
The Fruit Fly, *Drosophila melanogaster*: The Making of a Model (Part I)

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Abstract

The fruit fly, *Drosophila melanogaster* (Meigen, 1830) has been established as a cornerstone for research into a wide array of subjects including diseases, development, physiology, and genetics. Thanks to an abundance of genetic tools, publicly available fly stocks, and databases, as well as their considerable biological similarity to mammalian systems, *Drosophila* has been solidified as a key model organism for elucidating many aspects of human disease. Herein is presented an overview of what makes *Drosophila* such an appealing model organism. In Part I of this chapter, basic *Drosophila* biology is reviewed and the most relevant genetic tools available to *Drosophila* researchers are covered. Then in part II, we outline the use of *Drosophila* as a model organism to study a wide array of pathologies in which *Drosophila* has been used, along with key advances made in the specific field using the fly as a model organism.

Keywords: animal model, cancer, diseases, *Drosophila*, genetic techniques, heart, immunology, kidney, metabolic disorders, neurodegeneration

1. Introduction

Searching PubMed with the key words “*Drosophila melanogaster* for model diseases”, we find more than 2800 papers describing the use of this small and friendly invertebrate to study human diseases. This is quite remarkable considering that the genome of this animal is separated from ours by 795 million years. But what makes this organism so significant for the study of human diseases? First of all, the entire *Drosophila* genome has been sequenced [1] making it very simple to study and manipulate a particular gene. The *Drosophila* genome is 60% homologous to ours; in addition about 75% of the genes responsible for human diseases have a homolog in flies [2]. In addition, their small size (2–3 mm), short generation time, the

easy and inexpensive way to culture them in the laboratory, and their powerful genetic tools have established *Drosophila* as one of the leading animal models for education and biomedical research [3]. Indeed *Drosophila* can be used anywhere from teaching basic genetics, to primary school, to understanding the more complicated metabolic pathways controlling fundamental physiological and pathological conditions.

It is little more than 100 years since Thomas Hunt Morgan and his colleagues, including his pupil Calvin Bridge and his wife Lillian Vaughan Morgan, redefined important concepts of *Drosophila* in the famous “fly-room” at Columbia University (**Figure 1**). He clarified the theory of inheritance previously defined by Mendel and the identification of the gene *white* earned him the Nobel Prize for Physiology and Medicine in 1933 for the role of chromosomes in heredity [4]. Since then research using fruit flies has contributed to numerous discoveries allowing for the identification of components of fundamental pathways that regulate the biology of animals as well as humans. Accomplishments that have been recognized over the years by subsequent other Nobel Laureates in Medicine and Physiology using *Drosophila* include:

- In 1946 to Hermann Joseph Muller for the use of X-ray irradiation to produce in vivo mutations.
- In 1995 to Edward B. Lewis, Christine Nüsslein-Volhard and Eric F. Wieschaus for their contribution to the discovery of the genetic control of early embryonic development.
- In 2011 to Bruce A. Beutler and Jules A. Hoffmann, for their success in defining innate immunity.
- In 2017 to Jeffrey C. Hall, Michael Rosbash and Michael W. Young for their contributions to the molecular mechanisms that control the circadian rhythm.

Fly work has also benefitted from the strong commitment of *Drosophila* researchers to follow what is called the fly worker ethos, by adhering to the definition established by Bilder and Irvine [5]. This social behavior already set by Morgan and described by Kohler in 1994, describes a set of principles defined by the sharing of unpublished and published reagents,



Figure 1. Photograph of Morgan’s Fly Room at Columbia University, around 1920. Courtesy of American Philosophical Society.

an open communication among members of the community, and the distribution of advanced genetic tools that have greatly helped the rapid advance of *Drosophila*'s powerful genetics. Since *Drosophila* strains cannot be maintained as frozen embryos, the obvious problem is that they must be kept as living cultures with routine work called "fly pushing". The ethos philosophy allowed the creation of several stock centers around the world that provide more than 80,000 *Drosophila* stock variants to customers. The Bloomington *Drosophila* Research Center (BDSC), hosted by the Indiana University in the USA, maintains the largest public collection of *Drosophila* lines and also supports fly research with basic protocols of fly work. In parallel, the *Drosophila* Genomics Resource Center (DGRC) collects vectors and cDNAs to further distribute them at a small fee to the community. There are also centers in Japan, China, and Europe that provide useful lines for screening, including an RNAi library, the large Vienna *Drosophila* RNAi Center (VDRC) and the TRIP-RNAi Harvard collections, all available through BDSC. FlyBase is the first database of integrated genetic and genomic data about *Drosophila melanogaster*, that also includes data from other species of *Drosophilidae*, created as an initiative to have all information about the methods for gene expression, development and physiology of *Drosophila*. FlyBase provided an example for the more specific databases like the Berkeley *Drosophila* Genome Project (BDGP) and modEncode. Nowadays the scope is to create a database for all Model Organisms that includes information not only from *Drosophila melanogaster* but also database information from *Rattus rattus* (Linnaeus, 1758), *Mus musculus* (Linnaeus, 1758), zebrafish *Danio rerio* (Hamilton, 1882), the worm *Caenorhabditis elegans* (Maupas, 1900) and the yeast *Saccharomyces cerevisiae* (Meyen ex E.C. Hansen, 1883), reposted at the National Institute of Health (NIH).

Finally, *Drosophila* is also used as a model organism for educational purposes to illustrate in classrooms or to the public the relevance of genetics in biomedical research, or to explain concepts like inheritance. Sites like droso4school a promotion from the Manchester Fly Facility [6] or the Journal of Visual Experiments with the JoVe Science Education Data Base and Flymove are aimed at disseminating the relevance of *Drosophila* research to encourage and facilitate scientists to engage with primary and middle schools and with lay people.

In this two-part chapter, some of the many aspects that make *Drosophila* such a fundamental model organism are covered. Here, Part I outlines the basic biology and life cycle of the fly before summarizing some of the remarkable genetic tools available to *Drosophila* researchers. Part II will provide an overview of key disease states that *Drosophila* is used to model and some significant advances made in those fields.

2. Basic biology/life cycle

As a holometabolous insect, *Drosophila melanogaster* undergoes several drastic changes in body plan throughout its life. Progressing from an egg (embryo), to larva, then pupa, and finally adult, each stage provides a unique platform for studying a wide variety of diseases and conditions. Moreover, this animal's development is the result of a tight coordination between signals from hormones, (*in primis* ecdysone and prothoracicotropic hormone), nutrients (amino acids) and *Drosophila* insulin like peptides (Dilps) that act together to allow proper physiological growth of the animal.

2.1. Life cycle and regulation of development

The *Drosophila* life cycle lasts approximately 10 days at 25°C (**Figure 2**). A single fertile female can lay hundreds of eggs and *Drosophila* embryogenesis lasts approximately 24 h. During that time, the entire larval body plan is established through the expression of a number of critical genes, starting with several proteins transcribed from maternally derived mRNAs that were deposited at specific locations in the embryo such as *bicoid* and *dorsal* [7]. These proteins diffuse across it to establish both the anterior-posterior axis (e.g. *bicoid*) and the dorsal-ventral axis (e.g. *dorsal*). The diffusion of these proteins across the embryo forms gradients of each, and the varying levels of each protein will activate the transcription of specific cascades of genes including *gap genes*, *pair-wise genes*, *segment polarity genes*, and *hox genes* that will divide the embryo into segments, regions, and eventually structures [8]. Upon completing embryonic development, a first instar larva hatches from the egg and begins to eat. At this stage it is necessary for the larva to consume food not just for growth, but also to convert into storage as fats and sugars in the fat body, from where it will be used to sustain the larva through metamorphosis. As larvae grow, they shed their exoskeleton through a process called molting that is controlled by a fine-tuned consequential series of events involving the hormones ecdysone, juvenile and the prothoracicotropic hormone (PTTH) (**Figure 3**) that control animal growth. With each molt, the larva will enter a new instar stage, progressing through three instars before a final molt to form a pupa. Each instar stage is regulated by the level of PTTH that rises to control the release of ecdysone allowing larvae to grow [9]. PTTH is released from neurosecretory cells in the brain, a process that is remotely controlled by growth of the larval organs and imaginal discs, sac-like structures of monoepithelial cells that become organs after metamorphosis, with the release of *dilp8*, a member of the secreted insulin-like peptide family, in a negative feed-back loop that controls PTTH production [10–12]. PTTH stimulates the release

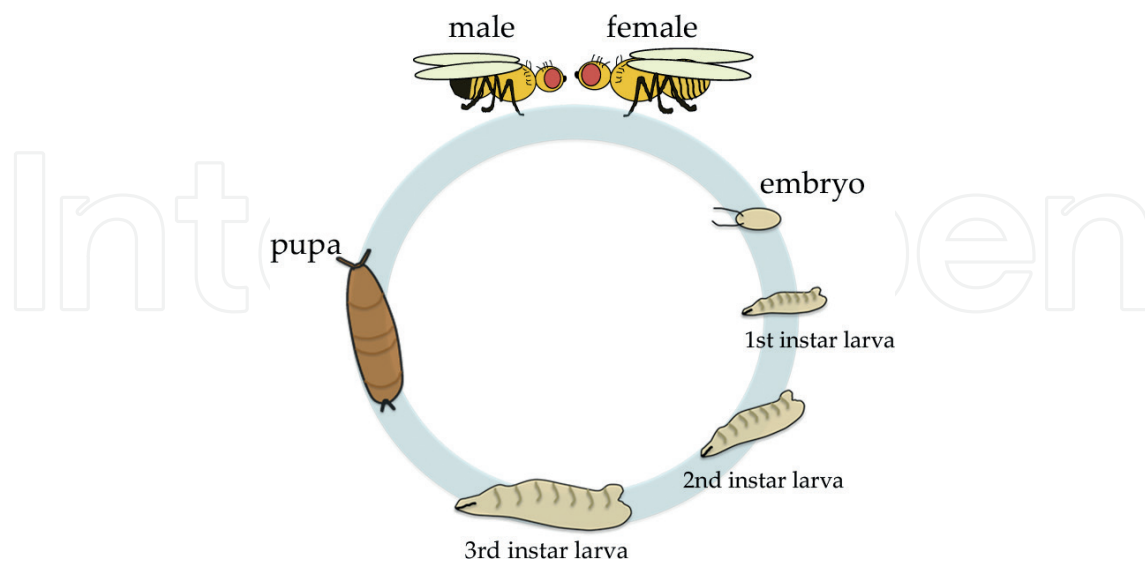


Figure 2. *Drosophila melanogaster* life cycle. The entire life cycle lasts approximately 10 days at 25°C. Flies complete embryonic development as eggs before hatching as first instar larvae. The larvae eat, grow, and molt through three instar stages before pupariating. Flies undergo metamorphosis during the pupal stage and adult structures are formed. Upon completing metamorphosis, an adult fly hatches.

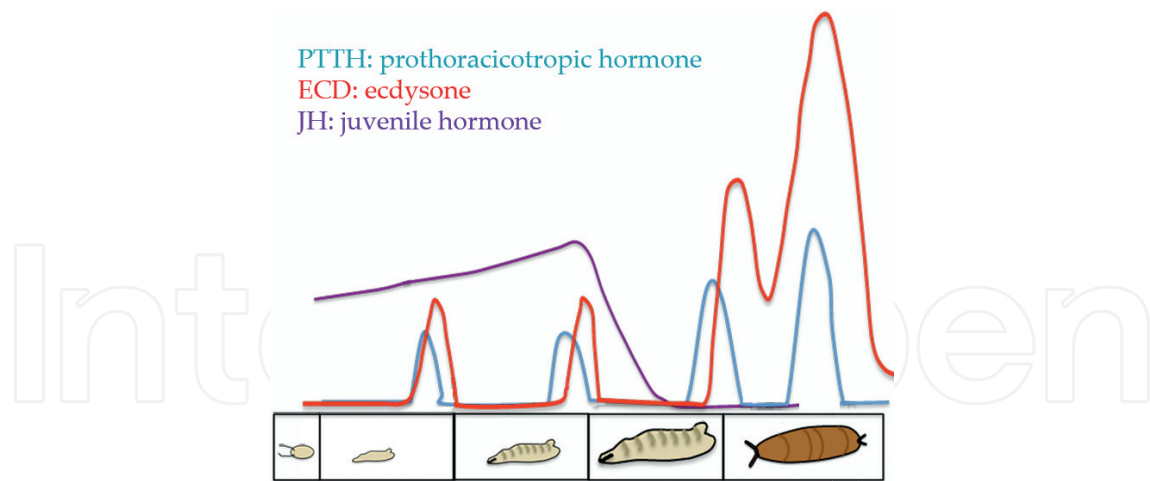


Figure 3. Hormonal control of molting and metamorphosis. This chart illustrates the levels in the hemolymph of hormones involved in the regulation of molting and metamorphosis. Before each molt, there is a burst of PTTH, triggering the release of ecdysone from the prothoracic gland. JH is present through most of larval development and instructs the larva to maintain the larval stage during each molt. When the critical weight is reached JH levels begin to fall, which occurs during the third instar stage. A large amount of growth occurs during the third instar stage until feeding stops and larvae begin to wander to pupariate, a stage triggered by a burst of PTTH and, subsequently ecdysone. A second larger burst of PTTH and ecdysone starts the final molt, and due to the absence of JH, the larva molts to the pupal stage.

of the molting hormone ecdysone from the prothoracic glands into the hemolymph causing the formation of a new cuticle (exoskeleton). As ecdysone levels fall again, another hormone, eclosion hormone (EH) initiates the actual molt where the larva sheds its exoskeleton and enters a new instar stage [13]. At the end of the third instar stage, larvae begin to wander to find a place to pupate, and are appropriately referred to as “wandering larvae.” As adults do not grow, their final body size is primary regulated by the growth occurring after the critical size is reached [13]. Generally, larvae must attain a specific critical size where organs must be properly developed, for pupation to occur [14], a process highly regulated by both genetic and environmental conditions (see below). Metamorphosis arises during the pupal stage and most embryonic and larval structures degrade during this time while the imaginal discs, which consist mostly of undifferentiated epithelial cells, differentiate into the adult organs. During this transition, the animal cannot feed from the external environment and in order to survive activates a process called developmental- autophagy, a “self-eating” signaling mechanism that converts the stored nutrients (fats and sugars) from the fat body into nutrients and macromolecules necessary to produce the energy required for the animal survival [15, 16].

2.2. Regulation of body size the interplay between hormones and growth factors

Larval growth is regulated by the interplay of the function of different organs (**Figure 4**), among which the fat body works as a hub to regulate several important processes. First, by sensing the amino acid concentration in the hemolymph, the fat body remotely controls the release of Dilps, in particular dilp2, from the Insulin Producing Cells (IPCs) in the brain [17]. This mechanism depends on the release into the hemolymph of secreted factors, like the Growth-Blocking Peptide-1 (GBP1) and CG11395 (GBP2) [18] and Stunted [19] with a mechanism that is dependent on the activation of the Target of Rapamycin (TOR) pathway in

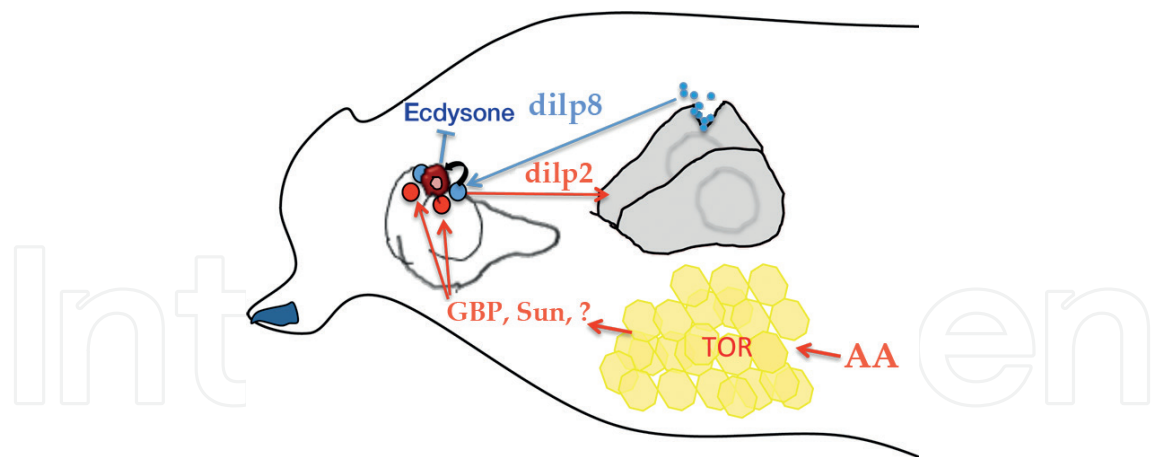


Figure 4. Integration of signals to control growth and metamorphosis. This schematic illustrates the coordination between nutritional status and developmental status to control the timing of metamorphosis and growth. The fat body senses amino acid concentration through TOR signaling and releases factors (in red) including growth-blocking peptides (GBPs), stunted (Sun), and other unknown factors (X?) into the hemolymph. These are sensed by the insulin producing cells of the brain, which then release dilp2 to cause growth. To ensure all organs are properly developed before metamorphosis, dilp8 is released from damaged or regenerating tissues to inhibit the production of PTTH in the brain, thereby blocking ecdysone levels to delay metamorphosis.

the fat cells. Second, the fat body controls animal survival with the activation of autophagy, consuming the fats and sugars that accumulated during the feeding phase. Third, the fat body responds to reduced ecdysone signaling from the brain by restraining metabolism and protein synthesis cell-autonomously before each molt by controlling the expression of the growth regulator Myc [20], which was shown to also regulate growth and Dilp2 secretion [21] constituting a regulatory loop that controls animal growth. Insulin signaling is the foremost important growth signal that in flies controls both growth/development and metabolism, with a unique and conserved pathway [22]. Dilps are produced by different organs and activate the Insulin Receptor (InR). Among Dilps (1-8), Dilp2, 3, 5 are produced by the IPCs in the brain and control animal growth and development [22, 23] while Dilp6, produced by the fat body and regulated by FOXO, functions to indirectly restrain Dilp2 secretion from the IPCs and to regulate longevity in the adult flies, a function similar to mammalian InR in aging [24]. A novel and exciting function was recently identified for dilp8, the last member of the Dilp family, to indirectly control ecdysone levels [25, 26]. *Dilp8* is a gene that encodes for a protein in the insulin/relaxin like family of peptides, originally identified for its control of bilateral symmetry [25, 26]. It is also produced by damaged or regenerating tissues, and is released into the hemolymph to remotely bind the *Drosophila* Leucine-rich repeat containing G protein-coupled receptor 3 (Lgr3), a member of the relaxin receptor expressed by specific neuronal cells located in a cluster in both sides of the brain [10–12]. These cells are part of a newly identified neuroendocrine circuit that ultimately acts on the hormone PTTH to reduce the levels of ecdysone during development. The growth of the imaginal discs times pupariation; indeed the time of pupariation is delayed with a reduction of ecdysone levels if a disc is injured. Thus, dilp8 activity seems to coordinate other signals to control the timing of pupariation and growth of the discs to ensure proper development of the animal [11]. These data on the function of dilp8 are described as part of the neurosecretory cell-rich *pars intercerebralis*, an

axis that mediates the larval to pupal transition, highlighting the presence in *Drosophila* of a mechanism similar to the hypothalamic pituitary axis in vertebrates to control development.

3. Fly genetics

3.1. Generation of transgenes

Drosophila melanogaster have four pairs of chromosomes, the first pair (X or Y) are also the sex chromosomes. Generally, having two X chromosomes designates the fly as female, whereas an X and Y will designate a male; however, the Y chromosome is extremely small and contains very few genes. Of the 2nd, 3rd, and 4th pairs, the 4th is the smallest and less commonly noticed because of the difficulty to insert transgenes and to obtain balancer lines [27]. A huge array of genetic techniques exists to allow researchers to manipulate the fly genome to overexpress, knock-down, mutate, tag, or alter the expression of a gene or genes [28]. Nearly all techniques are based on the ability to insert a foreign piece of DNA into the fly genome, generating what are referred to as transgenes. This foreign DNA can consist of an entire gene, a promoter region, gene fragments, mutated genes or almost any DNA sequence a researcher desires.

3.1.1. P-element transposons

Several commonly used techniques exist to integrate DNA into the genome. Transposon mediated integration, first utilized by Rubin and Spradling in 1982 [29] is one of the most commonly employed methods [28]. This technique capitalizes on the action of the P-element transposon. Transposons are pieces of DNA with specific sequence characteristics that have the ability to cut themselves out of the genome and reintegrate in another location through the action of the transposase enzyme [30]. The transposons can be modified through cloning to contain a desired piece of DNA. Plasmids containing the modified P-element constructs are injected into the *Drosophila* embryo germline cells together with plasmids encoding for the P transposase, the latter of which will subsequently chop out the P-element backbone from the first plasmid and insert the entire segment into a random point in the germ cell genome [31]. When these animals reach adulthood, they will produce a number of offspring that contain the modified transgene containing chromosomes in every cell. To determine whether the foreign DNA has been integrated, it is often necessary to link a marker gene to the desired transgene. This marker gene is usually inserted in the plasmid linked to the desired transgene and produces an easily observable phenotype, such as eye or body color, to clearly identify which flies contain the transgene (**Figure 5**). Marker genes are also used with many of the other techniques described below.

3.1.2. Homologous recombination

P-element transposon mediated transgenesis has several drawbacks, including that the location of the insertion cannot be selected and sometimes the transgene may be inserted within the regulatory or coding region of another gene and disrupt its function [32]. Rong and Golic in 2000 pioneered a procedure that can target specific genes in the *Drosophila* genome using

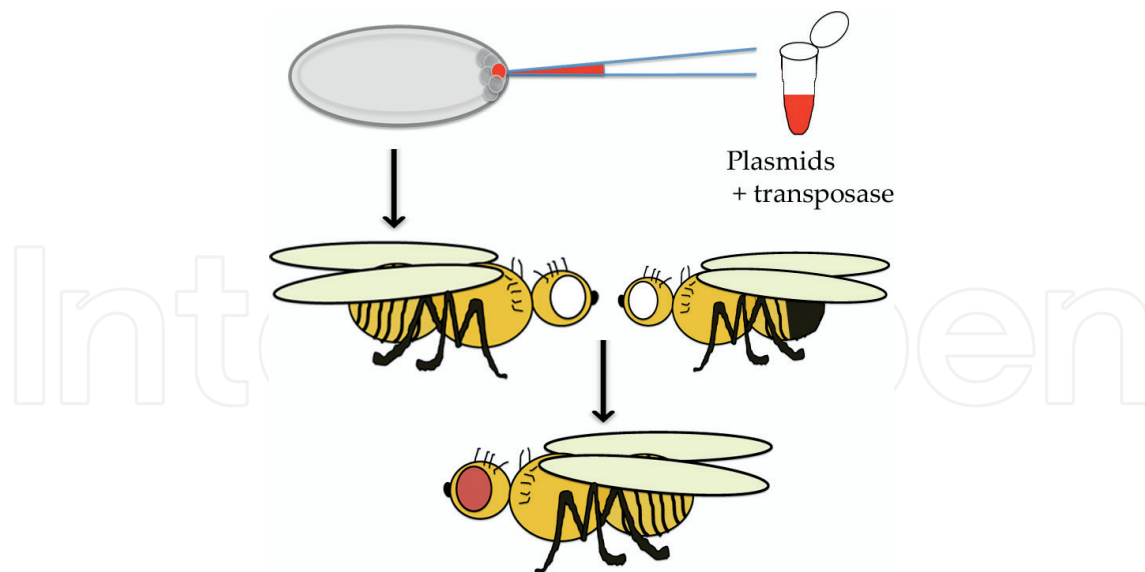


Figure 5. Microinjection of *Drosophila* embryos to generate transgenic flies. In this schematic, the embryo of a white eyed fly is injected with two types of plasmids: plasmids containing a P-element transposon with a desired transgene linked to a marker gene that will produce red eye pigment, and a plasmid containing the P-transposase enzyme. The injection occurs in the location of the embryo that will become the germline cells. Once both constructs are injected, the transposase enzyme will be produced and chop the entire transposon construction out of the other plasmid then, hopefully, insert it into the genome of the developing germ cells. The embryos will undergo development and the adult flies will now potentially contain sperm or eggs with the desired transgene. The flies can be mated, and if their offspring contain the transgene, they will have red eyes thanks to the expression of the marker gene.

homologous recombination [33]. This technique uses the cell's own DNA repair machinery and homologous recombination to swap out one allele or piece of DNA for another and can even be used to knockout genes [34]. Fly lines must first be generated, often by using P-element transposons, that express: 1- the site-specific recombinase flippase (FLP), an enzyme that recognizes specific sequences known as FLP recombination targets (FRT) and will excise DNA contained between FRT sequences to catalyze recombination; 2- a site-specific endonuclease (I-Sce1), which is an enzyme that generates a double stranded break in DNA at a specific sequence; and 3- a transgenic donor construct that contains the FRT sites on either end, a recognition site for the endonuclease, and some sequence similarity to the gene or area that is to be targeted [33]. When all three of these elements are brought together in a single fly, generally by mating, the FLP recombinase can excise the DNA contained between the two FRT sites creating a circular exogenous piece of DNA that the endonuclease will then cleave at its recognition site to linearize it. The broken piece of exogenous DNA will then recombine with the genomic area that it has been designed to target, effectively inserting itself into the genome at the desired area. In this way, an endogenous gene can be removed (knockout) or modified [33, 35]. While this method is effective at targeting specific genes for modification, it is very labor intensive, often requiring the generation of at least three different transgenic fly lines to target just one gene.

Several other methods that generate double stranded breaks to trigger homologous recombination have been developed [28]. These include using zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALEN), which are enzymes that can target specific

DNA sequences and cause double stranded breaks, however each gene requires generating a new specific enzyme and can be challenging [36].

3.1.3. *phiC31 integrase: site-specific integrase insertion*

Another method to target specific locations in the genome uses the bacteriophage ϕ C31 integrase which can insert a transgene at a specific recognition site in the genome [37]. Bacteriophages are viruses that target bacteria. The ϕ C31 integrase is an enzyme that recognizes specific attachment sites in both the bacteriophage genome (designated *attP*) and in its bacterial host's genome (*attB*) and catalyzes recombination between the two to insert itself into the bacteria's genome [38]. *Drosophila* lines have been generated with *attP* sites inserted in their genome using P-element transposons, allowing the ϕ C31 integrase to effectively insert a large segment of DNA from a plasmid containing the *attB* site via recombination at the *attP* site in the *Drosophila* genome [37]. This technique is an effective method for inserting large pieces of DNA, something that is problematic for P-element transposons, and allows researchers to insert their transgene at a specific site [28].

3.1.4. *Bacterial artificial chromosomes*

Bacterial artificial chromosomes (BACs) and recombination engineering (recombineering) are gaining traction in the *Drosophila* community because they allow the insertion of very large pieces of DNA [28]. Systems been developed that use specially modified BACs that are easy to amplify [39]. Genes or other DNA fragments are inserted into these constructs using recombineering, a process much easier than cutting DNA with restriction enzymes and reattaching it with ligases [40]. One BAC in particular combines several technological platforms and contains P-element transposase recognition sites and ϕ C31-mediated integration sites [40].

3.1.5. *CRISPR/Cas9*

The CRISPR/*Cas9* system is another rapidly expanding technology for altering the genome [41]. First coined in 2002, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) arrays are sets of repetitive nucleotide sequences with interspaced non-repetitive sequences that function in bacterial immunity against viruses [42, 43]. There are also a number of CRISPR-associated genes (*Cas* genes). Parts of the CRISPR arrays are transcribed and processed into shorter CRISPR RNA's (crRNA) and noncoding trans-activating crRNA (tracrRNA) [44, 45]. After being infected with a bacteriophage, *cas* enzymes (encoded by several *Cas* genes) allow bacteria to keep copies of viral DNA within their genome in the form of CRISPR arrays. When the virus attacks again, these copies are transcribed and processed into crRNA and tracrRNA that together target the virus to be cleaved by other *cas* enzymes like *Cas9* [42, 45–47]. In 2013, researchers began to exploit this technology to modify the mammalian genome [48, 49] and the *Drosophila* genome [50]. By taking advantage of the fact that *Cas9* is guided by crRNA and tracrRNA to cleave a specific DNA sequence, artificial constructs can be designed to target any desired gene. The double strand break generated by *Cas9* is then repaired either by homologous recombination with an exogenous piece of DNA containing a mutated form of

the gene or any other desired piece of DNA, or through error-prone non homologous end joining [48–50]. In this way, any gene or part of the genome can be easily modified.

3.2. Generation of mutants

In addition to ectopically expressing or reducing the function of a gene using the classic binary system (see last section in methods), another useful way to study gene function is to generate mutations in the genome and observe the resulting phenotypes, and then work backwards to figure out what gene was modified. The function of this gene can be inferred by the phenotype that occurred when the gene was destroyed. These studies involve mutating a large number of genes in many flies, then screening through the phenotypes and determining what genes were altered. There are a number of ways to generate mutants, including using P-element transposons and chemical mutagens like Ethyl Methanesulfonate (EMS).

3.2.1. *P*-element mutagenesis

This technique utilizes P-elements (usually containing gene markers as described above), or other transposable elements, to move around in the genome to disrupt gene function. This is possible either by inserting themselves in a new position that could interfere with a gene or removing it from a gene and degrading a little bit more of the DNA sequence from where it was removed [51, 52]. Though P-elements show certain preferences for where they reinsert, they cannot be directed to a specific location and have no precise recognition sequence [30]. It is therefore necessary after P-element mobilization to screen the flies that show an altered phenotype to determine which gene or genes were disrupted and use PCR to identify where the insertion occurred [51].

3.2.2. EMS mutagenesis

This method uses the chemical EMS to generate random mutations in the genome [53]. EMS produces a form of guanine, O⁶-methylguanine, that incorrectly base pairs with thymine during DNA replication, usually generating GC to AT transitions that potentially alter codons or destroy splice sites, which will damage the function of a gene product. These mutations are generated at random and while some create visible phenotypes, or even lethality, others show no obvious changes, so extensive screening is needed to determine which mutation or mutations caused the observed phenotypes [53].

3.3. Genetic screens

The large use of *Drosophila* as a model organism is also due to the ability to perform genetic screens aimed at the identification of novel genes important for understanding biological process. In order to do so, Drosophilists have developed balancer chromosomes that are essential to maintain mutant fly stocks and for mating design. These chromosomes carry multiple inversions and gene rearrangement that firstly suppress recombination, and secondly contain a dominant phenotypic marker visible in the larvae or adults. Because they segregate normally during meiosis, they can be followed using the dominant phenotype. All the information on balancer stocks are available from *Drosophila* stock centers.

A genetic screen can follow two main strategies: Forward or Reverse Genetics [54]. A Forward Genetics approach is based on random, genome-wide mutagenesis to generate a large progeny with aberrant phenotypes and allows the identification of individual genes involved in any given process. Traditional forward genetic screens in *Drosophila* use X-rays, chemical EMS and transposon mutagenesis [53]. Reverse Genetics instead is a targeted mutagenesis applied to a gene of a known visible phenotype and is normally used to understand the gene's biological function. The goal is to find new mutations that enhance the preexisting mutant phenotype or that suppress it [55, 56], but these genetic screens are often laborious and time consuming. The best strategy is to start with a strong phenotype. This type of mutagenesis can be accomplished via numerous mechanisms, such as classical loss of function mutant alleles, transposable P-element insertions existing for virtually all gene loci, knock-down using RNA interference strategies, or more recently using the CRISPR/Cas9 techniques (see previous section).

3.4. Most common techniques in *Drosophila*

A huge step forward on the feasibility of genetic screens was improved by the generation of the UAS/GAL4 system [57] that allowed the expression of transgenes within specific tissues of interest.

3.4.1. The UAS/GAL4 system and its modifications

This system requires the use of lines that are generated and maintained in separate stocks and targeted gene expression will be visible only in the progeny of the cross. Using the yeast transcription factor GAL4 cloned into a P-element vector, a tissue specific promoter is cloned upstream of the GAL4 gene. In parallel, a line is generated that includes a P-element vector containing the upstream activating sequences (UAS) to which GAL4 protein can bind [58]. This binary expression system is used to drive the expression of a gene of interest in any tissues where the promoter GAL4 is expressed (**Figure 6**). Because experimental design may demand expression in a more limited time window (i.e. in adult only or if the expression of the gene of interest is detrimental), the UAS/GAL4 system is often accompanied by the use of the yeast *GAL80*, a gene that encodes for a protein that physically binds to GAL4 and represses its activity [59]. This strategy was improved by the use of the ubiquitously expressed temperature-sensitive allele of GAL80 (*GAL80^{ts}*), that is active and binds GAL4 at the permissive temperature of 18°C, while at the restrictive temperature of 29°C GAL80 is degraded [60]. Another method to modulate the activity of GAL4 is to use the hormone inducible variant of GAL4, either the GAL-ER, where GAL4 is fused with the domain of the human estrogen receptor and activated by estradiol [61], or the GeneSwitch system [62] where GAL4 is fused with the domain that binds the human progesterone receptor and is activated by RU486. Finally, a more sophisticated system is the Split GAL4 [58], which allows a better control of the timing of the expression. This method is based on the use the DNA binding domain of GAL4 and its transcriptional activation domain fused separately to a promoter or hemi drivers. Only when their expression domain overlaps is the active GAL4 reconstituted and activation occurs [63].

Nowadays, the number of GAL4 lines available is constantly growing. There are UAS lines both for overexpression or RNAi interference targeting almost for all the genes in the fly. UAS- lines

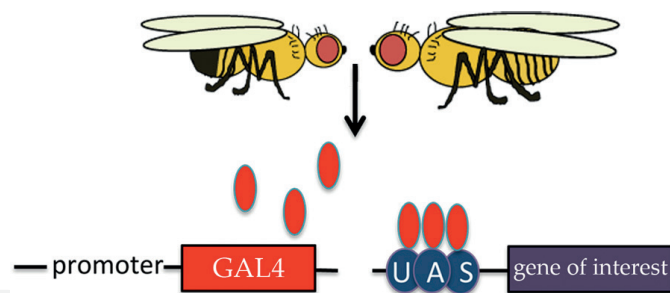


Figure 6. Gene expression using the GAL4/UAS system. This diagram illustrates how to drive expression of a gene of interest in a specific tissue using the GAL4/UAS system. Here a female fly is carrying the transgene for the GAL4 transcription factor (indicated in red) downstream of a tissue specific promoter region. This female fly is mated with a male fly carrying another transgene in which a UAS (upstream activating sequence) is upstream of a “gene of interest,” indicated in purple. The UAS sequence is the binding site for the GAL4 transcription factor. The offspring of these flies will now have both constructs. In these flies, a tissue specific transcription factor will bind to the promoter region of the GAL4 transcription factor and that GAL4 will be transcribed then translated (indicated in red) and subsequently bind to the UAS (indicated in blue) upstream of the gene of interest and activate the transcription of this gene. In this way, the gene of interest will only be produced in a designated tissue because of the specificity of the promoter placed upstream of the GAL4 transcription factor.

with more applied specific modifications, like the enhancer-trap GFP vectors, include those from the Janelia Farm Fly light project that created more than 7000 driver lines with an intergene overlapping sequence of 3 kb fragment near the gene of interest. These lines have been characterized for their expression pattern in embryos, larvae brain and adult CNS [64] and in the larval imaginal discs [65], available at the BDRC stock center. The use of binary systems is continually evolving to provide even more inter-exchangeable systems. Indeed the recent design of the LexA/lexAop [66] and the Q system [67], both inducible systems that can be used in combination with GAL4/UAS gene expression, allows researchers to perform screens in a tissue using the UAS/GAL4, with the specific patterns of expression determined by the LexA/LexAop or Q system. They can be used simultaneously in the same animal because neither of these systems cross-react to each-other.

3.4.2. The FLP/FRT system and Mosaic Analysis with a Repressible Cell Marker

To characterize the role of a gene in a small group of cells and not in the whole compartment, or to analyze the role of a mutation, it is possible to create mosaics that have homozygous mutant cells (clones) in an otherwise heterozygous animal via mitotic recombination. These studies were made possible with the combined use of the UAS/GAL4 system with the *Saccharomyces cerevisiae* enzyme FLP, a recombinase that recognizes 34 bp recombination target sequences (FRTs) on DNA [68]. FRTs on chromosomes enable mitotic recombination between homologous chromosomes in the presence of the FLP recombinase (FLP/FRT system) [68, 69]. Ubiquitous promoters like actin5C or tubulin1 α were cloned separated from GAL4 by a FLP-out cassette containing an inert gene between the two FRTs [70]. When flies carrying the cassette are crossed with flies expressing the FLP recombinase under control of the hsp70 heat shock promoter, a heat shock temporally activates the recombination event in their progeny; FRTs remove the cassette allowing the expression of GAL4 and results in random clone-induction in all tissues of the animal. The timing and duration of the heat shock determines the number of cells in which the recombination event occurs and can be recombined with GAL80ts to restrict GAL4 expression. The FLP/FRT system can be used

to manipulate gene expression when associated with UAS lines (overexpression or RNAi) and to generate loss-of-function clones in heterozygous mutant tissues [71]. Mosaic Analysis with a Repressible Cell Marker (MARCM) [72] is a technique that allows the expression of a marker or of a gene of interest in mutant clones, and is based on the ability of GAL80 to inhibit GAL4 activity and to produce positively labeled clones. In this case, GAL4 and GAL80 need to be expressed ubiquitously using the tubulin 1 α promoter. The *tubGal80* transgene is in trans with the mutation and distal to a FRT site. The event of recombination results in two populations of daughter cells, in which one inherits two copies of tubGAL80 and is wild-type, while the other one that loses the tubGAL80 and is homozygous mutant. Loss of GAL80 de-represses GAL4 allowing the expression of a marker or of a gene of interest in the mutant clone.

4. Conclusions

As illustrated in Part I of this chapter, *Drosophila melanogaster* provides a very useful platform for studying a variety of diseases and conditions. The basic lifecycle and biology of *Drosophila* combined with a wide array of genetic tools allows researchers to easily and quickly manipulate the function of any gene or genes of interest. In Part II of this chapter, it will be shown how *Drosophila* is employed as a model to study a variety of pathologies and to uncover potential therapies.

Conflict of interest

The authors declare no conflict of interest.

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References

- [1] Adams MD, Celniker SE, Holt RA, Evans CA, Gocayne JD, Amanatides PG, Scherer SE, Li PW, Hoskins RA, Galle RF, et al. The genome sequence of *Drosophila melanogaster*. Science. 2000;**287**(5461):2185-2195
- [2] Pandey UB, Nichols CD. Human disease models in *Drosophila melanogaster* and the role of the fly in therapeutic drug discovery. Pharmacological Reviews. 2011;**63**(2):411-436

- [3] Millburn GH, Crosby MA, Gramates LS, Tweedie S, FlyBase C. FlyBase portals to human disease research using *Drosophila* models. *Disease Models & Mechanisms*. 2016;**9**(3):245-252
- [4] Kenney DE, Borisy GG. Thomas Hunt Morgan at the marine biological laboratory: Naturalist and experimentalist. *Genetics*. 2009;**181**(3):841-846
- [5] Bilder D, Irvine KD. Taking stock of the *Drosophila* research ecosystem. *Genetics*. 2017;**206**(3):1227-1236
- [6] Patel S, Prokop A. The Manchester Fly Facility: Implementing an objective-driven long-term science communication initiative. *Seminars in Cell & Developmental Biology*. 2017;**70**:38-48
- [7] Nusslein-Volhard C, Frohnhofer HG, Lehmann R. Determination of anteroposterior polarity in *Drosophila*. *Science*. 1987;**238**(4834):1675-1681
- [8] Gebelein B, McKay DJ, Mann RS. Direct integration of Hox and segmentation gene inputs during *Drosophila* development. *Nature*. 2004;**431**(7009):653-659
- [9] Nijhout HF, Williams CM. Control of moulting and metamorphosis in the tobacco hornworm, *Manduca sexta* (L.): Growth of the last-instar larva and the decision to pupate. *The Journal of Experimental Biology*. 1974;**61**(2):481-491
- [10] Colombani J, Andersen DS, Boulan L, Boone E, Romero N, Virolle V, Texada M, Leopold P. *Drosophila* Lgr3 couples organ growth with maturation and ensures developmental stability. *Current Biology : CB*. 2015;**25**(20):2723-2729
- [11] Garelli A, Heredia F, Casimiro AP, Macedo A, Nunes C, Garcez M, Dias AR, Volonte YA, Uhlmann T, Caparros E, et al. Dilp8 requires the neuronal relaxin receptor Lgr3 to couple growth to developmental timing. *Nature Communications*. 2015;**6**:8732
- [12] Vallejo DM, Juarez-Carreno S, Bolivar J, Morante J, Dominguez M. A brain circuit that synchronizes growth and maturation revealed through Dilp8 binding to Lgr3. *Science*. 2015;**350**(6262):aac6767
- [13] Nijhout HF, Riddiford LM, Mirth C, Shingleton AW, Suzuki Y, Callier V. The developmental control of size in insects. *Wiley Interdisciplinary Reviews Developmental Biology*. 2014;**3**(1):113-134
- [14] Edgar BA. How flies get their size: Genetics meets physiology. *Nature Reviews Genetics*. 2006;**7**(12):907-916
- [15] Neufeld TP, Baehrecke EH. Eating on the fly: Function and regulation of autophagy during cell growth, survival and death in *Drosophila*. *Autophagy*. 2008;**4**(5):557-562
- [16] Anding AL, Baehrecke EH. Autophagy in cell life and cell death. *Current Topics in Developmental Biology*. 2015;**114**:67-91
- [17] Geminard C, Rulifson EJ, Leopold P. Remote control of insulin secretion by fat cells in *Drosophila*. *Cell Metabolism*. 2009;**10**(3):199-207

- [18] Koyama T, Mirth CK. Growth-blocking peptides as nutrition-sensitive signals for insulin secretion and body size regulation. *PLoS Biology*. 2016;**14**(2):e1002392
- [19] Delanoue R, Meschi E, Agrawal N, Mauri A, Tsatskis Y, McNeill H, Leopold P. *Drosophila* insulin release is triggered by adipose Stunted ligand to brain Methuselah receptor. *Science*. 2016;**353**(6307):1553-1556
- [20] Delanoue R, Slaidina M, Leopold P. The steroid hormone ecdysone controls systemic growth by repressing dMyc function in *Drosophila* fat cells. *Developmental Cell*. 2010;**18**(6):1012-1021
- [21] Parisi F, Riccardo S, Zola S, Lora C, Grifoni D, Brown LM, Bellosta P. dMyc expression in the fat body affects DILP2 release and increases the expression of the fat desaturase Desat1 resulting in organismal growth. *Developmental Biology*. 2013;**379**(1):64-75
- [22] Partridge L, Alic N, Bjedov I, Piper MD. Ageing in *Drosophila*: The role of the insulin/Igf and TOR signalling network. *Experimental Gerontology*. 2011;**46**(5):376-381
- [23] Gronke S, Clarke DF, Broughton S, Andrews TD, Partridge L. Molecular evolution and functional characterization of *Drosophila* insulin-like peptides. *PLoS Genetics*. 2010;**6**(2):e1000857
- [24] Bai H, Kang P, Tatar M. *Drosophila* insulin-like peptide-6 (dilp6) expression from fat body extends lifespan and represses secretion of *Drosophila* insulin-like peptide-2 from the brain. *Aging Cell*. 2012;**11**(6):978-985
- [25] Colombani J, Andersen DS, Leopold P. Secreted peptide Dilp8 coordinates *Drosophila* tissue growth with developmental timing. *Science*. 2012;**336**(6081):582-585
- [26] Garelli A, Gontijo AM, Miguela V, Caparros E, Dominguez M. Imaginal discs secrete insulin-like peptide 8 to mediate plasticity of growth and maturation. *Science*. 2012;**336**(6081):579-582
- [27] Stocker H, Gallant P. Getting started: An overview on raising and handling *Drosophila*. *Methods in Molecular Biology*. 2008;**420**:27-44
- [28] Venken KJ, Bellen HJ. Transgenesis upgrades for *Drosophila melanogaster*. *Development*. 2007;**134**(20):3571-3584
- [29] Rubin GM, Spradling AC. Genetic transformation of *Drosophila* with transposable element vectors. *Science*. 1982;**218**(4570):348-353
- [30] O'Hare K, Rubin GM. Structures of P transposable elements and their sites of insertion and excision in the *Drosophila melanogaster* genome. *Cell*. 1983;**34**(1):25-35
- [31] Karess RE, Rubin GM. Analysis of P transposable element functions in *Drosophila*. *Cell*. 1984;**38**(1):135-146
- [32] Bellen HJ, Hugo J, Bellen. *Current Biology: CB*. 2004;**14**(6):R218
- [33] Rong YS, Golic KG. Gene targeting by homologous recombination in *Drosophila*. *Science*. 2000;**288**(5473):2013-2018

- [34] Rong YS, Golic KG. A targeted gene knockout in *Drosophila*. *Genetics*. 2001;**157**(3):1307-1312
- [35] Rong YS, Titen SW, Xie HB, Golic MM, Bastiani M, Bandyopadhyay P, Olivera BM, Brodsky M, Rubin GM, Golic KG. Targeted mutagenesis by homologous recombination in *D. melanogaster*. *Genes & Development*. 2002;**16**(12):1568-1581
- [36] Gaj T, Gersbach CA, Barbas CF 3rd. ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. *Trends in Biotechnology*. 2013;**31**(7):397-405
- [37] Groth AC, Fish M, Nusse R, Calos MP. Construction of transgenic *Drosophila* by using the site-specific integrase from phage phiC31. *Genetics*. 2004;**166**(4):1775-1782
- [38] Thorpe HM, Smith MC. In vitro site-specific integration of bacteriophage DNA catalyzed by a recombinase of the resolvase/invertase family. *Proceedings of the National Academy of Sciences of the United States of America*. 1998;**95**(10):5505-5510
- [39] Wild J, Hradecna Z, Szybalski W. Conditionally amplifiable BACs: Switching from single-copy to high-copy vectors and genomic clones. *Genome Research*. 2002;**12**(9):1434-1444
- [40] Venken KJ, He Y, Hoskins RA, Bellen HJ. P[acman]: A BAC transgenic platform for targeted insertion of large DNA fragments in *D. melanogaster*. *Science*. 2006;**314**(5806):1747-1751
- [41] Hsu PD, Lander ES, Zhang F. Development and applications of CRISPR-Cas9 for genome engineering. *Cell*. 2014;**157**(6):1262-1278
- [42] Barrangou R, Fremaux C, Deveau H, Richards M, Boyaval P, Moineau S, Romero DA, Horvath P. CRISPR provides acquired resistance against viruses in prokaryotes. *Science*. 2007;**315**(5819):1709-1712
- [43] Jansen R, Embden JD, Gaastra W, Schouls LM. Identification of genes that are associated with DNA repeats in prokaryotes. *Molecular Microbiology*. 2002;**43**(6):1565-1575
- [44] Brouns SJ, Jore MM, Lundgren M, Westra ER, Slikhuis RJ, Snijders AP, Dickman MJ, Makarova KS, Koonin EV, van der Oost J. Small CRISPR RNAs guide antiviral defense in prokaryotes. *Science*. 2008;**321**(5891):960-964
- [45] Deltcheva E, Chylinski K, Sharma CM, Gonzales K, Chao Y, Pirzada ZA, Eckert MR, Vogel J, Charpentier E. CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III. *Nature*. 2011;**471**(7340):602-607
- [46] Deveau H, Barrangou R, Garneau JE, Labonte J, Fremaux C, Boyaval P, Romero DA, Horvath P, Moineau S. Phage response to CRISPR-encoded resistance in *Streptococcus thermophilus*. *Journal of Bacteriology*. 2008;**190**(4):1390-1400
- [47] Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science*. 2012;**337**(6096):816-821
- [48] Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, Hsu PD, Wu X, Jiang W, Marraffini LA, et al. Multiplex genome engineering using CRISPR/Cas systems. *Science*. 2013;**339**(6121):819-823

- [49] Andersen DS, Colombani J, Palmerini V, Chakrabandhu K, Boone E, Rothlisberger M, Toggweiler J, Basler K, Mapelli M, Hueber AO, et al. The *Drosophila* TNF receptor Grindelwald couples loss of cell polarity and neoplastic growth. *Nature*. 2015;**522**(7557): 482-486
- [50] Gratz SJ, Cummings AM, Nguyen JN, Hamm DC, Donohue LK, Harrison MM, Wildonger J, O'Connor-Giles KM. Genome engineering of *Drosophila* with the CRISPR RNA-guided Cas9 nuclease. *Genetics*. 2013;**194**(4):1029-1035
- [51] Hummel T, Klambt C. P-element mutagenesis. *Methods in Molecular Biology*. 2008;**420**:97-117
- [52] Cooley L, Berg C, Spradling A. Controlling P element insertional mutagenesis. *Trends in Genetics : TIG*. 1988;**4**(9):254-258
- [53] Bokel C. EMS screens : From mutagenesis to screening and mapping. *Methods in Molecular Biology*. 2008;**420**:119-138
- [54] St Johnston D. The art and design of genetic screens: *Drosophila melanogaster*. *Nature Reviews Genetics*. 2002;**3**(3):176-188
- [55] Moulton MJ, Letsou A. Modeling congenital disease and inborn errors of development in *Drosophila melanogaster*. *Disease Models & Mechanisms*. 2016;**9**(3):253-269
- [56] Hales KG, Korey CA, Larracuente AM, Roberts DM. Genetics on the fly: A primer on the *Drosophila* model system. *Genetics*. 2015;**201**(3):815-842
- [57] Brand AH, Perrimon N. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development*. 1993;**118**(2):401-415
- [58] Elliott DA, Brand AH. The GAL4 system: A versatile system for the expression of genes. *Methods in Molecular Biology*. 2008;**420**:79-95
- [59] Nogi Y, Shimada H, Matsuzaki Y, Hashimoto H, Fukasawa T. Regulation of expression of the galactose gene cluster in *Saccharomyces cerevisiae*. II. The isolation and dosage effect of the regulatory gene GAL80. *Molecular & General Genetics : MGG*. 1984;**195**(1-2):29-34
- [60] Zeidler MP, Tan C, Bellaiche Y, Cherry S, Hader S, Gayko U, Perrimon N. Temperature-sensitive control of protein activity by conditionally splicing inteins. *Nature Biotechnology*. 2004;**22**(7):871-876
- [61] Mondal K, Dastidar AG, Singh G, Madhusudhanan S, Gande SL, VijayRaghavan K, Varadarajan R. Design and isolation of temperature-sensitive mutants of Gal4 in yeast and *Drosophila*. *Journal of Molecular Biology*. 2007;**370**(5):939-950
- [62] Han DD, Stein D, Stevens LM. Investigating the function of follicular subpopulations during *Drosophila* oogenesis through hormone-dependent enhancer-targeted cell ablation. *Development*. 2000;**127**(3):573-583
- [63] Luan H, Peabody NC, Vinson CR, White BH. Refined spatial manipulation of neuronal function by combinatorial restriction of transgene expression. *Neuron*. 2006;**52**(3):425-436

- [64] Manning L, Heckscher ES, Purice MD, Roberts J, Bennett AL, Kroll JR, Pollard JL, Strader ME, Lupton JR, Dyukareva AV, et al. A resource for manipulating gene expression and analyzing cis-regulatory modules in the *Drosophila* CNS. *Cell Reports*. 2012;**2**(4):1002-1013
- [65] Jory A, Estella C, Giorgianni MW, Slattery M, Lavery TR, Rubin GM, Mann RS. A survey of 6,300 genomic fragments for cis-regulatory activity in the imaginal discs of *Drosophila melanogaster*. *Cell Reports*. 2012;**2**(4):1014-1024
- [66] Lai SL, Lee T. Genetic mosaic with dual binary transcriptional systems in *Drosophila*. *Nature Neuroscience*. 2006;**9**(5):703-709
- [67] Potter CJ, Tasic B, Russler EV, Liang L, Luo L. The Q system: A repressible binary system for transgene expression, lineage tracing, and mosaic analysis. *Cell*. 2010;**141**(3):536-548
- [68] Golic KG, Lindquist S. The FLP recombinase of yeast catalyzes site-specific recombination in the *Drosophila* genome. *Cell*. 1989;**59**(3):499-509
- [69] Andrews BJ, Proteau GA, Beatty LG, Sadowski PD. The FLP recombinase of the 2 micron circle DNA of yeast: Interaction with its target sequences. *Cell*. 1985;**40**(4):795-803
- [70] Struhl G, Basler K. Organizing activity of wingless protein in *Drosophila*. *Cell*. 1993;**72**(4):527-540
- [71] Xu T, Rubin GM. Analysis of genetic mosaics in developing and adult *Drosophila* tissues. *Development*. 1993;**117**(4):1223-1237
- [72] Lee T, Luo L. Mosaic analysis with a repressible cell marker for studies of gene function in neuronal morphogenesis. *Neuron*. 1999;**22**(3):451-461