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Drosophila as a screening tool to study human neurodegenerative diseases

Sarah Lenz[1], Peter Karsten[1], Jörg B. Schulz[1,2], Aaron Voigt[1]

[1]Department of Neurology, University Medical Center, RWTH Aachen, Pauwelsstrasse 30, 52074 Aachen, Germany

[2]JARA – Translational Brain Medicine

Correspondence should be addressed to:

Aaron Voigt, PhD

Department of Neurology

University Medical Center

RWTH Aachen

Pauwelsstrasse 30

D-52074 Aachen

Germany

Phone: +49-241-808-5054

Fax: +49-241-808-2582

E-mail: **avoigt@ukaachen.de**

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Abstract

In an aging society, research involving neurodegenerative disorders is of paramount importance. Over the past few years, research on Alzheimer's and Parkinson's diseases has made tremendous progress. Experimental studies, however, rely mostly on transgenic animal models, preferentially using mice. Although experiments on mice have enormous advantages, they also have some inherent limitations, some of which can be overcome by the use of *Drosophila melanogaster* as an experimental animal. Among the major advantages of using the fly is its small genome, which can also be modified very easily. The fact that its genome lends itself to diverse alterations (e. g. mutagenesis, transposons) has made the fly a useful organism to perform large-scale and genome-wide screening approaches. This has opened up an entirely new field of experimental research aiming to elucidate genetic interactions and screen for modifiers of disease processes *in vivo*. Here, we provide a brief overview of how flies can be used to analyze molecular mechanisms underlying human neurodegenerative diseases.

Introduction

Neurodegenerative diseases like Alzheimer's disease, Parkinson's disease (PD) or frontotemporal lobar degeneration are devastating age-related disorders. The mechanisms leading to the development and onset of such diseases are still elusive. To date, we have found neither a cure for these diseases nor any means to arrest their progression. Thus, patients suffering from a neurodegenerative disease only receive symptomatic treatment. An in-depth understanding of disease etiology and the mechanisms involved in disease onset and progression, therefore, are a prerequisite for a rational design of potential therapies.

In order to shed light on the pathogenesis of neurodegenerative disorders, researchers utilize animal model organisms. Invertebrate model systems like *Drosophila melanogaster* are particularly suited

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to address certain questions pertaining to neurodegenerative disorders. Although evolutionarily separated, flies and humans share basic molecular mechanisms. In the context of neurodegenerative diseases, the fact that around 70% of disease-associated human genes have a fly homolog (Bier 2005) makes research with this organism feasible. Moreover, there is a reasonable similarity between the central nervous systems of flies and humans, with both consisting of neurons and glia and utilizing the same neurotransmitters. In combination with the great variety of established genetic tools, these similarities render *Drosophila* a useful model organism to study the etiology of human neurodegenerative diseases (Shulman *et al.* 2003). In this review, we would like to provide a brief overview of how research using *Drosophila* has led to novel insights into human neurodegenerative diseases.

Screens: Unbiased high throughput analysis in *Drosophila*

Among the advantages of using flies for research are low costs and efficient handling in terms of required room and time (Greenspan 2004), which allow maintenance of large collections of fly lines in stock centers with public access. Owing to the small and by now fully sequenced genome of flies (Adams *et al.* 2000, Rubin *et al.* 2000), large-scale screening approaches are easy to perform. Consequently, a plethora of classical forward and backward genetic screens have been used to identify specific genes in flies and elucidate their functions.

In forward screens, randomly mutagenized flies, e. g. by chemicals or X-ray radiation, are screened for disturbances of a pre-defined phenotype/process. Such screens have proved very helpful as highlighted by the Nobel prize to C. Nüsslein-Volhard, E. Wieschaus and E.B. Lewis in 1995. Here, forward genetics were used to screen for genes involved in early development, namely the segmentation of the embryo (Nusslein-Volhard & Wieschaus 1980). A drawback of such screens was the difficult and laborious fine-mapping of random mutations (usually loss-of-function mutations) to specific genes. In former times, mapping of mutations was only possible if the mutant allele caused

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an obvious, easy-to-score phenotype or lethality. This problem has been partially overcome by the development of single nucleotide polymorphism maps (Berger *et al*. 2001) or whole-genome sequencing (Blumenstiel *et al.* 2009). Another way of generating loss-of-function mutations in flies is transposon-mediated mutagenesis (e. g. by P-elements see (Bellen *et al.* 2004, Cooley *et al.* 1988). Here, transposable elements are mobilized with the aim of generating new insertion sites and thereby disrupting genes (Figure 1). The benefit of using transposable elements is the straight-forward mapping of the insertion site, which allows identification of the disrupted gene. However, in this way only a small percentage of the *Drosophila* chromosomes can be mutagenized because transposable elements do not randomly integrate into the genome but do so preferentially in specific hot-spots (Spradling *et al.* 1995, Bartolome *et al.* 2002, Peter *et al.* 2002). Despite that, non-random integrating transposons have been generated, for example minos (Metaxakis *et al.* 2005) or piggyBac (Thibault *et al.* 2004). Nevertheless, transposable elements are still useful tools, as they can be modified in a fashion to suit specific requirements and applications (e. g. gene disruption, enhancer trapping, introduction of recombination sites, over-/misexpression etc. (see for example (Ryder & Russell 2003, Akimoto *et al.* 2005, Hoehne *et al.* 2005, Hummel & Klambt 2008, Venken & Bellen 2012,)). This is why nowadays large collections of transposon-inserted fly lines are freely available (O'Kane & Gehring 1987, Bier *et al.* 1989, Thibault et al. 2004, Bellen *et al.* 2011), e. g. at the Bloomington *Drosophila* Stock Center (http://flystocks.bio.indiana.edu/), the Exelixis Collection at the Harvard Medical School (https://drosophila.med.harvard.edu/) and the *Drosophila* Genetic Resource Center in Kyoto (http://www.dgrc.kit.ac.jp/en/index.html).

Reverse genetic screens explore the functions of predefined genes (St Johnston 2002). After the disruption of a targeted gene, the resulting phenotype is analyzed. The fly provides a large variety of methods to disrupt target genes. Mobilization of transposable elements allows random generation of mutant alleles (Figure 1) (Ryder & Russell 2003, Kim *et al.* 2012, Kao & Lee 2013). In addition,

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classical homologous recombination can be employed for knockout or replacement of genes/alleles (Rong *et al*. 2002, Choi *et al*. 2009). Gene silencing by RNA interference (RNAi) has been established (for a review see (Kennerdell & Carthew 2000, Rao *et al.* 2009)), representing means of mimicking classical knockout strategies. Using the binary UAS/Gal4 expression system (Figure 2), short hairpin RNAs (shRNAs) can be expressed to efficiently induce RNAi-mediated silencing of endogenous genes in a spatiotemporal manner (Brand & Perrimon 1993). This permits analysis of the detrimental effects of gene inactivation in postmitotic cells of the nervous system. The Vienna *Drosophila* RNAi Center (VDRC, http://stockcenter.vdrc.at/control/main) implemented a collection of more than 22,000 transgenic *Drosophila* strains, each containing an inducible UAS-shRNA construct targeting a single protein-coding gene. More than 12,000 genes, or 88.2% of the fly genome, are represented in this collection (Dietzl *et al.* 2007).

One intriguing example of how reverse genetics can provide novel insights into human neurodegenerative diseases is the PINK1/Parkin pathway. To date, both PD-linked gene products are well accepted in mitochondrial quality control and mitophagy (removing dysfunctional mitochondria by authophagy). However, first *in vivo* evidence that PINK1 and Parkin might act in the same cellular pathway was derived from fly research. Analyzing loss-of-function mutations in *Drosophila Pink1* (generated by imprecise excision of a P-element, see Figure 1), it became evident that *Pink1*-deficient flies display almost identical phenotypes as described for *parkin*-deficient flies. This already suggested that both gene products might participate in the same pathway. Two groups independently confirmed this assumption by showing that overexpression of Parkin (using the UAS/Gal4-system) was able to rescue phenotypes observed in *Pink1*-deficient flies (Clark *et al.* 2006, Park *et al.* 2006).

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Modifier screens combine the advantages of forward and reverse genetic screens (St Johnston 2002). Modifier screens require predefined phenotypes that are easily accessible and sensitive to genetic modifications. In the context of neurodegenerative diseases, for example, the expression of a toxic, disease-linked gene product is targeted to the fly eye. This might result in a so-called rough eye phenotype (REP, for a review see Kumar (2012)) caused by degeneration of eye-specific cells, e. g. photoreceptors. Usually, these REPs are quite robust and display only small variability. Additionally, the severity of the REP correlates with the degree of cell loss. As the fly eye is easily accessible from the outside, and the enhancement or suppression of photoreceptor loss is reflected by changes in REP appearances, REPs provide an ideal readout for screens. An example of such a screening approach is provided in Figure 3.

In modifier screens, flies displaying a REP are crossed with flies with either loss-of-function mutations or misexpression of endogenous genes under UAS control. The F1 generation is then screened for obvious changes in REP appearance. Key benefits of such screens are: first, they can be easily and quickly conducted, allowing the screening of large collections of potential modifiers *in vivo*. Second, by external investigation of the REP, the degree of photoreceptor loss as an indicator for neurotoxicity can be evaluated. Third, epistatic interactions can be revealed and therefore even genes that would not be normally detected in a traditional forward screen may be identified. Modifier screens are the current standard and variations mainly exist in the choice of effector lines. A rough summary of performed screens on neurodegenerative diseases in flies is presented in Table 1. In the following paragraphs, we present some screens in more detail to illustrate the general screening approaches, providing examples of how results from such screens have enriched our knowledge of neurodegenerative diseases.

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Several examples of modifier screens in *Drosophila* are summarized below. In one of the first modifier screens, a fly line with eye-specific expression of a polyglutamine (polyQ) tract derived from Huntingtin harboring 127 glutamine repeats was generated. These flies were crossed with 7,000 *de novo*-generated autosomal P-element insertion strains. The F1 generation was analyzed for changes in the polyQ-dependent REP, which served as readout for genetic interactions. In this screen, dHDJ1 (*Drosophila* NH_2-terminal J domain with homology to human HSP40/HDJ1) and dTPR2 (*Drosophila* tetratricopeptide repeat protein 2) were identified as suppressors of toxicity (Kazemi-Esfarjani & Benzer 2000). Shulman and Feany used a very similar approach to identify modifiers of REP generated by Tau-induced toxicity. In their screen, alterations of a Tau-induced REP served as a readout (Shulman & Feany 2003). In contrast to the screen by Kazemi-Esfarjani and colleagues, Shulman and Feany used a collection of roughly 2,000 lines containing EP elements. Inserted in close vicinity to a given gene, UAS sites within the EP element allow gene overexpression or silencing (depending on the orientation of the gene with respect to the EP element) under GAL4 control (Figure 1). Accordingly, the screen by Shulman and Feany allowed the identification of gain-of-function and loss-of-function modifiers of Tau toxicity. Interestingly, one third of the modifiers identified in the screen encoded for protein kinases and phosphatases, of which some were shown to phosphorylate Tau *in vitro.* One of these modifiers was the kinase Par-1. In subsequent studies, Par-1 has been shown to play an initiator role in Tau phosphorylation, triggering additional, temporally ordered phosphorylations of Tau by downstream kinases like Cdk5 and GSK-3β. In summary, the sequential phosphorylations result in the generation of toxic Tau species (Nishimura *et al.* 2004, Chatterjee *et al.* 2009, Ambegaokar & Jackson 2011). To date, GSK-3β is accepted to be one of the main kinases in Tau phosphorylation. Of note in this context, Jackson and co-workers established a direct link of altered GSK-3β levels and Tau toxicity *in vivo*. The authors were able to show that GSK-3β overexpression enhanced Tau phosphorylation and toxicity, while reducing GSK-3β levels suppressed Tau toxicity (Jackson *et al.* 2002).

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An elegant variation of the classical modifier screens using EP elements was used by Bilen and Bonini (2007). They performed a genome-wide screen for new modifiers of the REP generated by polyQ-induced toxicity. Initially, they performed a screen with a subset of 2,300 available EP-element insertion lines. Furthermore, *de novo* EP-element insertions were generated and only those novel insertions that modified polyQ toxicity were selected for further analysis (Bilen & Bonini 2007). In continuation of this work, Lessing and Bonini showed that toxicity induced by a truncated form of Ataxin-3 with a polyQ expansion is dependent on normal activity of Ataxin-2. This interaction depends on a conserved protein-interaction motif of Ataxin-2 and binding of cytoplasmic poly(A)-binding protein (PABP) to this motif. These findings suggest that the normal roles of Ataxin-2 and PABP regulate translation of specific mRNAs, which are critical to SCA3 disease (Lessing & Bonini 2008). Finally, Voßfeldt and co-workers were the first to perform a large-scale screen using a collection of UAS-shRNA lines (Figure 3). The question that was addressed by their study was whether or not eye-specific silencing of almost 7,000 genes representing human orthologues had an impact on the REP induced by the expression of a toxic Ataxin-3 species. The candidate interactor genes (roughly 500, involved in various cellular processes) obtained in this study constitute a valuable pool for future research on modifiers of genes involved in neurodegenerative disorders (Voßfeldt *et al.* 2012).

In summary, modifier screens using alterations in REPs induced by eye-specific expression of disease-linked, toxic gene products are valuable tools. In addition to the REP, there are certainly multiple other readout systems to address neurodegeneration and neuronal dysfunction in *Drosophila.* Some of these readout systems address parameters of fly behavior like locomotion, flight, vision and longevity. Electrophysiological recording of neurons allows direct assessment of neuronal dysfunction (e. g. recording of synaptic transmission in the giant fiber pathway or retinogram). Moreover, histological analysis is frequently used to address neurodegeneration and

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cell death (e. g. vacuolization of fly brains upon neuron loss). In the context of neurodegenerative diseases, these assays are more significant than the REP, which explains their frequent use ín the verification of genetic interactions found in REP-based screens. However, in conjunction with large-scale screen, the REP is the easiest to score, facilitating the fastest readout.

Depending on the disease model analyzed, other readouts might allow for large-scale screens as well. One such example is described below. Eye-specific expression of the well-known PD-linked protein α-Synuclein does not cause a REP. Nevertheless, expression of α-Synuclein in aminergic (serotonergic and dopaminergic) neurons of the fly is detrimental. Depending on the strength of α-Synuclein expression, flies show an age-dependent decline in locomotion and earlier mortality (Butler *et al.* 2012). Interestingly, dopamine (DA) levels in heads derived from flies with aminergic α-Synuclein expression are reduced, indicating that α-Synuclein causes dysfunction of DA neurons. DA levels in fly heads are easy to determine by HPLC. Butler and coworkers used the α-Synuclein-induced decline in DA as readout to conduct a screen (Butler *et al*. 2012). One candidate derived from this screening approach was the HSP90-like mitochondrial chaperone TRAP1. The authors showed that reduction of TRAP1 significantly enhanced DA decline and other detrimental effects of α-Synuclein in flies (reduced climbing ability, decline in longevity, loss of DA neurons), while expression of human TRAP1 provided rescue. As an extension of this work, two groups independently showed that TRAP1 (fly and human) is also able to rescue PINK1 but not Parkin loss-of-function phenotypes (Costa *et al.* 2013, Zhang *et al.* 2013). These results were confirmed in cultured human cells, emphasizing the conserved role of TRAP1 in detrimental effects of α-Synuclein and its function within the PINK1/Parkin pathway (Butler *et al*. 2012, Zhang *et al*. 2013). In summary, the data suggest that mitochondrial TRAP1 is an important factor in α-Synuclein and PINK1-induced PD and that enhancing TRAP1 activity might represent a strategy for therapeutic approaches. The

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data also tightly link α-Synuclein toxicity to mitochondrial dysfunction, implicating that dominantly inherited forms of PD may involve mitochondrial pathology.

All in all, *Drosophila melanogaster* is now an established model organism to study human neurodegenerative diseases. The high degree of conservation in molecular pathways between flies and humans has led to the discovery of novel pathomechanisms in disease, which we have sought to emphasize here. In conclusion, future biased and unbiased research using *Drosophila* will help shed light on disease mechanisms in human neurodegenerative diseases.

Conflict of interest

The authors declare no conflicts of interest.

References

Adams, M. D., Celniker, S. E., Holt, R. A. et al. (2000) The genome sequence of Drosophila melanogaster. *Science,* **287,** 2185-2195.

Akimoto, A., Wada, H. and Hayashi, S. (2005) Enhancer trapping with a red fluorescent protein reporter in Drosophila. *Dev Dyn,* **233,** 993-997.

Ambegaokar, S. S. and Jackson, G. R. (2011) Functional genomic screen and network analysis reveal novel modifiers of tauopathy dissociated from tau phosphorylation. *Hum Mol Genet,* **20,** 4947-4977.

Bartolome, C., Maside, X. and Charlesworth, B. (2002) On the abundance and distribution of transposable elements in the genome of Drosophila melanogaster. *Mol Biol Evol,* **19,** 926-937.

Bellen, H. J., Levis, R. W., He, Y. et al. (2011) The Drosophila gene disruption project: progress using transposons with distinctive site specificities. *Genetics,* **188,** 731-743.

Bellen, H. J., Levis, R. W., Liao, G. et al. (2004) The BDGP gene disruption project: single transposon insertions associated with 40% of Drosophila genes. *Genetics,* **167,** 761-781.

Berger, J., Suzuki, T., Senti, K. A., Stubbs, J., Schaffner, G. and Dickson, B. J. (2001) Genetic mapping with SNP markers in Drosophila. *Nat Genet,* **29,** 475-481.

This article is protected by copyright. All rights reserved.

Accepted Article

Bier, E. (2005) Drosophila, the golden bug, emerges as a tool for human genetics. *Nat Rev Genet,* **6,** 9-23.

Bier, E., Vaessin, H., Shepherd, S. et al. (1989) Searching for pattern and mutation in the Drosophila genome with a P-lacZ vector. *Genes Dev,* **3,** 1273-1287.

Bilen, J. and Bonini, N. M. (2007) Genome-wide screen for modifiers of ataxin-3 neurodegeneration in Drosophila. *PLoS Genet,* **3,** 1950-1964.

Blard, O., Feuillette, S., Bou, J., Chaumette, B., Frebourg, T., Campion, D. and Lecourtois, M. (2007) Cytoskeleton proteins are modulators of mutant tau-induced neurodegeneration in Drosophila. *Hum Mol Genet,* **16,** 555-566.

Blumenstiel, J. P., Noll, A. C., Griffiths, J. A., Perera, A. G., Walton, K. N., Gilliland, W. D., Hawley, R. S. and Staehling-Hampton, K. (2009) Identification of EMS-induced mutations in Drosophila melanogaster by whole-genome sequencing. *Genetics,* **182,** 25-32.

Brand, A. H. and Perrimon, N. (1993) Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development,* **118,** 401-415.

Butler, E. K., Voigt, A., Lutz, A. K. et al. (2012) The mitochondrial chaperone protein TRAP1 mitigates alpha-Synuclein toxicity. *PLoS Genet,* **8,** e1002488.

Cao, W., Song, H. J., Gangi, T., Kelkar, A., Antani, I., Garza, D. and Konsolaki, M. (2008) Identification of novel genes that modify phenotypes induced by Alzheimer's beta-amyloid overexpression in Drosophila. *Genetics,* **178,** 1457-1471.

Chang, H. C., Dimlich, D. N., Yokokura, T. et al. (2008) Modeling spinal muscular atrophy in Drosophila. *PLoS One,* **3,** e3209.

Chatterjee, S., Sang, T. K., Lawless, G. M. and Jackson, G. R. (2009) Dissociation of tau toxicity and phosphorylation: role of GSK-3beta, MARK and Cdk5 in a Drosophila model. *Hum Mol Genet,* **18,** 164-177.

Choi, C. M., Vilain, S., Langen, M., Van Kelst, S., De Geest, N., Yan, J., Verstreken, P. and Hassan, B. A. (2009) Conditional mutagenesis in Drosophila. *Science,* **324,** 54.

Clark, I. E., Dodson, M. W., Jiang, C., Cao, J. H., Huh, J. R., Seol, J. H., Yoo, S. J., Hay, B. A. and Guo, M. (2006) Drosophila pink1 is required for mitochondrial function and interacts genetically with parkin. *Nature,* **441,** 1162-1166.

Cooley, L., Kelley, R. and Spradling, A. (1988) Insertional mutagenesis of the Drosophila genome with single P elements. *Science,* **239,** 1121-1128.

Costa, A. C., Loh, S. H. and Martins, L. M. (2013) Drosophila Trap1 protects against mitochondrial dysfunction in a PINK1/parkin model of Parkinson's disease. *Cell Death Dis,* **4,** e467.

Dietzl, G., Chen, D., Schnorrer, F. et al. (2007) A genome-wide transgenic RNAi library for conditional gene inactivation in Drosophila. *Nature,* **448,** 151-156.

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Accepted Article

Doumanis, J., Wada, K., Kino, Y., Moore, A. W. and Nukina, N. (2009) RNAi screening in Drosophila cells identifies new modifiers of mutant huntingtin aggregation. *PLoS One,* **4,** e7275.

Esposito, G., Vos, M., Vilain, S., Swerts, J., De Sousa Valadas, J., Van Meensel, S., Schaap, O. and Verstreken, P. (2013) Aconitase causes iron toxicity in Drosophila pink1 mutants. *PLoS Genet,* **9,** e1003478.

Fernandes, C. and Rao, Y. (2011) Genome-wide screen for modifiers of Parkinson's disease genes in Drosophila. *Mol Brain,* **4,** 17.

Fernandez-Funez, P., Nino-Rosales, M. L., de Gouyon, B. et al. (2000) Identification of genes that modify ataxin-1-induced neurodegeneration. *Nature,* **408,** 101-106.

Greene, J. C., Whitworth, A. J., Andrews, L. A., Parker, T. J. and Pallanck, L. J. (2005) Genetic and genomic studies of Drosophila parkin mutants implicate oxidative stress and innate immune responses in pathogenesis. *Hum Mol Genet,* **14,** 799-811.

Greenspan, R. J. (2004) *Fly Pushing - The Theory and Practice of Drosophila Genetics*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Higashiyama, H., Hirose, F., Yamaguchi, M., Inoue, Y. H., Fujikake, N., Matsukage, A. and Kakizuka, A. (2002) Identification of ter94, Drosophila VCP, as a modulator of polyglutamine-induced neurodegeneration. *Cell Death Differ,* **9,** 264-273.

Hoehne, M., de Couet, H. G., Stuermer, C. A. and Fischbach, K. F. (2005) Loss- and gain-of-function analysis of the lipid raft proteins Reggie/Flotillin in Drosophila: they are posttranslationally regulated, and misexpression interferes with wing and eye development. *Mol Cell Neurosci,* **30,** 326-338.

Hummel, T. and Klambt, C. (2008) P-element mutagenesis. *Methods Mol Biol,* **420,** 97-117.

Ito, K., Sass, H., Urban, J., Hofbauer, A. and Schneuwly, S. (1997) GAL4-responsive UAS-tau as a tool for studying the anatomy and development of the Drosophila central nervous system. *Cell Tissue Res,* **290,** 1-10.

Jackson, G. R., Wiedau-Pazos, M., Sang, T. K., Wagle, N., Brown, C. A., Massachi, S. and Geschwind, D. H. (2002) Human wild-type tau interacts with wingless pathway components and produces neurofibrillary pathology in Drosophila. *Neuron,* **34,** 509-519.

Kao, C. F. and Lee, T. (2013) Reverse genetics by loss-of-function mosaic analysis in Drosophila. *Cold Spring Harb Protoc,* doi:10.1101/pdb.prot071670.

Kazemi-Esfarjani, P. and Benzer, S. (2000) Genetic suppression of polyglutamine toxicity in Drosophila. *Science,* **287,** 1837-1840.

Kennerdell, J. R. and Carthew, R. W. (2000) Heritable gene silencing in Drosophila using double-stranded RNA. *Nat Biotechnol,* **18,** 896-898.

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Accepted Article

Kim, H., Kim, K., Kim, J., Kim, S. H. and Yim, J. (2012) Mutagenesis by imprecise excision of the piggyBac transposon in Drosophila melanogaster. *Biochem Biophys Res Commun,* **417,** 335-339.

Kumar, J. P. (2012) Building an ommatidium one cell at a time. *Dev Dyn,* **241,** 136-149.

Latouche, M., Lasbleiz, C., Martin, E. et al. (2007) A conditional pan-neuronal Drosophila model of spinocerebellar ataxia 7 with a reversible adult phenotype suitable for identifying modifier genes. *J Neurosci,* **27,** 2483-2492.

Lawal, H. O., Terrell, A., Lam, H. A. et al. (2012) Drosophila modifier screens to identify novel neuropsychiatric drugs including aminergic agents for the possible treatment of Parkinson's disease and depression. *Mol Psychiatry*.

Lessing, D. and Bonini, N. M. (2008) Polyglutamine genes interact to modulate the severity and progression of neurodegeneration in Drosophila. *PLoS Biol,* **6,** e29.

Li, L. B., Yu, Z., Teng, X. and Bonini, N. M. (2008) RNA toxicity is a component of ataxin-3 degeneration in Drosophila. *Nature,* **453,** 1107-1111.

Llamusi, B., Bargiela, A., Fernandez-Costa, J. M., Garcia-Lopez, A., Klima, R., Feiguin, F. and Artero, R. (2013) Muscleblind, BSF and TBPH are mislocalized in the muscle sarcomere of a Drosophila myotonic dystrophy model. *Dis Model Mech,* **6,** 184-196.

Metaxakis, A., Oehler, S., Klinakis, A. and Savakis, C. (2005) Minos as a genetic and

genomic tool in Drosophila melanogaster. *Genetics,* **171,** 571-581.

Nishimura, I., Yang, Y. and Lu, B. (2004) PAR-1 kinase plays an initiator role in a temporally ordered phosphorylation process that confers tau toxicity in Drosophila. *Cell,* **116,** 671-682.

Nusslein-Volhard, C. and Wieschaus, E. (1980) Mutations affecting segment number and polarity in Drosophila. *Nature,* **287,** 795-801.

O'Kane, C. J. and Gehring, W. J. (1987) Detection in situ of genomic regulatory elements in Drosophila. *Proc Natl Acad Sci U S A,* **84,** 9123-9127.

Osterwalder, T., Yoon, K. S., White, B. H. and Keshishian, H. (2001) A conditional tissue-specific transgene expression system using inducible GAL4. *Proc Natl Acad Sci U S A,* **98,** 12596-12601.

Park, J., Al-Ramahi, I., Tan, Q. et al. (2013) RAS-MAPK-MSK1 pathway modulates ataxin 1 protein levels and toxicity in SCA1. *Nature,* **498,** 325-331.

Park, J., Lee, S. B., Lee, S. et al. (2006) Mitochondrial dysfunction in Drosophila PINK1 mutants is complemented by parkin. *Nature,* **441,** 1157-1161.

Peter, A., Schottler, P., Werner, M. et al. (2002) Mapping and identification of essential gene functions on the X chromosome of Drosophila. *EMBO Rep,* **3,** 34-38.

This article is protected by copyright. All rights reserved.

Accepted Article

Rao, D. D., Vorhies, J. S., Senzer, N. and Nemunaitis, J. (2009) siRNA vs. shRNA: similarities and differences. *Adv Drug Deliv Rev,* **61,** 746-759.

Rong, Y. S., Titen, S. W., Xie, H. B. et al. (2002) Targeted mutagenesis by homologous recombination in D. melanogaster. *Genes Dev,* **16,** 1568-1581.

Rubin, G. M., Yandell, M. D., Wortman, J. R. et al. (2000) Comparative genomics of the eukaryotes. *Science,* **287,** 2204-2215.

Ryder, E. and Russell, S. (2003) Transposable elements as tools for genomics and genetics in Drosophila. *Brief Funct Genomic Proteomic,* **2,** 57-71.

Shulman, J. M. and Feany, M. B. (2003) Genetic modifiers of tauopathy in Drosophila. *Genetics,* **165,** 1233-1242.

Shulman, J. M., Shulman, L. M., Weiner, W. J. and Feany, M. B. (2003) From fruit fly to bedside: translating lessons from Drosophila models of neurodegenerative disease. *Curr Opin Neurol,* **16,** 443-449.

Spradling, A. C., Stern, D. M., Kiss, I., Roote, J., Laverty, T. and Rubin, G. M. (1995) Gene disruptions using P transposable elements: an integral component of the Drosophila genome project. *Proc Natl Acad Sci U S A,* **92,** 10824-10830.

St Johnston, D. (2002) The art and design of genetic screens: Drosophila melanogaster. *Nat Rev Genet,* **3,** 176-188.

Tan, L., Schedl, P., Song, H. J., Garza, D. and Konsolaki, M. (2008) The Toll-->NFkappaB signaling pathway mediates the neuropathological effects of the human Alzheimer's Abeta42 polypeptide in Drosophila. *PLoS One,* **3,** e3966.

Thibault, S. T., Singer, M. A., Miyazaki, W. Y. et al. (2004) A complementary transposon tool kit for Drosophila melanogaster using P and piggyBac. *Nat Genet,* **36,** 283-287.

Venken, K. J. and Bellen, H. J. (2012) Genome-wide manipulations of Drosophila melanogaster with transposons, Flp recombinase, and PhiC31 integrase. *Methods Mol Biol,* **859,** 203-228.

Voßfeldt, H., Butzlaff, M., Prüßing, K. et al. (2012) Large-scale screen for modifiers of ataxin-3-derived polyglutamine-induced toxicity in Drosophila. *PLoS One,* **7,** e47452.

Yang, Y., Gehrke, S., Haque, M. E. et al. (2005) Inactivation of Drosophila DJ-1 leads to impairments of oxidative stress response and phosphatidylinositol 3-kinase/Akt signaling. *Proc Natl Acad Sci U S A,* **102,** 13670-13675.

Zhang, L., Karsten, P., Hamm, S. et al. (2013) TRAP1 rescues PINK1 loss-of-function phenotypes. *Hum Mol Genet*.

Zhang, S., Binari, R., Zhou, R. and Perrimon, N. (2010) A genomewide RNA interference screen for modifiers of aggregates formation by mutant Huntingtin in Drosophila. *Genetics,* **184,** 1165-1179.

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Figure legends

Figure 1: Use of transposable elements. Schematic representation of *Drosophila* chromosomes with a transposable element (e. g. a P-element, grey triangle) inserted on 3rd chromosome. In mutagenesis screens using transposable elements (here on 3rd chromosome), the element is mobilized (arrow) and new integrations (black triangle) are selected. With the determination of the exact integration site, potential mutagenized genes (exemplified gene, exons schematically indicated as boxes: untranslated regions in white, coding regions in grey) are identified. An integration of the transposable element in coding regions of a gene usually causes gene disruption. Gene disruption is less evident if the insertion site is located in untranslated regions or an intron of a gene. Such insertion might cause the full variety of effects, ranging from no effect at all to amorphic mutations. Even if such a mutation has no effect on gene function, it allows mutagenesis of the gene. Repeated excision (arrow) of the element in rare cases might result in removal of neighboring sequences (imprecise excision), thus creating (partial) gene deletions.

Transposable elements have been modified to fulfill multiple functions (for detailed description see (Hummel & Klambt 2008)). One example is the introduction of UAS sites into transposable elements (e. g. EP-elements). Depending on the site and direction of integration, such an element (blue triangle) might allow overexpression (upper) or RNAi-mediated silencing (lower) of neighboring genes in a Gal4-dependent manner.

Figure 2: The UAS/Gal4 system. The binary UAS/Gal4 system consists of the yeast transcription factor Gal4 and its specific binding sites, the so-called Upstream Activating Sequences (UAS). Upon Gal4 binding to UAS sites expression of downstream sequences is activated. The *Drosophila* genome is devoid of the *Gal4* gene and UAS sites, and fly transcription factors do not activate expression of sequences under UAS control. Thus, this transcriptional activation system was genetically modified to generate an artificial expression system in *Drosophila* (Brand & Perrimon 1993, Ito *et al.* 1997,

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Osterwalder *et al.* 2001). Therefore, two different species of transgenic fly lines were generated. The first one is the so-called Gal4 driver line. In these flies Gal4 expression is controlled by a specific endo- or exogenous promoter (element) resulting in a characteristic spatio-temporal Gal4 expression pattern. Hundreds of different Gal4 driver lines are available to researchers at public stock centers. The other is the UAS fly line. There are almost no limits with regard to the UAS-targeted sequences. This could be any protein-coding cDNA or DNA coding for short hairpin (sh) forming RNAs. Expression of such shRNAs will initiate a pathway finally causing gene-specific mRNA degradation by RNA interference (RNAi). By mating flies transgenic for a Gal4 driver and a UAS construct, respectively, Gal4 and its cognate UAS binding sites will be present in the offspring. Consequently, only the F1 generation will display Gal4 activated expression of UAS-controlled sequences. Thus, by choosing a specific Gal4 driver line, expression of UAS-controlled sequences can be directed to various tissues or cell types even with temporal resolution.

Figure 3: Large-scale modifier screens. We use the work of Voßfeldt and colleagues as an example to illustrate large-scale modifier screens in *Drosophila* (Voßfeldt *et al.* 2012). Shown is a flow chart to illustrate the different steps of the screening procedure. In this screen, Gal4 activated eye-specific expression of an Ataxin-3-derived polyQ stretch induced a rough eye phenotype (REP). This REP was found to be sensitive towards genetic modification (see eye pictures). As effector lines, a collection of 7,488 UAS-shRNA fly lines was used. These lines represent a selection of shRNA lines, capable of silencing almost all fly genes known to have an ortholog in humans (6,930 genes, roughly 50% of all protein coding genes in the fly genome). In a first step, the authors excluded those shRNA lines of which eye-specific expression altered external eye structures. The remaining 6,644 lines were analyzed for their ability to modify the polyQ-induced REP. In sum, 508 shRNAs were identified to either enhance or suppress the polyQ-induced REP. In flies, such a large-scale and high throughput analysis can be easily performed by one person within a year.

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Table 1: Overview and summary of screens identifying modifiers of neurodegenerative disease pathology in *Drosophila*.

List of modifier screens in neurodegeneration		
a) Protein **b) Screened system**	**Short description**	**Reference**
Alzheimer's Disease (including tauopathies)		
a) Tau[V337M] b) GOF	**Readout:** REP induced by eye-specific (GMR) expression of Tau[V337M] **Screened:** 2,276 EP insertion strains	(Shulman & Feany 2003)
a) Tau[V337M] b) GOF	**Readout:** REP induced by eye-specific (GMR) expression of Tau[V337M] **Screened:** ~1,200 P-Mae-UAS.6.11 insertion lines , facilitating RNAi or overexpression of neighboring genes	(Blard *et al.* 2007)
a) Tau[WT] b) GOF	**Readout:** REP induced by eye-specific (GMR) expression of Tau[WT] **Screened:** ~1,000 P-lethal and 900 EY insertion strains	(Ambegaokar & Jackson 2011)
a) Aβ42 b) GOF	**Readout:** REP induced by eye-specific (GMR) expression of Aβ42 **Screened:** >200 chromosomal deficiencies (autosomal)	(Tan *et al.* 2008)
a) Aβ42 b) GOF	**Readout:** REP induced by eye-specific (GMR) expression of Aβ42 **Screened:** 1,963 EP insertion strains	(Cao *et al.* 2008)
Parkinson's Disease		
a) DJ-1 b) LOF	**Readout:** REP upon RNAi-mediated silencing of DJ-1 **Screened:** biased selection of potential interactors (PI3K/PTEN/Akt pathway)	(Yang *et al.* 2005)
a) Parkin b) LOF	**Readout:** flight defect and reduced viability in *parkin*-deficient flies **Screened:** 2,400 EP insertion strains	(Greene *et al.* 2005)
a) PINK1 and Parkin	**Readout:** abnormal wing posture	(Fernandes &

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b) LOF	**Screened:** >200 chromosomal deficiencies (autosomal)	Rao 2011)
a) α-Synuclein[A53T] b) GOF	**Readout:** dopamine level in fly heads **Screened:** >270 chromosomal deficiencies (genome-wide)	(Butler *et al.* 2012)
a) VMAT b) LOF	**Readout:** locomotion deficits in larvae induced by partial loss of the vesicular monoamine transporter (VMAT) **Screened:** ~1,000 known drugs	(Lawal *et al.* 2012)
a) PINK1 b) LOF	**Readout:** flight defect in *Pink1*-deficient flies **Screened:** 193 EMS alleles	(Esposito *et al.* 2013)
Polyglutamine (polyQ) Diseases		
a) polyQ b) GOF	**Readout:** REP induced by eye-specific (GMR) expression of polyQ **Screened:** 7,000 *de novo*–generated autosomal P-element insertion strains	(Kazemi-Esfarjani & Benzer 2000)
a) SCA1-Q82 b) GOF	**Readout:** REP induced by eye-specific (GMR) expression of Ataxin 1 (Q82) **Screened:** 1,500 P-lethal and 3,000 EP insertion strains	(Fernandez-Funez *et al.* 2000)
a) polyQ b) GOF	**Readout:** REP induced by eye-specific (GMR) expression of polyQ **Screened:** unknown number P-element insertion strains	(Higashiyama *et al.* 2002)
a) SCA7 derived polyQ b) GOF	**Readout:** Longevity induced by pan neural (*elav*) expression of polyQ **Screened:** biased selection of 36 modifiers identified in previous REP-based screens	(Latouche *et al.* 2007)
a) SCA3-derived polyQ b) GOF	**Readout:** REP induced by eye-specific (GMR) expression of polyQ **Screened:** 2,300 EP insertion lines and an unknown number of *de novo* EP insertion lines	(Bilen & Bonini 2007)
a) SCA3-derived polyQ b) GOF	**Readout:** REP induced by eye-specific (GMR) expression of polyQ **Screened:** unknown number of EP insertion lines	(Li *et al.* 2008)

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a) Huntingtin (exon 1) with different length of polyQ b) GOF	**Readout:** polyQ aggregation in BG2-c2 cells **Screened:** BG2-c2 cell-based aggregation screen using 7,200 dsRNAs, candidates confirmed in flies (changes in REP induced by eye-specific (GMR) expression polyQ)	(Doumanis *et al.* 2009)
a) Huntingtin (exon 1) with different polyQ length b) GOF	**Readout:** polyQ aggregation in S2 cells **Screened:** genome-wide RNAi screen on aggregation in S2 cells, candidates confirmed in flies (changes in REP induced by eye-specific (GMR) expression polyQ)	(Zhang *et al.* 2010)
a) SCA3 derived polyQ b) GOF	**Readout:** REP induced by eye-specific (GMR) expression of polyQ **Screened:** collection of roughly 8,000 RNAi lines (VDRC)	(Voßfeldt et al. 2012)
a) SCA1-Q82 b) GOF	**Readout:** REP induced by eye-specific (GMR) expression of Ataxin 1 (Q82) **Screened:** biased selection of 704 alleles effecting 337 kinases	(Park *et al.* 2013)
Motor Neuron Diseases		
a) *survival motor neuron* (*smn*) linked to SMA b) LOF	**Readout:** lethality induced by *smn*-LOF **Screened:** Exelixis collection, unknown number of diverse transposon integration lines	(Chang *et al.* 2008)
a) *Dystrophia Myotonica Protein Kinase* gene (*DMPK*) linked to Myotonic Dystrophy Type 1 (DM1) b) GOF	**Readout:** REP induced by expression of non-coding CTG repeats in the 3′ untranslated region (UTR) of the *DMPK* gene **Screened:** 1,215 randomly chosen RNAi lines (NIG-Fly collection)	(Llamusi *et al.* 2013)

GOF: gain-of-function. LOF: loss-of-function. REP: rough eye phenotype. GMR: glass multimer reporter. Elements: EP, EY and Mae-UAS.6.11 are transposable elements, randomly integrated in the fly genome in which UAS sites facilitate either overexpression or RNAi-mediated silencing of neighboring genes. EMS: ethyl methanesulfonate used for mutagenesis. SCA: Spinocerebellar ataxia. S2 cells: Schneider 2 cells, derived from a primary culture of late stage (20–24 hours old) *Drosophila melanogaster* embryos, likely from a macrophage-like lineage. BG2-c2 cells: cell line derived from central nervous system of 3rd instar larvae. VDRC: Vienna *Drosophila* RNAi Center. UTR: untranslated region. NIG-Fly: fly stock collection at the National Institute of Genetics, Japan.

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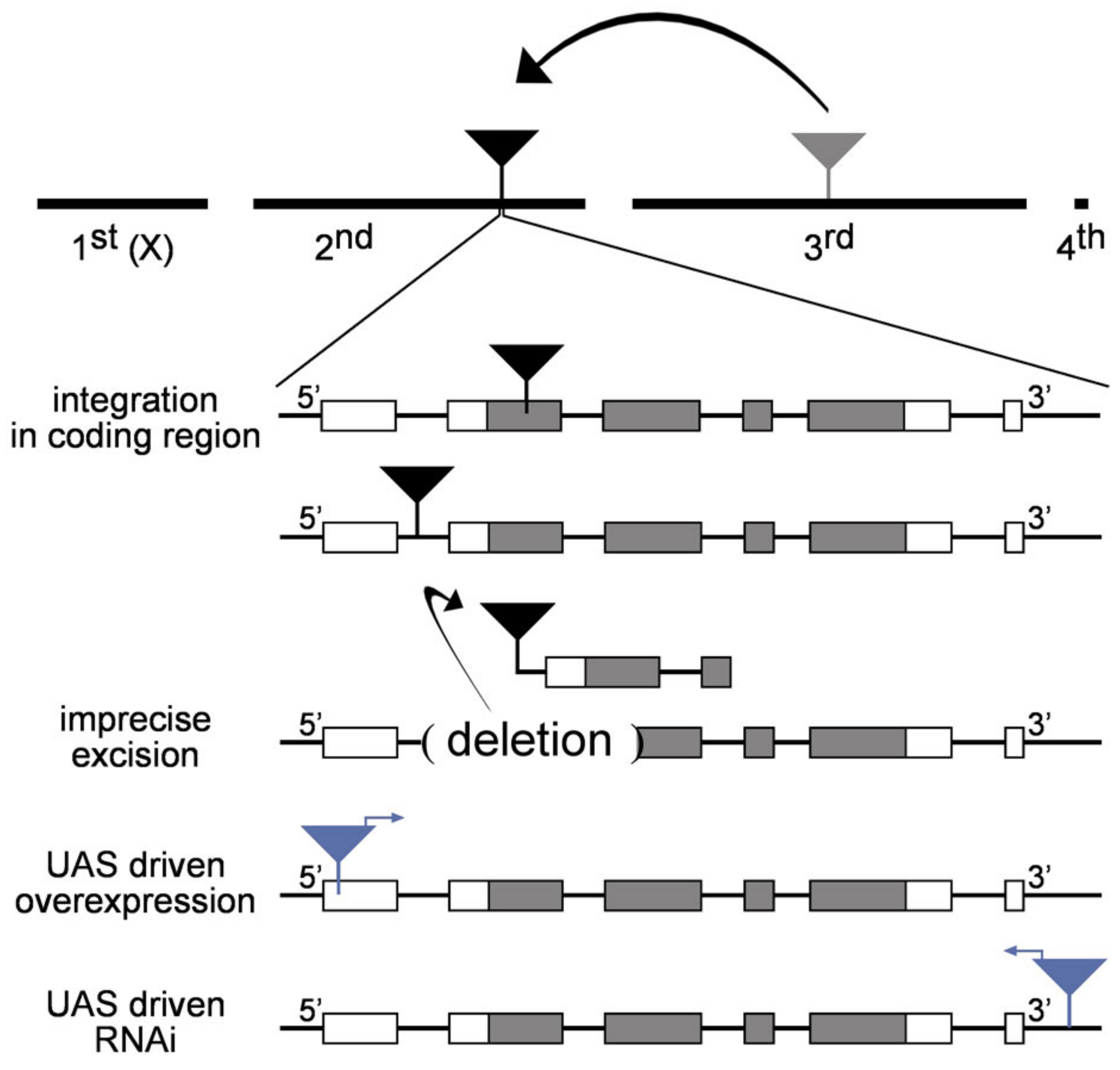

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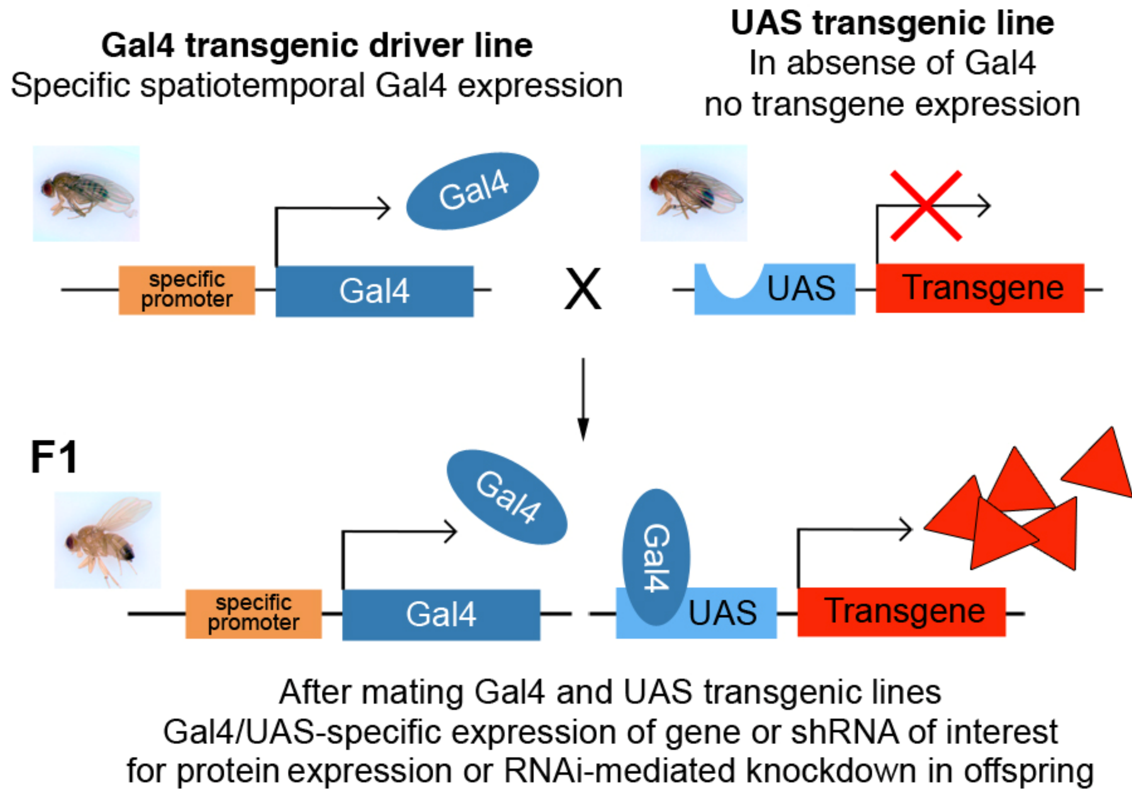

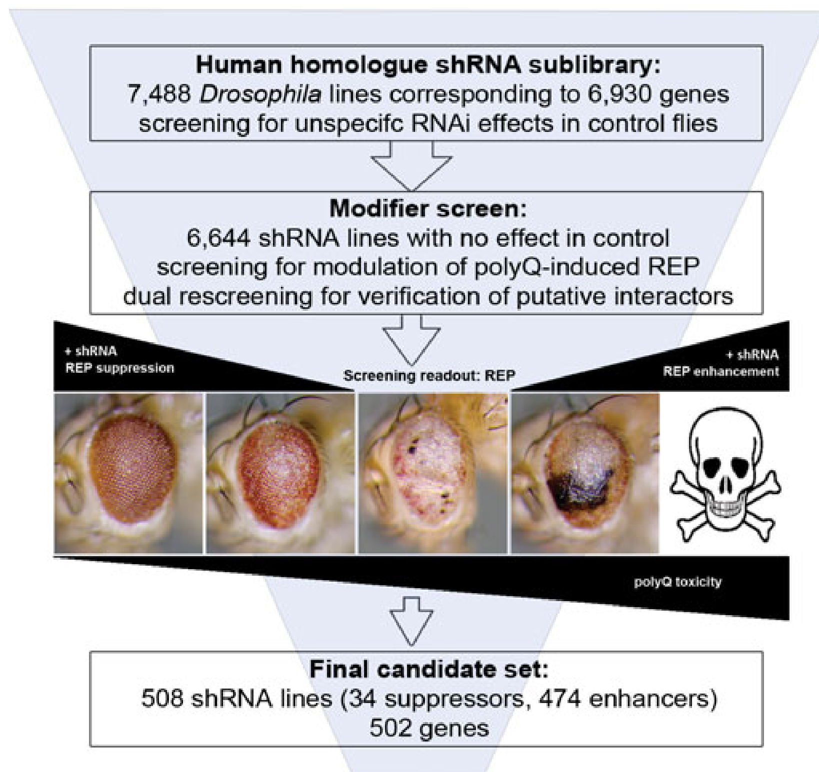

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