Chapter 7
Understanding the Functions of Longevity Genes in *Drosophila*

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Abstract The fruit fly, *Drosophila melanogaster*, has been an excellent model organism to study aging and longevity. A number of genes affecting longevity have been identified by forward and reverse genetic approaches. Historically, antioxidant genes were the first target to study their roles in aging and longevity, as predicted by the "free radical theory of aging." Superoxide dismutase (SOD), catalase (Cat), and thioredoxin (TRX) have been examined with transgenic flies. SOD and TRX have multiple copies, each of which has a unique expression pattern and functional property. The next target was the insulin/insulin-like growth factor-1 (IGF-1) signaling pathway, which controls growth, body size, oxidative stress resistance, and longevity. The Jun N-terminal kinase (JNK) signaling pathway plays a critical role in regulating organismal physiology upon oxidative stress and longevity. More recently, an emerging target is epigenetic mechanisms, which appear to control longevity with novel pathways.

Keywords Antioxidant · Stress resistance · Insulin/IGF-1 signaling · JNK signaling · Epigenetic mechanism

7.1 *Drosophila melanogaster* as a Model System to Study Aging

The fruit fly, *Drosophila melanogaster*, has been used as a model organism to study aging and longevity. Its genome is relatively small (180 Mbp), containing approximately 14,000 genes (FlyBase, http://flybase.org/). More than 50% of them have homologs in humans and share 75% of known human disease-related genes. It takes

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10–14 days from eggs to adult flies, which produce the next generation within a few days. A mated female can lay 50–100 eggs per day, and because of its small body size, it is easy to collect a large number of flies and perform experiments in a limited space. The longevity of adult flies is from 1 to 2 months, depending on the genetic and environmental conditions. Finally, advanced genetic techniques and resources are available.

Identification of mutants is the first step of the genetic approach to the mechanism of aging and longevity determination. Genes affecting longevity have been identified by forward and reverse genetic approaches. The gene functions have been assessed using gain-of-function (overexpression/misexpression), loss-of-function mutants, or RNA-mediated knockdown. In this chapter, we will review some of the representative longevity genes, which are related to (1) antioxidant, (2) insulin/IGF-1 signaling, (3) JNK signaling, and (4) epigenetic mechanism.

7.1.1 Antioxidant

Oxidative stress is the primary cause of aging and is implicated in many age-associated diseases, including Parkinson's disease and Alzheimer's disease (Dawson and Dawson 2003; Finkel and Holbrook 2000; Harman 1956). Oxidative stress can be induced by external or internal factors, such as UV radiation or the respiratory system in mitochondria. It can damage cellular macromolecules, such as nucleic acids, proteins, and lipids, which may interfere with normal cellular functions and ultimately lead to cell death (Imlay 2003). Therefore, antioxidant defense systems must have critical roles in maintaining normal cellular processes during aging. Organisms carrying mutations in genes responsible for antioxidant defense mechanisms likely shorten longevity, whereas its enhancement may extend longevity. We focus on the most studied antioxidant genes encoding superoxide dismutase (SOD), catalase, and thioredoxin in *Drosophila*.

SOD scavenges superoxide anion radicals and thus protects cells from oxidative damage. The *Drosophila* genome contains three genes *Sod1*, *Sod2*, and *Sod3*. Localization of the protein products is different; SOD1, SOD2, and SOD3 are in the cytoplasm, mitochondria, and extracellular space, respectively. SOD1 and SOD3 are copper/zinc (Cu/Zn) SODs, whereas SOD2 is a manganese (Mn) SOD.

7.1.1.1 Cytoplasmic SOD (Sod1)

Flies with a null mutation of *Sod1* are hypersensitive to paraquat, a free radical generator, and have shortened longevity (Phillips et al. 1989). The mutant flies were hypersensitive to hyperoxia, glutathione depletion, and ionizing radiation, and all these phenotypes were rescued by a wild-type *Sod1* transgene (Parkes et al. 1998a). The introduction of additional copies of the *Sod1* gene did not show a marked

increase in longevity (Seto et al. 1990). A slight rise in longevity was observed when bovine Cu/Zn SOD was expressed using the *actin5c* promoter (Reveillaud et al. 1994). In contrast, transgenic flies overexpressing human SOD1 in adult motoneurons dramatically extended longevity by up to 40% and rescued other defects of the null mutant (Parkes et al. 1998b). Sun and Tower (1999) developed an "FLP-OUT" system to overexpress a gene at desired life cycle stages with a controlled genetic background. The longevity of flies overexpressing SOD1 extended up to 48% (Sun and Tower 1999). The longevity extension by SOD1 overexpression was striking in the experiments, where control flies were relatively short-lived (Orr and Sohal 2003). Thus, the effects of SOD1 overexpression appear to be dependent on the experimental context.

7.1.1.2 Mitochondrial SOD (Sod2)

SOD2, a manganese superoxide dismutase (Mn-SOD), detoxifies superoxide radicals (O^{-}_{2}) in mitochondria. The role of this enzyme must be critical for cells to protect from oxidative damage during aging. Transgenic overexpression of Mn-SOD reduced the longevity by 4–5% (Mockett et al. 1999). There was no difference in the hydrogen peroxide-releasing rate of mitochondria, protein oxidative damage, or resistance to 100% oxygen between wild-type and flies overexpressing Mn-SOD. When Mn-SOD was overexpressed using the FLP-OUT technique, the mean longevity of flies increased by an average of 16% (Sun et al. 2002). The maximum longevity increased by 15%, but the one line showed a 37% increase. Simultaneous overexpression of catalase and Mn-SOD had no additional benefit, consistent with the previous observations that catalase is present in excess in adult flies through longevity. The RNA interference (RNAi)-mediated knockdown of *Sod2* causes mortality in young adults and enhances sensitivity to paraquat toxicity (Kirby et al. 2002). Knocking down of *Sod2* did not cause overtly harmful effects on larval and pupal development. A null mutation, $Sod2^{n283}$, was generated by imprecise excision of a *P*-element transposon inserted in the locus (Duttaroy et al. 2003). Adult flies homozygous for the mutation died within 24 h after eclosion, indicating a critical role of *Sod2* in adult survival. Flies heterozygous for the mutation ($Sod2^{n283/+}$) are sensitive to oxidative stress induced by paraquat treatment. The adult lethality of $Sod2^{n283}$ was exclusively due to the loss of *Sod2* function since a wild-type *Sod2* transgene rescued this phenotype.

7.1.1.3 Extracellular SOD (Sod3)

The *Drosophila Sod3* gene encodes a functional extracellular SOD. The physiological role of *Sod3* has been investigated using a loss of function mutant and the RNA-mediated knockdown (Jung et al. 2011). Neither the mutation nor knockdown of *Sod3* shows any apparent defects during development. However, longevity was significantly reduced in these flies at 25 and 29 °C, indicating that *Sod3* is required

for normal longevity in adult flies. Since they also show reduced viability against paraquat treatment, *Sod3* appears to play a significant role as a superoxide anion scavenger.

Sod3 is one of the highly upregulated genes in a fly model of amyloid β (Aβ) toxicity (Favrin et al. 2013). RNAi-mediated knockdown of *Sod3* improved the phenotype associated with Aβ-expressing flies, namely, climbing performance and survival. The results indicate that *Sod3* in Aβ model flies increases Aβ toxicity. Since there was no increase in catalase expression in Aβ flies, the upregulation of *Sod3* may increase toxic H_2O_2.

7.1.1.4 Catalase (Cat)

Flies with a hypomorphic mutation in *Cat* had only 14% catalase activity in the parent control flies that had average longevity (Orr and Sohal 1992). Transgenic flies overexpressing *Cat* with increased levels of catalase activity (up to 80%) showed enhanced resistance to hydrogen peroxide but did not extend longevity (Orr and Sohal 1994). The overexpression of both Cu/Zn SOD (*Sod1*) and *Cat* exhibited a one-third extension of longevity and reduced the accumulation of 8-hydroxydeoxyguanosine during aging and in response to the exposure of live flies to X-rays (Sohal et al. 1995). On the contrary, there was no significant increase in flies' longevity overexpressing *Cat* and flies co-overexpressing Cu/Zn SOD (*Sod1*) and Cat (Sun and Tower 1999). Catalase was targeted ectopically to the mitochondria matrix by fusing a leader peptide derived from ornithine aminotransferase with its N-terminus (Mockett et al. 2003). There was no impact of this targeted expression of catalase on the longevity of the flies. However, they became more resistant to exogenous hydrogen peroxide, paraquat, and cold stress (Mockett et al. 2003).

7.1.1.5 Thioredoxin (Trx-2, TrxT, dhd)

Thioredoxin (TRX) is an antioxidant molecule conserved from bacteria to humans (Arner and Holmgren 2000). The sequence containing the redox-active site, Cys-Gly-Pro-Cys-Lys, is conserved among all TRX family proteins (Holmgren 1985). It is a major cellular protein disulfide reductase carrying a conserved active site with a pair of cysteine residues, and it serves as an electron donor to enzymes, such as thioredoxin-dependent peroxide reductase (Miranda-Vizuete et al. 2000; Chae et al. 1994) and ribonucleotide reductase (Thelander and Reichard 1979; Holmgren 1985). Upon substrate reduction, two sulfhydryl (SH) groups in the active center of reduced thioredoxin, Trx-(SH)2, are converted to disulfide in the oxidized form, Trx-S2. TRX is induced by various oxidative stimuli, including UV irradiation, inflammatory cytokines, and chemical carcinogens, and plays crucial roles in regulating cellular responses such as gene expression, cell proliferation, and apoptosis (Nishinaka et al. 2001).

The *Drosophila* genome contains three TRX family genes, *Trx-2*, *TrxT*, and *deadhead* (*dhd*), all of which have a characteristic active center for TRX and show a similar extent of sequence homology to human TRX. *Trx-2* is expressed ubiquitously, whereas *dhd* and *TrxT* are sex-specific, predominantly expressed in females and males, respectively (Svensson and Larsson 2007; Svensson et al. 2003; Pellicena-Palle et al. 1997; Salz et al. 1994).

Trx-2 is one of the longevity-extending genes identified in a systematic gain-of-function screen in *Drosophila* (Seong et al. 2001a). *Trx-2* was overexpressed ubiquitously under the control of an *hsp70* promoter. Later it was shown that neural-specific overexpression of any of TRX (*Trx-2*, *TrxT*, *dhd*) is sufficient for extending longevity and improving locomotor activity in aged animals (Umeda-Kameyama et al. 2007). Besides longevity, the overexpression of *Trx-2* increases resistance to oxidative stress in adult flies (Svensson and Larsson 2007).

Studies on loss-of-function mutants are necessary to understand the role of endogenous TRX in oxidative stress resistance and longevity. Loss-of-function mutation in *Trx-2* has been generated by *P*-element imprecise excision and used for biochemical and physiological characterization (Tsuda et al. 2010b). The loss of *Trx-2* reduced longevity, hypersusceptibility to paraquat, and accumulation of protein carbonyl, an oxidative stress marker in aged animals. The mean longevity of the mutant was 36% shorter than that of wild-type flies. In addition, *Trx-2* mutants expressed high levels of antioxidative genes, such as *Sod1*, *catalase*, and *glutathione synthetase*, suggesting that they are exposed to high levels of oxidative stress.

The overexpression of any of the *Drosophila* TRX genes has been shown to suppress the accelerated neurodegeneration that occurs in the *Drosophila* Parkinson's disease model, in which the human Parkin-associated endothelin receptor-like receptor (Pael-R) is expressed in all neurons (Umeda-Kameyama et al. 2007). The Pael-R-induced phenotype includes the selective loss of dopaminergic neurons and reduced locomotor activity, and all of these were suppressed by *Drosophila* TRX as efficiently as human Parkin (Yang et al. 2003). The mechanism of suppression could be complex since TRX has a wide variety of cellular functions, including a cytoprotective effect against oxidative stress (Nakamura et al. 1994; Andoh et al. 2002), a neuroprotective activity (Hori et al. 1994), a neurotrophic activity (Endoh et al. 1993), the regulation of the stability of apoptosis signal-regulating kinase 1 (ASK1) through ubiquitination-proteasomal degradation (Liu and Min 2002), and to interact with unfolded and denatured proteins as a molecular chaperone (Kern et al. 2003). To assess the role of the redox activity of TRX in suppressing Pael-R-induced neurotoxicity in flies, redox-defective mutants, TrxT (C35A) and TrxT(D26A/K57I), have been generated. The TRX mutants could suppress the neurodegenerative phenotype, indicating that the redox activity of TRX is dispensable for inhibiting Pael-R-induced neurotoxicity. Also, the neuroprotective function of wild-type and redox-defective TRX was observed in a *Drosophila* model of Machado-Joseph disease (MJD) expressing polyglutamine (Warrick et al. 1998). Since the redox-defective TRX mutants were active as a chaperone, its activity could be necessary to suppress Pael-R or polyglutamine-induced neurotoxicity.

7.1.2 Insulin/IGF-1TOR Pathway

Insulin is an evolutionally conserved peptide hormone secreted from the pancreas and promotes glucose uptake in muscle and adipose tissue. Insulin also stimulates cell growth and differentiation and promotes the storage of glucose and lipids by stimulating amino acid uptake, protein synthesis, glycogenesis, and lipogenesis (Saltiel and Kahn 2001). The insulin/IGF-1 and target of rapamycin (TOR) pathways are among the signaling pathways that control cell and organismal growth, body size, and longevity. Dietary restriction or mutations that reduce the insulin/IGF-1/TOR signaling activity produce a small body size and extend longevity.

In *Drosophila*, a mutation-reducing body size was first identified among a collection of *P*-element insertion lines (Bohni et al. 1999). The gene was named *chico*, which means small body in Spanish. It encodes a protein similar to the vertebrate insulin receptor substrate (IRS), and *chico* mutants are less than half the size of wild-type flies, owing to fewer and smaller cells. The mutants show metabolic abnormalities such as delayed development and abnormal accumulation of lipids (Bohni et al. 1999). Then, *chico* mutants were found to be long-lived (Clancy et al. 2001).

Insulin-like receptor (*InR*) mutants were also long-lived (Tatar et al. 2001). Heteroallelic combinations of *InR* alleles were used to produce viable and dwarf adults with a substantially low level of INR kinase activity. Among four distinct alleles, only InR^{p5545}/InR^{E19} females showed extended longevity. The *InR* dwarf female flies appear to be very much affected by the endocrine system. Juvenile hormone (JH) synthesis was significantly reduced in mutant females. In the mutant dwarf flies, the triacylglycerol level is elevated fourfold, as observed in diapause *D. triauraria* mutants and dwarf *D. melanogaster* mutants for *chico* and Cu/Zn SOD activity increases twofold. The topical application of a JH analog, methoprene, to the mutant females could induce vitellogenesis and revert the long-lived phenotype to the control level. Therefore, partial defects in JH synthesis account for infertility and extended longevity (Tatar et al. 2001).

The *Drosophila* genome encodes eight insulin-like peptides (*Drosophila* insulin-like peptides1–8: Dilp1–8). In adult flies, *Dilp2*, *Dilp3*, and *Dilp5* are expressed in median neurosecretory cells in the brain. The ablation of these cells leads to increased fasting glucose levels in the hemolymph of adults, similar to that found in diabetic mammals (Broughton et al. 2005). They also exhibit increased lipid and carbohydrate storage, reduced fecundity, and reduced tolerance to heat and cold. The ablated flies show extended longevity and increased resistance to oxidative stress and starvation, implying that these ligands are involved in the insulin/IGF-1 signaling (Broughton et al. 2005). The RNAi-mediated knockdown of *Sir2*, a mammalian SIRT1 homolog, upregulates *Dilp2* and *Dilp5* expression. These genes might be involved in the mechanism of longevity extension by dietary restriction (Banerjee et al. 2012, 2013). Since the expression of *Dilp3* and *Dilp5* is upregulated in *Dilp2* knockdown individuals, there may be a compensatory mechanism among these genes (Grönke et al. 2010).

The role of intracellular components of the insulin/IGF-1/TOR pathways, such as PTEN, FOXO, TOR, 4E-BP, and S6K, has been examined for their functions to regulate longevity (Partridge et al. 2011; Kannan and Fridell 2013). We identified *wdb* and *lkb1* as longevity-extending genes through a gain-of-function screen of those already screened for the ability to reduce wing and eye sizes (Funakoshi et al. 2011). The overexpression of *wdb* reduces the level of phosphorylated AKT, while the overexpression of *lkb1* increases the level of phosphorylated AMPK and reduces the level of dephosphorylated S6K. These results suggested that *wdb*- and *lkb1*-dependent longevity extension was mediated by the downregulation of S6K, a downstream component of the insulin/IGF and TOR signaling pathways.

Tsuda et al. (2010a) provided genetic evidence that insulin-degrading enzyme (IDE) antagonizes Dilp2 signaling and the human Aβ-induced neurotoxicity in *Drosophila* Alzheimer model. IDE, a zinc metalloendopeptidase, has been implicated in the pathogenesis of both DM2 and AD (Fakhrai-Rad et al. 2000). Overexpression of *Drosophila* Ide (dIde) in IPCs reduces to 92% and 94% of control flies, respectively. When dIde or human IDE (hIDE) was misexpressed in the developing wing imaginal discs, the wing size was significantly reduced in these flies: 82% and 83% of the control, respectively. These results indicate that both *dIde* and *hIDE* negatively regulate tissue growth. Misexpression of *Dilp2* in developing wing imaginal discs increases wing size. However, the co-overexpression of *dIde* suppresses the Dilp2-induced phenotype. *Dilp2* promotes growth through the insulin receptor, InR (Brogiolo et al. 2001). Overexpression of *InR* in the wing imaginal disc also increases wing size. However, unlike those induced by *Dilp2*, the InR-induced phenotype is not suppressed by co-overexpression of *dIde*, suggesting that dIde acts upstream of InR. PTEN is a negative regulator of the insulin signal by inhibiting PI3K activity. Loss-of-function mutations in PTEN increase body size by elevating PI3K activity (Goberdhan et al. 1999). dIde overexpression did not affect the large wing phenotype caused by the PTEN mutation. These genetic experiments suggest that dIde negatively regulates the insulin signaling pathway, most likely between the Dilp2 ligand and InR.

Human IDE is capable of digesting both β-amyloid (Aβ) and the Aβ precursor protein (APP) intracellular domain (AICD) in vitro. To examine whether overexpression of *dIde* can suppress the neurotoxicity induced by Aβ in vivo, we used the *Drosophila* AD model, in which human APP and the β-site APP-cleaving enzyme (BACE) are misexpressed in photoreceptor neurons (Greeve et al. 2004). In this model, a highly organized architecture of retinal photoreceptors degenerates in an age-dependent manner. Forced expression of *dIde* or *hIDE* suppresses neuronal degeneration in this model. In addition, pan-neural overexpression of APP and BACE using *elav*-GAL4 shortens the longevity of adult flies. The reduced life span was partially rescued by forced expression of *dIde* or *hIDE*, suggesting that dIde or hIDE can inhibit the pathological processes associated with Aβ and AICD accumulation in vivo.

7.1.3 JNK Signaling Pathway

Seong et al. (2001a) identified 25 genes whose overexpression extended the longevity through a misexpression screen. Among 13 genes whose functions are known or suggested, six were related to stress resistance or redox balance. We investigated the function of *plenty of SH3s* (*POSH*) in detail. In mammals, POSH has been shown to function as a scaffold protein need to activate the JNK signal (Saitoh et al. 1998; Villafania et al. 2000; Tapon et al. 1998). It has a RING finger domain and four SH3 domains, which were conserved between *Drosophila* and mammals. Neural-specific overexpression of *POSH* extended the longevity by 14% (Seong et al. 2001b). In addition, forced expression of *POSH* during development caused various morphological abnormalities, reminiscent of ectopic activation of the JNK signal. Overexpression of *POSH* induced *puckered* (*puc*) encoding a serine/threonine protein phosphatase induced by JNK activation. POSH is also required for terminating immune response after infection through degrading TAK1, an activator of both the JNK and the Relish pathways (Tsuda et al. 2005).

The JNK signaling cascade is triggered by a variety of insults, including UV radiation and oxidative stress. Wang et al. (2003) identified downstream target genes induced by JNK signaling and demonstrated the role of JNK signaling in oxidative stress tolerance. Longevity was dramatically extended in flies heterozygous for a loss-of-function allele of *puc*, a negative regulator of JNK. The *puc*-dependent longevity extension was suppressed by a mutation of the JNK activator, hep^{1}, demonstrating that an increase in JNK signaling activity extends the longevity phenotype.

JNK promotes nuclear translocation of Foxo and induces the expression of Foxo-dependent stress response genes that promote cell-autonomous stress defense and damage repair. Wang et al. (2005) demonstrated that Foxo is required for JNK to extend longevity. JNK also antagonizes the insulin/IGF-1 signaling systemically by activating Foxo and downregulating the expression of Dilp2 in insulin-producing cells (IPCs). JNK-dependent inhibition of insulin production has been observed in low-nutrient conditions (Agrawal et al. 2016). Eiger, the *Drosophila* homolog of TNFα, is produced by fat body cells, released in the hemolymph, and activates its receptor Grindelwald locally expressed in the brain IPCs, leading to JNK-dependent inhibition of insulin production.

7.1.4 Epigenetic Mechanism

Histone modification is one of the central epigenetic mechanisms that regulate gene expression. Trimethylated histone H3 lysine 27 (H3K27me3) is repressive methylation of histone H3 established by the polycomb repressive complex (PRC) through its core catalytic subunit, the H3K27-specific methyltransferase encoded by the *E(z)* gene in flies (Jones and Gelbart 1990). Flies heterozygous for mutations in *E(z)* or

esc encoding H3 binding protein increase longevity (33% or 45% longer than control), reduce H3K27me3 levels, and increase resistance to oxidative stress and starvation (Siebold et al. 2010). Mutations in the polycomb silencing antagonist *trithorax* suppressed the increased longevity and stress resistance. Also, the moderate reduction of H3K27me3 in long-lived *E(z)* heterozygotes partially derepress direct targets of polycomb silencing. Moskalev et al. (2019) observed 22–23% life span extension in *E(z)* heterozygous mutants for both sexes and higher levels of resistance to hyperthermia, oxidative stress, and endoplasmic reticulum stress. Genome-wide transcriptome analyses identified 239 genes whose expression level was altered more than twice by *E(z)* mutation. The affected genes include those involved in carbohydrate metabolism, lipid metabolism, drug metabolism, and nucleotide metabolism.

Ma et al. (2018) observed an age-associated decrease of H3K27me3 levels in muscles and analyzed changes of epigenome profiles obtained using the ChIP followed by high-throughput DNA sequencing. There was a dramatic shift in the pattern of H3K27me3 modification in aging. The role of PRCs in aging was explored using mutations in 24 genes encoding PRC components. The majority of mutants showed mild or no effect, but those bearing esc, *E(z)*, *Pcl*, *Su(z)12* of PRC2, and *Psc* and Su(z)2 of PRC1, lived substantially longer. The combination of *Pclc421* and *Su(z)12c253*, trans-heterozygote double mutants showed the most potent effect in H3K27me3-reduction and life extension. Transcriptome analyses identified several hundreds of upregulated genes. The analysis of 63 genes with known effects on aging, including genes in insulin/IGF-1, mTOR pathways, revealed no consistent changes in the expression in PRC2 mutants. Thus they are unlikely to contribute to PRC2-dependent longevity. Gene ontology analysis revealed that the "glycolytic process" and "closely related pathways" were highlighted for genes upregulated, while the "oxidation-reduction process" was enriched for genes downregulated. LC-MS-based untargeted metabolomics also demonstrated enhanced glycolysis in long-lived PRC2 mutants. Using weighted gene co-expression network analysis (WGCNA) (Langfelder and Horvath 2008), two glycolytic genes, *Tpi* (triosephosphate isomerase) and *Pgi* (phosphoglucose isomerase), whose expressions were upregulated in different tissue types across individual long-lived PRC2 mutants. Mutations in these genes mitigate or diminish the longevity benefits of PRC2 deficiency.

Conversely, transgenic expression of *Tpi* and *Pgi* in wild-type background improved life span, locomotion, and resistance to oxidative stress. Therefore, the upregulation of glycolytic genes alone is sufficient to mimic antiaging features of PRC2 mutants. This comprehensive study underscores the mechanistic link between epigenetic, transcriptional, and metabolic processes in aging, highlighting the role of glycolysis in promoting metabolic health and longevity (Ma et al. 2018).

7.2 Epigenetic Inheritance of Longevity

Maternal diet has impacts on the metabolism and longevity in offspring. Namely, a maternal diet (high sugar) increased carbohydrate storage and decreased cholesterol storage in developing offspring, and adult offspring accumulate increased triglyceride levels when challenged with a high-sugar diet (Buescher et al. 2013). The effects can be inherited through multiple generations. *Drosophila melanogaster* will continue to be a model system to explore the mechanism underlying the transgenerational inheritance of metabolic traits.

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