experiments. The use of such balancers prevents recombination between homologous chromosomes during the meiosis in the selected flies.

As an example for use of these balancer lines in \textit{Drosophila}, flies carrying the transgene A, (w+) / + (red eyes with normal shape, normal wings) at the second chromosome can be first crossed with the balancer line IF/CyO (white eyes with abnormal shape, curly wings) to obtain the heterozygous line A/CyO which is easily recognizable by having red normal eyes and curly wings (Greenspan, 2004). The offspring of these A/CyO flies can be still A/CyO or A/A (red eyes, normal wings) (Greenspan, 2004), allowing the maintenance of a population of flies respectively carrying the heterozygous or homozygous transgene. The line CyO/CyO is not viable (Greenspan, 2004). Similarly, flies carrying a transgene B at the third chromosome can be crossed with a balancer line.
such as MKRS/TM6B (Greenspan, 2004), to obtain the needed offspring B/TM6B for further crossing and experiments.

An expression system such as GAL4-UAS is constituted by two constructs (Fig.1): the first carries the cell-specific promoter (“driver”) that drives the expression of a transcriptional activator (the “trans-activator”), like the yeast-derived GAL4 protein; the second carries the sequence of the desired transgene, which is under the control of a promoter with an enhancer sequence, like the upstream activation sequence (UAS), that is specific to and recognized by the GAL4 protein. Importantly GAL4 (and trans-activators of other expression systems) lacks endogenous targets in D.melanogaster. The fly with the construct with the driver-GAL4 can next be crossed with another line with the construct UAS-gene of interest. In the offspring lines carrying both the constructs, the GAL4 protein will now bind the UAS sequence and activate the gene expression in the cells that the driver is specific for. Besides the possibility to study the expression of the desired gene in specific cells by using the various cell-specific promoters available, one of the advantages of this system is also that the transgenes are not expressed in the parental lines, but only in the offspring (first generation) obtained after the crossing. In this way, the parental lines carrying a toxic gene (e.g. a line with UAS-PolyQ-HTT) do not express it and can survive normally.

Figure 1: the GAL4-UAS system. See main text for explanation.
In our project, we required a second, independent system for which we selected the LexA-LexO binary expression system (Fig. 2). This system is based on the use of a trans-activator named LexA - a bacterial transcription factor - that binds specific sequences called LexO (LexA Operator). Similar to the GAL4-UAS system, here the cell-specific promoter drives the expression of LexA that can bind LexO in the promoter of the transgene of interest, allowing its expression. For our investigation, we made use of a refined LexA-LexO that was designed to be used independently in combination with the GAL4-UAS system (Brand and Perrimon, 1993). In additions, for this system two different LexA trans-activators are available: LG for moderate expression and LhG for stronger expression. In the latter, the transcriptional activity was improved by introducing the hinge region (H) of GAL4 in the original sequence of LG (Yagi et al., 2010).

Figure 2: the LexA-LexO system. See main text for explanation.

As previously said, in our investigation, we used GAL4-UAS and LexA-LexO in combination to express two transgenes in different cell types in the same fly. The combined use of the two systems to co-express two transgenes in different cell types in the same fly is illustrated in Fig. 3. By making use of the balancer lines with a distinct phenotype (see above), we first obtained the needed parental flies. By crossing the first parental line carrying the two drivers constructs (e.g. elav-GAL4 and alrm-LexA) with the second parental line carrying the two transgene constructs (e.g. UAS-eGFP and LexO-RFP), we obtained an offspring in which each transgene is driven by its respective cellspecific promoter (i.e. eGFP in neurons and RFP in astrocytes of the Drosophila brain) (Fig. 3).
3.3. Verification of the expression systems

To indeed verify that the two expression systems can drive the expression of the transgenes and in a completely independent and non-overlapping manner in the generated *Drosophila* lines, we monitored the expression of the fluorescent model protein eGFP expression as highly sensitive readout. To do so, we crossed the parental lines to obtain an offspring carrying a combination of constructs which can be positive or negative for the expression of eGFP. Whereas positive combinations (i.e. promoter-GAL4 > UAS eGFP or promoter-LexA > LexO eGFP) should lead to expression of eGFP, a negative combination (i.e. promoter-GAL4 > LexO eGFP or promoter-LexA > GAL4 eGFP) should not (Fig.4A).

Next, we verified in the experimental flies, by western blot analyses, that the inactive combinations (i.e. GAL4-LexO or LexA-UAS), in contrast with the active one (GAL4-UAS), do not lead to eGFP expression in the generated *D.melanogaster* lines (Fig.4B). Importantly, we verified this for the neuronal (*elav*), astrocytic (*alm*) and pan-gliial (*repo*) promoters, both in male and female flies (Fig.4B). These data indicate that the two systems are independent each other, implying that our *Drosophila* system can be used as a tool to investigate the interplay between neurons and astrocytes *in vivo*. In effect, these results indicate that each transgene is expressed in the desired cell types, therefore allowing the investigation of mechanisms such as the intercellular prion-like spreading of...
the disease-causing protein (section 2.6 of Chapter 2) or the release of chaperones via vesicle release from one cell type to another (Takeuchi et al., 2015), excluding that this might instead due to a leakage of the expression systems.

**Figure 4:** Validation of the Gal4-UAS and LexA-LexO expression systems

A) Schematic explanation and experimental confirmation (by using eGFP expression) of the independent Gal4-UAS and LexA-LexO expression systems. In the D. melanogaster lines carrying an “active combination” of a binary expression system, a cell-specific promoter (i.e. neuronal-, glial- or astrocytic- specific) drives the expression of the trans-activator (GAL4 or LexA) that recognizes and binds a specific sequence in the promoter of eGFP (UAS or LexO respectively), leading to the final expression of the transgene. In the “inactive combination”, the trans-activator does not recognize such sequence (i.e. in a line in which the trans-activator LexA is combined with the UAS sequence). B) Western blots of total head lysates of 15-day-old adult male and female flies in which the transgene UAS-eGFP or LexO-eGFP is driven (or not) by the Gal4 or LexA (LG or LhG) promoters (neuronal: elav, lanes 2-5; astrocytic: alrm, lanes 6-8; glial: repo, lanes 9-10). Active and inactive combinations, as defined in Fig.4A, are shown. Anti-GFP antibody for eGFP detection. Tubulin was used as loading control. Number of fly heads per each lysate sample and genotypes in Materials and Methods.

We also investigated the two different LexA trans-activators lines, LG for moderate and LhG for stronger expression. In Fig.5A-B, the levels of different eGFP expression, using moderate LG promoters or stronger LhG promoters (for neuronal, glial or astrocytic expression), are shown and compared with the GAL4-UAS mediated expression. Again, we verified this for the neuronal (elav), astrocytic (alrm) and pan-glial (repo) promoters, both in male and female flies (Fig.5A and 5B). The blots confirm that LhG promoters are stronger than the LG promoters, as also previously reported (Yagi et al., 2010). The possibility of titrating the level of expression of the transgene under the control of the LexA-LexO system opens to the possibility to compare how such level can influence the experimental read-outs. For instance, in our research the chaperone is under the control of the LexA-LexO system. By using the LG or the LhG drivers, we can investigate and compare how the level of expression of the HSP in neurons or astrocytes affects the phenotype of the Huntington’s model, exploring cell-autonomous and non-cell autonomous mechanisms. Chaperones can affect the aggregation and therefore toxicity of disease-causing PolyQ proteins and in vitro experiments indicate that the level of the HSPs is a key factor for protection and anti-aggregation activity (Månsson et al., 2014). Our model represents an advance because it allows to explore such aspects in an in vivo context.
**Fig.5: Level of transgene expression using the GAL4-UAS or LexA-LexO expression systems.** A) Western blots of total head lysates of 15-day-old male adult flies in which the transgene UAS-eGFP or LexO-eGFP is driven by the GAL4 or LexA promoter (LG or LhG) in neurons (elav) or astrocytes (alrm). eGFP lines with different chromosome insertion positions (Table T1) are shown. Anti-GFP antibody for eGFP detection. Tubulin was used as loading control. Number of fly heads per each lysate sample and genotypes in Materials and Methods.

B) Western blots of total head lysates of 15-day-old adult male and female flies in which the transgene UAS-eGFP or LexO-eGFP is driven by the GAL4 or LexA (LG or LhG) promoters (neuronal: elav, lanes 1-8; glial: repo, lanes 9-11; astrocytic: alrm, lanes 12-14). Levels of eGFP expression in different genders and promoters are shown. Anti-GFP antibody for eGFP detection. Tubulin was used as loading control. Number of fly heads per each lysate sample and genotypes in Materials and Methods.

In conclusion, we generated a *D.melanogaster* model that allows the independent and non-overlapping expression of two different transgenes of interest in different population of cells - neurons and astrocytes (or all glial cells) - of the fly brain, by using different cell-specific promoters and the GAL4-UAS and LexA-LexO systems. The use of the *attP*-site specific PhiC31 integrase system (Bischof et al. 2007) to generate these lines avoids “position effects” due to random integration of the transgenes in the fly genome. Moreover, such pre-determined insertion of the constructs in equivalent positions into the genome consents high comparability between the different lines. Finally, the use of two different trans-activators LG or LhG - also allows to titrate the level of expression of the transgene under the control of the LexA-LexO system (Yagi et al., 2010). All together, the data above indicate that the generated *Drosophila* model is ideal to answer our research questions and open to intriguing further perspectives.

### 3.4 Perspectives

The transgenic expression in neurons or astrocytes opens to the possibility to investigate the *in vivo* effects of such genetic manipulation when the same gene is expressed in the two different cell types (e.g. the reduction in lifespan of a toxic PolyQ protein when expressed in neurons or glial cells). More importantly, two different transgenes can be expressed at the same time in the two cell populations and this allows to investigate both cell-autonomous and non-cell autonomous effects.

Our model can find use in investigating the role of chaperones in the brain, against the toxicity mediated by the expression of disease-causing proteins associated with neurodegenerative diseases.
(as it will be illustrated in the Chapters 4 and 5). As previously explained, the protective chaperone DNAJB6 (Hageman et al., 2010; Kakkar et al., 2016) can be co-expressed with PolyQ Huntingtin in the same neurons. In a parallel experiment, using a different set of driver lines, the same chaperone DNAJB6 can be expressed in astrocytes whereas PolyQ Huntingtin is expressed in neurons. Hence, the non-cell autonomous effects and interplay between neurons and astrocytes can be explored and comparison with the effects observed in other combinations can be made. Nonetheless, the model can be used to explore other mechanisms and pathways between neurons and astrocytes, not only by over-expression of transgenes but also by the cell-specific silencing of certain genes.

In conclusion, our new established model highlights the potential of D.melanogaster as in vivo research tool to investigate the complex interlinks between neurons and astrocytes and paves the way to explore possible therapeutic options that exploit the crucial role of astrocytes in maintaining the neuronal fitness and viability in normal conditions, but also during disease (Chapter 2).
REFERENCES

SUPPLEMENTARY DATA

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UAS:LexO-TRANSGENE Lines

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<td>ZH-attP-58A3</td>
</tr>
<tr>
<td>UAS:eGFP (D)</td>
<td>w(-); UAS eGFP; +/+</td>
<td>2</td>
<td>R</td>
<td>ZH-attP-58A3</td>
</tr>
<tr>
<td>LexO V5-DNAJB6</td>
<td>w(-); LexO V5-DNAJB6; +/+</td>
<td>3</td>
<td>L</td>
<td>ZH-attP-68E1</td>
</tr>
<tr>
<td>LexO eGFP (A)</td>
<td>w(-); LexO eGFP; +/+</td>
<td>3</td>
<td>L</td>
<td>ZH-attP-68E1</td>
</tr>
<tr>
<td>LexO eGFP (B)</td>
<td>w(-); LexO eGFP; +/+</td>
<td>3</td>
<td>L</td>
<td>ZH-attP-62B</td>
</tr>
<tr>
<td>LexO eGFP (C)</td>
<td>w(-); LexO eGFP; +/+</td>
<td>3</td>
<td>L</td>
<td>ZH-attP-75D</td>
</tr>
</tbody>
</table>

**Table T1. D.melanogaster lines generated in this study.** T1 D.melanogaster lines generated by injection and transformation of embryos with attB plasmids, based on attP-site specific PhiC31 integrase system, by Best Gene Inc.

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