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Invited Mini Review

The role of insulin/IGF-1 signaling in the longevity of model invertebrates, *C. elegans* and *D. melanogaster*

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Insulin/insulin-like growth factor (IGF)-1 signaling (IIS) pathway regulates aging in many organisms, ranging from simple invertebrates to mammals, including humans. Many seminal discoveries regarding the roles of IIS in aging and longevity have been made by using the roundworm *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster*. In this review, we describe the mechanisms by which various IIS components regulate aging in *C. elegans* and *D. melanogaster*. We also cover systemic and tissue-specific effects of the IIS components on the regulation of lifespan. We further discuss IIS-mediated physiological processes other than aging and their effects on human disease models focusing on *C. elegans* studies. As both *C. elegans* and *D. melanogaster* have been essential for key findings regarding the effects of IIS on organismal aging in general, these invertebrate models will continue to serve as workhorses to help our understanding of mammalian aging. [BMB Reports 2016; 49(2): 81-92]

INTRODUCTION

The roundworm *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster* have been used as two most popular invertebrate models for studying aging and longevity (1, 2). In particular, their short lifespan together with their low cost and easy handling has established these invertebrates as excellent systems for research on molecular mechanisms regulating animal aging. Many important discoveries regarding evolutionarily conserved aging-regulatory pathways have been made using *C. elegans* and *D. melanogaster*. One of such pathways is the insulin/insulin-like growth factor (IGF)-1 signaling (IIS) pathway, which was first shown to regulate longevity in *C. elegans*, and subsequently confirmed by using *D. melanogaster*. Importantly, the findings using these two invertebrate model organisms stimulated research on the role of IIS in mammalian aging, and led to discoveries showing that IIS also regulates aging in mammals, including mice and humans (3, 4). In this review, we will describe which components of IIS regulate lifespan, and how IIS modulates aging processes in these two model organisms. We will also review endocrine signaling and the importance of insulin-like peptides (ILPs) for systemic longevity regulation. Overall, our review will provide useful information regarding the conserved roles of IIS pathway in the aging of model organisms, which will eventually pave the way for understanding the mystery of human aging.

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THE ROLE OF INSULIN/IGF-1 SIGNALING IN *C. elegans* AGING

Insulin/IGF-1 signaling pathway components that regulate the lifespan of *C. elegans*

The insulin/IGF-1 signaling (IIS) pathway contains many evolutionarily conserved components that regulate aging (Fig. 1). The gerontogenes *daf-2* and *age-1* encode the sole insulin/IGF-1 receptor and phosphatidylinositol-3-OH kinase (PI3K) (5, 6), respectively. DAF-2 and AGE-1 are two key upstream components of IIS that regulate various physiological aspects, including aging and adult lifespan. Two of the most important discoveries in the field of aging research were perhaps the findings demonstrating that inhibition of *daf-2* or *age-1* dramatically extended lifespan in *C. elegans* (7-9). These discoveries stimulated many subsequent studies on the role of IIS in lifespan regulation, not only in *C. elegans* but also in *D. melanogaster* and mammals.

IIS transduces signals through a combination of well-organized sequential events, depending on environmental conditions. Under favorable conditions, IIS is activated and this confers normal development and adult lifespan. Specifically, agonist insulin-like peptides (ILPs) bind to their receptor, DAF-2, which in turn recruits an insulin receptor substrate (IRS)/IST-1 (10). This leads to the activation of the AGE-1/PI3K, which increases the level of phosphatidylinositol (3,4,5)-trisphosphate (PIP_3) (5, 11); this event is antagonistically balanced by DAF-18/PTEN phosphatase that promotes the conversion of PIP_3

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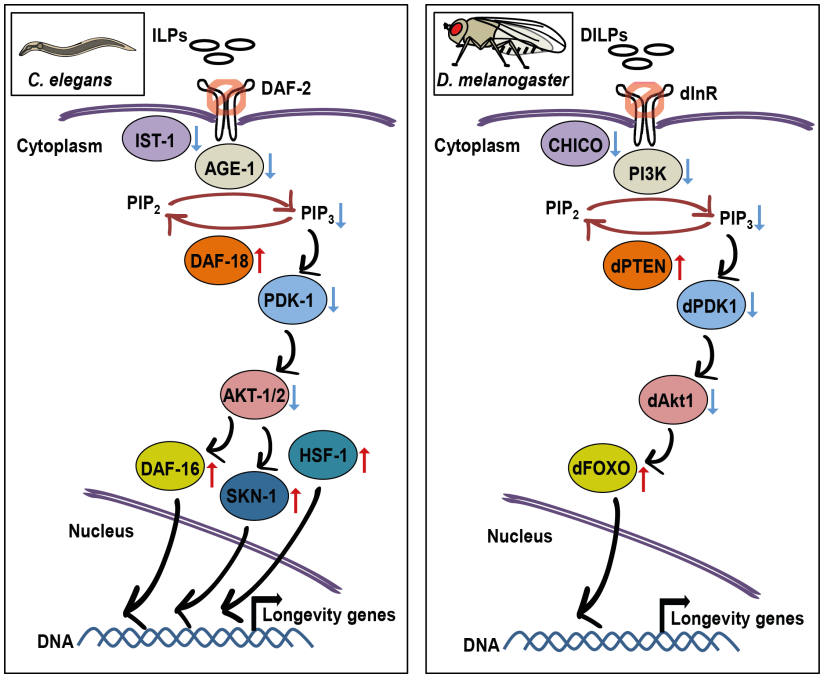

Fig. 1. Conserved longevity-regulatory components of insulin/IGF-1 signaling pathway in *C. elegans* and *D. melanogaster*. Insulin-like peptides (ILPs in *Caenorhabditis elegans* and DILPs in *Drosophila melanogaster*) bind to insulin/IGF-1 receptor (DAF-2 in *C. elegans* and dInR in *D. melanogaster*) and lead to its phosphorylation. Inhibition of insulin/IGF-1 receptor results in decreased binding to the insulin receptor substrate (IST-1 in *C. elegans* and CHICO in *D. melanogaster*), which in turn decreases the activity of phosphoinositide-3 kinase (AGE-1 in *C. elegans* and PI3K in *D. melanogaster*) that converts PIP_2 to PIP_3; conversely, the PTEN phosphatase (DAF-18 in *C. elegans* and dPTEN in *D. melanogaster*) functions to antagonize the activity of the phosphoinositide-3 kinase by converting PIP_3 to PIP_2. Decreased PIP_3 levels lead to decreased activities of phosphoinositide-dependent kinase 1 (PDK-1 in *C. elegans* and dPDK1 in *D. melanogaster*) and the serine/threonine-specific protein kinase B (AKT-1/-2 in *C. elegans* and dAkt1 in *D. melanogaster*), and the activation of downstream transcription factor FOXO (DAF-16 in *C. elegans* and dFOXO in *D. melanogaster*). Reduced insulin/IGF-1 signaling in *C. elegans* also increases the activities of heat shock transcription factor-1 (HSF-1) and SKN-1 (NRF2). These transcription factors regulate the expression of target genes, which contribute to longevity.

to phosphatidylinositol (4,5)-bisphosphate (PIP_2) (12-19). The signals provided by PIP_3 activate the downstream kinase cascade, composed of 3-phosphoinositide-dependent protein kinase 1 (PDK-1) (20), protein kinase B (AKT-1/-2) (21), and serum- and glucocorticoid-inducible kinase-1 (SGK-1) (22; but see also 23, 24). This in turn phosphorylates and inactivates DAF-16/FOXO transcription factor, by promoting its nucleus-to-cytosol translocation (22, 25-30). Conversely, in unfavorable conditions, IIS is down-regulated and leads to the activation of DAF-16/FOXO via enhancing its translocation from the cytoplasm to the nucleus, where it switches on the expression of genes that promote longevity. Thus, *C. elegans* IIS pathway acts as a system in which many components transduce signals to modulate the aging processes, depending on extracellular conditions.

Three most important downstream lifespan-regulatory transcription factors of IIS that have been identified so far are DAF-16/FOXO, heat shock transcription factor 1 (HSF-1) and SKN-1/nuclear factor erythroid 2 (NRF2). DAF-16/FOXO regulates aging processes downstream of the canonical IIS cascade as described above. In addition, Jun-N-terminal kinase (JNK/JNK-1) (31), AMP-activated protein kinase (AMPK/AAK-2) (32-34), and Ste20-like protein kinase (MST1/CST-1) (35) activate DAF-16/FOXO via phosphorylation. Other non-kinase proteins have been shown to regulate *C. elegans* DAF-16/FOXO. A serine/threonine-protein phosphatase 4-regulatory subunit SMK-1 (36), and an RNA helicase HEL-1 (37), extend longevity by acting together with DAF-16/FOXO. DAF-16/FOXO is acetylated by an acetyl-transferase CBP-1/CREB binding protein (CBP), whose inhibition leads to constitutive nuclear localization of DAF-16/FOXO (38). Host cell factor 1 (HCF-1) and enhancer of *akt-1* null 7 (EAK-7) are other regulatory factors that inhibit DAF-16/FOXO activity without altering its subcellular localization (39-41). DAF-16/FOXO also interacts with two highly homologous 14-3-3 protein family members, FTT-1/PAR-5 and FTT-2 (42, 43). The 14-3-3 proteins modulate the interaction between DAF-16/FOXO and other co-factors, such as SIR-2.1/sirtuin 1, an NAD-dependent

deacetylase (42, 44). These diverse interactions and post-translational modifications may help differentially regulate the activity of DAF-16/FOXO upon various environmental changes.

Downstream targets of DAF-16/FOXO were identified by using various approaches such as chromatin immunoprecipitation, bioinformatics, microarray and mRNA sequencing (31, 45-51). The DAF-16/FOXO target genes collectively contribute to longevity by enhancing cellular maintenance in animals with reduced IIS. Since many regulatory modes and targets of FOXO transcription factors are conserved among species, the longevity-regulatory modes of *C. elegans* DAF-16/FOXO are likely to be recapitulated in IIS-medicated longevity in mammals.

SKN-1, an oxidative stress-responsive NRF transcription factor, also contributes to the longevity conferred by reduced IIS (52, 53). Similar to DAF-16/FOXO, SKN-1 is sequestered in the cytoplasm by phosphorylation via the canonical IIS protein kinases, including AKT-1/-2 (52). SKN-1 mediates the expression of genes involved in detoxification and stress responses (52, 54-63). Overexpression of constitutively nuclear SKN-1 extends lifespan in a DAF-16/FOXO-independent manner (52). SKN-1 also promotes protein homeostasis through regulating proteasome production, which contributes to a longer lifespan (55, 57, 64). In addition, SKN-1 promotes longevity of animals with reduced IIS through remodeling of extracellular matrix (65).

HSF-1 is another important transcription factor acting downstream of IIS, and is essential for the longevity of animals with reduced IIS (66-70). The function of HSF-1 in promoting longevity and reducing proteotoxicity is closely associated with the conserved IIS pathway. Genetic inhibition of *hsf-1* accelerates tissue aging, thereby shortening the lifespan (71). Knockdown of *hsf-1* also suppresses the longevity phenotype of *daf-2* and *age-1* mutants; conversely, the overexpression of *hsf-1* is sufficient to extend lifespan (51, 66, 67, 69, 72, 73). HSF-1 binds to specific regions of DNA containing heat shock elements (HSEs) (74-76). The binding of HSF-1 to HSEs triggers the induction of genes encoding molecular chaperones, such as HSP-70 and HSP-16, whose overexpression extends lifespan (77, 78). Thus, HSF-1 appears to lead to longevity by up-regulating the chaperone network that enhances the proper folding of various proteins (66, 67). DDL-1 (the *C. elegans* homolog of human coiled-coil domain-containing protein 53: CCDC53), DDL-2 (the *C. elegans* homolog of human Wiskott-Aldrich syndrome protein and SCAR homolog: WASH2), and HSB-1 (heat-shock factor binding protein-1), form a complex with HSF-1 and regulate lifespan by inhibiting the activity of HSF-1 (69). Overall, HSF-1 and SKN-1 appear to promote longevity mainly through the induction of target genes that increase resistance to various stresses.

Systemic regulation of insulin/IGF-1 signaling-mediated longevity in *C. elegans*

As the IIS pathway consists of many potential endocrine components, it is likely that IIS regulates lifespan in a systemic manner. The *C. elegans* genome encodes 40 ILPs, which appear to act as extracellular endocrine signals in *C. elegans* (79, 80). Functional studies on several ILPs, including *ins-6* (81, 82), *ins-7* (47, 83, 84) and *daf-28* (80, 85, 86), have been conducted. However, the majority of the 40 ILPs, which potentially regulate longevity and development, are yet to be characterized in detail. This is perhaps because many possible combinations of the interactions between ILPs and DAF-2/insulin/IGF-1 receptor make it difficult to dissect the specific functions of each ILP. A recent study indicates that ILPs can function in a combinatorial manner to coordinate various physiological processes (87). This finding is different from the previous notion that ILPs generally confer a functional redundancy due to their structural similarities (79, 80, 88-91). Therefore, some individuals or a group of ILPs may have a profound effect on longevity.

Most of the ILPs are expressed in neurons, although some ILPs are expressed in non-neuronal tissues such as hypodermis and intestine (79-83, 88-90, 92-94). Overexpression of *ins-7* in the intestine decreases the activity of DAF-16/FOXO in non-intestinal tissues and shortens lifespan (83), suggesting an endocrine tissue-nonautonomous role of INS-7 in longevity. The expression of *daf-2* in neurons is largely responsible for the longevity of *daf-2* mutants (95, 96), pointing to the endocrine regulation of longevity by neuronal IIS. Together, it appears that IIS can systemically regulate lifespan from various tissues, via endocrine signaling.

The role of insulin/IGF-1 signaling in *C. elegans* physiology and age-related disease models

C. elegans that is exposed to unfavorable environmental conditions such as reduced food availability, extreme temperatures and a high population during development, enters an alternative diapause stage called dauer (97, 98). IIS is one of extensively studied signaling pathways that govern this dauer developmental decision (Fig. 2). Genetic inhibition of *daf-2* or *age-1*, which extends adult lifespan, can cause constitutive dauer formation even under favorable conditions (97, 98). This dauer formation requires key downstream effectors in IIS, including DAF-18/PTEN and DAF-16/FOXO (97, 98). Reduced IIS activates a transcriptional program through DAF-16/FOXO, which leads to dauer formation. These findings raise the possibility that IIS may regulate dauer decision and lifespan using same effectors. However, the regulation of longevity and dauer formation by IIS can be uncoupled. Neuronal DAF-16/FOXO plays a more important role in the dauer decision than in lifespan regulation, whereas intestinal DAF-16/FOXO has a more profound effect on the lifespan extension than on the dauer decision (99). In addition, IIS pathway regulates the lifespan exclusively during adulthood, while it regulates the dauer formation during early larval development (100). Thus, spatiotemporal regulation of IIS differentially influences two separate aspects of animal physiology, development and adult lifespan.

IIS also regulates resistance to a variety of stresses. *C. elegans*

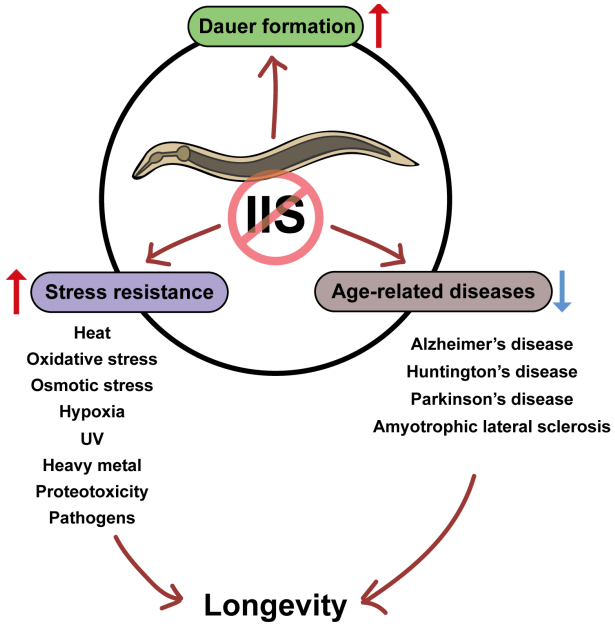

Fig. 2. The role of insulin/IGF-1 signaling in *C. elegans* physiology and age-related disease models. Insulin/IGF-1 signaling (IIS) regulates dauer formation, stress resistance, and the models of age-related diseases in *C. elegans*. Reduced IIS promotes dauer formation and enhances resistance to various external and internal stresses, and pathogens. Inhibition of IIS also ameliorates defects associated with various human disease models. These protective effects of reduced IIS contribute to organismal longevity.

with reduced IIS displays enhanced resistance to environmental stresses such as oxidative stress (Fig. 2) (52, 101-103), heat stress (104-106), hypoxic stress (107, 108), osmotic stress (109, 110), ultraviolet (UV) stress (36, 111), and heavy metal toxicity (112). Moreover, reduced IIS promotes better maintenance of internal homeostasis against cytosolic proteotoxicity (66, 113) and endoplasmic reticulum (ER) stress (114). The key downstream transcription factors of IIS that contribute to longevity, including DAF-16/FOXO (25, 26, 36, 66, 103, 106-116), HSF-1 (66, 67) and SKN-1 (52, 53), regulate these stress resistance phenotypes as well. Thus, proper regulation of IIS is crucial for the protection of *C. elegans* from both external and internal stresses.

Bacteria serve as a major food source for *C. elegans*, and are likely to be abundant in the natural habitats of *C. elegans*, such as rotten fruits. Therefore, it seems likely that *C. elegans* constantly comes in contact with various bacterial species, which may include pathogenic bacteria. To combat infection by pathogens, *C. elegans* is equipped with an innate immune system, and IIS is one of the most prominent innate immune signaling pathways (117). *C. elegans* with reduced IIS displays enhanced pathogen resistance, which is mediated by DAF-16/FOXO, HSF-1, and SKN-1 (84, 118-122). Reduced IIS leads to the induction of several antimicrobial genes (47), and reduction in bacterial packing in the intestine (123). Interestingly, *Pseudomonas aeruginosa*, a popular model bacterial pathogen in *C. elegans*, activates IIS to counteract the host immunity (124). Therefore, IIS may be located at the front of constant battles between the host *C. elegans* and its bacterial pathogens.

Because of its powerful genetics, *C. elegans* has also been widely used for modeling various human diseases, especially neurodegenerative diseases. The disease models of *C. elegans* were established by generating transgenic animals expressing various human disease-associated proteins; these include β-amyloid peptides (Aβ) for Alzheimer's disease (125-127), polyglutamine (polyQ) proteins for Huntington's disease (128-132), α-synuclein for Parkinson's disease (133-137), and a mutant superoxide dismutase 1 (SOD1) for amyotrophic lateral sclerosis (ALS) (138-141). The Alzheimer's disease model *C. elegans*, which expresses $A\beta_{1-42}$ in body wall muscles, is paralyzed and displays the accumulation of protein aggregates (68, 125, 142). Reduced IIS relieves these phenotypes via activating DAF-16/FOXO and HSF-1 (68), and inducing autophagic degradation of the protein aggregates (142). Reduced IIS also suppresses the short lifespan of $A\beta_{1-42}$-expressing animals (68). The *C. elegans* model for Huntington's disease has been widely used for studying proteotoxicity caused by aggregation of polyQ proteins (113, 128-131, 143-150). The polyQ-expressing worms display progressive neurodegeneration, neuronal dysfunction, retarded development, and defective motility (113, 128-131, 143-150). The *daf-2* and *age-1* mutations ameliorate a gradual age-dependent increase in toxicity resulting from polyQ aggregation through HSF-1 and DAF-16/FOXO (66, 113, 132, 146, 149). Parkinson's disease patients suffer from degeneration of dopaminergic neurons, which display accumulated protein inclusions that contain α-synuclein (151). Similarly, the *C. elegans* models for Parkinson's disease, which express wild-type or mutant human α-synuclein proteins in neurons, display the loss of dopaminergic neurons (133, 134, 137, 152). Reduced IIS by *daf-2* mutations dramatically suppresses this neurodegeneration phenotype (152). ALS, which is characterized by progressive motor neuron degeneration (153), has also been studied using a *C. elegans* model (138-141). Familial ALS is associated with mutations in the gene encoding SOD1 (154, 155). Neuronal expression of a mutant human SOD1 causes locomotion defects (140) and paralysis (141) in *C. elegans*. *daf-2* mutations protect the ALS model worms from the paralysis (141). Collectively, the results using *C. elegans* models indicate that IIS plays a crucial role in the pathophysiology of a majority of neurodegenerative diseases (Fig. 2). These findings imply that IIS modulates protein homeostasis to regulate normal neuronal functions, which may be essential for a long and healthy life.

INSULIN/IGF-1 SIGNALING PATHWAY AND *Drosophila melanogaster* AGING

Insulin/IGF-1 signaling components implicated in the longevity of *D. melanogaster*

The IIS pathway of *Drosophila melanogaster* consists of many components (Fig. 1), including the insulin/IGF receptor (dInR), the insulin receptor substrate (CHICO), the phosphatidylinositol 3-kinase (PI3K) Dp110/p60, 3-phosphoinositide-dependent protein kinase 1 (dPDK1) and the protein kinase B (PKB), also known as dAkt1, and the transcription factor *Drosophila* FOXO (dFOXO) (156-171). The activation mechanism of the IIS pathway in *Drosophila* has substantial similarities to that in *C. elegans*. Basically, the activation of dInR leads to up-regulation of a cascade of intracellular phosphorylation events, subsequently leading to the phosphorylation of dFOXO protein (160-162). dInR conveys signals from *Drosophila* insulin-like peptides (DILPs), directly to PI3K or to CHICO, the insulin receptor substrate (156, 172). PI3K, which converts PIP_2 to PIP_3, has a catalytic subunit, Dp110, and a regulatory subunit, Dp60 (158, 159). The action of PI3K is antagonized by the activity of dPTEN (173-175), which catalyzes PIP_3 to PIP_2. PIP_3 acts as an intracellular second messenger that activates a cascade of protein kinases, including dPDK1 and PKB/dAkt, which subsequently lead to the phosphorylation and the nuclear exclusion of dFOXO (162, 170). Conversely, reduced IIS through *dInR* or *CHICO* mutations, or overexpression of *dPTEN*, causes the translocation of dFOXO from the cytoplasm to the nucleus, where it up-regulates genes involved in longevity and stress resistance (160, 162, 176, 177).

In *Drosophila*, the IIS pathway regulates various physiological processes, including lifespan, stress responses, growth and development. Genetic inhibition of negative regulators of dFOXO, including several DILPs (178, 179), dInR (180), the IRS/CHICO (181, 182), or 14-3-3 epsilon (183), extends the lifespan of *Drosophila*. Conversely, overexpression of antagonistic IIS regulators, such as dPTEN or dFOXO, also extends the lifespan and/or delay heart aging (177, 184-186; but see also 187). Overall, these findings using *Drosophila* have remarkable similarities with those of *C. elegans*, highlighting the evolutionarily conserved nature of lifespan regulation by IIS components.

Endocrine regulation of lifespan by *Drosophila* insulin/IGF-1 signaling

The *Drosophila melanogaster* genome encodes eight DILPs (172, 188-190). The *dilp* genes display distinct temporal expression patterns. For example, *dilp2* is expressed from embryo to adult stages, whereas *dilp4* is expressed only during development prior to adulthood (172, 179, 191-195). In addition, the expression sites of the eight *dilp* genes are diverse (196). Notably, the major site of DILP production is the median neurosecretory cells (mNSCs) in the brain, also called insulin-producing cells (IPCs), where *dilp1*, *dilp2*, *dilp3* and *dilp5* are expressed (172, 179, 191-194).

Cell non-autonomous regulation of lifespan by *Drosophila* IIS was proposed based on findings using tissue-specific overexpression of IIS components. Up-regulation of dFOXO in the adult head fat body is sufficient to promote longevity and oxidative stress resistance (177). Muscle-specific overexpression of either dPTEN, dFOXO, or 4E-BP (a dFOXO target), also significantly increases lifespan (184). The ablation of IPCs lengthens lifespan (179, 197), which corroborates the endocrine regulation of lifespan by IIS; this is also reminiscent of lifespan extension by the ablation of sensory neurons in *C. elegans* (reviewed in 198). Among the DILPs expressed in the IPCs, DILP2 has been extensively explored for its implication in lifespan regulation, since it has the highest homology with human insulin (172, 177-179, 199-201). The *dilp2* null-mutant flies live long (178), and the expression of *dilp2* in the IPCs is reduced by the activation of dFOXO (177, 199). Therefore, *Drosophila* IIS appears to regulate the expression of DILPs and longevity via a feedback mechanism. Furthermore, reduced *dilp2* expression and inhibited IIS in the fat body are associated with lifespan extension conferred by transgenic expression of a dominant-negative p53 (200). DILP6, which is predominantly produced in the fat body, is another endocrine lifespan regulator (202-204). Surprisingly, overexpression of *dilp6* in the abdominal fat body leads to the repression of *dilp2* in the brain, suggesting synergistic effects on lifespan regulation by a potential dInR antagonist DILP6 and an agonist DILP2 (204). Collectively, lifespan regulation by IIS is controlled systemically by the action of DILPs that transmit signals, at least between the brain and fat body.

CONCLUSIONS

In this review, we have described findings regarding mechanisms by which IIS influences lifespan in two representative invertebrate models, *C. elegans* and *D. melanogaster*. The roles of many IIS components in aging are remarkably well conserved between *C. elegans* and *D. melanogaster*, and the intervention of the IIS leads to an extended lifespan in both animals. This suggests that the role of IIS in aging is likely to be conserved across phyla beyond these two species. Indeed, many findings using these invertebrate models have led to the discoveries demonstrating that changes in IIS can extend lifespan in mammals. For example, heterozygous IGF1 receptor- knockout mice ($Igf1r^{+/-}$) live longer than wild-type (205), and lower circulating IGF1 level correlates with mouse longevity (206). In addition, genetic variants of IIS components, including IGF1 receptor and FOXO3A, are associated with human longevity (4, 207). Thus, the evidence for the evolutionarily conserved nature of IIS-mediated longevity is extremely strong, ranging from invertebrates to humans.

Both *C. elegans* and *D. melanogaster* have been invaluable for the identification of IIS components and their roles in aging at the organism level. Still, much remains to be discovered

regarding the regulatory mechanisms of aging and longevity at the molecular level. As we can make new discoveries regarding organismal aging using these invertebrate models much faster than using vertebrates, *C. elegans* and *D. melanogaster* will continue to serve as indispensable tools for broadening our knowledge in aging. The progresses made by using these invertebrate models will eventually lead to the promotion of long and healthy human lives, and the prevention of age-associated diseases.

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