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# *DROSOPHILA*: THE CENTURY-LONG FLIGHT FROM THE WILD TO THE PATIENT

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## ABSTRACT

**Background:** Evolutionary conservation of key biological pathways between the fruit fly *Drosophila melanogaster* and humans and reduced genetic redundancy have long made flies a valuable genetic model organism. Thanks to the arsenal of sophisticated genetic tools developed and refined by the fly community, the use of *Drosophila* has expanded well beyond basic research. From the fundamental notion that genes are located on chromosomes to modeling human complex diseases such as cancer and neurological disorders, to designing fly "avatar" lines that precisely reproduce the specific mutations found in single cancer patients for personalized medicine, *Drosophila* continues to fuel biomedical advances. Numerous examples of drug testing in flies have yielded novel drug candidates, new uses for approved drugs, and applications for rapid drug optimization in modern approaches combining biology with medicinal chemistry. Thanks to the effectiveness of "fly pharmacology" approaches, *Drosophila* is also proficiently used to study the mechanism of action of environmental pollutants that represent a serious concern to human health. This review traces the history of some of the main advances in *Drosophila* biomedical and cancer research.

### Keywords

disease models; Drosophila; drug testing; cancer; neoplasia; polycystic kidney disease; fly avatars; mutagenesis; personalized medicine



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## Background

The fruit fly *Drosophila melanogaster*, also called vinegar fly or midge, has been a genetic model for over 100 years. With a rapid generation time and ease of rearing in the laboratory settings, this miniature whole animal has easily distinguished male and female individuals, which has allowed performance of controlled genetic crosses. Clearly identifiable organs and appendages have simplified the isolation of mutants, so accelerating the development of several research tools and collections which, in turn, extended the use of *Drosophila* to translational research (reviewed in [1,2]). The completion of its genome sequence has greatly increased its value as a genetic model, allowing functional and morphological investigation of communal traits: about 60 percent of the midge genome is indeed homologous to that of humans, and about 75 percent of genes involved in human diseases have a *Drosophila* homolog [3,4,5]. Importantly, the way these genes are connected functionally into pathways is also conserved from flies to humans [6]. *Drosophila* was instrumental to reveal that genes are found on chromosomes [7]. Then, J. H. Muller first demonstrated that X-rays could induce new phenotypes in the fruit fly [8]. These discoveries ushered in the early mutational studies using ionizing radiation to induce random mutations followed by observation, recording, and preservation of the resulting phenotypes (reviewed in [9]).

*Drosophila* was fundamental to discovering how innate immunity works. Toll-like receptors were found in humans after their characterization in fruit flies [10,11,12]. Toll receptors were first discovered in the 1980s as factors establishing the fly embryonic dorsal-ventral axis. Toll activation generates a ventral-to-dorsal gradient that induces nuclear translocation of the factor Dorsal which differentiates gene expression along the dorsal-ventral axis and creates a morphogenetic gradient directing the development of distinct structures [13,14]. Jules Hoffman noticed that *Toll* mutants were often infected by the mold *Aspergillum*. However, *dorsal* mutants appeared as robust as wild type, suggesting that toll may function beyond embryonic patterning in the adult, where it may protect from microbial infections [15]. In the presence of fungal, bacterial or viral infection, pathogen-associated molecular patterns (PAMPS) or damage-associated molecular patterns (DAMPS) activate Spätzle, a group of isoforms of a homodimeric glycoprotein that binds Toll and induces a cascade aimed at fighting the infection [16]. The Spätzle-toll receptor complex, similar to human TLRs, recruits a heterotrimeric complex formed by the MyD88 adaptor protein, Tube, and the kinase Pelle, eventually priming Cactus degradation. Cactus, like its human homolog IkB, sequesters the fly NF-kB homolog Dorsal in the cytoplasm and represses signaling. Upon Cactus degradation, Dorsal and Dorsal-related immunity factors translocate into the nucleus and activate genes encoding innate immunity components [17,18]. The *Drosophila* genome contains nine *Toll-like* genes with *Toll-1* being the first discovered, *Toll-5* the one most similar to *Toll* itself, and *Toll-9* the most similar to the mammalian homologs [18,19,20,21]. In humans, toll-like receptors are one of five classes of pattern recognition receptors (PRRs) linked to innate immunity as in *Drosophila* [22,23]. Like flies, in humans ten TOLL-LIKE receptors that can induce gene activation via NF-kB and IRFs have been found, confirming the translational potential of the fruit fly (reviewed in [24]).

*Drosophila* was instrumental to discovering the mechanisms regulating circadian rhythms. mammals and other metazoans have internal mechanisms adapted to a 24-hours day/night cycle (reviewed in [25]). In the fruit fly, the protein Period (PER) is responsible for the cellular clock. PER accumulates in cells by night and it is degraded by day [26]. In particular, the *per* and *timeless* (*tim*) genes are transcribed in the morning and translated into cognate proteins. The PER protein is slowly phosphorylated and degraded. The TIM protein is deactivated by light and becomes more stable with the coming of night, when it progressively accumulates into the cytoplasm and dimerizes with PER. The TIM-PER complex enters the nucleus and inhibits *per* and *tim* transcription at night, resulting in a feedback loop regulated by light [27,28,29]. This mechanism is conserved in mammals. In mice, there are three PER homologs called mPER1, mPER2 and mPER3, with a negative feedback loop equivalent to the *Drosophila* one [30,31]. These are two of many exemplary cases in which the fly enabled the discovery of fundamental mechanisms with translational value that would have been too complicated to experiment in vertebrate systems. Underscoring the translational importance of the *Drosophila* model, as of today, six Nobel Prizes were awarded to ten scientists for discoveries in *Drosophila melanogaster* which include the Toll and PER/TIM discoveries described above (Table 1).

To review the use of *Drosophila* models in bio-medicine and personalized medicine, we relied on a key historical perspective [2] and searched PubMed for research articles and reviews published until October 2024 with the keyword strings “fly avatars”, “humanized flies”, and “*Drosophila* and personalized medicine”. The search results were vetted to focus on exemplary applications to elucidate the molecular mechanisms of neoplasia, tumorigenesis and innovative drug testing. Alongside the original primary references, this section also included modern perspectives matured after years of successful applications that were presented in review articles. We apologize to the colleagues whose contributions could not be included due to length limitations. To introduce these successful examples of modeling human disease in *Drosophila*, we illustrated key genetic tools and techniques that make the fly an exceptionally valuable model and presented the nuances of deciphering complex biology by focusing on the evolutionarily conserved “core engines” in the fly’s streamlined model system to make educated deductions of translational value.

## Modelling disease in *Drosophila*

Extensive *Drosophila* genetic research and a genome less redundant than the human one enabled the creation of numerous genome manipulation techniques reviewed in [38, 39, 40]. Using these tools, new genotypes can be engineered to model a wide variety of diseases such as multiple cancers, heart diseases, diabetes, and Fragile X syndrome [41]. The enormous potential of the fly model is evident: between 2000, when the first *Drosophila* genome sequence was released, and August 2024, PubMed reports over 1,700 reviews and many more primary reports on different fly disease models. *Ad hoc* transgenics complement existing collections of spontaneous and chemically- or radiation-induced mutants and can be used in combination. The small size of the fly enables the breeding and testing of large populations in contained spaces with low costs, as well as screening for rare phenotypes (reviewed in [2,42]). Larger animal models, like mice, are also useful to study the specific mechanism of single mutations of interest, but their complexity, genetic redundancy, slow

breeding cycle and expensive rearing conditions greatly complicate functional studies, the analysis of rare phenotypes, and population studies. In contrast, *Drosophila* enables definition of core conserved pathways effectively and rapidly, generating translational knowledge that can be applied to interpret the complexity and redundancy of vertebrate pathways. Moreover, the well-documented fly genetics and phenotypic markers makes it possible to monitor the consequences of genetic manipulations in the entire organism. For instance, Yusuff and colleagues [43] screened 79 fly homologs for rare pathogenic copy number variants (CNVs) associated with neurodevelopmental disorders. CNVs are duplications or deletions that can be linked to healthy genetic variability or to pathogenic conditions like cardiac defects, kidney malformations, autism and neurological conditions [44, 45, 46]. CNVs result from non-allelic homologous recombination, while non-recurrent CNVs result from errors during DNA replication without consistency throughout the genome [47]. Considering the complexity of the genetic interactions, large populations are needed to screen the contributions of every gene, that, unlike vertebrates, can be effectively done in *Drosophila*. Powered by large populations, this study correlated CNVs with developmental aberrations in wings, eyes, and internal organs. Altered phenotypes can be linked to developmental defects [2,48]. Even if the fly and human anatomy differ, *Drosophila* contains the streamlined equivalent of most human organs, and its development is driven by genes with human homologs, that have made *Drosophila* a powerful model for developmental biology and genetics that generates translatable knowledge.

## Drug and compound testing in *Drosophila*

The fly-human conservation extends to pharmacological and toxicological pathways [49]. This allows meaningful drug and compound testing and screening in a miniature whole organism with controlled genetic makeup. *Drosophila* can be used to discover new drug candidates, test FDA-approved drugs for novel uses [50], decipher toxicity mechanisms, drug mechanism of action, conserved target organ specificity, and identify developmental windows of susceptibility. Dose-response effects can be studied in the fly, remaining aware that for each molecule, effective dosage shows species-specificity, and within the same species, sex and individual variation [51,52]. Indeed, this biological variability has been used to advocate for personalized drug dosage optimization in human therapy. “Humanized” fly models, in which the human cDNA of choice is expressed in lieu of the fly ortholog, allow to test how specific protein variants respond to drugs in a whole animal. Unlike cell culture systems, these models also reproduce developmental effects. Humanized flies can be designed to duplicate single or constellations of mutations typical of individual cancer patients. These “avatar” flies were used to test diverse drug combinations and identify effective chemotherapy cocktails rapidly enough to inform therapy and benefit the patient. Specific examples applied to oncology are discussed below. With underlying mechanistic conservation, drug assays, dubbed “fly pharmacology”, can directly translate to the patient when the molecule tested is either an approved drug or a widely used supplement like vitamin B5 for TANGO2 Deficiency Disorder [53] or melatonin for reducing cysts in polycystic kidney disease [54]. Novel compounds can be discovered using fly models tailored to testing biological activity in a selective whole animal system that accelerates the drug discovering pipeline by reducing false positives occurring in cell culture systems [55]. Once active molecules are identified, structure activity relationship studies can be performed

efficiently in the fly using analytical amounts of synthesized compounds that accelerates the phase 0 pre-clinical testing. Finally, compared to other animal models, *Drosophila* husbandry and genetic manipulation are economic and may especially suit the study of rare diseases, for which the prospect of a small market often discourages substantial research investments.

An emerging line of investigation is to leverage the conservation of the fly metabolism and apply metabolomics to the discovery of how molecules and toxicants interfere with metabolic pathways, including those related to disease [56]. Having highly inbred and homozygous fly lines facilitates the readout of pharmacological assays that favors mechanistic studies. On the other hand, sensitivity to drugs and toxicants varies in the natural populations because of the segregation of sensitive genetic variants. Identifying these genetic variants in humans is challenging because of uncontrolled genetic backgrounds and diverse environmental, developmental, life and exposure histories. Although rodent models could mimic such human complexity, their expensive rearing and lengthy generation time and transgenesis limit use to small populations. Moreover, only a few genetic backgrounds are available, that hinders the assignment of drug response phenotypes to genetic variants. In contrast, genetic resources such as the *Drosophila* Genetic Reference Panel (DGRP) have been developed specifically to study the effects of natural variants on gene expression and phenotypes, including drug and toxicant response [57]. Consisting of 1,300 highly inbred lines with fully sequenced genomes, the *Drosophila* Genetic Reference Panel has been used to identify, among others, the genetic contributors to alcohol sensitivity [58, 59], and the toxicity of methylmercury [60] and heavy metals as lead and cadmium [61]. The DGRP can therefore be applied to study the genetics and toxicology of conserved genes of interest and, once combined with classical genetics, also the underlying mechanisms. These studies in the fly routinely yield knowledge that can be translated to humans because of the genomic conservation. Importantly, the fly is the only model in which this type of genome-wide association studies can be done.

## *Drosophila* genetic tools and techniques for disease and cancer research

**P-element mediated transformation.—**A classic genetic tool to manipulate the *Drosophila* genome is the transposable P elements that were first discovered in 1970 [62]. Many abnormalities *e.g.*, chromosomal rearrangements, high rates of mutation, and sterility brought on by rudimentary gonad development were found when male flies from wild strains were mated with female flies from laboratory strains. However, normal and fertile offspring were produced by the reciprocal cross. This group of characteristics was referred to as hybrid dysgenesis [63]. P elements are transposons thought to cause all these abnormalities, that now we know are regulated by Piwi-interacting RNAs (piRNAs). The small piRNAs confer sequence-specificity to PIWI proteins by complementary Watson-Crick base pairing with their targets, primarily transposable elements and other genomic repeats, similar to the RNA interference (RNAi) systems [64]. In *Drosophila*, as in mammals, piRNAs are limited to the gonads. In wild type females, piRNAs are found in the egg cytoplasm which inhibit P element transposition. This does not happen in strains showing hybrid dysgenesis, causing mutagenic P element mobilization (reviewed by [65]). Leveraging on the P element ability to insert into the genome, their transposition has been

tailored to insert genes of choice in the fly genome. Like bacterial insertion sequences, P elements are composed of two terminal inverted repeats (IR) flanking a transposase gene containing three introns [66]. The transposase catalyzes genomic insertion at the IR sequences. to use P elements for genetic engineering, the gene of interest is placed between two IRs, replacing the transposase gene, and cloned into a transformation vector containing a selectable marker, usually a mini-*white* gene rescuing the white eye phenotype. The resulting construct is microinjected into *white* mutant eggs together with a helper plasmid carrying the transposase sequence, which activates the insertional process but cannot be hosted into the genome and is eventually degraded. While insertion occurs in 25–40% of all cells [67,68], only insertions in the germ cells can be passed on to the next generation. Once these embryos have developed into adults, germline insertions are highlighted through genetic crossing by mating single injected flies with un-injected flies of the same genotype. Any progeny expressing the mini-*white* marker gene is then recognized based on the eye color and stabilized into transgenics lines for further studies [68].

**The UAS/GAL4 system.—**In 1993 Andrea Brand and Norbert Perrimon [69,70] used P element-mediated insertion to generate fly transgenics expressing respectively the yeast transcription factor GAL4 and its recognition site, the upstream activation sequence (UAS). GAL4 regulation is unique to yeast, which prevents activation in the absence of either element and constitutes a tight regulation. When GAL4-expressing flies are crossed with UAS-containing transgenics, the transcriptional regulation system is reconstituted and any gene downstream of UAS is expressed under GAL4 control. The UAS/GAL4 system is now available in many different modifications allowing to express coding genes or RNAs (e.g., interfering RNAs targeting specific genes by RNAi, RNA interference) in the whole organism or specific tissues or cells (reviewed in [71]). Modifications of this system include the inducible expression of GAL4, *e.g.*, under the control of the heat shock protein 70 enhancer (*hsp70*) which allows pulsed activation of the GAL4-dependent expression of any gene of interest [72].

**Mosaic techniques.—**One of the powerful *Drosophila* genetic tools that has enabled modern understanding of how molecular interactions affect cell-cell relationships and the entire organism is the mosaic technique, which accounts on mitotic recombination to generate homozygous mutant cells in a tissue or organ. Certain mutations are indeed lethal in the whole animal, which prevents their experimental probing. Instead, introducing these mutations in a patch of cells in an otherwise wild type animal usually allows experimentation. Moreover, several genes have stage-specific roles in diverse tissues and developmental stages, thus are pleiotropic. This complicates the elucidation of each cell-autonomous function. Finally, cell-cell interactions are fundamental in healthy epithelia and are disrupted in diseases such as cancer, where healthy and mutated cancerous cells become neighbors. In the early 80’s, genetic mosaics were induced by X-rays, which caused chromosomal aberrations that, with the help of specific recessive markers linked to the mutation of interest, allowed to observe the phenotype resulting from the mitotic recombination throughout the adult body [73, 74]. Later, the FLP/FRT binary system from yeast permitted to exchange chromosomal arms by site-specific recombination and observe the mutant phenotypes in both larval and adult tissues and organs thanks to the use of *in*

*vivo* reporter genes such as GFP [75]. Finally, in the mosaic Analysis with a Repressible Marker (MARCM), specific cell populations can bear combined loss-of-function (LOF) and gain-of-function (GOF) mutations, which allows to investigate complex phenotypes resulting from genetic interactions [76] (Figure 1). At the end of the process, marked cells bear the mutations of interest. See the legend to Figure 1 for details.

## *Drosophila* to model neoplastic growth and cancer

*Drosophila* has proven itself an excellent model to study cancer and oncogenic mechanisms [77]. Many genes involved in carcinogenesis were first identified in *Drosophila* and, only later, in humans (reviewed in [78]). For example, the oncogenic protein Myc is conserved from flies to humans and is functionally interchangeable [79]. Several developmental processes orchestrated by Myc were first characterized in flies and then found in humans, such as cell growth regulation through the PI3K/AKT/mTORC1 signaling, the central-peripheral axis linking nutrition and systemic growth, and cell competition (reviewed in [80]). The early discovery of *lgl* mutants that manifested cancer-like hyperproliferation and disorganized tissue architecture indicated that the *lethal giant larvae* (*lgl*) gene was a candidate tumor suppressor gene [81,82]. In fact, almost 80% of human cancers display mutations that cause loss of cell polarity [83]. Of note, *lgl* mutant cells deprived of the Myc protein are unable to form a tumor [84], establishing a direct link between cell polarity regulation and cell growth [85]. In flies, *lgl*, *discs large* (*dlg*) and *scribble* (*scrib*) gene products interact with each other to maintain apical-basal cell polarity [81,82]. As those genes are functionally conserved in humans, many studies have addressed their role in human cancers; for example, *scrib* mutations in patients with cancer are linked to loss of cell polarity and metastasis [86]. Another example is the protein Warts (Wts), first characterized in fruit fly epithelia. Wts is a serine-threonine kinase of the Hippo pathway, critical in regulating the development of epithelial-derived organs such as eyes, wings, legs, and genitalia. When phosphorylated, Wts inhibits Yorkie, a pro-proliferative factor. If Wts is mutated, this inhibitory role falters and cells over-proliferate [87,88]. Soon after being characterized in the fruit fly, the Hippo pathway has been found to coordinate cell polarity and cell growth also in mammalian development and tumorigenesis, with the final effector YAP (Yki homolog) hyperactivated in the majority of human cancers [89]. Because of their involvement in cell metabolism and growth, several “cancer pathways” were found to be dysregulated also in lesser-known non-malignant neoplastic pathologies like the cystic degeneration of polycystic kidney disease (PKD). Similar to human PKD, a *Drosophila* model of polycystic kidney disease displayed *d-myc* upregulation and mTOR activation and similar pharmacological responses to rapamycin [90] and some mimics of the second mitochondria-derived activator of caspases (Smac) [91]. Therefore, mechanistic and pharmacologic knowledge from cancer models in *Drosophila* and other systems could help unravel the molecular events underscoring cystic degeneration and, possibly, inform on crucial differences between benign growth and malignancy (reviewed in [92]).

Besides studying single tumor-related pathways, *Drosophila* can be made to develop tumors. The classic method for inducing tumors in *Drosophila* started from the so-called two-hits hypothesis formulated by A. Knudson in 1971, proposing that both alleles of a tumor suppressor should be mutated to obtain cancer (reviewed in [93]). The two-hits model has

been reconsidered over time, and now we know that the loss of oncosuppressors combined with oncogene activation cooperate in cancer initiation (reviewed in [77]). Today, plenty of *Drosophila* cancer models exist. Next, we will present selected examples of organotypic cancer models and their translational impact on the clinic.

**Colorectal cancer (CRC).—**Similar to humans, flies possess a gut that is morphologically divided into fore-, mid- and hind-gut with conserved cell populations. In fact, there are enterocytes, that deal with digestion and absorption, entero-endocrine cells, that release signaling factors to regulate gut functionality, and stem cells [94]. In addition, humans display mucipar cells, that secrete mucus to lubricate the digestive tract epithelium. *Drosophila* CRC models exhibit typical hallmarks such as cell hyperproliferation, altered tissue architecture, evasion of apoptosis and senescence, as well as invasive migration and metastasis [95, 96]. In vertebrates, the most common forms of CRC are due to mutations in the *WNT*, *Apc (Adenomatous polyposis coli)*, and *RAS* (rat sarcoma) genes [97], the fly homologs of which are *dwnt* (also referred to as *wingless*, *wg*), *Apc 1/2*, *Dras 1/2/3* (*Drosophila Ras*) respectively. In the *Drosophila* gut, loss of *Apc* leads to cell hyperproliferation [98]. Moreover, *RAS* loss can reduce the hyperproliferation phenotype of *Apc* mutants; conversely, overexpression of the activated form of RAS, $RAS^{v12}$, exacerbates the *Apc* mutant phenotype and leads to malignant transformation [98], showing the important level of conservation of these functions from the fruit fly to humans. Based on these results, Adams and colleagues [99] developed a *Drosophila* CRC model using the MARCM technique to repress *Apc* and simultaneously hyperactivate *Dras* in the gut tissue. This allowed comparing the phenotype of the clones with adjacent normal cells in the same organ. Four weeks after, the fly guts resembled human CRC. Authors observed hyperplastic growth, large variability in cell morphology and shape and increased nuclear density and size. This model also contained a clever engineered feature to allow the study of anticancer drugs in that the mutant cells also expressed the luciferase enzyme under UAS control. Luciferase produces light while modifying its luciferin substrate. Therefore, in a gut lysate the level of luminescence was proportional to the number of clones generated and thus to the proliferation of tumor cells. Feeding the CRC flies two anticancer drugs used to treat human CRC, oxaliplatin or 5-fluorouracil, significantly reduced the dimensions of the mutant clones induced in the intestine as measured by the luciferase assay.

Using the P element-based pUAST transformation vector, Pfeifle and colleagues [100] introduced a missense mutation that is lethal at the larval stage, so the transformants were unable to reach adulthood. This model can be used to assess drugs that rescue the ability to get to the adult stage. In the study, the authors focused on the mitogen-activated kinase pathway (MAPK), also known as the RAS/RAF/MEK/ERK signaling cascade (reviewed in [101]). In particular, they expressed the activated form of the serine-threonine kinase dRaf $^{A572E}$ using a gut-specific and temperature sensitive driver, *escargot esg*$^{ts}$*>GAL4*, and a transgene expressing the dRaf $^{A572E}$ protein from the GAL4-binding sequence UAS. This method induces GAL4 expression, and consequently dRaf $^{A572E}$, in the intestinal stem cells after a heat shock. When these flies were treated with cobimetinib, a specific MEK1/2inhibitor, about 37% of the larvae reached the pupal stage versus 1% of those treated with DMSO as negative control [100].

Quintero and Bangi [102] produced a genetic platform named PromoterSwitch that can generate small cancer clones derived from individual stem/progenitor cells in *Drosophila* adults. Like other solid tumors, CRC typically contains all the cell types of the original tissue, but cell fate is disrupted in transformed clones, that creates unique genetic heterogeneity and drug sensitivity. PromoterSwitch uses the UAS/GAL4/GAL80 system and FLP-FRT site-specific recombination to target the expression of transgenes in specific cell lineages. First, cell-specific promoters can be activated to drive the transgenes and induce transformation and then, clones are created in those same cells by either deactivating the UAS-GAL4 system (in the majority of cells) or expressing GAL4 under the ubiquitous actin promoter yielding few cell-type-specific clones to examine. The existing fly model recapitulating the most common CRC genetic landscape, called RPA (*KRAS* activation+*TP53* and *APC* LOF) was used to introduce in intestinal stem cells (ISCs) and progenitor cells two additional mutations found in CRC: RPPA (*PTEN* LOF to model PI3K pathway activation) and RPMA (*SMAD4* LOF to model loss of TGF-β signaling). Compared to the RPA background, both RPPA and RPMA clones reproduced key aspects of tumorigenesis, *e.g.*, faster proliferation, heterogeneous activation of several tumor markers, and induction of non-autonomous responses in the neighboring cells. In particular, the enteroendocrine cells (EEs) were more abundant and expressed markers denoting changed EE differentiation and cell fate. The EEs derive from ISCs, secrete hormones that alter their surroundings, induce ISC proliferation, and participate in inter-organ signaling. Importantly, in human CRC, EEs abundance is associated with ISC proliferation and poor prognosis. The heterogenous PromoterSwitch-induced clones expressed variable amounts of several EEs-specific hormones that likely contribute autonomously to tumor growth and affect the neighboring tissues, mimicking the nuances of CRC tumorigenesis. Thus, this system can reproduce the cell fate disruption typical of tumorigenesis and critical CRC features that enable the study of how the genotype may affect CRC outcome in a whole organism.

**Breast and other cancers.—**In another study [103], a humanized *Drosophila* P-cadherin (P-cad) line was used to understand the effects of its overexpression. In breast and other cancers, P-cad over-expression induces “stem-like” properties promoting cell survival and malignancy [104,105]. The endogenous *Drosophila* E-cad (Shotgun, DE-cad) forms hybrid complexes with the human P-cad to be tested in the wing and eye discs as models of epithelial tissue [103]. P-cad and related E-cadherin (E-cad) are often co-expressed and regulate embryonic development and adult tissue architecture and modulate stem signaling pathways [106,107]. This system recapitulated the key functions of the P-cad in human cells [103], where the signaling networks downstream of P-cad depend on cell and tissue context [106, 107]. Alike human breast cancer, in which the loss of E-cad is compensated by P-cad [108], DE-cad knockdown also inhibited wing development in flies, and P-cad expression partially rescued the phenotype [103]. Finally, the P-cad humanized fly revealed two other functionally conserved factors related to cytoskeleton-regulated gene expression that were confirmed in a human breast cancer cell line: myocardin-related transcription factor A (Mrtf) and serum response factor (Srf) [103]. human and fly MTRF-3 can interact with monomeric G-actin, that shields a nuclear localization signal, maintaining MTRF-3 in the cytoplasm. When F-actin polymerizes, *e.g.*, in response to P-cad, the free G-actin monomers decreased and MTRF could translocate to the nucleus, interact with SRF and activate the transcription

of several genes, including some regulating actin nucleation. The fly readout, shape and size of the adult wing, showed that the hybrid model largely reproduced the human system. However, in the wing blade, P-cad reduced tissue growth, in contrast to breast cancer cells, in which P-cad fuels tumorigenesis, which underscores the existence of tissue-specific nuances alongside numerous conserved fundamental similarities [103]. Importantly, this study also showed that, in addition to the known role to maintain tumorigenesis, P-cad also functions in early cancer by transiently activating *MTRF-3/SRF* transcription in response to changes in actin nucleation. This model will be important to further examine P-cad effectors and cytoskeletal changes during carcinogenesis.

**Flies and personalized medicine.—**Cancer cells accumulate several mutations. Moreover, as cancer progresses, new mutations are acquired in cell subsets, or clones, which makes carcinogenesis a very individualized process. While there are recurrent mutations in most cancer types, the combination of mutations and the number of cells that carry them are not only specific to the individual, but also to the different metastases colonizing secondary organs. This high genetic heterogeneity deeply affects the response to therapy, as different cell clones may interact with drugs differently. Thus, personalized medicine is gaining momentum, in which specialized chemotherapy cocktails are ideally designed to target the collection and ratio of cells with (potentially variable amounts of) mutated proteins in a particular patient. To improve rapid detection of effective chemotherapy cocktails, Bangi and colleagues [109] have set up *Drosophila* patient-specific avatar models. In one application of this ingenious strategy, a mutation in KRAS (G13A) was found using next-generation whole-exome sequencing from the tumor from a 53-year-old individual with colorectal adenocarcinoma that had received several rounds of ineffective chemotherapy treatments. As the pathology progressed, whole exome sequencing detected the accumulation of numerous mutations. The corresponding fly avatar contained the original KRAS (G13A) mutation and others known to be most relevant for tumorigenesis such as the biallelic loss of *APC*, *TP53*, and *FBXW7*, and heterozygous somatic mutations of *SMARCA4*, *FAT4*, and *MAPK14*. The UAS/GAL4 system was used to mimic patient complexity by making transgenic flies expressing the mutated genes in the posterior hindgut while inhibiting the corresponding fly orthologs using RNAi constructs under UAS/GAL4 control. This fly avatar was used to rapidly screen 121 FDA-approved drugs either individually or in combination to find that the trametinib/zoledronate combination reduced lethality. When this same combination was administered to the patient, cancerous lesions were reduced by 45% [109].

Another successful application of the fly avatar model was made for a 54-year-old male with adenoid cystic carcinoma (ACC) [110]. DNA from a frozen specimen of a lung metastasis, RNA and DNA from a blood sample and whole exome sequencing were used to map the patient's "genomic landscape". Four genes: *FAT4*, *FAT1*, *FAT3*, and *ERCC2*, which had heterozygous missense mutations predicted to be deleterious, were expressed in the tumor. The *Drosophila* orthologs of *FAT1/3*, *FAT4*, and *ERCC2* were simultaneously targeted by RNAi mediated knockdown and the resulting fly avatar used to screen combinations of FDA-approved drugs from a library. Confirming the relative efficacy of docetaxel previously observed in the patient, both tofacitinib and docetaxel were found to have low efficacy also in the fly avatar. However, a combination of vorinostat, pindolol and tofacitinib revealed

to be the most effective and was later tried on the patient. Once the relative dosage was adjusted to minimize side effects, the patient stabilized, showing no new bone lesions and mild regression of existing lung lesions. Over time, the treatments halved glucose uptake in pulmonary and bone metastases, signaling reduced cancer cell metabolism [110].

In another study, Sonoshita and colleagues [111] used an established medullary thyroid carcinoma *Drosophila* model driven by oncogenic REarranged during Transfection (RET) expression to tackle the pharmacological challenge of identifying FDA approved kinase inhibitors to target specific kinase networks. Expressing the M955T isoform of the *Drosophila* RET using the *patched* (*ptc*) promoter first expressed in the embryo, the *ptc>dRet[M955T]* transgenic fly model displayed some phenotypic traits specific to the human oncogenic M918T RET isoform, including the activation of the early steps of metastatic transformation, and did not reach adulthood. This model was used to screen for kinase inhibitors that rescued development. Several hits specific for members of the mitogen-activated protein kinase (MAPK) network were identified, including sorafenib, regorafenib and trametinib, three structurally similar molecules. Although sorafenib was effective in both flies and patients, its efficacy is low, and it displayed severe adverse effects. To explore the kinase networks modulated by sorafenib and create novel molecules with unique kinase targeting, a combined genetics and medicinal chemistry approach with structure activity relationship studies were carried out by synthesizing analogs that modified the spacer, linker and cap regions of the sorafenib molecule, while maintaining a fourth one, the hinge binder, constant. When tested in flies and tissues with genetically modulated expression of specific kinases, one analog specifically rescued both lethality and the metastatic index, while displaying reduced toxicity. Importantly, these properties were maintained in a murine model. Notably, fruit flies do not have a thyroid. Thus, this successful example of fly modeling illustrates how the power of experimental design can accelerate the exploration of the chemical space of approved drugs and optimize desired properties.

In one study [112] on lung tumor development, an *EGFR* fly mutant was used to screen an FDA-approved compound library. A constitutively active form of the *Drosophila* EGFR, $EGFR^{CA}$ (A887t) was expressed in the cells of the fly airway, the tracheas, using the *ppk4-GAl4* driver. This caused lethality at the third instar stage larvae, with thickened and shortened tracheal dorsal trunks and a compressed inner tracheal lining. However, the tyrosine kinase inhibitors afatinib, gefitinib, and ibrutinib could rescue such lethality. Specifically, over the course of seven days, both afatinib and gefitinib increased the number of both pupae and eclosed adults compared to ibrutinib. The morphological characteristics seen in the fly trachea, such as abnormalities of the chitinous layer, cellular structure, epithelial thickness, and number of nuclei, could likewise be reversed by both afatinib and gefitinib, making them promising drug candidates. These results are consequential, because somatic *EGFR* mutations are found in 10% of non-small cell lung cancers and are known oncogenic drivers. Moreover, about half of non-small cell lung cancers develop drug resistance, making combination therapy a promising approach due to its simultaneous targeting of multiple pathways.

### *Drosophila* to study the impact of environmental contaminants

Considering that *Drosophila* is an excellent model to rapidly study carcinogenesis and the mechanism of action of molecules of interest, it would be interesting to design assays to test the effects of environmental toxicants and pollutants. Wild type flies could be used to study the biological effects of pollutants and their mixtures on healthy organisms, while disease models could reveal enhanced sensitivities. Genetic collections such as the DGRP could yield insight on the naturally occurring variants that augment sensitivity to or protect from such effects. Common contemporary pollutants include industrial byproducts, fertilizers and pesticides used agriculturally, and their breakdown molecules, as well as degradation products such as micro- and nano-plastics [113,114,115]. Several pollutants are genotoxic. A common assay to study mutagenicity is the somatic mutation and recombination test (SMART) assay which by analysis of wing or eye markers allows characterization of the type of induced genetic damage, such as deletion, point mutation and recombination [116]. Additional pollutants may also include natural and man-made radiation from radioactive isotopes used in diagnostic medicine, medical treatments and industry. Research into the mechanistic consequences of low and very low doses of radiation (LDR, VLDR) exposure will improve comprehension of the biological consequences, molecular mechanisms, and potentially adverse outcomes and risks associated with these unavoidable exposures [117]. Interestingly, *Drosophila* has also been the first complex organism in the study of how the (low dose) natural environmental radiation impacts living organisms that may lead to mechanistic breakthroughs in the understanding of these processes [118]. *Drosophila* has proven itself as an excellent model to study low dose radiation [119], the developmental neurotoxicity of radiation therapy used to treat pediatric brain tumors [120] and radioresistance [121]. Moreover, *Drosophila* is used as a model organism in space research. Flies were the first animal sent to space in 1947 to test the feasibility of space travel for living organisms. Launched to an altitude of about 109 km from earth on a V-2 rocket, flies ushered in the space exploration era. The United States National Aeronautics and Space Administration (NASA) maintains a permanent *Drosophila* laboratory called the Fruit Fly Lab (FFL) on the International Space Station [122] and launched the first FFL-01 mission in 2014. The latest Fruit Fly Lab-03, FFL-03 mission aims to study immune system dysfunctions and infections that pose major risks to astronauts on long space exploration missions [123].

The use of disease models first and then avatars that best mimic the degree of complexity of specific conditions may be useful in preventing and understanding the impact that increasingly complex mixes of pollutants may have on the human body and their potential influence on disease progression.

## Conclusions

*Drosophila* has been a source of translational biological knowledge for over a century. The exceptionally collaborative fly research community has developed and maintains several valuable resources, among which the Bloomington Stock Center and Flybase [124,125,] and a vast array of genetic tools. *Drosophila* researchers have founded consortia to undertake challenging projects such as the complete sequencing of the fly genome that

served as a blueprint for developing bioinformatics tools for the human genome project, which, ultimately, had intrinsic translational value. In cancer research, *Drosophila* and mice are generally recognized as the top whole-animal models that can reproduce both the detailed genetic makeup and the cell environment typical of cancers. Historically, flies have been instrumental in deciphering single-hit [126] and complex [127] cancer mechanisms, identifying most of the signaling pathways and oncogenic drivers, and in discovering cancer cell cooperation and competition mechanisms (reviewed in [127]), the tumor environment [128] and metastases [129] to produce knowledge that has been translated to mice and, eventually, humans. Flies have more recently been used in personalized medicine approaches to rapidly and successfully identify pharmacological inhibitors for single or combinations of pathways found disrupted in cancer. However, fruit flies, like every model organism including mice, are not humans, and differences are to be expected and must be factored in both experimental strategy and data interpretation. Model organism research can overcome intrinsic limitations of human subject research. For example, unlike the highly inbred strains of model animals used in the lab, natural populations, including human populations, have genomic variants that confer individual differences and personal susceptibility to disease and drug response among other traits. These variants affect disease epidemiology, underlie the variability of drug response in clinical trials, and can only be studied in whole organisms and natural populations. Candidate variants are identified by genome-wide association studies (GWAS) in humans and flies, however they can only be validated experimentally in flies. *Drosophila* and mice are highly complementary in cancer research and have been used synergistically [103,130,131,132]. Transgenesis and genome editing to produce *ad hoc* genetic backgrounds are more rapidly and effectively performed in *Drosophila* than mice. While both models have innate immunity, only mice have adaptive immunity. However, even humanized mice do not completely reproduce human adaptive immune responses due for example to cytokine species-specificity and different mucosal immunity [133,134]. Additionally, most humanized mice are patient-derived xenografts (PDX) made in immunocompromised strains that cannot reproduce healthy immunity. *Drosophila* shines in high throughput screening applied to both drug discovery and genetic network studies. Its small size and fast generation time coupled with ingenious phenotypic and biochemical readouts make it a statistically robust system with demonstrated value in drug testing, high-throughput screening and the identification of genetic modifiers [135,136,137,138]. Unlike organoids and complex cell culture, whole animal models, including the fly, reproduce the complexity of a whole organism, with distinct organs and cell environments. Whole animal models can reveal undesirable properties of molecules of interest, *e.g.*, developmental and physiological disruptions that are ground for the elimination from the drug development pipeline. *Drosophila* can quickly reveal such problems, saving costs and effort. Furthermore, the effects of the environment on cancer and other diseases can be studied effectively first in the fly and then validated in mice. How diet affects the tumor environment has been first demonstrated in a fly model of cachexia (tumor wasting) [139,140,141]. Conserved toxicological pathways encourage the use of *Drosophila* to test the biological effects and genotoxicity of environmental pollutants like micro- and nanoplastics in personalized backgrounds and identify their respective genetic, dietary, and environmental modifiers, all fields that will become more and more important in the future. Thanks to an exceptionally collaborative research community, expansive tools for rigorous

experimental design, and its desirable biological features, the fly has and will continue to yield valuable basic and translational knowledge to directly benefit the patient.

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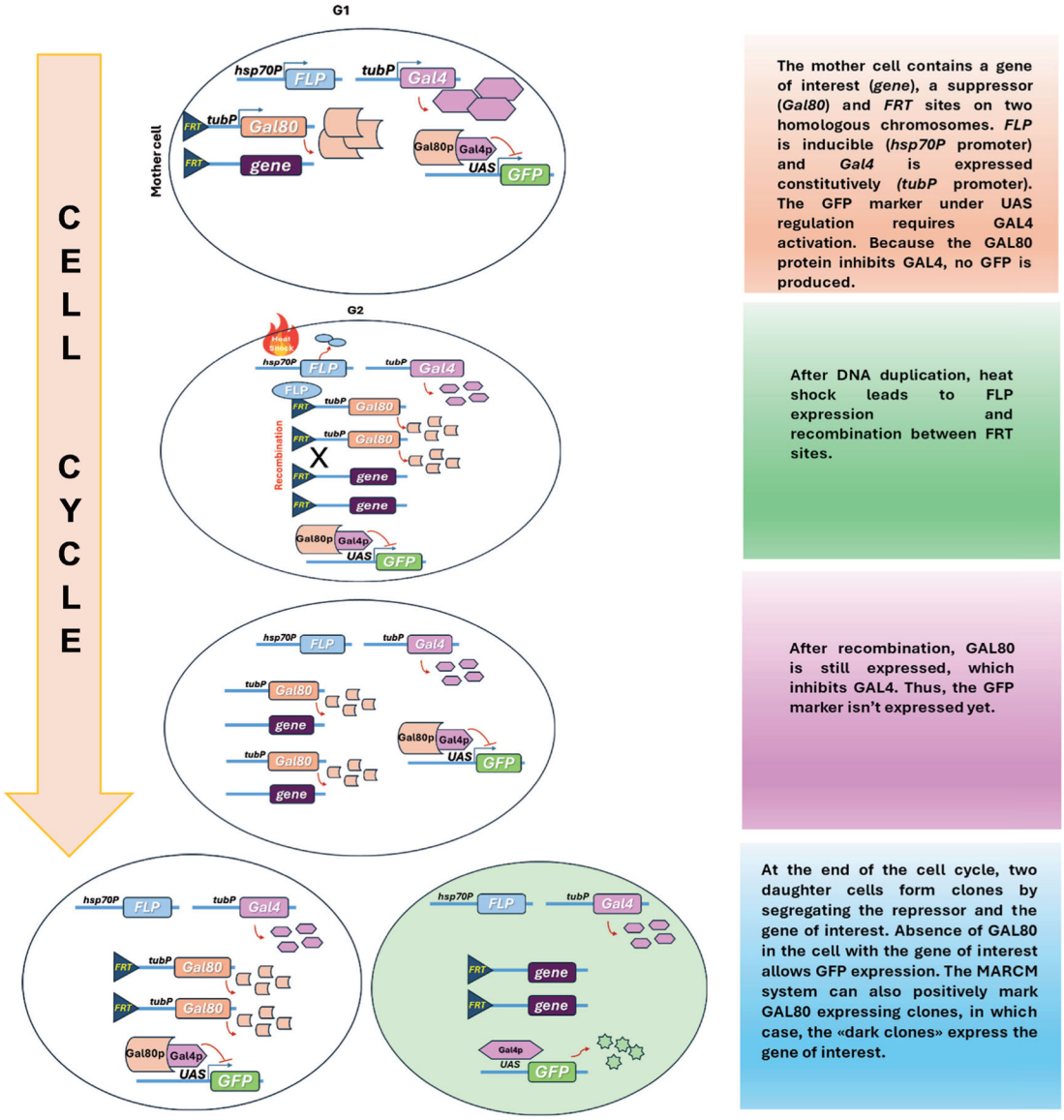


**Figure 1.**

Principles of the MARCM systems

Using complex gene expression control, MARCM is based on the inducible expression a recombinase enzyme, Flippase (FLP) to exchange parts of chromosomes containing genes, markers and/or mutations of interest flanked by short specific Flippase Recognition Target (FRT) sequences. Mitotic recombination in a heterozygous progenitor cell generates two distinct daughter cells, one of which inherits a gene or mutation of interest in double dose and is fluorescently marked with green fluorescent protein (GFP), while the other cell inherits neither mutation nor marker. Flexibility of the existing genetic tools allows the design of complementary setups in which the unlabeled cell harbors the gene of interest. A common inducible promoter for FLP is *Hsp70p*, which is activated upon heat shock. Note, both the exact temperature and heat shock duration must be determined case by case. tissue-specific promoters can also be used to restrict MARCM and clone formation

only to tissues of interest. GAL4 is a yeast-specific transcriptional activator that recognizes upstream activating sequences (UAS) naturally found upstream of some yeast genes. Both GAL4 and UAS can be respectively expressed and engineered in other contexts to induce specific genes e.g., here, GFP. In this example, GAL4 is expressed constitutively in the progenitor cell. Similarly, Gal80p, a GAL4 inhibitor, is also constitutively expressed, which prevents GAL4 from activating gene expression. Mitotic recombination produces daughter cells homozygote for either the gene of interest or the *Gal80p* gene, which enables GAL4 function (and GFP expression) only in the daughter cell that inherited the gene or mutation of interest. Descendants of both daughter cells will form recognizable clones that can be studied, for example by high resolution confocal microscopy. Because the GAL4 system is unique to yeast, cells of other species may only contain engineered UAS sequences, thus targeting GAL4 activation exclusively to the inducible gene of interest. A gene of interest can be a mutated gene, a marker itself or an RNAi construct.

**Table 1.**

Nobel Awards for discoveries first made in *Drosophila*. Six Nobel Prizes were awarded for research using the *Drosophila melanogaster* model to deconvolute knowledge gaps in human biology

| Nobel winners | Year | Scientific Reason | Relevant publications |
|---|---|---|---|
| Thomas Hunt Morgan | 1933 | The role played by chromosomes in heredity | Morgan, 1910 [32] |
| Hermann Joseph Muller | 1946 | The production of mutations by means of X-ray irradiation | Muller, 1928 [8] |
| Edward B. Lewis, Christiane Nüsslein-Volhard, Eric F. Wieschaus | 1995 | The genetic control of early embryonic development | Lewis, 1978[33]; Nusslein-Volhard and Wieschaus, 1980 [34] |
| Richard Axel | 2004 | Odor receptors and the organization of the olfactory system | Buck and Axel, 1991 [35] |
| Jules Hoffmann | 2011 | The activation of innate immunity | Imler and Hoffman, 2002[15] |
| Jeffrey C. Hall, Michael Rosbash, Michael W. Young | 2017 | Molecular mechanisms controlling the circadian rhythm | Bargiello at al., 1984 [36]; Hardin et al., 1990 [37] |