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The Fruit Fly *Drosophila melanogaster* as a Model for Aging Research

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Abstract Average human life expectancy is increasing and so is the impact on society of aging and age-related diseases. Here we highlight recent advances in the diverse and multidisciplinary field of aging research, focusing on the fruit fly *Drosophila melanogaster,* an excellent model system in which to dissect the genetic and molecular basis of the aging processes. The conservation of human disease genes in *D. melanogaster* allows the functional analysis of orthologues implicated in human aging and age-related diseases. *D. melanogaster* models have been developed for a variety of age-related processes and disorders, including stem cell decline, Alzheimer's disease, and cardiovascular deterioration. Understanding the detailed molecular events involved in normal aging and age-related diseases could facilitate the development of strategies and treatments that reduce their impact, thus improving human health and increasing longevity.

Keywords Adult stem cells · Age-related diseases · Aging · Dietary restriction · *Drosophila melanogaster* · Drug discovery · Hutchinson–Gilford progeria syndrome

Abbreviations

APH-1	Anterior pharynx-defective 1
aPKC	Atypical protein kinase C
APP	Amyloid precursor protein
Aβ	Amyloid-β
BACE	β-site APP cleaving enzyme 1
bp	Base pairs
GFP	Green fluorescent protein

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HGPS	Hutchinson–Gilford progeria syndrome
KCNQ	Potassium channel, KQT-like subfamily
LMNA	Lamin A gene
MHC	Myosin heavy chain
PDGF	Platelet-derived growth factor
PEN-2	Presenilin enhancer 2
PVF2	PDGF/VEGF-like factor 2
RNAi	RNA interference
UAS	Upstream activating sequence
VEGF	Vascular endothelial growth factor
ZMPSTE24	Zinc metalloproteinase homologous to yeast Ste24

Contents

1 Aging Research

The good news is that human life expectancy is steadily increasing in the developed world, as improved health care and hygiene mean that people stay healthier and thus live longer [1]. However, this also means that more people live long enough to experience the drawbacks of aging, for example, physical and mental decline and higher risks of cardiovascular deterioration, cancer, and neurodegenerative disorders [2]. Although many genes are known to coordinate cell growth and differentiation during development, none are known that exclusively cause damage and aging. Therefore aging mechanisms could be less conserved than developmental and metabolic pathways. However, there is growing evidence that modulators of the rate of aging are conserved over large evolutionary distances [3].

Table 1 Comparison of model organisms used in aging research

		C. elegans	*D. melanogaster*	*M. musculus*
Practical issues	Generation time	3–5 days	10–14 days	3–4 weeks
	Adult size	1 mm	3 mm	10 cm
	Lifespan	2–3 weeks	4–6 weeks	Years
	Maintenance costs	Low	Low	High
Similarity to humans	Number of genes (approx.)	19,000	13,000	25,000
	Conservation with human genome	>50 %	>60 %	>90 %
	Anatomical similarity to humans	Low	Medium	High
Molecular tools	Targeted gene knockout/ time	No	Yes	Yes/month
	Reverse genetics tools	Yes	Yes	Targeted knockout
	Generation of transgenic line	Weeks	Weeks	Months

(modified after 37)

The fruit fly *Drosophila melanogaster* is an excellent model system in which the genetic and cellular basis of important biological processes such as aging can be dissected, allowing the parallel mechanisms in vertebrates to be deciphered [4]. Understanding the details of the molecular events involved in the aging process will eventually help to reduce the impact of age-related diseases, thus improving human health and increasing longevity.

2 *Drosophila melanogaster* in Aging Research

Drosophila melanogaster was introduced as a model species in genetics, developmental biology, signal transduction, and cell biology in the early 1900s [5, 6]. The *D. melanogaster* genome is only 5 % of the size of a typical mammalian genome, but most gene families and pathways are shared with mammals, as well as many tissues and organ systems (Table 1) [7, 8]. Aging research in *D. melanogaster* benefits from a comprehensive range of methods to perturb gene function, such as mutagenesis screens, RNA interference (RNAi), and transgenesis [9, 10]. There are abundant publicly available resources, including thousands of *D. melanogaster* strains provided by the Bloomington Stock Center, as well as many cell lines, clone libraries, antibodies, and microarrays. There is also an exhaustive database containing information relevant to *D. melanogaster* genetics, development, and molecular biology (for review see Refs. [7] and [11]).

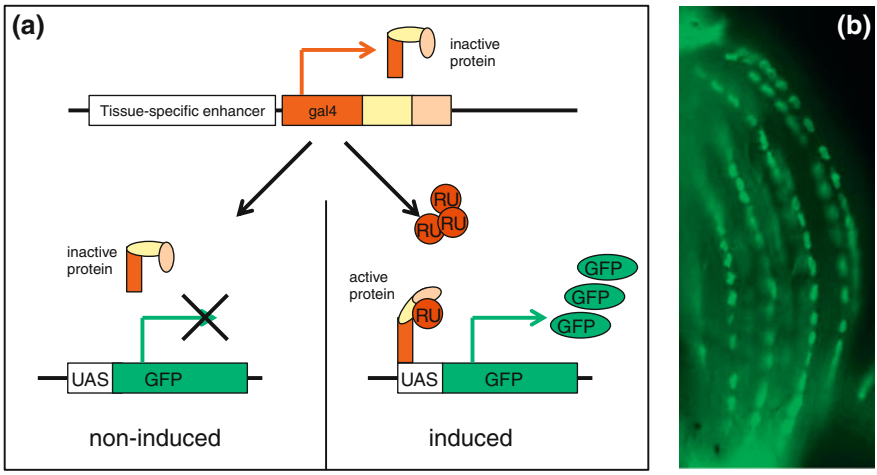

Fig. 1 The GeneSwitch GAL4 system. a The GeneSwitch-*Gal4* gene is expressed in the target tissue(s), according to the upstream tissue-specific promoter/enhancer (*upper* panel). In the absence of the activator RU486 (RU, *red circle*), the GeneSwitch-GAL4 protein remains transcriptionally inactive and cannot activate the downstream reporter gene, for example, encoding green fluorescent protein (GFP), which is linked to an upstream activating sequence (UAS) that responds to GAL4 (uninduced, *left* panel). However, by spiking food with RU486 (induced, *lower right* panel), the GeneSwitch-GAL4 becomes transcriptionally active, and the downstream UAS-linked gene is activated (modified after 15). **b** UAS-Kugelkern-GFP (*green*) is expressed in the nuclei of adult fruit fly leg muscle cells using the muscle-specific GeneSwitch-myosin heavy chain (MHC) promoter

2.1 Transgenic Systems for Longevity Analysis

D. melanogaster is a valuable model organism for the investigation of aging and age-related human diseases because its short life span of 4–8 weeks (depending on temperature and diet) allows the studies to be completed rapidly. Large numbers of flies can be cultivated in small bottles, so maintenance is straightforward and inexpensive. The high fecundity of the species allows large numbers of genetically homogeneous animals to be produced rapidly, which is essential for survival assays (Table 1).

It is possible to investigate gene function in fruit flies by conditional transgene overexpression. For lifespan studies, conditional gene expression systems have several advantages, for example, transgene expression is triggered in the Tet-on system by spiking food with the drug doxycycline, and in the GeneSwitch system transgene expression is similarly triggered using the drug RU486/mifepristone [12–18]. The GeneSwitch system combines several advantages (Fig. 1). First, it provides powerful control over genetic background effects on longevity, because flies overexpressing the transgene of interest have an identical genetic background to control flies, differing only in the presence or absence of the inducer. Second, it allows the tissue-specific control of transgene expression, such as expression in the

nervous system or muscles, and also ubiquitous expression using an actin driver line [18]. Third, transgene expression can be limited to specific lifecycle stages such as larval development or adulthood, which is required, for example, to circumvent lethal effects during development [12–16].

2.2 Multipotent Adult Stem Cells in D. melanogaster

Adult stem cells are tissue-restricted cells with the unique ability to self-renew and to differentiate into all the specific cell types of a particular tissue (reviewed in Ref. [19]). As a result, they provide a continuous supply of differentiated cells in their tissue compartment. The renewal of differentiated cells is particularly important for tissue homeostasis in adult organisms, because this maintains adult organs and facilitates repair after injury or disease [20, 21]. The capacity of adult stem cells for cellular renewal and tissue homeostasis is thought to decline with age. This functional decline may be responsible for many tissue-specific phenotypes associated with aging (reviewed in Refs. [22 and 23]). In contrast to mammals, most adult *D. melanogaster* tissues are thought to be postmitotic. However, the aging of *D. melanogaster* stem cells, for example, in the midgut epithelium and the gonads, provides excellent models for the study of stem cell renewal and aging.

The aging of adult stem cells in the *D. melanogaster* gonad causes a loss of fecundity that becomes more severe with age [24]. The rate of adult stem cell division in the male gonad declines significantly with age, and this correlates with a reduction in the number of somatic hub cells that contribute to the stem cell niche. Interestingly, the stem cell division rate does not decline in *methuselah* (*mth*) mutant flies, which have a prolonged lifespan and greater stress resistance [25].

The digestive systems of vertebrate and invertebrate species show extensive similarities in terms of development, cellular architecture, and genetic regulation. Enterocytes form the majority of the intestinal epithelial cells and are interspersed with hormone-producing enteroendocrine cells. Human intestinal cells are continuously replenished by adult stem cells, and the deregulation of this process may underlie some common digestive diseases and cancers [21]. Recently, somatic stem cells have also been discovered in the midgut of the adult fruit fly, and like their vertebrate counterparts these proliferating progenitor cells reside within the midgut epithelium [20, 21]. Genetic mosaic analysis and lineage labeling has shown that differentiated midgut epithelial cells arise from a common lineage [20, 21]. These adult stem cells are multipotent, and Notch signaling is required to produce the correct proportion of enteroendocrine cells. Furthermore, the Notch signaling pathway is necessary for homeostatic proliferation in the midgut epithelium. The hyperactivation of Notch signaling suppresses adult stem cell proliferation whereas the inhibition of Notch signaling induces proliferation [20, 21]. Choi et al. [23] reported an age-related increase in the number and activity of adult stem cells in the *D. melanogaster* midgut. Furthermore, oxidative stress induced by

N,*N*′-dimethyl-4,4′-bipyridinium dichloride (Paraquat) or the loss of catalase activity mimicked the changes associated with aging in the midgut, and this was associated with the overexpression of PVF2 (*D. melanogaster* PDGF/VEGF-like factor 2), which was required for the age-related changes in midgut adult stem cells. Goulas and colleagues [26] found that the integrin-dependent adhesion of stem cells to the basement membrane was responsible for the asymmetric segregation of the signaling factors Par-3, Par-6, and atypical protein kinase C (aPKC) to the daughter cells. Perturbing this mechanism or altering the orientation of stem cell division resulted in the formation of tumors in the intestinal epithelium [26]. The identification and characterization of *D. melanogaster* midgut stem cells with striking similarities to their vertebrate counterparts will facilitate the genetic analysis of normal and age-related abnormal intestinal functions in humans.

2.3 Dietary Restriction

Dietary restriction extends the lifespan of many different organisms including yeasts, worms, and flies, as well as mammals [24]. Dietary restriction can increase the longevity of *D. melanogaster* by up to 30 % and reduce the reproduction rate, for example, by maintaining adults on a cornmeal–sugar–agar diet topped with a dilute concentration of yeast [27, 28]. However, adult flies maintained on highly restricted diets are short-lived and infertile. Therefore, longevity is only maximized by providing an intermediate diet in which restricted food intake is combined with adequate nutrition (reviewed in Ref. [28]).

It is now clear that specific nutrients rather than calories mediate longevity. Therefore *D. melanogaster* mutants that show no extension of lifespans on restricted diets should help to identify the genetic pathways through which dietary restriction controls aging. However, the genetic analysis of dietary restriction is difficult because longevity tests across a range of diets must be carried out using genetic screens [28]. Wang and colleagues showed that a transporter of Krebs cycle intermediates, encoded by the *I'm not dead yet* (*Indy*) gene, interacts with dietary restriction mechanisms to increase longevity [28, 29]. Although dietary restriction extends the lifespan of numerous species, the precise mechanisms have not been determined for any of these organisms. It is therefore unclear whether dietary restriction reflects evolutionary conservation or convergence [24].

3 Using *D. melanogaster* to Model Human Diseases

Age-related diseases are becoming increasingly prevalent in industrialized societies due to the greater average life expectancies. The biological aging process is one of the major risk factors for virtually all of the common diseases of developed societies, including Alzheimer's disease, Parkinson's disease, stroke, age-related

macular degeneration, type 2 diabetes mellitus, osteoporosis, sarcopenia, arteriosclerosis, and most types of cancer [30]. More than 100 years of *D. melanogaster* research has provided a wealth of genetic, genomic, cellular, and developmental data, as well as tools, techniques, and reagents, resulting in a well-characterized system that is easy to manipulate but complex enough to be relevant as a model for human diseases [30]. Many basic biological, physiological, and neurological characteristics are conserved between flies and mammals, and the *D. melanogaster* genome sequence shows that more than 60 % of human genes (including disease genes) have functional orthologues in the fruit fly [31–34], but the fly genome has only minimal genetic redundancy which makes it much easier to study gene function [35]. *D. melanogaster* has therefore become a popular model organism for the investigation of human diseases [35–38].

3.1 Alzheimer's Disease

Alzheimer's disease is a neurodegenerative disorder characterized by the functional impairment and destruction of neurons, resulting in a progressive loss of memory and other cognitive functions, leading to dementia [39]. Despite the much greater complexity of the human brain, the *D. melanogaster* central nervous system generates complex behaviors, including learning and memory, and comprises neurons and glia that operate on the same fundamental principles as their vertebrate counterparts; many neurotransmitter systems, including dopamine, glutamate, and acetylcholine, are conserved between flies and humans [40].

The pathology of Alzheimer's disease includes the formation of neuritic plaques, which are extracellular deposits primarily comprising the protein amyloid-β (Aβ), and internal neurofibrillary tangles primarily comprising aggregates of the neuronal microtubule-associated protein Tau [39]. Aβ is 40 or 42 amino acids in length and is formed by the proteolytic cleavage of the larger amyloid precursor protein (APP) [41]. The N-terminus of Aβ is generated by β-secretase activity whereas γ-secretase activity defines its length, with Aβ40 being more common and Aβ42 representing the more fibrillogenic and neurotoxic form. The activity of γ-secretase activity depends on four components: Presenilin, Nicastrin, Anterior pharynx-defective 1 (APH-1), and Presenilin enhancer 2 (PEN-2). In contrast, β-secretase activity has been attributed to the individual protein β-site APP-cleaving enzyme 1 (BACE1) [42, 43].

A typical strategy for establishing a human disease model in *D. melanogaster* is to investigate whether the gain or loss of function of a given gene known to be involved in the disease can enhance or suppress the disease phenotype in the fly. Two complementary approaches are often implemented: (i) the candidate gene approach tests a specific hypothesis that a given gene or genetic pathway plays an important role in a particular disease process; or (ii) genetic screens can be used to conduct unbiased surveys for genetic modifiers.

D. melanogaster neurons are sensitive to Aβ toxicity. The targeted expression of Aβ42 in flies causes neurodegenerative phenotypes, amyloid deposits, and learning defects, whereas the targeted expression of Aβ40 only induces learning defects [44, 45]. Human APP expressed in *D. melanogaster* is cleaved by endogenous γ-secretase activity because the fly contains homologues of APP and all four components of the γ-secretase complex. The *D. melanogaster* homologues of *presenilin* and *nicastrin* were both identified in genetic screens for mutations that produce *Notch*-like phenotypes, and both proteins are required for the proteolytic cleavage and release of the Notch intracellular fragment [46–49].

Mutations in the human *tau* gene, encoding the protein found in Alzheimer's neurofibrillary tangles, are associated with familial frontotemporal dementia syndromes. A *tau* homologue has been identified in *D. melanogaster* [50], and the expression of human *tau* in the fly causes progressive neurodegeneration as well as a truncated lifespan. Unlike human *tau*-related diseases, neurodegeneration in the fly can occur without Tau aggregating into neurofibrillary tangles [51]. This suggests that Tau acquires its toxic properties before it forms macromolecular aggregations, and therapies could be developed that target pretangle forms of Tau [40].

3.2 *D. melanogaster* Age-Related Cardiovascular Disease Model

Cardiac dysfunction is the most common cause of death among the elderly in industrialized societies, and it is therefore useful to develop models that provide insight into the progression and genetic control of age-related changes in heart function [52]. The fruit fly is the only invertebrate genetic model organism with a heart. The genetic basis of cardiac dysfunction associated with age and disease in the fruit fly has been studied by developing heart function assays using high-speed video cameras to capture heart wall movements in semi-intact preparations with the fly heart surgically exposed [52]. Like the human heart, the performance of the fly heart deteriorates with age; that is, there is a progressive increase in electrical pacing-induced heart failure and arrhythmias [52]. These defects are exacerbated in *Dmel\KCNQ* deletion mutants, which experience episodes of prolonged heart contraction and fibrillation aggravated by age [52]. Therefore, despite the anatomical differences between flies and humans, the *D. melanogaster* heart is an emerging and promising genetic model of age-dependent cardiovascular deterioration.

4 *D. melanogaster* Premature-Aging Models

The molecular mechanisms underlying human aging have been investigated by considering progeroid syndromes such as Hutchinson–Gilford progeria syndrome (HGPS), in which a dominant point mutation in the *LMNA* gene (encoding lamin A, a component of the nuclear lamina) causes a premature accelerated aging-like

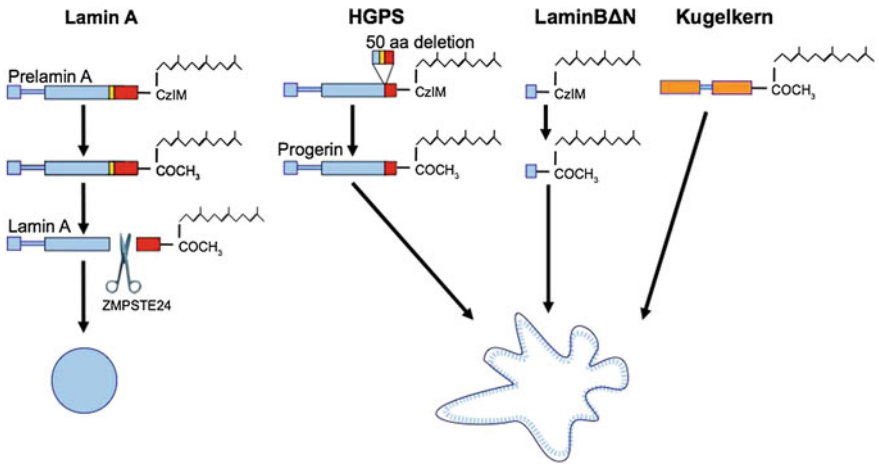

Fig. 2 Farnesylated nuclear proteins induce nuclear shape changes. The premature Prelamin A protein is farnesylated. The C-terminal region, including the farnesyl residue (*red*), is cleaved by the endoprotease ZMPSTE24 at the endoprotease binding site (*yellow*). In HGPS cells, a point mutation leads to the activation of a cryptic splicing site resulting in a 150-bp deletion, removing 50 amino acids including the endoprotease binding site. The resulting protein (Progerin) is permanently farnesylated and induces aging-like phenotypes as related nuclear shape changes, DNA damage, and reduced heterochromatin. A highly truncated form of Lamin B (LaminBΔN) can induce similar phenotypes [62, 68, 69]. LaminBΔN comprises only the C-terminal region of Lamin B, including the nuclear localization signal and the farnesylation site. The *D. melanogaster* protein Kugelkern contains a putative coiled-coil region (*small blue bar*), a nuclear localization signal, and a C-terminal farnesylation site but no other conserved features (*orange*). Even so, Kugelkern can induce aging-like phenotypes similar to those described for Progerin and LaminBΔN

disorder in children [53, 54]. Affected children appear normal at birth, but soon develop symptoms and pathologies associated with normal human aging. Children with HGPS generally die from myocardial infarction or cerebrovascular accidents at an average age of 13 years [55, 56].

The mutation responsible for HGPS influences the splicing of the primary *LMNA* transcript such that a normally rare splicing variant is produced constitutively (Fig. 2). The normal splicing variant loses its farnesyl group and partly relocates from the nuclear lamina to the nucleoplasm, but the rare variant (known as Progerin) retains its farnesyl group and is permanently inserted into the nuclear lamina where it affects nuclear shape, DNA integrity, and chromatin architecture [55, 56]. Even in healthy individuals, this cryptic splicing site is sporadically active and Progerin is present in aged human cells. Although *progerin* mRNA is only found at low levels, the protein accumulates in the skin in a subset of dermal fibroblasts [57], and in coronary arteries [58], thus participating in the physiological aging process [59]. Inhibiting the *progerin* splicing variant can reverse the nuclear defects observed in aging cells [53, 54, 59–61].

HGPS is characterized by lobulated wrinkled nuclei, but these are also present in healthy aging humans, in nematodes, and also in *D. melanogaster* [59, 62, 63].

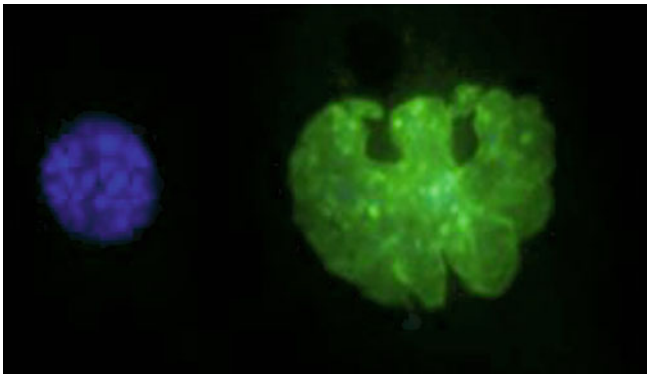

Fig. 3 The nucleus in aging fibroblasts. Mouse fibroblasts transfected with *D. melanogaster kuk* (*green*, *right* panel) undergo nuclear shape changes similar to those observed in aging fibroblasts and HGPS cells. Untransfected control cells have round and smooth nuclei (*blue*, *left* panel)

In wildtype flies, the nuclei of flight-muscle cells become larger with age and they adopt an aberrant shape [62]. This aging-like phenotype can be prematurely induced by expressing farnesylated lamina proteins (Figs. 2, 3). The overexpression of genes encoding *Lam* (*D. melanogaster* Lamin B) or Kugelkern (*kuk*) induces aberrant nuclear shapes early in adult life and reduces the fly lifespan, correlating with an early decline in age-dependent locomotor behavior [62]. Lobulation of the nuclear membrane induced by the insertion of farnesylated nuclear proteins can lead to premature aging-like phenotypes in cultured mammalian cells and in adult flies [62].

5 The Role of *D. melanogaster* in Drug Discovery

Understanding the molecular mechanisms of aging may facilitate the development of novel strategies to attenuate or delay the process in humans. Research using popular vertebrate model organisms such as mice (*Mus musculus*) and zebrafish (*Danio rerio*) has provided important insights into vertebrate aging [64, 65]. However, both species live for 3 years or longer under laboratory conditions, making longevity screens time-consuming and expensive. In contrast, small and prolific organisms with a lifespan of only few weeks, such as the fruit fly and the nematode *Caenorhabditis elegans* provide the basis for large and unbiased screens allowing the investigation of novel genes and substances that influence aging in a physiological context [66].

Drug discovery usually begins with the identification of a target protein implicated in the disease, followed by high-throughput screens of chemical compound libraries to identify substances that interact with the targets and alter

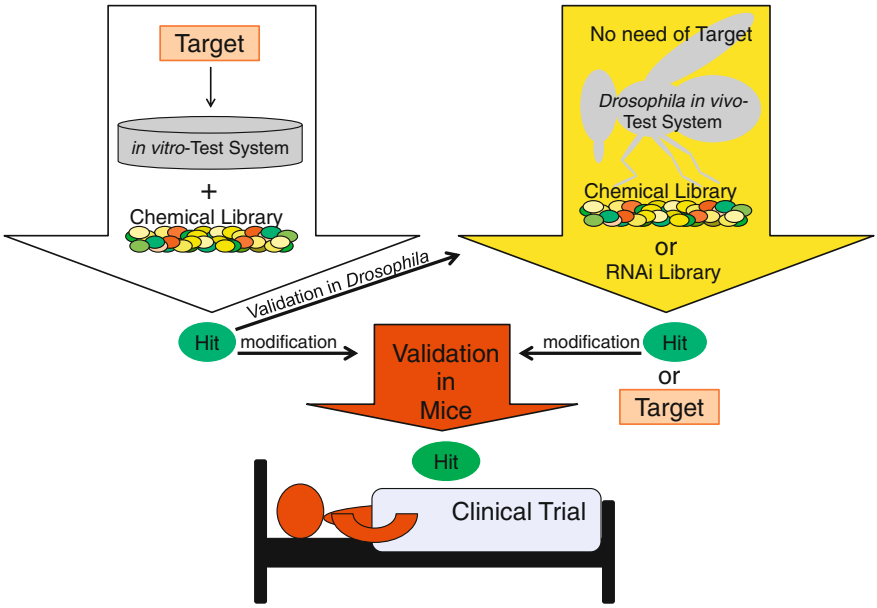

Fig. 4 A schematic view of two alternative drug discovery pathways based on high-throughput screens. The *white arrow* shows the traditional drug discovery process, which is based on the identification of a Target (e.g., enzyme, receptor, or ion channel) implicated in a human disease. High-throughput screening of a large chemical library is carried out to identify Hits, but these large-scale screens are typically based on in vitro cell culture, biochemical assays, or receptor binding assays (in vitro Test System). Hits are optimized by medicinal chemistry (modification) and subsequently tested in rodent models (*red arrow*, Validation in Mice) before clinical development (Clinical Trial). Alternatively, the high-throughput screens are performed in whole-animal models (*Yellow arrow*; *D. melanogaster* in vivo-Test System). This also allows the screening of large libraries based on chemical compounds, RNAi, or genetic modifiers. Furthermore, genetic manipulation can be used to produce a phenotype, for example, by expressing the disease-causing human protein in the fly. Depending on the purpose of the screen, the appropriate output parameter can be chosen (e.g., biochemical, cellular, tissue, behavioral parameters, or even longevity). The outcome of such a screen can be a positive compound (Hit) or a novel gene implicated in the disease or aging process (Target) revealing new molecular mechanisms. *D. melanogaster* may also bridge the gap between traditional high-throughput screening and validation in mammalian models (Validation in *D. melanogaster*)

their activity (Fig. 4). Traditionally, these large-scale screens have involved in vitro cell culture, biochemical assays, or receptor-binding assays. Positive compounds (hits) are optimized by medicinal chemistry and then tested in rodent models. Despite significant investment, most drug candidates fail before they reach the market, for example, due to unpredicted toxicity, off-target effects, or clinical inefficacy (reviewed in Ref. [35]). The attrition rate is high because of the poor selectivity of hits in the initial test systems, which have only limited predictive value for clinical performance because they cannot take into account the complexity of living organisms. To address this challenge it would be useful to develop

primary drug screening methods applied directly in whole animals, where all relevant systems are present and functioning together in a manner that is more relevant to human pathology. However, it is infeasible to use the common rodent models for whole-animal primary screening because millions of animals would be required to screen tens of thousands of small molecules in each experiment, and in the case of aging-relevant compounds this would also take many years. Innovative new screening platforms are therefore required to identify hits relevant to aging-related disease targets [35].

Recently, small invertebrate animal models such as *D. melanogaster* and *C. elegans* have been used for high-throughput drug screening [67]. *D. melanogaster* in particular allows whole-animal, high-throughput screening in a model that is relevant to humans, so that unbiased primary screens can be carried out without requiring the prior identification of a target protein [38]. The genetic and physiological conservation between invertebrates and humans suggests that human diseases can be modeled in flies and worms, although anatomical differences mean that only a partial picture of the human aging process/disease can be achieved. The use of a model organism such as the fly offers speed and high-throughput screens in the whole animal, and significantly reduces overall costs that together should result in enhanced drug discovery rates [38].

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