Chapter 7

# The Fruit Fly, *Drosophila melanogaster*: Modeling of Human Diseases (Part II)

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Additional information is available at the end of the chapter

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**Abstract**

The fruit fly, *Drosophila melanogaster* (Meigen, 1830) has been established as a key model organism thanks in part to their considerable biological similarity to mammals and an abundance of available genetic tools. *Drosophila* have been used to model many human disease states and have been critical in elucidating the genetic mechanisms contributing to them. Part I of this chapter covered basic *Drosophila* biology and relevant genetic tools available to *Drosophila* researchers. Here in part II, we review the use of *Drosophila* as a model organism to study neurodegenerative disorders, cardiovascular diseases, kidney diseases, cancer, metabolic disorders, and immune disorders, as well as key findings made in those fields thanks to *Drosophila* research.

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

## 1. Introduction

Please refer to the Introduction of Part I, The fruit fly, *Drosophila* melanogaster: The Making of a Model.

In this two-part chapter, some of the many aspects that make *Drosophila* such a fundamental model organism are covered.

Part I covered the basic fly biology and key genetic tools.

Here, Part II provides an overview of important disease states that *Drosophila* is used to model and some significant advances made in those fields.

## 2. *Drosophila melanogaster* as model to study human diseases

*Drosophila melanogaster* is a widely used model organism to understand many molecular and developmental processes common to higher eukaryotes. A prerogative for a good model system is to share higher physiology within the molecular pathways with humans, and it is remarkable that approximately 75% of genes associated with human diseases have *Drosophila* homologs and share similarities in their functions, which is of particular interest for medical purposes [2]. Based on this genetic similarity, the fly is a valid tool for understanding the function of genes involved in human disorders. Clearly, *Drosophila* has the limitation of being an invertebrate system, as some biological processes evolved only within the vertebrate lineage. Despite this, *Drosophila* exhibits complex behaviors, and each phenotype observed must be contextualized considering that mammalian physiology is not very different from that of the tiny fly. It is not easy to choose an appropriate organism to model a disease due to the higher complexity of humans, and it is necessary to evaluate the nature of the pathology before choosing. *Drosophila* provides a good background for genetic and biological studies of different pathological conditions such as neurological, cardiac, and metabolic disorders (**Table 1**).

| Organ system | Diseases |
|---|---|
| **Brain and nervous system**<br>The Drosophila brain is two-lobed and contains approximately 100,000 neurons. It is organized into several main structures including: supraesophageal ganglion (optic lobes and cerebrum) and a subesophageal ganglion. Flies also have a segmented nerve cord similar to a mammalian spinal cord (FLYBRAIN neuron Database) | • Neurodegeneration<br>○ Huntington's disease<br>○ Amyotrophic lateral sclerosis<br>○ Spinocerebellar ataxia<br>○ Alzheimer's disease<br>○ Parkinson's disease<br>• Cancer |
| **Immune system**<br>Circulating immune cells called hemocytes (consisting of plasmatocytes, lamellocytes, and crystal cells) fight pathogens by encapsulating them, generating ROS, and/or producing antimicrobial peptides (AMPs). Many tissues are also capable of generating AMPs including the gut and fat body [72] | • Wound healing<br>• Cancers, including acute myeloid leukemia<br>• Autoimmune diseases<br>• Allergies |
| **Digestive system**<br>Consists of mouth parts for chewing, salivary glands to produce saliva, a crop (similar to a stomach), the proventriculus for grinding food, and a gut (midgut and hindgut) for digestion and nutrient and water absorption | • Intestinal infections<br>• Intestinal inflammation<br>• Cancer |
| **Excretory system**<br>Structures called Malpighian tubules and nephrocytes function similar to kidneys and filter nitrogenous waste from hemolymph. The tubules connect to the hindgut and excretory waste is eliminated along with digestive waste in the form of uric acid | • Nephrotic syndrome<br>• Polycystic kidney disease<br>• Kidney stones |

| Organ system | Diseases |
|---|---|
| **Circulatory system**<br>*Drosophila* has an open circulatory system. The tube-like heart (consisting of the dorsal vessel and the aortic arches) circulates hemolymph (insect blood) around the body cavity | • Congenital heart defects<br>• Cardiomyopathies<br>• Arrhythmias<br>• Channelopathies<br>• Heart failure |
| **Respiratory system**<br>*Drosophila*, like many other insects, does not carry oxygen in their hemolymph. Instead, a system of trachea connects directly with organs for gas exchange. Trachea open to the environment though tiny holes in the exoskeleton called spiracles | • Viral infection<br>• Respiratory disorders, including asthma and COPD (not discussed here) |
| **Energy storage**<br>Flies store glycogen and triglycerides in a specialized structure called the fat body. The fat body has functions similar to the mammalian liver and adipose tissue and is heavily involved in regulating growth, metabolism, and the immune system [16, 73–75]. | • Metabolic disorders<br>• Non-alcoholic fatty liver disease<br>• Diabetes<br>• Cancer |
| **Reproductive system**<br>Flies have ovaries for egg production in females, and testes for sperm production in males. These structures develop from imaginal discs in the larva. A fertile female fly can lay hundreds of eggs | • Female reproductively<br>• Cancer<br>• Aging<br>• Epigenetics |
| **Musculoskeletal system**<br>Flies have an exoskeleton composed mostly of a chitinous cuticle and an outer waxy coating. The cuticle is produced by epithelial cells and can be hard like bone or softer (as in the case of larvae). Muscles attach to points inside the exoskeleton and allow the fly to move | • Parkinson's disease (and other neurodegenerative diseases affecting movement)<br>• Musculoskeletal disorders (not discussed here) |

**Table 1.** The "Organ-Disease".

*Drosophila* has certain characteristics unique to insects such as an open circulatory system, exoskeleton, and tracheal system for gas exchange; however, they also share many similar organs and biological processes with mammals. The following summarizes the major organ and physiological systems in *Drosophila* and their comparative function to human diseases.

## 2.1. Neurodegenerative disorders

The *Drosophila* central nervous system (CNS) is composed of a bilaterally symmetrical brain with two cell types, neurons and glia, both originating from neural progenitors named neuroglioblasts. The fly CNS is considerably simpler than that of vertebrates and the neurodevelopment pattern is conserved among the organisms. Wnt, the mammalian homolog of the *Drosophila wingless* plays an important function during neuronal development [7] and Notch signaling, which plays a pivotal role during neurogenesis and neuronal differentiation, is also evolutionary conserved [8]. Neurons attend to neurotransmission while glia sustain the neurons during development and adult life mainly by providing

trophic factors [9, 10]. When studying neuropathies, it is relevant to consider the interaction between neurons and glia, and research in *Drosophila* is contributing to this. In fact, the power of the neurodegenerative fly model is in the ability to explore the disease in a physiological context. While glia support neuronal survival and promote recovery in cases of neuronal damage, impairment of glial function induces non-autonomous neuronal death. Glial anti-neurodegenerative functions suggest using them as targets in human neurodegeneration [11]. The *Drosophila* brain, in particular the visual system, is widely employed for research related to neurodegenerative diseases [12]. The nervous system of people suffering from these debilitating conditions exhibits the progressive loss of neurons. The origins are disparate, and in many cases, they are unknown so it is necessary to intensify the research, aiming to understand how to treat them. Interestingly, insects lack the human hematoencephalic barrier allowing for pharmacological screening directed at the central nervous system. Depending on the mechanisms inducing the disorder and the symptomatology, we can differentiate several types of human neurodegeneration. Most neurodegenerative disorders are characterized by the presence of protein aggregates in the neurons that are different for the various classes of diseases. Despite identifying many causative factors, it remains to be determined how these proteins become neurotoxic. Thanks to the precious genetic tools available, the fly is an excellent model to explore the function of the genes coding for the proteins involved. In addition, the molecular pathways are remarkably conserved allowing for parallels with humans [13]. The simpler fruit fly CNS allows for a better understanding of the function of a gene involved in a disease and its relationship with the other neuronal patterns.

In order to characterize neuronal dysfunction in *Drosophila,* several approaches can be used including testing motility, individual and social behaviors, hearing, learning, and memory [14–16]. A histological method based on measuring the vacuoles in adult fly brains allows for the quantification of neuronal degeneration [17]; moreover, electrophysiological assays enable the analysis of synapse functionality [18]. Fruit flies affected by neurodegeneration share behavioral defects and reduced lifespans.

*Drosophila* is already used to investigate proteinopathies (protein misfolding diseases) such as Huntington's disease, amyotrophic lateral sclerosis, and spinocerebellar ataxia [19–21]. The cause of Huntington's disease (HD) is the expansion of CAG repeats in the *huntingtin* gene, leading to a polyglutamine (poliQ) repeats in the huntingtin (htt) protein. Htt is required for axonal transport and synapsis, and the fly homolog shares the same expression pattern and function [22]. The poliQ expansion is toxic also for *Drosophila* neurons; in fact, the fly gradually loses photoreceptors when human htt is expressed in the eye compartment. When human mutated genes encoding for polyQ are expressed in *Drosophila*, there is a phenotype comparable to the human disease, for instance late onset, progressive loss of neurons and motility, and premature death, and the formation of large protein aggregates of mutant Htt visible also in neurons of *Drosophila* (**Figure 1**). The *Drosophila* HD model has contributed to some findings, for instance it uncovered that the histone deacetylase (HDAC) controls the level of neurodegeneration, making it an important achievement for

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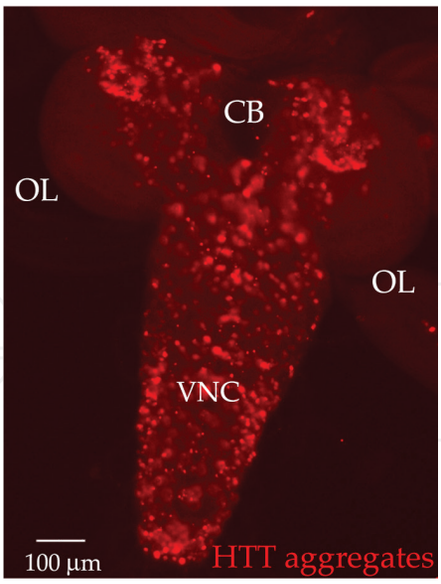


**Figure 1.** Human huntingtin aggregates in neurons**.** Photograph of a larval brain showing the formation of aggregates of mutant human huntingtin (HTT) with 93-polyQ repeats (red) in neurons using *Elav-Gal4* to express *UAS-HTTQ93*. HTT aggregates are visualized by immunofluorescence with anti-HTT antibodies. OP: optical lobe, CB: central brain, and VNC: ventral nerve cord.

human poliQ diseases [23]. In the fly, as in humans, the neurodegeneration rate is related to polyQ repeat length [24]. Spinocerebellar ataxia (SCA) is another disorder originating from abnormal CAG repeats. Humans can be affected by several types of SCA and *ataxin* is the mutated gene. Autophagy is a fundamental process to limit the poliQ aggregation, and in a fly model of SCA3, autophagy proteins are overexpressed allowing for a rescue of the toxicity [25]. Amyotrophic lateral sclerosis (ALS) is a disease characterized by loss of cortical and spinal motor neurons [26]. Several genes are involved in ALS and most of them can be expressed in *Drosophila* to assess their contribution to neurodegeneration. A causative factor of ASL is a mutation in superoxide dismutase SOD1 [27], and interestingly, loss of *Drosophila* SOD1 causes neuronal death while human SOD1 expression increases the fly lifespan [28, 29].

Tauopathies, including Alzheimer's, Parkinson's, and others, refer to disorders caused by aberrant accumulation of the microtubule-associated protein tau [30]. *Drosophila* has a tau homolog and the pathways involved in tau neurotoxicity such as wnt, JNK, and TOR are shared with humans [31–33]. More than 30 transgenic fly models have been established that express various forms of human wild-type and mutant tau and have uncovered many potential mechanisms for tau toxicity in a variety of neurodegenerative diseases [34]. Alzheimer's disease (AD) is one of the most common neurodegenerative disorders and yet its pathogenesis is still unclear. The tiny fly is once again a good organism to model this affliction because the AD-associated genes, such as APP and *presenilins*, are evolutionarily conserved. The brains of Alzheimer's patients are marked by aggregation of beta-amyloid

protein and neurofibrillary tangles (NFTs) originating from hyperphosphorylation of Tau [35]. Tau expression induces learning and memory deficits in *Drosophila*, mimicking AD in humans [36]. Some recent advances uncovered by *Drosophila* Alzheimer's models include: explaining the mechanisms behind the phosphorylation of tau and its toxicity [37–40] along with ways to reverse it [41, 42], as well as linking DNA damage and oxidative stress triggered by tau phosphorylation in causing neurotoxicity [33, 43]. Moreover, *Drosophila* models are helping researchers to uncover the interaction between beta-amyloid proteins and tau and how they cause neuronal death [34]. Parkinson's disease (PD) is characterized by the progressive loss of dopamine neurons in the substantia nigra, a part of the brain responsible for motor control, as well as the formation of protein accumulations known as Lewy bodies, which are composed primarily of alpha synuclein [44]. Many mechanisms have been proposed for the cause of this neuronal death including disruptions in protein degradation, oxidative stress, mitochondrial dysfunction, autophagy and lysosomal dysfunction, and problems with calcium homeostasis [45] Furthermore, phosphorylated tau has been found to be associated with alpha synuclein in Lewy bodies [46, 47] and the two may function together to destabilize microtubules and damage axonal transport, also contributing to cell death [48]. Many fly models exist to study Parkinson's disease [49]. The fly dopamine neurotransmitter is similar to the human version and its function in movement is conserved [50]. Homologs of several PD-related genes are present in *Drosophila*, allowing researchers to model this neurodegenerative disease [51]. *Drosophila* models are currently being used to test a variety of potential therapeutic approaches, including boosting antioxidant mechanisms, reducing the oxidative stress caused by dopamine metabolites, and using inhibitors for members of the TOR pathway to improve Parkinson's symptoms [49].

### 2.2. Cardiovascular diseases

*Drosophila melanogaster* and humans share some aspects of heart development and function making the fly a good model for studying cardiovascular diseases, which are the leading causes of death worldwide. The heart precursors of *Drosophila* originate in the lateral mesoderm and converge on the dorsal midline to form a linear tubular structure comparable to the early vertebrate embryo heart. In *Drosophila,* a simple contractile tube pumps the hemolymph through the larval body cavity in an open cardiovascular system and regulates cardiac rhythm (**Figure 2**). The cardiovascular system has an anteroposterior polarity and it consists of the posterior portion named the dorsal vessel, corresponding to the heart, and the narrow anterior portion named the aorta, which facilitates the transport of hemolymph to the head [52]. The dorsal vessel is made up of two cell types: the cardiomyocytes, which are the inner contractile muscle cells, and the pericardial non-contractile cells, which flank the cardiomyocytes. The human heart has four distinct chambers, likewise the fly heart is divided into four chambers, each one consisting of six myocardial cells [53] that have a sarcomere structure similar to mammalian cardiac cells. The hemolymph flow moves nutrients, immune cells, and molecules required for homeostasis; however, oxygen is transported through diffusion from spiracles that invaginate from the cuticle into the interior of the animal. Despite the fly dorsal vessel being much simpler than the mammalian looped heart, the signaling

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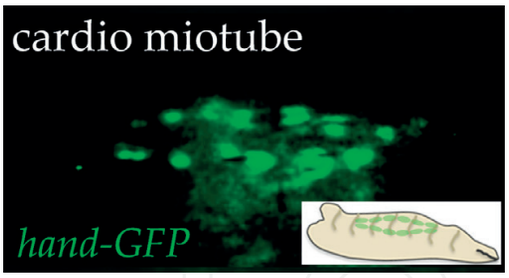


**Figure 2.** Cardiomyotube**.** Photograph of larval cardiomyotube with the cardiomyocytes visualized by the expression of the reporter *hand-GFP*.

pathways involved are remarkably conserved [54]. Cardiogenic genes required for the proper development of the *Drosophila* embryonic heart were identified through genome wide screens [55] showing that many molecules important for heart development and morphogenesis are conserved in humans [56]. *Tinman* is a homeobox transcription factor discovered in *Drosophila* and it is a master gene of cardiac development conserved in higher organisms [57, 58]. In addition, *pannier* and *hand,* which play crucial roles for heart specification as well as *neuromancer*, have counterparts in humans [59–61]. Moreover, these signaling pathways are required for some adult function both in *Drosophila* and in mammals suggesting that they have a conserved physiology [62].

Even if most studies are based on the embryonic development of the fly heart, nowadays the focus is shifting to the function and structure of the *Drosophila* adult heart as a model of human heart defects. Indeed, the great availability of genetic tools in *Drosophila* allows for the identification of elements important for heart functions and facilitates the analysis of mutant isoforms associated with congenital heart defects [63]. The physiological mechanisms are conserved among *Drosophila* and vertebrates supporting the utility of the fly to investigate cardiomyopathies and arrhythmias [52]. The improvement of techniques for the measurement of cardiac performance in *Drosophila* also permits the analysis of the effect of aging and the stress response on the heart [64]. Cardiac dysfunction can occur naturally in *Drosophila*, and this phenotype depends on age, just like in humans [64]. Some strategies allow heart rate monitoring in response to externally applied electric pacing in order to understand the effects of aging in adult flies. Insulin-IGF receptor (InR) and TOR signaling play an important role in regulation of age-dependent cardiac performance [65]. *Drosophila* is also one of the most efficient model organism used to discern the mechanism underlying channelopathies and cardiomyopathies as many impaired pathways are evolutionarily conserved [66]. Cardiomyopathies affecting *Drosophila* resemble those of humans both in terms of the genes responsible and the resulting effect. Such a similarity among the fly and humans is also found in the case of channelopathies and arrhythmias.

Several assay systems are helpful in characterizing *Drosophila* heart function, such as optical coherence tomography (OCT), an imaging of the *Drosophila* heart tube to observe contraction *in vivo* similar to clinical echocardiography [62]. In addition, semi-automated measurements allow researchers to record heart function to quantify cardiac impairment in *Drosophila*.

### 2.3. Kidney diseases

Despite millions of people suffering from kidney disorders, there is a disconcerting lack of therapies available to patients because the primary causes of kidney disorders are not completely characterized. *Drosophila* is advantageous to model renal disorders since many genes, proteins, and even some functions of the vertebrate kidney have parallels with the fruit fly. Despite many differences due the greater complexity of the human kidney, several orthologous genes have an important role in renal development and function, both in humans and in *Drosophila* [67]. For example, many genes encoding for electrolyte transporting proteins affected in congenital renal disorders have fly counterparts [68, 69].

The insect Malpighian tubules and the nephrocytes are functionally analogous to the vertebrate kidney; in fact, these two organs in *Drosophila* guide the metabolite homeostasis and the excretory process (**Figure 3**). Nephrocytes, which surround the heart and esophagus, are responsible for filtering the hemolymph, similar to the podocytes in the human glomerulus. In addition, nephrocytes have filtration diaphragms similar to the podocyte slit diaphragms that work as a filtration barrier in higher organisms [70, 71]. The Malpighian tubules, corresponding to the tubular part of nephrons, are two pairs of elongated and thin tubes connected to the hindgut that secrete urine after absorption of water, ions, solutes, and organic metabolites from the hemolymph. The principal cells and the stellate cells are the two main cell types in Malpighian tubules involved in excretion [72].

Nephrotic syndrome refers to ultrafiltration dysfunction leading mostly to extra protein in the urine and deficiency of protein in blood [73]. Given the evolutionary conservation of the diaphragms and their regulative mechanisms, *Drosophila* is a good option to look into this kind of disease. Some events during the renal development are shared

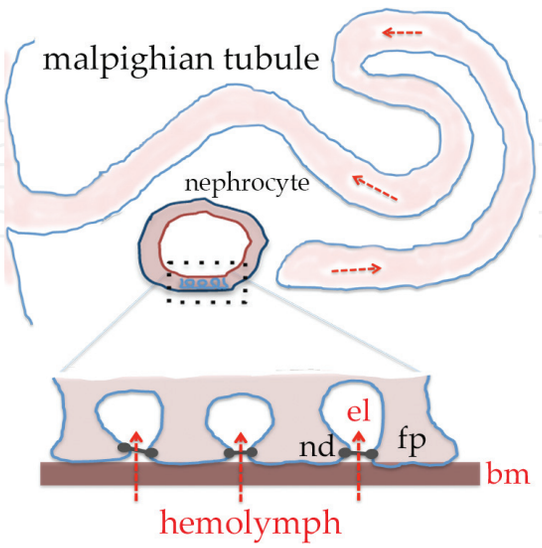


**Figure 3.** Excretory system in larvae. Malpighian tubule and nephrocyte are composing the filtration barrier; hemolymph is filtrated by nephrocyte. Nd: nephrocyte diaphragm, fp: foot process, bm: basal membrane, and el: extracellular lacunae.

between the fly and humans and the molecular pathways are conserved. All the genes playing a pivotal role in renal development, such as *Kruppel* and *Cut* involved in cell specification, *Dwnt* in tubulogenesis, and *Sns*, a nephrin-like protein, in cell differentiation, have a counterpart in mammals. One of the fundamental phases of Malpighian tubules formation is a mesenchymal-to-epithelial transition that resembles the steps of kidney development [74]. This makes the fruit fly organ able to provide insights on disorders affecting the tubular nephrons such as polycystic kidney disease and renal agenesis [75, 76]. *Drosophila* is also useful to study nephrolithiasis, also known as kidney stones, since insects also produce stone formations like calcium phosphate and calcium oxalate [77]. A simple method exists to score the filtration and the uptake of a secreted fluorescently tagged protein (ANFRFP) that accumulates in nephrocytes to assess the renal function in *Drosophila* [75].

The similarities among the species definitely allow the use of the *Drosophila* renal structure as a model to better understand the basis of human kidney impairments and consequently to develop personalized therapeutic agents. Furthermore, immune and inflammatory responses are trigger factors of kidney diseases so they should be taken into account when analyzing these pathologies [78].

### 2.4. Cancer and growth

The fly is a simple model to improve the understanding of tumor biology and progression [79–83] as the available genetic tools support the analysis of the mechanisms underlying growth regulation in an intact epithelium rather than in cell cultures. The advantage is remarkable since cell-cell and cell-environment interactions contribute to tissue size regulation. The *Drosophila* cell cycle can escape the normal control system leading to the typical cancer hyperproliferation. Reproducing human tumors in *Drosophila* allowed for the identification of many oncosuppressor genes that regulate cell division and differentiation [84]. In the fly, the tumor hallmarks mimic the human ones: autonomous proliferation signals and overgrowth, irregular cell morphology, bypassed apoptosis, and metastasis [85]. In spite of these similarities, there are several limitations including lack in flies of processes such as telomere maintenance and angiogenesis that participate in cancer development.

A great conservation across species is detected in regards to the signaling pathways affecting growth. Initial studies using activated proto-oncogenes such as the receptor tyrosine kinase (*ret*), a gene responsible for medullary thyroid carcinoma (MTC), allowed researchers to perform genetic screens for suppressors or enhancers of the rough eye phenotype, which indicates an overproliferation of cells in the eye [86]. These studies evolved to include tumors that were induced by the activation of growth signaling pathways, such as PI3K and EGFR in glia, which resemble human glioma [87], or studies involving tuberous sclerosis, an autosomal dominant disorder characterized by benign tumors in multiple organs induced by the loss of function activity of the TSC1 and 2 tumor suppressor genes [88]. A large number of studies also demonstrated how the Hippo pathway, which regulates growth through the activation of Yki, is highly conserved and required for cellular proliferation as well as for apoptosis, has a human counterpart that retains sequence

and function, and is mutated within the context of cancer [89, 90]. The same goes for *Salvador*, a gene promoting apoptosis, and *Archipelago* [91–94]. The two organisms also share PTEN, a tumor suppressor that plays a crucial role in carcinogenesis both in humans and in flies [95].

New studies defined how the loss of cell polarity could be considered a hallmark of malignancy [96]. Members of discs large (dlg) and lethal giant larvae (lgl) were identified as tumor suppressors in the fly by promoting cell invasion if mutated, with a similar role also seen in human neoplasm [97]. The role of proteins involved in cellular adhesion, such as Rho1 and E-cadherin, was also shown to be conserved and relevant for migration and invasion helping the study of the metastatic process [98, 99]. Other well-studied oncogenes in *Drosophila* that promote overgrowth and cell survival are *Ras* and *Notch* and were also shown to play a role in cellular polarity [100]. Dpp, the homolog of human bone morphogenetic protein/transforming growth factor-beta (BMP/TGF beta), is also responsible for epithelial integrity [101] and implicated in a model for cancer in *Drosophila*. All these parallelisms provide the potential to dissect in vivo the interacting patterns causing the tumor growth.

As anticipated, the communication between neighboring cells must be taken into consideration when analyzing a tumor tissue. Competitive interactions occur among cells with different growth rates in a process known as cell competition, which was first described in *Drosophila* using ribosomal proteins [102, 103] and then characterized using dMyc, the fly homolog of human cMyc [104]. Cells expressing higher levels of Myc behave as supercompetitors: they survive and acquire a proliferative advantage inducing apoptosis in the weaker nearby cells, termed losers [105–107]. The mechanisms controlling overproliferation and metastasis are comparable to those involved in cell competition since in human cancer, cells overexpressing Myc acquire the capacity to grow more than normal and to invade the neighboring normal cells. Since then, a few additional oncogenes and tumor suppressor genes have been associated with a competitive behavior, and cell competition is now thought to have an important role in human cancer [108–112]. This similitude underscores the utility of using flies for studying how cells compete for survival.

More studies are arising on the connection between the insurgence of tumors and diet or obesity. Recent studies linked the growth of prostate tumors and the status of obesity [113]. Caloric restriction reduces the growth of tumor cells in rodent models through reduced systemic insulin and IGF-1 signaling [114], while the activation of PI3K induces tumors to be resistant to diet restriction [115] suggesting an important relationship between PI3K signaling in tumors and the nutrients in the tumor environment. The exact link between obesity and cancer has not yet been established and the fly may facilitate this research thanks to the ability to combine obesity and tumor models in *Drosophila.* Insulin signaling is the main regulator of metabolic homeostasis, and it is also involved in cancer development and progression [116] but we have yet to understand how hyperinsulinemia promotes tumor formation. Interestingly, the oncogenes Src and Ras were overexpressed in a *Drosophila* model of obesity and increasing the level of insulin exacerbates the malignant phenotype due to *wingless* activity [117]. The interplay between obesity and cancer is an important area of study to understand the relevance of fat to tumor growth, since fatty acids are unable to penetrate the biological membranes and need to be cleaved by lipases (lipolysis). Recent studies indicate that in the peritumoural area, an increase in adipose triglyceride lipase

(ATGL) that mediates lipolysis results in tumor survival [118, 119]. The ability to manipulate flies genetically and the possibility to change the composition of lipids or nutrients in their food will likely put *Drosophila* as a key model to investigate the relationship between obesity and cancer and the mechanisms that control cellular overgrowth. Cancer research can only benefit from the ability to create specific disease models in *Drosophila.* This approach lets researchers detect oncogenes and tumor suppressors, allowing a detailed in vivo analysis of the mechanisms triggering cancer. From these findings, drug therapy compounds can then be developed and tested.

### 2.5. Metabolic disorders

Hepatic diseases affect a large proportion of the population worldwide making it crucial to investigate the underlying pathogenic mechanisms that still remain unclear. Identification of the molecular defects underlying liver disease requires studies in model organisms, and recently *Drosophila* has been proposed for this purpose [120].

The use of the fruit fly in the study of hepatic disorders is partially restricted due to the absence of a homologous organ for the liver. The fat body in *Drosophila* acts as storage for sugar and fat and also performs metabolic functions similar to those of the mammalian hepatocytes, regulated by insulin through an evolutionarily conserved mechanism [121, 122]. During starvation, triglycerides are transported from the fat body into the hemolymph where they are captured by the oenocytes, clusters of hepatocyte-like cells that are important for lipid metabolism [123]. Therefore, some functions of hepatocytes are performed by oenocytes, which are located near the body wall surface and play a prominent role in the fly lipid processing. *Drosophila* homologs of genes specifically expressed in human hepatocytes are expressed in larval oenocytes and the fat metabolism pathway is similar among the organisms [123]. An interesting aspect regarding lipid metabolism is the interaction between oenocytes and fat cells, as oenocytes control lipolysis in fat cells through a feedback similar to that in mammals [123]. Underfed *Drosophila* stores many fat droplets resulting in the accumulation of triacylglycerols in the liver, a condition called steatosis, and forms an excellent model for understanding human non-alcoholic fatty liver disease (NAFLD) [124]. Moreover, the relationship between oenocytes and fat cells needs to be elucidated because it contributes to the pathogenesis of metabolic syndrome [125], and fly modeling can be useful for this purpose.

It is necessary to improve assays examining the function of the fat body and oenocytes to solidify *Drosophila* as a liver disease model. To date, the analyses are based on evaluating lipid accumulation depending on different nutritional conditions. Fly lipid homeostasis can be monitored by Raman scattering microscopy that allows for the visualization of the lipid content in larval oenocytes and in the fat body by *in vivo* imaging [126]. Oil Red-O and BODIPY are dyes permitting the assessment of lipid content [123, 127].

Several proteins that contribute to lipid metabolism in *Drosophila*, including proteins responsible for lipid storage, transport, and utilization, have counterparts in higher organisms [128, 129]. This similitude makes the fruit fly helpful in describing the main pathways controlling homeostasis and provides an opportunity to examine metabolic disorders affecting humans such as diabetes and obesity [122]. For example, the main regulator of sugar and

fat metabolism is the nutrient-sensing target of rapamycin (TOR) both in *Drosophila* and in mammals [130]. Flies are able to regulate carbohydrate metabolism by cellular storage of excess nutrients. The hormone insulin controls hemolymph sugar levels and maintains carbohydrate homeostasis through a phylogenetically conserved signaling pathway [122, 131]. *Drosophila* insulin induces an increase in fat cell mass, just as in mammals, because insulin acts on triglyceride storage and on fat body cell number. Shaggy is a serine/threonine protein kinase orthologous to glycogen synthase kinase 3 (GSK3), and it is responsible for the lipid accumulation in *Drosophila* fat cells while the transcription factor *Drosophila* FOXO (dFOXO) influences the adipocyte cell number [121]. Both of these key factors are regulated by the conserved insulin pathway [121]. Dilp2, 3 and 5, members of the *Drosophila* insulin-like peptides (Dilps) are expressed in the insulin-producing cells (IPCs), a cluster of cells in the brain that function similarly to human pancreatic β cells [132]. Additionally, the adipokinetic hormone participates in fly glucose regulation with a glucagon-like function [55]. Functional changes to these metabolic regulators in *Drosophila* cause a phenotype similar to metabolic impairment as well as affecting body size [132, 133]. The resemblance between *Drosophila* and mammals helps to elucidate the main mechanisms of metabolic homeostasis involved in common pathologies such as type 2 diabetes mellitus (T2DM), which is characterized by insulin resistance, hyperglycemia, and defects in lipid metabolism [134]. High-glycemic diets promote obesity, a disorder characterized by excessive fat storage. *Drosophila* fed a high fat diet store fat in the fat body and in the midgut [135]. This condition changes the animal physiology and lifespan mainly due to insulin resistance [136, 137]. Moreover, obesity is considered among the risk factors for diabetes, cardiac diseases, and several types of cancer [138, 139]. Insulin resistance is also related to NFALD, the most frequent chronic hepatic disorder [140]. NAFLD originates from metabolic impairment highlighting the strong relationship between the liver and metabolism and the subsequent need to examine the pathways linking them [124].

*Drosophila* has facilitated the study of metabolic pathways thanks to the availability of several assays of metabolic function, including some that are available for use only in *Drosophila,* which allow for the quantification of lipids, sugars, ATP, and mitochondria. In spite of the anatomical differences between flies and humans, the identification of novel genes and pathways in the fruit fly could arrange for new therapies to treat metabolic disease in humans.

### 2.6. Immunological diseases

The mechanism of the innate immune system is fairly conserved across species, and *Drosophila* is a leading organism for elucidating the process of defense from pathogens and its evolution [141]. Since the adaptive immune response of vertebrates could hide some aspects of the innate immunity, it is beneficial to use *Drosophila* to detail the regulation of innate immunity because this organism does not have an adaptive one [141]. Pathogenic microorganisms, such as bacteria, fungi, nematodes, and viruses, can infect *Drosophila*, priming an immune reaction. Despite the greater refinement of mammalian immunity, *Drosophila* and humans share general defense strategies like epithelial barriers, phagocytosis, and antimicrobial peptides. The fly's first line of defense against to pathogens is a physical barrier represented by the epithelia of the epidermis, trachea, and gut. Clotting factors in the hemolymph provide a second barrier because they can entrap invaders by means of their protein filaments [142]. Epithelia then

release antimicrobial peptides (AMPs) and reactive oxygen species (ROS), triggering a local immune response [143, 144]. Beside their toxic activity, ROS are involved in wound healing and tissue repair both in *Drosophila* and mammals [145]. In addition to epithelia, blood cells and the fat body are also required for *Drosophila* immunity. The external agents are phagocytized by hemocytes; the circulating blood cells and different types of hemocytes are involved in this reaction. Plasmatocytes are monocyte-like cells, which able to phagocytose pathogens, apoptotic bodies, and other foreign particles. Crystal cells, another type of hemocyte, are involved in the production of melanin, a protein involved in both encapsulating and killing microorganisms as well as being implicated in wound healing. Hemocytes differentiate into lamellocytes if a more specialized response is required, and lamellocytes can trap larger parasites, producing a cellular capsule around it in a process named encapsulation [146, 147]. In *Drosophila*, the majority of blood cells have phagocytic activity.

Some fly macrophages originate via self-renewing and others from progenitor cells that are located in the lymph gland, a specialized hematopoietic organ. The great importance of the lymph gland in controlling the blood cell homeostasis makes this *Drosophila* organ comparable with the hematopoietic stem cell niche in the bone marrow [148, 149]. ROS levels have a crucial role in the regulation of *Drosophila* hematopoiesis [150]. Moreover, the signaling pathways regulating blood cell differentiation are conserved from *Drosophila* to humans [151, 152]. These similarities with vertebrate hematopoiesis underscore the utility of the fly to elucidate the basis of hematopoietic injury, necessary because an impairment in hematopoietic differentiation and homeostasis causes several diseases such as leukemia. *Drosophila* has already been used to study acute myeloid leukemia, a widespread form of leukemia, in particular to identify the genes promoting the disease. AML1 is one of the transcription factors activating myeloid differentiation and it has a counterpart in the fly [153]. When AML1 is fused with the repressor ETO, the differentiation is inhibited while the proliferation of multilineage progenitors is activated, leading to acute myeloid leukemia. AML1-ETO expression in *Drosophila* causes the same effect, confirming the fly as a good genetic model for leukemia [153, 154].

The great availability of genetic tools in the fly contributed to defining the innate immune system and to establishing that it is a specific mechanism. In fact, *Drosophila* can respond specifically to pathogens, discriminating between classes of surface molecules on different intruders. AMPs have different targets, for instance drosomycin acts on fungi, defensin on Gram-positive bacteria, and drosocin on Gram-negative bacteria [155]. Moreover, the sequences of AMPs are conserved between humans and insects [156]. Not only is the defense mechanism evolutionarily conserved, but also is the molecular pattern promoting innate immune reactions. *Toll* and *Imd* are the two master genes of *Drosophila* immunity, but *FoxO, JAK/STAT, and JNK* transduction also play a part [157]. After pathogen detection, *Toll* and *Imd* induce a cascade of events that finally release the antimicrobial peptides in fat body cells through the activation of the NF-κB transcription factors Dif, homolog of Dorsal, and Relish, respectively [155]. *Toll* encodes an interleukin 1 receptor-like protein that in *Drosophila* acts in parallel during two different processes: the dorsoventral specification and the immune response regulation [158]. *Toll* is activated by fungi and most Gram-positive bacteria and has a pivotal function both in the humoral response and in phagocytosis. Dissecting *Toll* signaling in *Drosophila* helped to understand toll-like receptors that play an important role

in inflammatory responses [159–161]. The Immune deficiency (*Imd*) signaling is mainly involved in the *Drosophila* reaction to Gram-negative bacterial infection [162]. The flies are also helpful in examining the defense against viral infection as they share with humans some proteins, named restriction factors, involved in the reaction to viral infection. Restriction factors, for instance Pastrel in *Drosophila,* are induced in host cells by virus infection and they can recognize specific viral elements, but the mechanism by which they act in insects is not very clear yet [163].

In order to examine immunity in the fly, an efficient and simple procedure has been developed to elucidate the physiological effect after infection and to quantify the pathogen load. It consists in scoring bacterial load, fly mortality, and also evaluating the effect on immune transcription factors after the direct introduction of bacteria in the fly body cavity, eluding the epithelial barrier [164].

The innate immunity contributes to *Drosophila* homeostasis and it is regulated by endocrine and metabolic systems. Since immune dysfunction leads to several human diseases, including autoimmune disorders, allergy, and intestinal infections, it is fruitful to use this model organism to better understand how all these systems are regulated. The fruit fly is also used to investigate the association between the microbiome and host, trying to characterize the resistance and tolerance mechanisms that are conserved in humans [165–167]. Circadian rhythms also participate in immune regulation both in *Drosophila* and in humans providing another similarity between organisms [168].

## 3. Conclusions

As illustrated throughout these two chapters, *Drosophila melanogaster* has been an invaluable tool for unlocking mechanisms contributing to the pathogenesis of many diseases such as cancer, diabetes, obesity, neurodegenerative disorders, kidney disease, immunological impairments, and many others. Given the advances in the field of genetics, new tools and techniques are continually being developed that will keep flies at the forefront of biomedical research.

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## Conflict of interest

The authors declare no conflict of interest.

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