

Drosophila as a Model for Analyzing of Human Genetic and Pathogenic Related Diseases

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ABSTRACT: The fruit fly, *Drosophila melanogaster*, has been used to analyze genetics, development, and signaling for nearly a century. About 60% of the genes that are believed to cause human disease have found to a recognizable match in the genetic code of the common fruit fly (*Drosophila melanogaster*), and 50% of *Drosophila's* protein sequences are similar to those of mammals. Fruit flies are mostly used in disease analysis of human because their gene and protein similarities are included in an organism with only four pairs of chromosomes, the X/Y sex chromosomes and three autosomes, numbered 2, 3 and 4. The advantages of using *Drosophila* are that they breed and mature rapidly, are inexpensive and easy to grow, produce several hundred offspring per generation, and need very little space. The fruit fly is also an ideal candidate for human disease studies because simple mutations cause obvious phenotype differences and its genome map has been fully sequenced.

KEYWORDS: Drosophila, model, human disease, genetic.

1 INTRODUCTION

In the recent year *Drosophila* is relied as a model organism and powerful genetic tools for the analysis of developmental genetics, signal transduction and cell biology studies. *Drosophila* was the model for Thomas H. Morgan's discoveries regarding the function of chromosomes in heredity. For his invaluable contribution he was awarded the Nobel Prize for Medicine in 1933 and expressed his gratefulness to Charles Woodworth and William Castle as pioneers of the *Drosophila* model [1]. In comparison with other organism fruit fly genetics presents a set of resources useful for gene discovery that are currently unavailable in other models' systems. It has a short life cycle, within 10 days from mating of adult flies, fertilized eggs are formed, embryo development to first instar larvae that continued to second and third instar larvae, then pupal formation and subsequent eclosion to produce the next adults gradually [2]. Generally adults acquire reproductive maturity within a few hours of emerging from the pupal case and have a life span of 60 to 100 days under standard laboratory conditions. Therefore, genetic crosses and the completion of inbred lines needs considerably less time than do those of mammalian models. The fly genetics has also the unique advantage of balancer chromosomes that include multiple inversions and suppress meiotic recombination with a corresponding non rearranged chromosome. Compared with mammalian genomes, the *Drosophila melanogaster* genome is significantly small. Therefore, *Drosophila* using as a model decreases the time and increases the efficiency of screening. The size of its genome is about 5% of the total human genome and comprising 5 chromosomes X, Y, 2L/R, 3L/R, and 4. These encode about 125 million base pairs of DNA that consist of 13,000 predicted gene products [3], [4]. *Drosophila* is smaller organism than mouse and human but the genome of this fly has multiple spliced sites and use different promoter start sites, and genes are sometimes contained within the intronic sequences of other genes. So, the compact fly genome capable to produces similar gene products as like higher vertebrates. In fact, studies of *Drosophila* and human genomes have revealed that about 80% of human diseases in which the disease-related gene has been identified have an orthologue in *Drosophila* [5], [6], [7]. Similar homologous of human neurodegenerative disease genes are recognized in the *Drosophila* genome, the function of these genes can then be analyzed

by generating mutations in the *Drosophila* homolog and then studying the resulting phenotypes. Cytogenetic analysis has directed to evaluate the complete mapping and sequencing of *Drosophila melanogaster* chromosomes, enabling it to use in an array of biological and biomedical investigations [8]. Thus, the study of human genetics and disease fruit fly can served as unique and sensitive model tools [9]. Its genetic screens have proceeded to the study of many mutant strains that have helped to understanding the visual and behavioral pathways, embryonic patterning, and the development of human diseases [9].

2 MAMMALIAN AND DROSOPHILA INTESTINAL PHYSIOLOGY

2.1 FOOD PASSAGE

Mammals swallowed food moves the esophagus to the stomach, where food accumulated and digestion occurred. It then passes to the small intestine for nutrient uptake and later to the large intestine for further nutrient, water and electrolyte uptake. Finally, it arrives to the rectum and anus for excretion [10], in case of *Drosophila*, similarly activity has been observed. At first the ingested food moves to the foregut that stored temporarily in the crop; then it passes to the anterior midgut where nutrient absorption proceeds [11]. Passing through the middle midgut, which holds the iron and copper cells (Fe/Cu cells), a region of low pH, it transports through the posterior midgut for further absorption and through the hindgut and rectum, where water and electrolytes are exchanged, and finally arrives the anus for excretion.

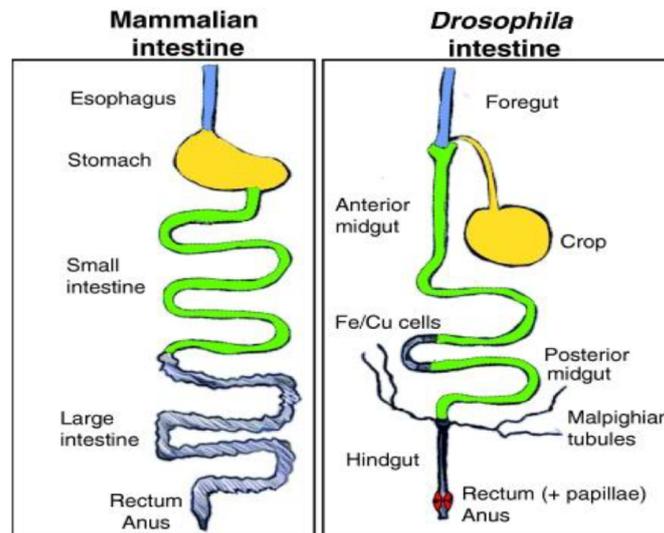


Fig. 1. Similarities between the mammalian and *Drosophila* intestines. The similarities were found between the esophagus and the foregut (blue), the stomach and the crop (yellow), the small intestine and the midgut (green), and the large-intestine–rectum–anus and the hindgut–rectum–anus (gray) in mammals and *Drosophila*. Dissimilarities include the presence of the fly kidney-like malpighian tubules that empty into the gut, the rectal papillae (red), which are responsible for water absorption (not present in mammals), and the fly Fe/Cu cells, which are found in a region of low pH that believes to be functionally distinct from the low-pH stomach of mammals [12].

2.2 INTESTINAL CELL DIFFERENTIATION AND REGENERATION

Both mammalian and fly guts have adult intestinal stem cells (ISCs) [13], [14], [15] and there are also similarities in cell composition and the signaling pathways that regulate intestinal regeneration (Figs 2 and and3).3). Enterocytes that uptake Nutrient and endocrine cells that produce hormone- are both present in flies and mammals [16], [17]. Other types of secretory cells that are found in the mammalian gut named mucus-producing goblet cells and the AMP-producing Paneth cells which have not been found in *Drosophila* midgut, but both the mucus and AMPs are originated from the *Drosophila* intestine [18], [19]. The *Drosophila* posterior midgut made up by a very simple lineage, only one type of mature absorptive cell, the enterocyte (as in mammals), and one main type of secretory cells called the enteroendocrine. On the other hand, the *Drosophila* midgut lacks of TA cells under normal homeostatic situations but such cells might exist under pathogenic conditions. Although asymmetric ISC divisions in the midgut make transient cells called enteroblasts, but these cells have no capacity to further cell division and become closely attach to ISC before maturation. Though the absence of crypts and villi, *Drosophila* midgut ISCs are situated basally and are broadly dispersed in the intestinal epithelium. But in case of mammals,

ISCs are typically intermingled with paneth cells and comprise a composite population of Lgr5-positive (Lgr5+) and Bmi1+ cells [12].

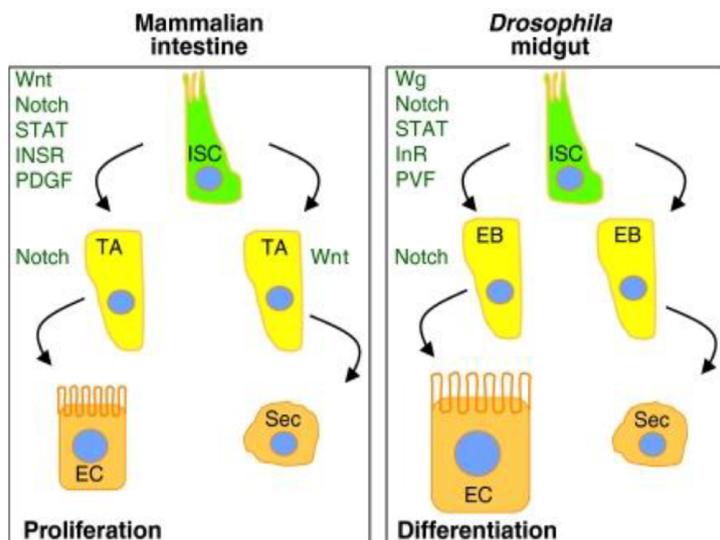


Fig. 2. ISC lineages of mammalian intestine and *Drosophila* midgut, and the common pathways that control them. The Wnt/Wg and STAT pathways are responsible for ISC division. The additional similarities between the mammalian and *Drosophila* midgut ISCs have been shown. The midgut ISCs divide when the InR pathway is activated (i.e. following drug exposure) and in the presence of PVF growth factors (during aging). In addition, ISCs are regulated by the Notch pathway; however, Notch exhilarates ISC proliferation in mammals but causes them to differentiate in *Drosophila* midgut. On the other hand, Notch signaling is similarly required for the specification of enterocyte (EC) versus secretory (Sec) fate during the commitment of TA cells and enteroblasts (EBs). Wnt/Wg, which is needed for determination of secretory cell fate differentiation in mammals, is apparently not crucial for similarly specifying cell fate in the fly midgut [12].

3 DROSOPHILA AS MODEL

3.1 ANALYSIS THE PARAOXONASE 1 EXPRESSION

The human Paraoxonases (PON) are a family of proteins (PON1, 2 and 3) and have a multiple enzymatic activities on distinct tissue expression profiles [20], [21]. Members of this gene family show it phosphotriesterase, esterase and lactonase activity to varying degrees [22], [23]. Among these PON, PON1 was originally named according to its capacity to hydrolyze paraoxon and other organophosphates, and has been found to associate with astounding number of traits and diseases that affect multiple organ systems [20]. While the phosphotriesterase function of PON1 is associated with organophosphate-degrading capacity [22]. Mutations in the human PON1 gene have been found to associate with aging and diseases of the cardiovascular, nervous, endocrine and gastrointestinal systems [24], [20]. PON1 can also hydrolyze acyl-homoserine lactones used by quorum-sensing bacteria to control multiple virulence factors during colonization of new environments [25], [26]. This association revealed that mutations in PON1 responsible for causing Crohn's disease and ulcerative colitis, or perhaps other phenotypes associated with the gut microbiota such as obesity and cardiovascular disease ([27], [28]. *Drosophila melanogaster* have no PON homologs that can offer an ideal model for studying the interactions in between PON genotype and host phenotypes [29]. Pezzulo et al. (2012) [30] hypothesized expression of PON1 in *D. melanogaster* and observed that PON1 alters the expression of multiple oxidative stress genes and decreases superoxide anion levels in the normal and germ-free *D. melanogaster*. It has also been noticed that the differences in the composition of gut microbiota, with a remarkable raise in levels of *Lactobacillus plantarum* and associated changes in expression of antimicrobial and cuticle-related genes. Generally expression of PON1 directly reduced the anionic levels of superoxide and altered bacterial colonization of the gut and its gene expression profiles, which highlights the complex nature of the interaction between host genotype and gut microbiota. Pezzulo et al. (2012) [30], also speculated the interaction between some genotypes and human diseases which were mediated by the presence of certain gut bacteria that induced specific immune responses in the gut and other surrounding host tissues.

3.2 GENETIC ANALYSIS OF INFLUENZA VIRAL-HOST INTERACTIONS

Influenza viruses are the family of orthomyxoviridae which infect vertebrates and are well known for the causative agents of the respiratory tract disease influenza. The outcomes of an influenza virus outbreak can be catastrophic, as evidenced by

the 1918 Spanish flu, which occurred approximately 50 million deaths worldwide [31],[32]. Generally, upper and lower respiratory tract were affected by the Influenza viruses when it invades and replicates inside the cells. Successful entry of the virus into a cell depends on two influenza virus proteins, one is hemagglutinin (HA) which binds to sialic acids on the surface of cells for attachment and another is matrix protein 2 (M2) which lowers the pH inside the virion, allowing for viral uncoating. M2 changes pH due to its proton-transport activity. M2 is a small, type III integral membrane protein that acts as a proton pump [33], [31]. Adamson et al. (2011) [34], developed a new tool to study virus–host interactions that play key roles in viral replication and multiplication and to help identification of novel anti-influenza drug targets. It has been expressed the influenza virus M2 gene in *Drosophila melanogaster* through the UAS/Gal4 system and generated dose-sensitive phenotypes in the eye and wing. Adamson et al. (2011) [34], confirmed that the M2 proton channel is properly targeted to cell membranes in *Drosophila* tissues and functions as a proton channel by altering intracellular pH. They demonstrated the anti-influenza drug amantadine, which target site is the M2 proton channel and suppressed the UAS-M2 mutant phenotype when fed to larvae. During screening of a candidate gene they identified mutations in components of the vacuolar V1V0 ATPase that change the UAS-M2 phenotype. Over expressing of these specific V1 subunits altered the replication capacity of influenza virus in cell culture. Finally, this study makes a clear concept for using of M2 ‘flu fly’ to identify new and previously unconsidered cellular genes as potential drug targets and better understanding of basic molecular mechanisms of influenza virology.

3.3 IDENTIFICATION AND ANALYSIS OF SERPIN-FAMILY GENES

The serpins family consists of more than 1,000 proteins, the main source those are plants, animals and viruses, but rarely found in fungi, bacteria, or archaea [35]. The serpin-fold contains of three β -pleated sheets with 8 or 9 short α -helical linkers whereas native serpins are in a metastable (stressed) configuration, which consists of a core structure with an exposed reactive centre loop (RCL) of 25-30 amino acids. The RCL express as ideal bait to the target proteinase which cleaves between two residues known as P1 and P1'. Serpins have been frequently studied in mammals where they regulate many extracellular proteolytic cascades including, coagulation, inflammatory and complement pathways are controlled by α 1-Antithrombin, α 1-Antitrypsin and C1-Inhibitor, respectively [36], [37] while Plasminogen Activator Inhibitor-1 alters angiogenesis, affecting both wound-healing and tumor growth [38]. There have a linked group of non-inhibitory serpin-fold proteins which contains molecular chaperones and have a diverse functions, [39], hormone transport, chromosome condensation [40], tumor suppression [41], and storage proteins [42]. Garrett et al. (2009) [43], collected 188 orthologues serpins from 11 *Drosophilid* genomes and used synteny for searching new family members, increase the total number to 226, or 71% of the number of orthologues are expected for assuming complete conservation of total 12 *Drosophilid* species. The critical Reactive Centre Loop (RCL) sequence contained the target proteinase cleavage site, which was rigorously conserved in inhibitory serpins, although three differential sets of orthologues found as looser. On the contrary, RCL of non-inhibitory serpin orthologues were less conserved, with 3 exceptions that presumably bind to conserved partner molecules. The derived consensus hinge motif of *Drosophilid* inhibitory serpins, differ a little from that of the vertebrate consensus. Clusters of three genes are appeared to have presented in the *melanogaster* subgroup, *Spn28D*, *Spn77B* and *Spn88E*, each of them contained one inhibitory serpin orthologue that was found in all *Drosophila*'s [43].

3.4 ANALYSIS OF INNATE IMMUNITY PATHWAYS

Drosophila melanogaster has been proved as a successful model organism for the study of innate immunity. The power of *Drosophila* genetics offered basic discoveries, such as for Toll-like receptors (TLRs) in immunity that has a great impact in case of mammalian immunity research [44]. *Drosophila* reacts to pathogens at several levels [45]. At first Pathogens closely contact with epithelial barriers and produce a local immune response on the basis of reactive oxygen species and antimicrobial peptides (AMPs). If pathogens somehow can across these barriers they produce two mechanisms of response. On one hand, there is a cellular-mediated immunity that plays phagocytosis of invading microorganisms which are leukocyte-like cells. On the other hand, a strong humoral immune response, that is activated on systemic infection. This is mainly a response of the fat body, an organ analogue to the mammalian liver, which secretes to the haemolymph a wide range of factors, including AMPs [46]. Toll is a transmembrane receptor which is a part of leucine-rich repeat subgroup of proteins that also hold Toll-IL-IR (TIR) domains [47], [48], [49]. The Toll pathway is facilitated by a serine protease cascade that proceeds to the processing of a Spaetzle. The physical interaction between Spaetzle and Toll commences an intracellular cascade that involves the adaptor proteins dMyD88 and Tube and the threonine-serine kinase Pelle. This leads to the degradation of Cactus and nuclear translocation of NF- κ B-like transcription factors Dorsal and Dif, which ultimately control the expression of antimicrobial peptides. The expression of antimicrobial peptides basically regulated by toll signaling pathway of Rel family transcription factor called Relish. Two well-defined cascades have been found to involve in the activation of Relish [50],[51]. One is mitogen activated protein kinase (MAPK) signaling pathway that involves TAK1 and IKK

which implicate the phosphorylation of Relish and another pathway involving the caspase Dredd which has been shown to subsequently proteolytically activate Relish. Recently, the MAPK Jun N-terminal protein kinase has been shown to be both down-regulated by Relish and in the expression of innate immunity-related genes [52].

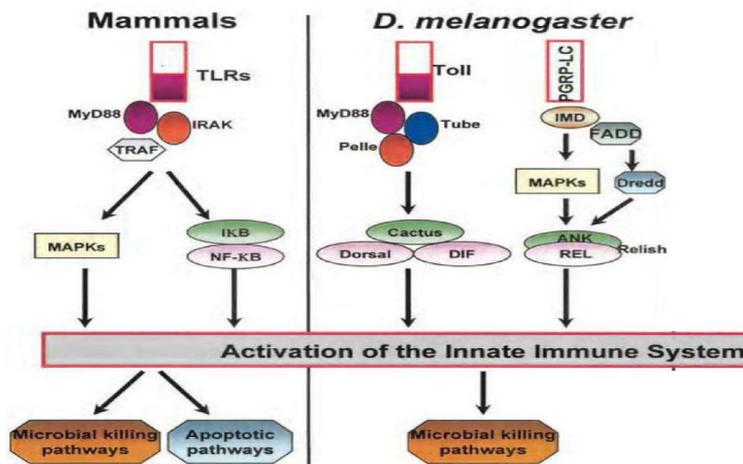


Fig. 3. Comparison of the *D. melanogaster* innate immunity pathway with homolog pathways in mammals. In mammals and *D. melanogaster*, Toll-like receptors are important in the recognition of microbial pathogens prior to the elicitation of innate immune responses. The *C. elegans* Tol-1 receptor is apparently not associated in this process. The intracellular TIR domain of Toll communicates with a similar domain contained in the MyD88 conserved protein. In mammals, this leads to the activation of both MAPKs and NF- κ B that ultimately accelerates the innate immune system. Similarly, Toll activation triggers innate immunity in *D. melanogaster* through the activation of the NF- κ B-like transcription factors Dorsal and DIF [53].

3.5 HUNTINGTON DISEASE

Huntington’s disease (HD) is the prototypic disease resulted by increasing of unstable CAG repeat, causing in expression of an extended polyglutamine tract near the amino terminus of a gene known as huntingtin [54]. *Drosophila* is reported as the first neurodegenerative disease model used a fragment of mutant ataxin-3/MJD10. This report was followed shortly by a model for HD using fragments of huntingtin [55]. Other researchers analyzed the effects of quasi-pure polyglutamine tracts expressed within the context of fly genes, such as *prospero* and *disheveled*. Models using expression of full-length ataxin-1 and -3 also have been revealed. Both candidate-based and unbiased genetic screens were launched in the fly to identify polyglutamine modifiers. Of them first observed modifier was *hsp70* which misexpression of a human *hsp70*, *HSPAL* and dramatically suppresses the eye phenotype of *SCA3* flies [56]. On the other hand, an unbiased transposon-based screen identified *Drosophila* HDJ1, an *hsp40* where misexpression of HDJ1 dramatically suppressed a quasi-pure polyglutamine phenotype [57]. *Drosophila* models have also been useful in validation of small molecule compounds predicted to suppress aggregation based on cell-based studies. Histone deacetylase inhibitors act as suppressors of mutant *htt* exon 1 toxicity in *Drosophila* and this analysis is used to validation of mouse models and several clinical trials of phenylbutyrate in Histone deacetylase [58], [59]. It was reported recently that inhibitor rapamycin can suppress *htt*-Q120 toxicity which has an acceptance autophagy role in polyglutamine disease [60]. Another studies identified that the Rho-associated kinase p160ROCK has an inhibitor role and act as a suppressor of mutant *htt* exon1 toxicity in the fly [61].

3.6 IDENTIFYING NEW ONCOGENE PARTNERS USING DROSOPHILA

Drosophila plays an important role for the screening of modifiers, enhancers or suppressors of human oncogenic protein functions on the basis of *in vivo* platform, illustrated by studies of AML. The pathogenesis of AML is thought to involve multistep genetic alterations in several genes that promote leukaemias [62]. Chromosomal translocations analyses in bone marrow of AML patients have found that several transcription factors that are crucial for haematopoiesis also play important roles in leukaemogenesis. These include AML1, LMO2, SALL, MLL and SCI/Tal1, all of which are proteins with clearly recognized *Drosophila* orthologues [63]. The *t(8;21) (q22;q22)* chromosomal translocation creates the AML1-ETO fusion product which is found in ~12% of AML patients. Under normal conditions, AML1, RUNX-domain proteins were found to responsible for tissue-specific transcriptional activation at multiple steps during haematopoiesis, whereas ETO (RUNX1T1 and MTG8) acted as a transcriptional repressor. It is believed that the fusion product of AML1-ETO acts as a constitutive repressor

of AML1 protein function and inhibits myeloid differentiation while promoting proliferation of multilineage progenitors [63]. Interestingly, the *Drosophila* RUNX protein Lozenge is necessary for the differentiation of crystal cells. Two independent groups revealed human AML1-ETO in *Drosophila* haemocyte precursors and found that this results in enhanced proliferation of circulating progenitors, the formation of 'melanotic tumours and prevention of crystal cell differentiation. One group expressed those mutations in components of the Wnt4-Frizzled and Ca²⁺ signalling pathways raised AML1-ETO activity [64]. The other group recognized eight suppressor candidates, including Calpain B (CalpB), a Ca²⁺-dependent protease [65]. In human cells CalpB was also found to interact with AML1-ETO [65], opening up the possibility that it might have a role in regulating leukaemogenesis. This functional investigation provides proof of principle that expression of human oncogenic factors in *Drosophila* haemocytes can assist to identify core regulatory networks whose dysregulation in humans responsible for the development of leukaemias.

3.7 DROSOPHILA MODELS OF PARKINSON DISEASE

Parkinson's disease (PD) is the second most common neurodegenerative disorder affecting more than 1% of the population over age 60. It is mainly characterized by the selective and progressive loss of dopaminergic neurons, accompanied by locomotor defects such as muscle rigidity, bradykinesia, postural instability, and tremor. Another pathological distinguishing mark of this disorder is the presence of cytoplasmic inclusions in the surviving DA neurons called Lewy bodies (LBs), which are mainly consisted of α -Synuclein and ubiquitin among other proteins [66], [67]. Although most PD cases are sporadic, several genes are found to associate with rare familial forms of the disease. Analyses of their function demonstrated that three types of cellular defects are found to involve in the formation and progression of PD and these are abnormal protein aggregation, oxidative damage, and mitochondrial dysfunction. Though PD models created in mice, fruit flies, and worms but *Drosophila* signed up as a very valuable model organism in the study of either toxin-induced or genetically linked PD. In *Drosophila*, several studies have expressed that pharmacological treatment could be used to model sporadic PD. One study revealed that chronic exposure to the pesticide rotenone, a mitochondrial complex I inhibitor, recapitulated key aspects of sporadic PD in *Drosophila* since it created neurodegenerative and behavioral defects [68]. Another study of rotenone-treated flies showed dose-dependent motor deficits quantified by a negative geotaxis test, which is commonly used to accomplish locomotor ability analyses in *Drosophila*, as well as selective loss of DA neurons in all the brain clusters. In a different study, paraquat exposure were found to reduce lifespan in flies as well as movement disorders such as resting tremors, bradykinesia, rotational behaviours, and postural instability, which mirror PD symptoms. The authors also revealed that such phenotypes were happened by selective loss of DA neuron clusters [69]. Thus, both studies robustly modelled environmental toxin-induced PD in *Drosophila* and provide useful tools for analyzing the mechanism of DA neurodegeneration.

3.8 THERAPEUTIC DRUG DISCOVERY

Drosophila has been extensively studied by researchers for decades, resulting in them ideal models for disease research and the screening of potential therapies. Genetic techniques have introduced reliable disease like fly models. Constructed models from these simple invertebrate organisms help to rapidly obtaining critical information and solving recent problem of molecule's effectiveness, toxicity and target before starting time-consuming clinical studies. Screening compounds of this manner plays an important role in the identification of pharmacological features directly, by which relevant potential effects for humans and other whole organisms can be assessed. Models for human diseases can be made in the fly by mutant generation, either by mutation in the fly homolog gene of a human disease-related gene or by expression of the human gene itself that introduce a scorable phenotype [70]. This *Drosophila* model can be directly used for the screening of small molecules that can rescue the phenotype or genetic screens that can identify the modifiers of the phenotype, all of which provide a new potential targets and models for a given disease. After preliminary screening, positive ideas can be achieved by testing of the additional fly models of the target disease. Significantly, the whole-organism validation research can be used for accomplishing the positive hits of traditional in vitro mammalian cell culture HTS and to rapid identification of compounds effective [70].

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