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[REVIEW]

Endocrine Regulation of Aging in the Fruit Fly *Drosophila melanogaster*

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The past few decades have witnessed increasing research clarifying the role of endocrine signaling in the regulation of aging in both vertebrates and invertebrates. Studies using the model organism fruit fly *Drosophila melanogaster* have largely advanced our understanding of evolutionarily conserved mechanisms in the endocrinology of aging and anti-aging. Mutations in single genes involved in endocrine signaling modify lifespan, as do alterations of endocrine signaling in a tissue- or cell-specific manner, highlighting a central role of endocrine signaling in coordinating the crosstalk between tissues and cells to determine the pace of aging. Here, we review the current landscape of research in *D. melanogaster* that offers valuable insights into the endocrine-governed mechanisms which influence lifespan and age-related physiology.

Key words: *Drosophila melanogaster*, aging, interorgan communication, insulin-like peptide, ecdysone, ecdysteroid, juvenile hormone, dopamine, serotonin, enteroendocrine hormone

INTRODUCTION

The endocrine system comprises multiple organs that produce and secrete hormones, which act as endogenous messengers, originating from one location, circulating in body fluids, and instructing another. Despite their exceptionally low amounts, hormones play a crucial role in orchestrating a plethora of physiological functions, including but not limited to growth, development, metabolism, and reproduction (Toivonen and Partridge, 2009).

Endocrine signaling is linked to the regulation of aging as well. The thrilling discovery that *C. elegans* with mutations in *daf-2*, a homolog of the mammalian insulin receptor, had unusually long lives, brought forward the concept that aging is subject to endocrine regulation (Kenyon et al., 1993; Kimura et al., 1997). To date, endocrine regulation of aging has been proven to be evolutionarily conserved in diverse organisms from worms to mice (Kenyon, 2011). Conversely, over the course of the lifespan, it is generally acknowledged that endocrine alterations, either incapability of the endocrine system in producing precise amounts of hormones, or insensitivity of receptors in responding to their controlling hormones, are responsible for the deterioration of body functions. Those changes are detrimental and can lead to frailty and severe diseases (van den Beld et al., 2018). Therefore, the endocrine system holds great promise as a target for therapeutic intervention to ameliorate aging, prevent diseases, and promote health-span.

The fruit fly *Drosophila melanogaster* represents an ideal model for endocrinological and aging studies. Flies and mammals share highly similar endocrinology and aging physiology; yet a short duration is required for fly aging and unparalleled genetic tools available in flies greatly facilitate research (Toivonen and Partridge, 2009; Piper and Partridge, 2018). Here, we review the current knowledge about the endocrine regulation of aging and the role of endocrine pathways in determining physiological functions during aging in *D. melanogaster*, with a main focus on the insulin/insulin-like growth factor signaling, ecdysteroids, juvenile hormones, biogenic amines, and gut-derived hormones. As no individual hormone works apart from others, and any given effect of aging is too complicated to be attributed to one hormone, we also discuss the signaling crosstalk between hormones in the regulation of aging, as summarized in Fig. 1. Despite significant advancements in recent research, there is still a substantial amount of information yet to be uncovered regarding the endocrinology of aging in *D. melanogaster.*

Insulin-like peptides

Drosophila possesses key elements of a highly conserved signaling system that closely resembles the insulin/insulin-like growth factor signaling (IIS) (Fig. 2) found in

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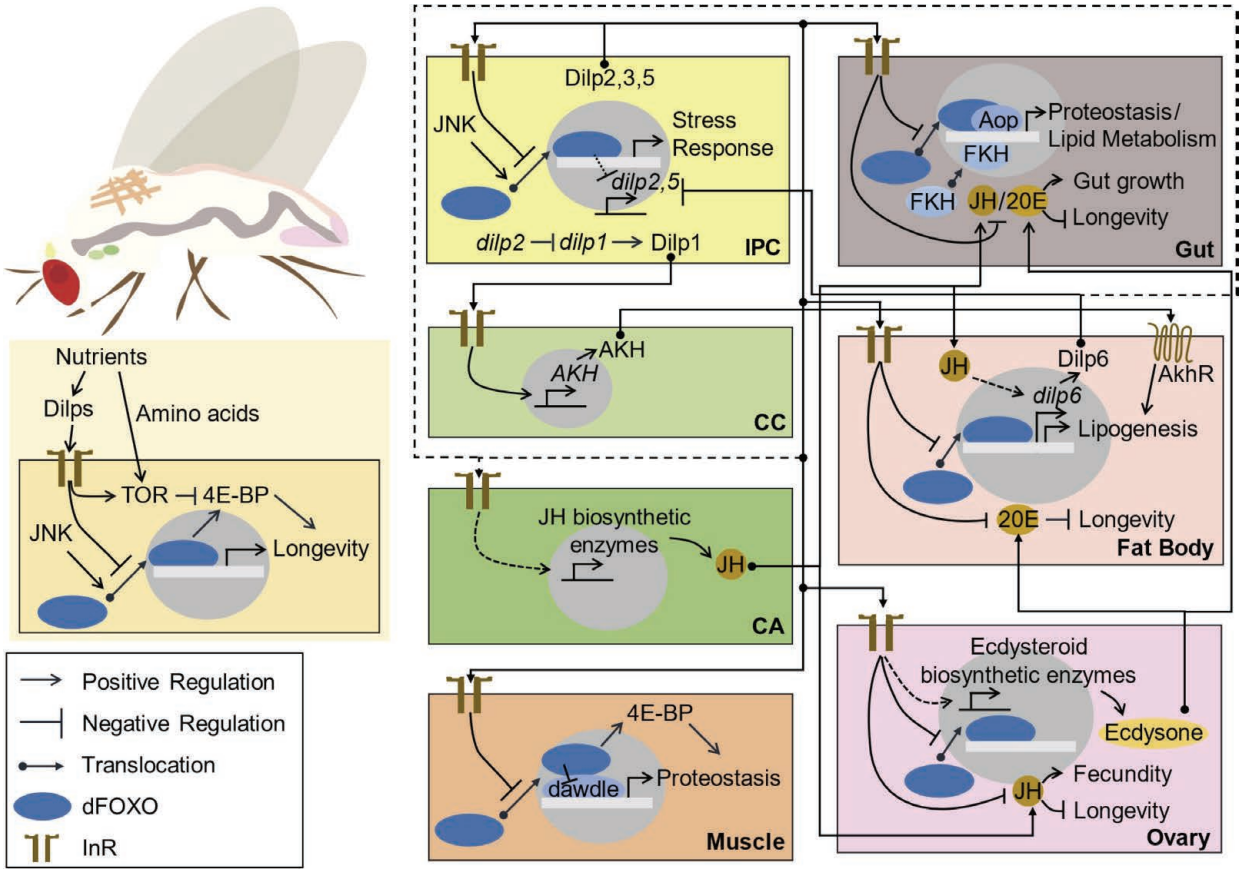

Fig. 1. Signaling crosstalk between pro-longevity pathways and their downstream effectors in *Drosophila melanogaster*. In the figure, solid lines indicate certain interactions, while dashed lines indicate that there is only circumstantial evidence. In general, Dilps are received by InR, thereby activating IIS. Reduced IIS induces nuclear translocation of dFOXO and confers longevity assurance. Dietary restriction, inhibition of TOR pathway, and mild JNK activation can prolong longevity in an IIS-dependent manner. Besides IIS, two other endocrine pathways, ecdysteroids and JHs, also regulate lifespan, either in parallel to IIS, or overlapping with IIS. Endocrine regulation of aging is coordinated between tissues and organs. IPCs produce Dilp1, Dilp2, Dilp3, and Dilp5, and the fat body produces Dilp6. *dilp1*, which is suppressed by *dilp2*, induces *AKH* expression in the CC to promote energy mobilization. Dilp2, Dilp3, and Dilp5 activate IIS in both IPCs and peripheral tissues, negatively impacting longevity. dFOXO directly represses dawdle in the muscle to improve proteostasis and interacts with Aop in the gut to prevent misregulation of lipid metabolism. In the fat body, dFOXO targets *dilp6* expression, and fat body-derived Dilp6 inhibits the expression of *dilp2* and *dilp5* in IPCs to promote longevity. In addition to dFOXO, FKH is another key transcriptional factor that lies downstream to attenuated IIS to mediate longevity. Ecdysteroids and JHs are biosynthesized in the ovary and the CA, respectively. Suppression of both pathways increases lifespan, and IIS might act upstream at both synthesis and effect level in their regulation of lifespan. In turn, JH might positively affect fat-body *dilp6* expression. In addition to IIS-dependent longevity assurance, JH might mediate the trade-off between fecundity and longevity in the ovary. Moreover, both JH and ecdysteroids have been implicated in the regulation of adaptive gut growth, whose dysregulation limits lifespan in aged individuals. Abbreviations: IPC, insulin-producing cells; CC, corpus cardiacum; CA, corpus allatum; Dilp, Drosophila insulin-like peptides; IIS, insulin/insulin-like growth factor signaling; dFOXO, Drosophila forkhead box-containing protein, O subfamily; InR, insulin receptor; FKH, FOXA ortholog Forkhead; Aop, anterior open; JNK, c-Jun N-terminal kinase pathway; TOR, target of rapamycin; 4E-BP, the eukaryotic translation initiation factor 4E binding protein; AKH, adipokinetic hormone; AkhR, adipokinetic hormone receptor; JH, juvenile hormone; 20E, 20-hydroxyecdysone.

vertebrates. *Drosophila melanogaster* has eight *Drosophila* insulin-like peptides (Dilps1–8), seven of which are likely to bind to the *D. melanogaster* insulin receptor (InR). InR transduces signals from Dilps to phosphoinositide 3-kinase (PI3K), via the insulin receptor substrate Chico (Biglou et al., 2021). The intracellular signaling involves activation of a series of kinases, exemplified by Akt/protein kinase B (PKB). The transcription factor *Drosophila* forkhead box-containing protein, O subfamily (dFOXO) is negatively regulated by Akt/PKB-mediated phosphorylation. Activated IIS promotes cytoplasmic retention of dFOXO, which otherwise translocates into the nucleus to upregulate the expression of various genes related to enhanced stress resistance (Biglou et al., 2021). IIS coordinates various physiological functions, one of them of relevance here being lifespan.

IIS components implicated in controlling aging

Attenuated IIS through genetic mutations of IIS pathway components has been consistently tied to extended fly lifespan (Partridge et al., 2011). Lifespan-extending genetic mutations of IIS pathway components in the fruit fly were first identified in 2001. Flies with a null mutation in *chico*, both homozygotes and heterozygotes, are long-lived; yet the heterozygous mutants are normal in their body size and fecun-

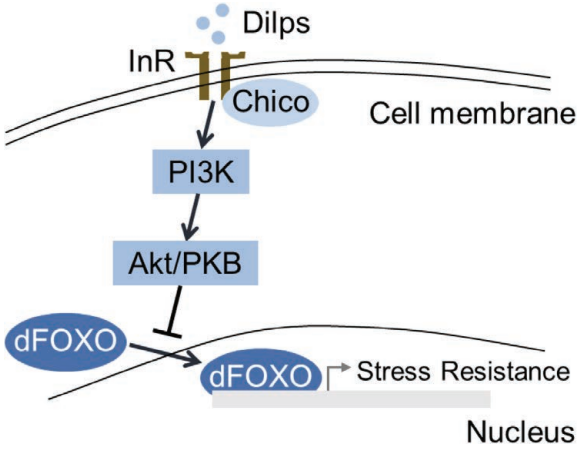

Fig. 2. Insulin/Insulin-like peptide signaling in *Drosophila melanogaster*. Drosophila insulin-like peptides (Dilps) bind to insulin receptors (InR) on the surface of target cells. The binding of Dilps to InR activates phosphoinositide 3-kinase (PI3K), via the insulin receptor substrate Chico. This further triggers a series of intracellular events involving the activation of various proteins through a cascade of phosphorylation reactions, exemplified by Akt/protein kinase B (PKB). Akt/PKB then phosphorylates the transcriptional factor Drosophila forkhead box-containing protein, O subfamily (dFOXO), promoting its retention in the cytoplasm. In the absence of IIS signaling, dFOXO freely translocates into the nucleus. Once in the nucleus, dFOXO binds to specific DNA sequences and upregulates the expression of genes involved in stress response. This results in enhanced stress resistance and increased lifespan.

dity (Clancy et al., 2001). A hypomorphic mutation in *InR*, which confers loss of InR function, also increases female lifespan by 85% (Tatar et al., 2001b). Subsequent studies have been devoted to understanding the role of tissue-specific intervention against IIS pathway components in longevity assurance. The tissues on which much progress has been made are the nervous system, the gut, and the fat body, which is the fly equivalent of the mammalian liver and white adipose tissue. *dilp2*, *dilp3*, and *dilp5* are mainly produced in a set of neurosecretory cells called insulin-producing cells (IPCs) in the brain, while *dilp6* is strongly expressed in the fat body (Semaniuk et al., 2021). Partial ablation of IPCs from the late larval stage results in an extension of lifespan (Broughton et al., 2005). Among the three neuronal *dilps*, *dilp2* is of particular interest due to its reduced expression level in long-lived IIS mutants (Hwangbo et al., 2004; Wang et al., 2005). Although IPC-specific *dilp2* knockdown does not result in any lifespan extension, *dilp2* mutants have an extended lifespan. The increase in *dilp3* and *dilp5* expression in both *dilp2*-reduced flies might run counter to modulation of overall neuronal *dilps*, obfuscating the role of each neuronal *dilp* in longevity regulation (Broughton et al., 2008; Grönke et al., 2010). On the other hand, overexpression of fat-body *dilp6* prolongs lifespan, via reducing neuronal *dilp2* and *dilp5* expression and circulating Dilp2 in the hemolymph, suggesting the presence of crosstalk between the fat body and the nervous system (Bai et al., 2012). Increasing the expression of *Imp-L2*, encoding a circulating Dilp-binding protein, ubiquitously or specifically in the gut and the fat body, phenocopies IIS reduction and enhances longevity, arguing that the gut can be a critical tissue as well (Alic et al., 2011b). Collectively, these findings show that IIS acts both autonomously and non-autonomously to control longevity.

Adult flies with dFOXO activated in the fat body have suppressed local IIS and decreased neuronal *dilp2* expression, which in turn reduces global IIS, implying a positive feedback mechanism in IIS' regulation of aging (Giannakou et al., 2004; Hwangbo et al., 2004; Alic et al., 2014b). Furthermore, mutation of *dFOXO* blocks the lifespan extension in flies with impaired IIS (Slack et al., 2011; Yamamoto and Tatar, 2011). These studies have proved both the necessity and the sufficiency of *dFOXO* in IIS-mediated lifespan regulation. Besides dFOXO, the *D. melanogaster* FOXA ortholog Forkhead (FKH) is another key transcriptional player that lies downstream of IIS. Gut-specific FKH upregulation is sufficient to prolong lifespan, and loss of FKH cancels IIS-mediated pro-longevity effects (Bolukbasi et al., 2017). In the nervous system, neuron-specific *FKH* overexpression and astrocyte-like glia cell-specific *dFOXO* overexpression independently extend lifespan (Woodling et al., 2020; Bolukbasi et al., 2021). Moreover, individual neurons respond differently to reduced IIS. While InR inactivation in serotonergic neurons increases lifespan, InR inactivation in cholinergic, dopaminergic, and glutamatergic neurons decreases lifespan (Dravecz et al., 2022). These findings have provided a cell type-specific insight into IIS-mediated lifespan regulation.

The mechanisms downstream of IIS to ameliorate aging

In parallel to increased longevity, repressing IIS influences various aspects of physiology, and a host of studies have focused on examining whether these physiological phenotypes are causal or simply correlated to lifespan extension (Giannakou and Partridge, 2007; Piper et al., 2008; Partridge et al., 2011; Kannan and Fridell, 2013). IIS mutants feature altered metabolism, in particular raised trehalose and glycogen levels (Broughton et al., 2005). With global phospho-proteomic analysis, *dilp2* has been found to decrease the phosphorylation of Ser15 of glycogen phosphorylase, the rate-limiting enzyme in glycogenolysis, thereby suppressing its enzymatic activity. Failure to deactivate the enzyme thus results in elevated glycogen levels in IIS mutants (Post et al., 2018). *dilp1* is required for *dilp2* mutants to live long, and this pro-longevity effect acts partially through the induction of adipokinetic hormone (AKH). AKH, produced in the corpus cardiaca (CC), acts as the functional homolog of mammalian glucagon, whose secretion induces mobilization of stored energy and increases lifespan (Post et al., 2019). Improved resistance to oxidative stress observed in long-lived IIS mutants is another possible mechanism to slow aging. Moderate upregulation of cellular detoxification via autophagy is necessary for IIS to increase lifespan (Bjedov et al., 2020). These studies have revealed critical causal outputs of IIS that guide longevity assurance.

More recent studies have taken approaches to characterizing the transcriptome and proteome of long-lived IIS mutants, to search for coordinated functional changes (Alic et al., 2011a, 2014a; Bai et al., 2013; Tain et al., 2017). Upon IIS reduction, the transcriptional response of dFOXO ensures correct expression of genes spanning various functions, including cell cycle, DNA repair, intracellular transport, and protein catabolism (Alic et al., 2011a). Chromatin immunopre-

cipitation analysis with dFOXO from long-lived IIS mutants has shown that dFOXO directly represses *dawdle*, encoding an activin-like ligand. Reduced activin signaling promotes autophagy, improves muscle performance, and reduces neuronal Dilp secretion, hence extending lifespan (Bai et al., 2013). In the gut, dFOXO regulates the expression of *Anterior open* (*Aop*), encoding an E-twenty six (ETS)-family transcriptional repressor, which counteracts ETS-family transcriptional activator to prevent detrimental drop in the lipid storage that limits lifespan (Alic et al., 2014a). In the gut and the fat body, Aop lies downstream to attenuated IIS to extend lifespan (Slack et al., 2015). Meanwhile, profiling of the proteomes in IPC-ablated and *dFOXO*-deleted flies has identified proteins whose expressions are dependent on IIS. These proteins can be grouped into diverse functional entities. Proteins associated with mitochondrial biogenesis increase in the fat body, while proteins associated with proteasomal assembly increase in the gut (Tain et al., 2017). Collectively, these studies have captured the complexity and tissue-specificity of cellular responses downstream of reduced IIS and proposed a consistent mechanism by which IIS employs autophagy and the ubiquitin-proteasome machinery to maintain proteostasis, thus promoting longevity.

Longevity pathways with which IIS interacts

Although IIS-mediated lifespan extension is mainly attributed to the downstream effects of dFOXO, dFOXO is only required for a part of transcriptional responses and physiological changes brought on by reduced IIS (Alic et al., 2011a; Slack et al., 2011). This suggests that IIS signals through additional longevity pathways to fine-tune survival. The two pathways of note are the target of rapamycin (TOR) pathway, and the stress-sensing c-Jun N-terminal kinase (JNK) pathway (Giannakou and Partridge, 2007). The TOR pathway is primarily associated with amino acid sensing and cellular responses to growth factors, mainly via direct phosphorylation of the key effector, the eukaryotic translation initiation factor 4E binding protein (4E-BP). Inhibiting the TOR pathway, either through overexpressing the downstream negative regulator, or rapamycin administration, extends lifespan (Kapahi et al., 2004; Bjedov et al., 2010). Reduced IIS represses the activity of the TOR complex 1, and 4E-BP is required for *chico* mutants to slow aging and increase stress resistance (Kapahi et al., 2004; Luong et al., 2006; Bai et al., 2015). Overexpression of *4E-BP* in muscles extends lifespan and preserves muscle proteostasis in response to dFOXO signaling (Demontis and Perrimon, 2010). The JNK pathway is another critical system in regulating longevity. JNK is activated in response to stresses to trigger the protective mechanism of cells and to promote survival. Mild JNK activation at an organismal level gives flies an extended lifespan and enhanced stress tolerance (Gan et al., 2021). Activation of JNK in the IPCs exerts pro-longevity effects through inducing nuclear translocation of dFOXO and downregulating *dilp2* expression, ultimately antagonizing IIS (Hwangbo et al., 2004; Wang et al., 2005; Karpac et al., 2009).

Besides the two signaling pathways, the nutrient-sensing IIS pathway extensively interacts with dietary restriction (DR), a conserved pro-longevity mechanism (Partridge et al., 2011). DR can be achieved by diluting the food medium, with the yeast component playing a major role (Mair et al., 2005). While flies with mutated *chico*, overexpressed *dFOXO*, or activated TOR can increase their lifespan to a similar extent as controls under DR, they reach their maximum lifespan at a higher food concentration than controls, implying that reduced IIS/TOR renders flies into a partially diet-restricted state (Clancy et al., 2002; Kapahi et al., 2004; Giannakou et al., 2008). Flies expressing a dominant-negative form of *InR* are longer-lived than controls even with DR (Grandison et al., 2009). In contrast, flies with deletion of three neuronal *dilp*s fail to respond to DR, such that their lifespan remains unchanged at all food concentrations, at a level the control can maximize under DR (Broughton et al., 2010; Grönke et al., 2010). It has been argued that a diet dilution series can overlook the effect of the nutrient ratio, which results in such mixed outcomes (Piper et al., 2011). Under the low protein-to-carbohydrate ratio that maximizes the lifespan of wild-type flies, *dilp5* mRNA is decreased and *dilp6* mRNA is increased (Min et al., 2008; Post and Tatar, 2016). These findings suggest that IIS and DR act through overlapping lifespan extension mechanisms (Kannan and Fridell, 2013; Tatar et al., 2014). However, DR prolongs the lifespan of *dFOXO*-null flies to the same magnitude as seen in controls (Giannakou et al., 2008; Min et al., 2008). Therefore, it has been proposed that in the absence of dFOXO, DR might foster longevity via the TOR pathway downstream to IIS (Grönke et al., 2010; Tatar et al., 2014).

Age-dependent changes of IIS signaling

Drosophila melanogaster aging can be detected by numerous physiological signs, including decreased locomotor activity, increased sleep fragmentation, and altered neuron functions (Piper and Partridge, 2018). Genetic manipulation of IIS can postpone such age-related deterioration of functions (Partridge et al., 2011). Systemic IIS reduction retards senescence of negative geotaxis, a reflex motor behavior related to escape, but not exploratory walking, a more complex locomotion involving the central nervous system, motor neurons, and muscles (Ismail et al., 2015). Lowered IIS/TOR ameliorates sleep fragmentation, improves night sleep quality, and enhances daytime activity (Metaxakis et al., 2014). Reducing IIS/TOR also suppresses the toxicity of beta-amyloid peptides, and microtubule-associated protein Tau, both contributing to the progression of Alzheimer's disease; however, it remains unclear how aging modulates IIS to increase the risk of this neuropathology (Huang et al., 2019).

Relatively limited work has been done on the contribution of age-related IIS alteration to age-related physiological changes; yet several lines of evidence suggest that IIS declines with age. Neuronal expression of *dilp3* is necessary for intermediate-term memory in young flies, and it decreases with age and its overexpression is sufficient to rescue memory defects in aged flies. This indicates that reduced IIS correlates to impaired memory in aged conditions (Tanabe et al., 2017). In the ovary, IIS is required for the maintenance of female germline stem cells (GSCs). While decreased levels of PI3K in aged ovaries imply IIS reduction with age, increasing local *dilp2* levels recovers age-dependent GSC loss (Hsu and Drummond-Barbosa, 2009). In the gut, dFOXO represses lipase expression in a dietary-responsive manner, which ensures balanced lipid uptake. In

aged guts, elevation of JNK signaling chronically causes dFOXO activation, yet Akt levels increase and become hypersensitive to insulin stimulation. IIS deregulation thus disrupts lipid homeostasis, which is reminiscent of insulin resistance in aged mammals (Karpac et al., 2013). Although lowered IIS is closely tied to a prolonged lifespan, its decrease in expression levels and responsiveness during normal aging might lead to an age-related decline in physiological functions, which awaits further comprehension.

Ecdysteroids

Ecdysteroids, including ecdysone and its active derivative 20-hydroxyecdysone (20E), are the only class of steroid hormones in insects. They are well known for their role in stimulating metamorphosis and maturation during development and regulating reproductive diapause, yolk protein synthesis, egg production, courtship behavior, and female GSC maintenance in adults (Gáliková et al., 2011; Ishimoto and Kitamoto, 2011; Schwedes and Carney, 2012; Uryu et al., 2015; Hoshino and Niwa, 2021; Okamoto and Watanabe, 2022). In larvae, ecdysone is synthesized from sterols in the endocrine organ called the prothoracic gland, released to the hemolymph, and distributed to the peripheral tissues, along with its conversion into 20E. Their binding to the heterodimeric nuclear receptor complex, the ecdysone receptor (EcR) and ultraspiracle (USP), initiates transcription (Niwa and Niwa, 2016). In adults, the prothoracic gland no longer exists, and ecdysone synthesis occurs in the female ovary and the male accessory gland (Yoshinari et al., 2019; Hutfilz, 2022). Notably, there is also evidence supporting the involvement of ecdysteroids in the regulation of aging (Simon et al., 2003; Tricoire et al., 2009; Gáliková et al., 2011).

Ecdysteroids in lifespan modulation

The first study characterizing the steroid regulation of longevity in *D. melanogaster* showed that several *EcR* heterozygote mutants were long-lived and resistant to various stresses (Simon et al., 2003). A similar longevity phenotype is also observed in the mutant deficient for the ecdysone biosynthesis, and 20E supplementation to these mutants brings their lifespan back to wild-type levels (Simon et al., 2003). Contradictorily, although a mild *EcR* inactivation from adulthood, either ubiquitously or restricted to the fat body and the gut, increases male lifespan, it is deleterious to female lifespan, arguing a striking sex-specific control of lifespan by ecdysteroids. In addition, the decreased lifespan of female *EcR* knockdown flies is rescued in germline-deficient ovo^{D1} mutants; thus it is tempting to speculate that the sex gland bridges between ecdysteroids and longevity (Tricoire et al., 2009).

Regulatory wiring between ecdysteroids and IIS

The lifespan-extending effect of both IIS and ecdysteroid signaling suggests that the two pathways might converge or mediate one another to regulate aging. Both long-lived and normal-lived *InR* mutants exhibit decreased ecdysteroid levels in the ovary (Tu et al., 2002). On the other hand, long-lived *chico* mutants have normal levels of ecdysteroid release from the ovary and circulation in the hemolymph (Richard et al., 2005). These studies indicate that there is no explicit link between reduced ecdysteroids and reduced IIS in promoting lifespan.

Nevertheless, it has been suggested that IIS and ecdysteroids have antagonistic actions that integrate larval growth with developmental transitions (Colombani et al., 2005). Ecdysone inhibits the mitogenic transcription factor Myc in the larval fat body, thereby activating dFOXO and attenuating IIS in the peripheral tissue (Delanoue et al., 2010). In turn, IIS relays nutrient signals to the PG to promote its growth, which together with other factors positively regulates ecdysteroidogenesis (Yamanaka et al., 2013). The work done in development would be informative for extrapolating how the two pathways interact in longevity.

Ecdysteroids in relation to aging phenotypes

Ecdysteroid signaling can be triggered when facing various stressors, which are more prevalent and more harmful at older ages, as response mechanisms to safeguard against damage (Hutfilz, 2022). Desiccation stress elevates the expression of peptidoglycan recognition protein-LC and antimicrobial peptides in the Malpighian tubules, to protect flies from infection. This is attributed to an upregulation of ecdysone synthesis. Aged flies exhibit dehydrated symptoms, and increased immune signaling and systemic 20E levels, highlighting the role of ecdysteroid in age-related immune defense against dehydration (Zheng et al., 2018). On the other hand, the elevated ecdysone level in aged flies promotes fibrosis, an excess extracellular matrix formation, in adult nephrocytes, leading to symptoms resembling mammalian kidney failure. This process is dependent on DopEcR, a G-protein coupled receptor for both dopamine and ecdysone, and the epidermal growth factor receptor (Zheng et al., 2020). Intestinal dysplasia and increased mitotic cells characterize the aging female gut. Suppressing *EcR* in midgut progenitor cells or ecdysone biosynthetic enzymes in the ovary in aged females curtails age-induced intestinal dysplasia and extends lifespan (Ahmed et al., 2020). In conclusion, age-related changes in ecdysteroid levels might bring either beneficial or detrimental effects to aged individuals, which might be dependent on the temporal and spatial patterns of its production.

Juvenile hormones

Juvenile hormone (JH) is a sesquiterpenoid originated from the endocrine gland called the corpus allata (CA). JH travels to peripheral tissues and directly binds to the intracellular receptor, Methoprene-tolerant (Met) and germ cell-expressed (Gce), to activate JH-inducible genes. The primary role of JH is to oppose the metamorphic action of ecdysone during development, with JH level maximizing at the larval stage and sharply dropping after pupariation (Riddiford, 2020; Zhang et al., 2021b). In adults, it is suggested that JH affects major aspects of reproductive fitness. JH promotes female ovarian maturation and post-mating gut remodeling, regulates male courtship behavior, and inhibits reproductive diapause, which is a physiological adaptation for enduring unfavorable conditions (Flatt et al., 2005; Wijesekera et al., 2016; Zhang et al., 2021a; Hutfilz, 2022; Okamoto and Watanabe, 2022). Therefore, the long-held idea states that JH mediates the trade-off between reproduction and lifespan (Tatar and Yin, 2001; Tatar et al., 2003; Flatt et al., 2005; Giannakou and Partridge, 2007; Partridge et al., 2011), while recent studies propose additional mecha-

nisms by which JH affects lifespan (Yamamoto et al., 2013; Giebultowicz and Long, 2015).

The role of JH in reproduction and aging

In *D. melanogaster*, a decreased JH level induces and maintains reproductive diapause. Diapausing flies exhibit reduced metabolism, arrested oogenesis, increased stress resistance, and negligible senescence, which, in other words, represents a state of cessation of aging (Flatt et al., 2005). Supplementation of JH analog (JHa) to dormant flies reverses diapause phenotypes and increases demographic senescence (Tatar et al., 2001a). Inhibiting JH production mimics diapause states in non-diapausing flies, and enhances the longevity of GSCs, indicated by their longer persistence (Easwaran et al., 2022). These discoveries underpin the argument that longevity can be linked to alterations in pathways that mirror natural changes during diapause, and to be more specific, decreased JH levels.

Indeed, the elimination of JH-producing CA from adulthood impairs female yolk protein production and increases survival in both sexes, with supplementation of JHa facilitating fecundity and reversing longevity (Yamamoto et al., 2013). Exposure of wild-type larvae to JHa during development promotes early-life fecundity, but negatively impacts adult survival and life expectancy (Flatt and Kawecki, 2007). Long-lived sterile *InR* mutants have ovaries resembling wild-type diapause flies and produce a low level of JH, with JHa treatment rescuing infertility but increasing mortality (Tatar et al., 2001b). These studies highlight the role of JH in mediating the trade-off between fecundity and longevity.

However, continuous JHa-feeding over generations selects JHa-resistant flies with normal fecundity and lifespan, which can in turn live slightly longer yet have unchanged fecundity in the absence of JHa (Flatt and Kawecki, 2007). Moreover, homozygous *chico* mutants, which are infertile, and heterozygous *chico* mutants, which are fertile, are both long-lived (Clancy et al., 2001). While one study concluded that both of them exhibit reduced JH synthesis (Tu et al., 2005), another study concluded that homozygous mutants exhibit a normal JH synthesis rate, and JHa fails to restore their fertility (Richard et al., 2005). Longevity and fecundity thus do not necessarily correlate with each other, making the JH-mediated trade-off coincidental. Complementing this notion, decreased JH levels even extend the lifespan of sterile ovo^{D1} mutants, suggesting that lifespan extension by JH reduction operates through mechanisms other than avoiding the reproductive cost (Yamamoto et al., 2013).

Potential mechanisms by which JH regulates aging

To further investigate the role of JH in aging, we still need to expand the relevance of conclusions regarding the role JH plays from diapause (Gruntenko and Rauschenbach, 2018; Kurogi et al., 2021). Since enhanced longevity in diapause is often tied to resistance to exogenous stress, the role of JH in stress tolerance has been well examined. Consistent with lifespan-extending effects of JH reduction, CA-ablated files are more resistant to heat and oxidative stress, via modulation of dopamine, a stress-responsive hormone (Gruntenko et al., 2010, 2012; Yamamoto et al., 2013; Gruntenko and Rauschenbach, 2018). The comparison of gene expression profiles between CA-ablated flies and controls showed that JH represses genes associated with oxidative reduction and induces processes of proteolysis, therefore suggesting several possible mechanisms by which reduced JH confers longevity assurance (Yamamoto et al., 2013).

It is suggested that JH depletion increases lifespan by employing the pro-longevity IIS pathway. Some IIS mutants have decreased JH (Tu et al., 2005), and reciprocally, elimination of JH via CA ablation reduces *dilp6* in the fat body but has no effect on neuronal *dilp2* or *dilp3* mRNA levels (Yamamoto et al., 2013). While *dilp6* overexpression extends lifespan (Bai et al., 2012), *dilp6* mutants exhibit a decrease in JH degradation and an increase in fecundity (Rauschenbach et al., 2017). These studies suggest that fat body *dilp6* is particularly relevant to JH, whereby JH positively regulates *dilp6* expression and in turn *dilp6* impacts JH production, and this feedback loop mediates lifespan extension. On the other hand, upregulated *dilp2*, *dilp3*, *dilp5*, and *dilp6* are reported in files subjected to diapause conditions (Kubrak et al., 2014), which complicates the comprehension of the relationship between JH and IIS in the regulation of aging. During development, larvae with CA ablated have increased *InR* levels and reduced growth rate, which occurs in a dFOXO-dependent manner (Mirth et al., 2014). At present, the precise mechanisms linking JH to IIS are still poorly defined, and the research into the molecular basis of the JH-IIS axis in the regulation of aging remains very limited.

JH, as its name “juvenile” suggests, increases from pupa to adults, but peaks right after adult emergence and decays drastically thereafter (Bownes and Rembold, 1987). Yet its function remains crucial in aged flies. Inhibition of JH synthesis over 4 weeks reduces the number of progenitor cells in the adult midgut, implying that JH plays a role in maintaining gut homeostasis during aging (Rahman et al., 2017). However, the research examining how age-related JH changes are related to aging phenotypes is still in its infancy.

Biogenic amines

Biogenic amines are a group of amino acid-derived compounds that act as neurotransmitters in the central nervous system and regulate various neurological and physiological processes. The two well-characterized biogenic amines, dopamine and serotonin, are implicated in the aging process of *D. melanogaster* (Toivonen and Partridge, 2009).

The role of dopamine in the aging brain

Dopamine (DA) has several roles in neural functions, from habituative learning, to regulation of female sexual receptivity, to coordination of motor behaviors. The perturbation in female sexual receptivity during aging coincides with a decrease in whole-body DA levels (Neckameyer et al., 2000). In contrast, despite the behavioral analysis proving an age-related decline in locomotion, age-related DA neuron cell loss is not detected. However, it is important to note that the functionality of DA neurons has not been thoroughly examined in this context (White et al., 2010).

Surprisingly, the mutants that lack tyrosine hydroxylase (TH), and thus DA synthesis, have a lifespan similar to wild-type flies while exhibiting age-dependent locomotor deficits, suggesting that there is some unaccounted factor besides DA at play (Riemensperger et al., 2011). Contrasting to the previous study, the mutants that are deficient in TH activities

or DA transport have a shortened lifespan, although they exhibit a similar age-related decline in circadian locomotor activities (Hanna et al., 2015; Bednářová et al., 2017). While elevating DA production in neurons produces no significant results, DA neuron-specific overexpression of the scaffold protein *Mask* extends lifespan and sustains locomotor abilities in aged flies, via enhancement of microtubule stability (Tian, 2021). These pieces of evidence support the notion that DA neuronal transmission contributes to improved DA-modulated behaviors, especially in the brain of aged flies.

Sensory cues linked to lifespan via dopamine and serotonin

Sensory inputs relay sensory cues to initiate physiological changes, thereby influencing aging (Miller et al., 2020). Gustatory perception of sweetness is required for a normal lifespan and stress resistance under nutrient-deprived conditions, and blockage of DA neurotransmission phenocopies loss of the sweet taste receptor *Gr64*, and thus the glucose-sensing ability. These data establish a model in which glucose-sensing signals are transmitted via DA neurons (Linford et al., 2015).

DR-mediated longevity intervention is mainly attributed to protein restriction (Piper et al., 2011). Serotonin signaling in the central nervous system confers flies' preference for protein over sugar following starvation. Flies with 5-HT2A serotonin receptor mutated are shielded from the lifespan-shortening effect brought about by voluntarily consuming more sugar than protein under a choice diet (Ro et al., 2016). Not only the neuronal response to the dietary choice but also the downstream reprogramming of metabolic pathways, require 5-HT2A (Lyu et al., 2021). Similarly, under nutrient-replete conditions, *5-HT2A* mutants exhibit decreased protein intake and prolonged lifespan (Munneke et al., 2022). Taken together, these findings indicate that blocking serotonin signaling abolishes protein sensibility and mimics the benefit of DR.

Besides sensing food, flies can perceive dead individuals of their species in their surroundings. Exposure to dead flies diminishes their starvation resistance, depletes their lipid stores, and reduces their lifespan. Interestingly, serotonin signaling through 5-HT2A is necessary for the influence of death perception on lifespan (Chakraborty et al., 2019).

Gut-derived hormones

The residence of nutrient-sensing and hormone-secreting enteroendocrine cells (EEs) in the intestinal epithelium highlights the gut as a key endocrine organ, both in flies and mammals. At least 14 different peptide hormones are released from distinct subtypes of EEs, which broadcast environmental stimuli, mediate energy balance, and coordinate physiological responses, both locally and systemically (Zhou et al., 2020; Guo et al., 2022).

The first evidence proving the requirement of EEs in longevity was the finding that mutants completely devoid of EEs have a shorter lifespan, together with reduced mitotic cells and midgut *dilp3* expression. As an increase in *dilp3* expression is linked to nutrient-stimulated midgut growth, it was proposed that EEs and EE-derived hormones serve as an important link between diets and gut adaptive growth via regulation of intestinal stem cell (ISC) proliferation (Amcheslavsky et al., 2014). Removal of two gut-derived hormones, Allatostatin A (AstA) or Diuretic hormone 31 (Dh31), decreases or increases lifespan, respectively. ISC hyper-proliferation is one of the gut senescence phenotypes that limit lifespan (Biteau et al., 2010), and consistently, *AstA* knockdown increases ISCs, whereas *Dh31* knockdown decreases ISCs in the aged intestine. The two gut-derived hormones thus have contrasting functions in midgut senescence and adult longevity (Takeda et al., 2018).

Aging is associated with the increase in EE cell lineages in the posterior midgut. Inhibiting EE lineage specification reduces EE cells in the aged intestine and ameliorates gut dysplasia, implicating a role of posterior EEs in disrupting intestinal homeostasis during aging (Tauc et al., 2021). Notably, Allatostatin C-producing EEs increase with age, whereas Tachykinin-producing EEs decrease with age (Tauc et al., 2021), even though both hormones are expressed in the posterior midgut (Guo et al., 2019). These studies provide circumstantial evidence supporting the possibility that age-related changes in gut-derived hormones are responsible for age-induced midgut deterioration. However, the contribution of specific hormones has not yet been thoroughly explored.

CONCLUSION AND OUTLOOK

Significant progress has been made with the model organism *D. melanogaster* since the discovery that aging can be subject to endocrine regulation. The endocrine pathways described above have far-reaching effects on various aspects, such as metabolism, reproduction, stress resistance, and survival, making them highly pleiotropic. While numerous studies have undoubtedly established IIS, ecdysteroid, and JH signaling pathways as contributors to direct lifespan modulation, emerging studies are setting out to test the role of other endocrine factors in aging, which allows us to paint a more accurate picture of endocrine regulation of aging. Notably, recent advances in single-cell RNA sequencing technologies have supported the characterization of aging phenotypes at the single-cell level in *D. melanogaster* (Lu et al., 2023). Insights into alterations of common age-associated pathways are believed to greatly facilitate our understanding of the endocrine regulation of aging.

Lifespan modulation is the outcome of multi-faceted physiological traits whose changes result from the coordination of various hormones. Although valuable studies have begun to connect different pieces of findings, more research will be needed to create a united circuitry of endocrine regulation of aging. Gaining a comprehensive understanding of these issues is crucial for identifying specific components in the signaling network that can be targeted for intervention and for developing potential drug targets for enhancing health during aging.

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COMPETING INTERESTS

The authors have no relevant conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

QQ and RN prepared, read, and approved the manuscript.

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