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Review On Versatile Drosophila Transgenic Models And Their Applications

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Abstract

Drosophila transgenic models are invaluable, particularly in the domains of genetics, molecular biology, and developmental biology. Researchers can learn a great deal about gene function, disease processes, and possible treatment approaches by inserting foreign DNA into the fruit fly's genome. The genetic manipulation where certain genes or mutations can be examined in detail, regulatory components may be uncovered, and the impact of genetic changes on different biological processes can be understood. These models go beyond basic genetic pathways to provide useful applications in the treatment of intricate problems as in neurodegenerative disorders, cancer biology, and organ regeneration. A revolutionary development in cancer research is the capacity to use Drosophila transgenic models to duplicate specific genetic changes linked to human tumors. This allows for the in-depth investigation of molecular pathways, the discovery of therapeutic targets, and the screening of anti-cancer drugs. The quick life cycle of Drosophila facilitates organ regeneration by enabling quick genetic screenings and insights into factors affecting organ development. Furthermore, these models are incredibly helpful in the investigation of neurodegenerative illnesses, offering vital information on the underlying mechanisms of conditions such as Parkinson's and Alzheimer's and supporting the development of treatment plans. The accuracy provided by Drosophila transgenic models addresses complex problems in a variety of biological domains and greatly useful in developmental biology and pharmacological screening.

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KEYWORDS: Drosophila, Transgenic model, Neurodegenerative, Cancer, Organ regeneration, Metabolism

INTRODUCTION

For more than a century, researchers have successfully utilized the model organism Drosophila to examine a variety of biological processes, such as genetics and heredity (Ringrose, 2009; *Transgenic Drosophila - an overview | ScienceDirect Topics*, no date). Drosophila melanogaster has been extensively investigated. Because the Drosophila genome has equivalents to 75% of the human disease genes(*How close are you to a fruit fly?*, 2015), There are many topics to discuss in this transgenic models such as new developments in our knowledge

of the systems regulating organ regeneration, neurodegeneration, cancer, and metabolic illnesses (Cheng, Baonza and Grifoni, 2018).

The capacity to use Drosophila transgenic models to duplicate particular genetic abnormalities linked to human tumours is a milestone in the field of cancer biology. This makes it possible to thoroughly examine the molecular mechanisms behind the growth of tumour's, find possible treatment targets, and test for anti-cancer drugs. Fruit flies are tiny, but it doesn't take away from their importance in advancing cancer research(Yamamura, Ooshio and Sonoshita, 2021).

Understanding organ regeneration using Drosophila transgenic models is beneficial for the study of organ regeneration, which has enormous potential for medicinal treatments. Examining the genetic influences on organ development and regeneration is made possible by these models. The results have potential uses in regenerative medicine in addition to adding to our theoretical knowledge of these processes(Fox, Cohen and Smith-Bolton, 2020).

Drosophila models have furthermore shown to be really helpful in deciphering the mysteries surrounding neurodegenerative illnesses like Alzheimer's and Parkinson's. Researchers can now see how these problems affect brain function and behaviour thanks to the introduction of genes linked to these disorders. This has allowed researchers to gain important insights into the underlying processes behind these diseases. Consequently, this facilitates the development of treatment approaches for these intricate and demanding ailments(Beira and Paro, 2016; Bolus *et al.*, 2020).

Transgenic models in Drosophila provide precise information that is essential for furthering our understanding of certain genes and their functions in biological processes. These studies have far-reaching implications that go well beyond the size of a fruit fly. They have made a substantial contribution to our knowledge of genetic principles and provided answers to complex problems in the fields of cancer biology, organ regeneration, and neurological illnesses.

Drosophila transgenic model for Cancer:

Different types of Drosophila cancer models, including gut, brain, hematopoietic, and carcinoma models, have been created and show typical cancer-related characteristics (16). Although some elements of cancer biology, such angiogenesis or immunosurveillance, are challenging to represent in flies, genetic and pharmacological screens employing Drosophila have provided fresh insights into the basic biology of cancer and the discovery of therapeutic targets. (17)

One among this holometabolous insect Drosophila goes through three phases of development: embryo, larva, and pupa. The quick generation time—11–12 days at 25°C—allows for the quick development and growth of novel strains for a range of assays(Giesecke *et al.*, 2023; Rozich, Randolph and Insolera, 2023).

Recently, a growing number of Drosophila scientists have started concentrating on research into the illness. Drosophila is suitable model for cancer, Since it is mostly a hereditary illness and one of the top causes of death globally.(Sonoshita and Cagan, 2017)

Scribble (scrib), Discs Large (dlg) and Lethal giant larvae (lgl), are among the fly neoplastic Tumour suppressor genes (TSG) that have been so far discovered. Their depletion results in the neoplastic development of both the ectodermal derivatives(Beaucher *et al.*, 2007).

Collagenase IV and MMP1(Matrix Metallo-Proteinase 1) production are two biochemical indicators that have been linked to aggressive human malignancies. Both contribute to the breakdown of basement membranes. Furthermore, it has been demonstrated that the latter is necessary for the spreading of lgl cells because lgl-MMP1 double mutant cells exhibit a significant decrease in their capacity to exit primary lesions. (Strand *et al.*, 1995; Beaucher *et al.*, 2007).

The FLP (Flippase) / FRT (Flippase Recognition Target) based method of mitotic clonal analysis is unquestionably one of the most beneficial and widely used investigation systems provided by Drosophila genetics. It makes it possible for scientists to produce distinct areas of homozygous mutant tissue in an animal that is ordinarily heterozygous..(Golic, 1991; Froldi *et al.*, 2008)

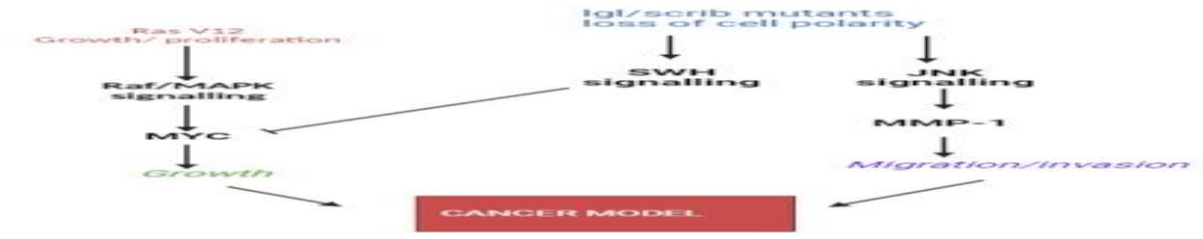

Figure 1: Major routes and pathway; Uncontrolled proliferation in Drosophila epithelial cells(Mirzoyan *et al.*, 2019)

SIGNALLING PATHWAYS

The atypical interaction of multiple signalling pathways that exhibit multiple tumour cancer hallmarks leads to cooperative oncogenesis, In scrib−/− benign tumors, There is an antitumorigenic role for the JNK pathway. TNF Eiger binds to the TNFR Wengen (Wgn) and activates it. Increased endocytosis levels help to activate the Eiger/Wgn complex, which in turn triggers a cascade of phosphorylation events that involves the JNKKKK, JNKKK, and JNKK in succession. In the end, phosphorylation of the JNK Basket triggers the activation of its downstream AP-1 TF effectors. The proapoptotic factors rpr and hid, which carry out apoptosis, as well as paxillin and mmp1, which cause the degradation of the basement membrane, are all stimulated by AP-1 TFs. Concurrently, the impairment of aPKC signaling caused by the loss of scribble function causes a loss of cell polarity, which activates the SWH target Yki. When Yki and Sd combine, they activate cyclin E expression, leading to overproliferation. Lastly, the expression of DE-cadherin declines, which adds even more to the phenotypic loss of cell polarity (Fig. 2A). In RasV12 hyperplastic tumour's, The adherens junction is typically where the Scrib and aPKC apical complexes are found, whereas the apical portion is where they are typically found. RasV12 that is aberrantly active stimulates the downstream pathways of Raf/Mek/MAPK and PI3K/AKT, which are in charge of overexpressing cyclin E (which causes overproliferation) and dmyc (which causes cell growth), respectively (Fig. 2B). In the RasV12; scrib−/− neoplastic tumors, JNK is protumorigenic. The upstream JNK signaling is not fully explored in the eye-antennal disc tumors yet. It appears to be related to Eiger binding primarily to Grnd (and Wgn), which further activates the JNKKK Tak1, the JNKKK Hep, the JNKKK Msn, and the Bsk. Hep's activity is increased upstream of Bsk by a parallel Src-dUev1/Ben/dTraf2 axis. It's possible that the nonreceptor tyrosine kinase FER and the novel JNKK MMK3 influence Bsk activity as well. Bsk causes the TFs Fos, Ets21c, and Ftfz1 to become activated. This in turn causes the expression of the BTBZF to increase, including dilp8 and upd (developmental delay), mmp1 and cher (basement membrane degradation and invasion), and chinmo and abrupt (resistance to neuron differentiation). It is unclear if they improve hid transcription as well. Yki is activated concurrently with the reduction of SWH signaling. Together with Sd, Yki activates the expression of dmyc (cell growth), cyclinE (proliferation), dilp8 and upd (developmental delay, not demonstrated) and of some other signaling components such as upd and stat (JAK-STAT pathway) as well as fos and ftzf1 (JNK pathway). RasV12 activates the Raf/Mek/MAPK and PI3K/AKT pathways, contributing to the expression of dmyc (cell growth) and cyclinE (proliferation). It also inhibits hid expression and Hid activity, protecting RasV12; scrib−/− cancer cells from apoptosis. Finally, tumor-secreted Upds activate the JAK-STAT pathway in an autocrine manner and contribute to ROS production and tumor growth through an unknown mechanism..(Dillard, Reis and Rusten, 2021)(Fig. 2C).

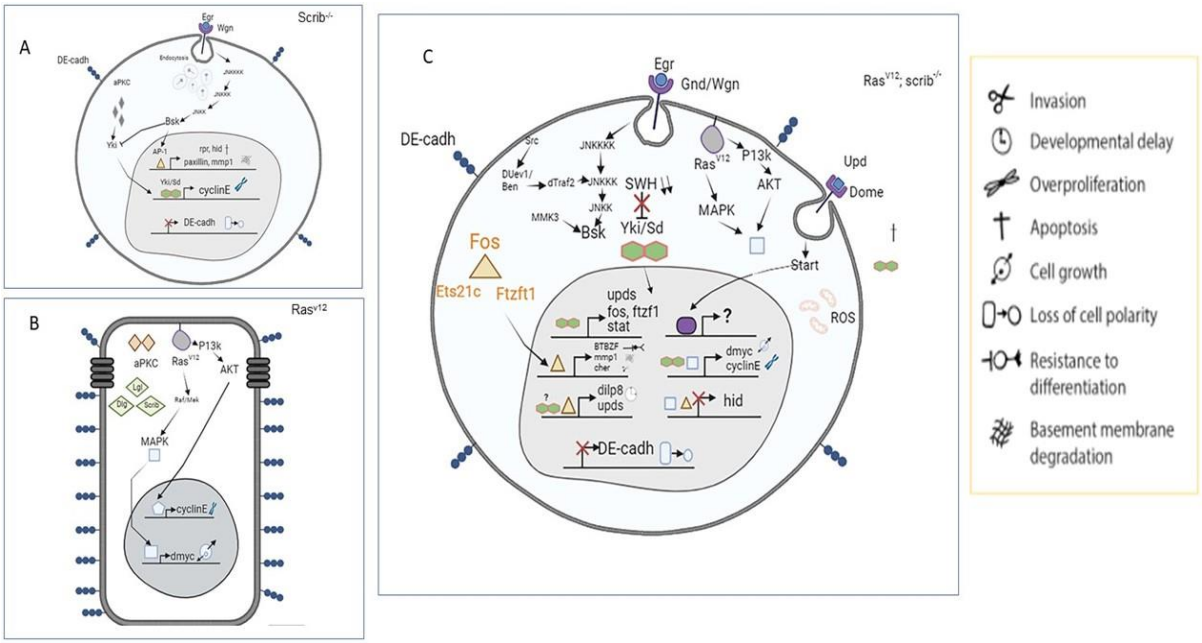

Figure 2: (A) signalling pathway in scrib benign tumour's, antitumorigenic role for the JNK pathway (B) RasV12 hyperplastic tumour's and MAPK and PI3K/AKT pathways and (C) In the RasV12; scrib−/− neoplastic tumour's and MAPK and PI3K/AKT pathways.

Drosophila transgenic model for Organ regeneration

One of the many arthropods that have been employed in studies on regeneration as model organisms is the drosophila. Because of their short adult lifetime (40 days at 25°C) and swift life cycle (egg to adult in about 10 days), regeneration tests, such as genetic screens, may be completed rapidly(Razzell, Wood and Martin, 2011; Tsai, Wang and Galko, 2018). The early Drosophila regeneration research required isolating and surgically fragmenting imaginal discs, then cultivating those discs in an adult's belly(Jin *et al.*, 2017; Sawyer, Cohen and Fox, 2017). For instance, the Gal4/UAS system for the expression of genes. that promote apoptosis, like reaper or eiger, to ablate wing disc regions that are spatially confined (Bergantiños, Corominas and Serras, 2010; Repiso, Bergantiños and Serras, 2013). In imaginal disc epithelia of Drosophila, Cell fates are decided upon in the third instar stage of the larva, but complete cell differentiation does not occur until metamorphosis(Khan, Schuster and Smith-Bolton, 2016).

The discovery of new essential signalling elements, including reactive oxygen species (ROS), was made possible through tissue ablation research. kinases like MAP kinase (p38) (Fox, Cohen and Smith-Bolton, 2020). Oxidative stress and the consequent activation of stress-activated protein kinases can be brought on by damage. Reactive oxygen species (ROS), which have often been thought to be detrimental and are produced by numerous redox metabolic activities, are increasingly being recognized as active participants in cell signalling events. Superoxide O2 -, peroxide H2O2, and hydroxyl radicals OH are ROS byproducts of aerobic metabolism that are necessary for inflammatory cell recruitment(Santabárbara-Ruiz *et al.*, 2015; Khan *et al.*, 2017; *Trithorax regulates systemic signaling during Drosophila imaginal disc regeneration - PubMed*, no date). Numerous redox-sensitive signal transduction cascades, such as the Jun-N Terminal kinase (JNK) and the stress-activated MAP kinases p38, are known to be triggered by ROS acting as second messengers(Santabárbara-Ruiz *et al.*, 2019). In Drosophila, both MAPK have been connected to stress reactions. Distinct environmental cues and stressors have distinct effects on the Drosophila p38 pathway. Additionally, raising ROS above the baseline level causes Drosophila hematopoietic progenitors to differentiate prematurely here JNK signalling plays a major role.

Gal4 & UAS are different stains of drosophila. Gal4 construct activates UAS-rpr beneath the direction of an enhancer particular to a wing (sal E/Pv >rpr), allowing examination of mature wings without harming the remainder of the living being. Sal E/Pv >rpr larvae were given a meal laced with antioxidants to reduce intracellular ROS.

Tissue repair required p38 signalling: After inducing cell death with sal E/Pv >rpr in many p38 pathway altered settings, were able to scored wing regeneration. Due to the fact that most alleles in homozygosis are fatal or semi-lethal. In this study, alleles from the p38a and p38b Drosophila p38 genes were employed. We discovered that p38b d27 heterozygous animals recovered their complete wings(Santabárbara-Ruiz *et al.*, 2019). Parameters like wing regeneration score is measured to check regeneration.

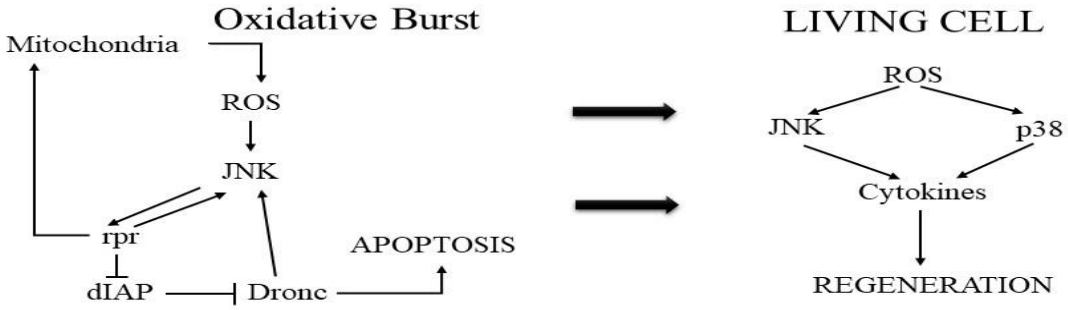

Figure 3: Cell death or injury triggers the activation of the cell protection module. (Santabárbara-Ruiz *et al.*, 2015)

Drosophila transgenic model for Metabolism

Drosophila is a crucial organism for biology because key discoveries in humans and other vertebrates have frequently been predicted by foundational research in fruit flies. The necessity of using up energy stores like lipids and glycogen when under calorie restriction or during pregnancy are common aspects of animal metabolism, as is the requirement to modify feeding behavior to correspond to dietary requirements(Mattila and Hietakangas, 2017; Zandawala *et al.*, 2018). A high-sugar diet results in hyperglycemia, hyperinsulinemia, and insulin resistance in Drosophila larvae and adults, as well as obesity in adults. Similar to humans, high-fat

diet-fed flies also exhibit insulin resistance, triglyceride levels that are increased, and heart dysfunction(*Dietary cysteine drives body fat loss via FMRFamide signaling in Drosophila and mouse - PubMed*, no date)(Kim *et al.*, 2021).
Microbes shape the physiology; Commensal bacteria encourage host growth via a variety of strategies. First, in situations where resources are sparse, Drosophila larvae can obtain extra nutrients from the biomass of inactive microorganisms(Musselman and Kühnlein, 2018; Zandawala *et al.*, 2018). Second, by enhancing the intestinal peptidases activity in the host, living bacteria can enhance amino acid absorption. The last point is that commensal bacteria actively generate and release vital nutrients that are lacking or insufficient in the diet(Bayliak *et al.*, 2019).

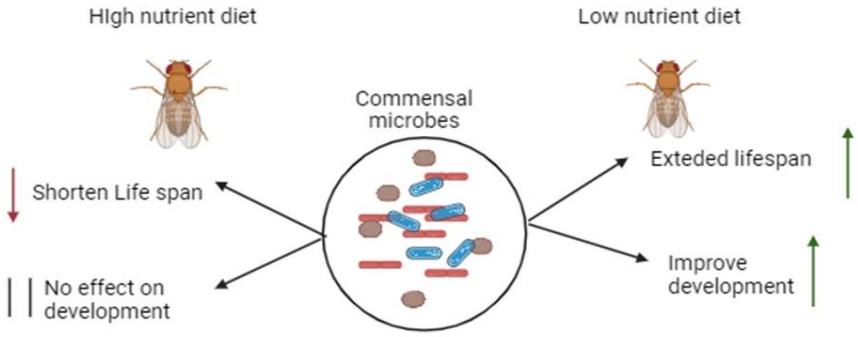

Figure 4: Impact of commensal microbes on larval development (bottom) and adult lifespan (top) when fed either a low- or high-nutrient diet (left/right) (Grenier and Leulier, 2020).

Understanding how fly insulin, glycogen, and other hormones are regulated in the endocrine regulation of metabolism(Kim *et al.*, 2021). In order to better understand the mechanisms that control fat metabolism, distribution, and deposition, Models such as Drosophila melanogaster have been used. Despite possessing eight partially redundant insulin-like peptides, Drosophila insulin signalling is remarkably comparable to the human insulin pathway and has been used as a model to research a variety of topics related to diabetes and the diabetic condition(Vatashchuk *et al.*, 2022).
Method: On cornmeal-molasses agar media, flies were grown between 22°C and 29°C. The mutant strains employed in this work that are sensitive to bangs include kdn1, tko25t, eas1, bas1, bss1, and sesB1. Genomic deficient lines Df(1)dx81, Df(1)5D, Df(1)G4e[l]H24i[R], and Df(1)JF5 in the 5D-F region.(Fergestad, Bostwick and Ganetzky, 2006)

Drosophila transgenic model for Neurodegeneration(ND):
A class of human illnesses with a few elements in common are called neurodegenerative disorders(Lu, 2009). Currently ND not only often to the geriatric it can be seen in adults also. Because of advancements in human genetics and genomics that have made it possible to identify the genes linked to familial forms of diseases which are like Alzheimer's disease (AD) and Parkinson's disease (PD), When it came to human illnesses, neurodegenerative diseases were once considered to be some of the most mysterious and challenging to treat.(Bonini and Fortini, 2003b; Lu and Vogel, 2009; Cheng, Baonza and Grifoni, 2018)
The aim of this strategy is to identify possible candidate genes associated with human disease by using the powerful genetic tools found in Drosophila. A series of triplet repeat disorders in humans known as the polyglutamine diseases are characterized by an enlarged CAG repeat in the open reading frame of the corresponding gene.(Bonini and Fortini, 2003a)
The Drosophila models that are available for disorders like Alzheimer's disease (AD), Parkinson's disease (PD), This provides opportunities for the identification of molecular mechanisms influencing the course of disease and tools for the identification of therapies(Bolus *et al.*, 2020).
Spinocerebellar ataxia 3 (SCA3)'s The first transgenic Drosophila model of a neurodegenerative disease in humans was first reported in 1998(*Mutation in the tau gene in familial multiple system tauopathy with presenile dementia - PMC*, no date). Transgenic flies that have the upstream activator sequence of yeast joined to the

target gene (UAS) crossed with flies that have the yeast GAL4 transcription factor linked to a tissue-specific promoter (Hua *et al.*, 2011).

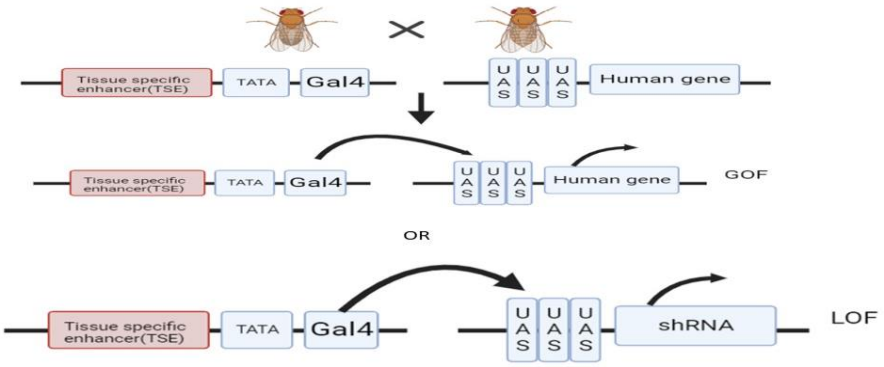

Figure 5: Humanized Flies Use the Binary GAL4/UAS System to Express Human Genes in Drosophila.

Genes can be either mutant disease genes or modifiers of human disease genes. For instance, tau overexpression amplifies tau-induced neurotoxicity when in addition to being phosphorylated by the GSK-3 ortholog (Shaggy) of Drosophila(Jackson *et al.*, 2002; Ambegaokar and Jackson, 2011). A number of teams have exploited A misexpression in flies to replicate amyloid-plaque development in a genetically feasible model, providing models for researching the molecular mechanisms behind determining potential pharmacological and genetic moderators and a toxicity. Targeted expression of Aβ42 resulted in learning deficits, amyloid deposits, and neurodegenerative phenotypes, whereas Aβ40 expression simply resulted in learning deficits(Burdick *et al.*, 1992; Finelli *et al.*, 2004; *Dissecting the pathological effects of human Aβ40 and Aβ42 in Drosophila: A potential model for Alzheimer's disease - PMC*, no date).

Drosophila transgenic model for Alzheimer's Diseases(AD):

AD affects more than 24 million people globally, which is characterized by increasing neurodegeneration and cognitive impairment(Ferri *et al.*, 2005).

It is well established that lowering Tau protein levels improves the learning and memory deficits brought on by exposure to asbestos(Roberson *et al.*, 2007). Another advantage for its use in biological studies, especially in the field of neurodegenerative diseases, is its short lifespan.. It can last for a maximum of 120 days on average, depending on your diet and level of stress. Drosophila is a perfect creature to research neurodegenerative illnesses like AD because of all these factors(Lenz *et al.*, 2013). Either the ratio of Aβ1-42 to Aβ1-40 is increased or the overall concentration of Aβ1-42 is enhanced in sporadic and most familial Alzheimer's disease cases(Crowther *et al.*, 2005). In flies concurrently expressing Aβ42, It has been discovered that elevated epidermal growth factor receptor (EGFR) exacerbates short-term memory loss. By using well-known EGFR inhibitors, the harmful impact of EGFR overexpression on Aβ42-induced memory loss was demonstrated(Wang *et al.*, 2012). The Drosophila UAS/Gal4 expression system is frequently used to express both endogenous and exogenous sequences in the target tissue.

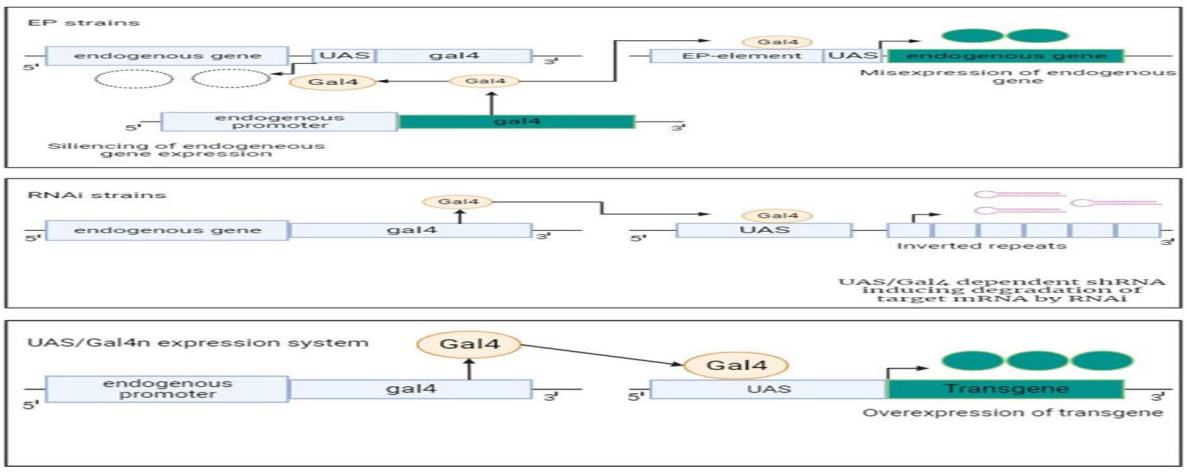

Figure 6: Genetic tools in Drosophila of the UAS/Gal4 expression system(Prüßing, Voigt and Schulz, 2013)

Drosophila transgenic model for Parkinson Diseases(PD):

After Alzheimer's disease, Parkinson's disease (PD) is acknowledged as the most prevalent neurodegenerative ailment and the most frequent movement illness (Lees, Hardy and Revesz, 2009). Drosophila has a distinct nervous system. especially in the brain of an adult, Drosophila exhibits complex behaviours that resemble some of the neurotransmitter dopamine(DA). DA-dependent human behaviours and have been shown to have discrete DA neuronal clusters containing roughly 200 DA neurons. Transgenic and deletion techniques have been used to generate Drosophila models of Parkinson's disease. A potent tool for directed transgene expression, the Drosophila Gal4/UAS system has been utilized to control the expression of mutant PD genes(Xiong and Yu, 2018).

The most common genetic factor that is known to induce Parkinson's disease (PD) is a mutation in the LRRK2 gene (PARK8, dardarin), That leads to late-onset, clinical features and age-related penetrance of autosomal dominant Parkinson's disease that are identical to late-onset sporadic PD(Paisán-Ruíz *et al.*, 2004). About 280 kD in size, LRRK2(leucine-rich repeat kinase 2) is a big multifunctional protein(Cookson, 2010). Numerous methods of vesicle trafficking, such as endocytosis, retromer trafficking and ER-Golgi, and autophagy-lysosomal pathways, may include LRRK2 in Drosophila models of the gene(Xiong and Yu, 2018). Melatonin reduces sleep problems and synaptic dysfunction caused by hLRRK2(Sun *et al.*, 2016).

GLUCOCEREBROSIDASE (GBA) model:

The human GBA1 gene has two homologs in Drosophila: CG31148 and CG31414, also known as dGBA1a and dGBA1b, and sharing a 32% amino acid similarity. These two genes exhibit varied tissue expression and are situated within the same chromosome as the CG31413 gene. While dGBA1b is expressed at low levels in both the adult brain and the adult fat body, dGBA1a is primarily expressed in the adult fly gut but not in the adult brain (Robinson *et al.*, 2013). Heterozygous changes the most common GBA mutations in Parkinson's disease are L444P and N370S, which are considered to be dominant-negative mutations. Human WT, N370S, and L444P are expressed by transgenic Drosophila were created in order to study how GBA operates in Parkinson's disease(Xiong and Yu, 2018).

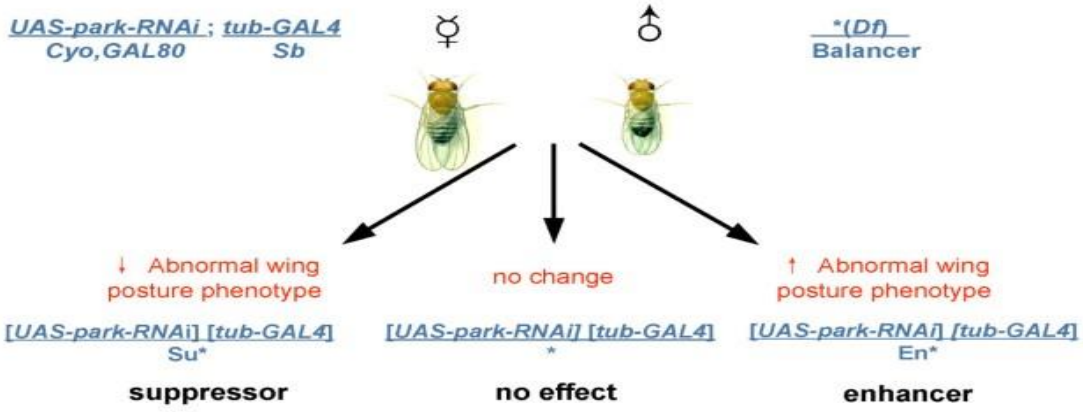

Figure 7: Isolating modifiers of PD genes in drosophila(Fernandes and Rao, 2011)

Conclusion

The versatile Drosophila model has proven to be an effective instrument in a variety of biomedical studies. From neurological diseases to cancer research, Drosophila provides a special platform for deciphering intricate biological mechanisms. Because of its quick life cycle and short lifespan, Drosophila is a great model organism for studies on neurological diseases, metabolism, and regeneration, and it makes for efficient experimentation. Drosophila has contributed significantly to our understanding of neoplastic tumor suppressor genes (TSG) and pathways in cancer research, allowing for a more thorough understanding of tumor genesis. The Flippase-based technique for mitotic clonal analysis has been very helpful in our understanding of the genetics of Drosophila cancer.

Studies on Drosophila regeneration have provided insight into critical signaling components, such as reactive oxygen species (ROS), and how they function in tissue repair.. Through the clever use of the Gal4/UAS system, fundamental insights into regenerative mechanisms have been revealed through spatial ablation of tissues.

The molecular processes behind diseases like Alzheimer's and Parkinson's have been uncovered by neurodegenerative disease models in Drosophila.. The relevance of insulin signaling pathways in studies relevant to diabetes is highlighted by their conservation between Drosophila and humans.
The molecular processes behind diseases like Alzheimer's and Parkinson's have been uncovered by neurodegenerative disease models in Drosophila. The ease of genetic manipulation has facilitated the creation of transgenic models, offering a platform for drug discovery and understanding disease progression.
In conclusion, Drosophila's significance in biomedical research lies in its ability to provide rapid, cost-effective, and translatable findings across various disciplines. As technology advances, Drosophila will likely continue to be a cornerstone in elucidating fundamental biological processes and advancing therapeutic interventions. The fruit fly's small size conceals its immense impact on our understanding of complex biological phenomena, making it a cornerstone in the edifice of biomedical research.

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