Chapter 4
Longevity Regulation by Insulin/IGF-1 Signalling

Seon Woo A. An*, Murat Artan*, Sangsoon Park*, Ozlem Altintas*, and Seung-Jae V. Lee

Abstract For the past three decades, many ageing-regulatory pathways have been identified using *C. elegans* as a model organism. The insulin/insulin-like growth factor (IGF)-1 signalling (IIS) pathway is one of the most evolutionarily well-conserved ageing-regulatory pathways ranging from worms to mammals. Here, we review the molecular mechanism and the functional significance of IIS in *C. elegans* ageing. Specifically, we describe the roles of key components of IIS in ageing, systemic ageing regulation by IIS, and other known physiological functions of IIS that contribute to longevity. We also discuss possible implications of IIS in mammalian health and ageing.

Keywords Ageing • Longevity • *C. elegans* • Insulin/IGF-1 signalling • *daf-2* • FOXO • Systemic regulation • Sensory neurons

4.1 Introduction

C. elegans insulin/insulin-like growth factor (IGF)-1 signalling (IIS) is one of the most established ageing-regulatory pathways, whose components have been extensively studied. In *C. elegans*, IIS is also important for resistance against various stresses, and this is consistent with many findings showing that enhanced stress resistance contributes to longevity. In addition, decreased levels of IIS prevent protein aggregation and delay the onset of many ageing-associated disease models in *C. elegans*. The function of IIS as a lifespan-regulatory pathway is evolutionarily conserved in *Drosophila*, mice, and very likely, in humans [1, 2]. In this chapter, we will describe mechanisms by which IIS plays roles in the regulation of ageing, stress resistance, and age-associated disease models. Further, we will discuss the implications that these findings in *C. elegans* have on human ageing.

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4.2 Components That Influence Lifespan in the Insulin/IGF-1 Signalling Pathway

The IIS pathway is composed of various signal-transducing factors, and the role of each component in lifespan regulation is relatively well-characterized in *C. elegans* (Fig. 4.1). *age-1* mutants were the first long-lived IIS mutants identified through a genetic screen [3, 4]. Subsequently, *daf-2* mutants, which have been known to display phenotypes in the development of dauer (an alternative diapause larva, discussed in Chap. 3), were shown to live twice as long as wild-type *C. elegans* [5]. *age-1* and *daf-2* were eventually shown to encode a phosphoinositide-3 kinase (PI3K) and an insulin/IGF-1 receptor, respectively [6, 7]; these are the key upstream components of IIS. Since then, many more factors that act downstream of the IIS pathway have been identified in *C. elegans*.

Inhibition of IIS promotes long lifespan in *C. elegans*. Specifically, the reduced function of DAF-2 results in the inactivation of the downstream kinase cascade, starting from AGE-1/PI3K [[8]; reviewed in [9]]. Down-regulation of AGE-1 then leads to the inactivation of 3-phosphoinositide-dependent kinase 1 (PDK-1) [10], likely through a decrease in the PI(3, 4, 5)P_3/PI(4, 5)P_2 ratio [11]. This, in turn,

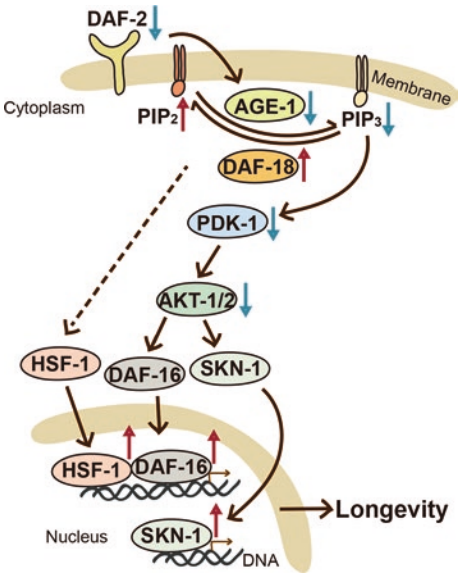

Fig. 4.1 Reduced IIS increases lifespan in *C. elegans*. Inhibition of DAF-2/insulin/IGF-1 receptor decreases the PI(3,4,5)P_3/PI(4,5)P_2 ratio through down-regulation of AGE-1/PI3 kinase, whose function is antagonized by the activation of DAF-18/PTEN. This decrease leads to the inactivation of PDK-1 and AKT-1/2, which subsequently promotes the nuclear translocation and activation of DAF-16/FOXO, and SKN-1/NRF2 transcription factors. HSF-1/heat shock factor 1 also collaborates with DAF-16 in the nucleus. These transcription factors regulate the expression of various genes that contribute to longevity in *C. elegans*

down-regulates the Akt/protein kinase B (PKB) family members, AKT-1 and AKT-2 [10, 12]. The PI(3, 4, 5)P_3/PI(4, 5)P_2 ratio can also be decreased by the activation of DAF-18/phosphatase and tensin (PTEN) phosphatase, which mediates dephosphorylation of PI(3, 4, 5)P_3 and increases lifespan [8, 13–17]. Down-regulation of IIS also leads to the activation of transcription factors, which up-regulate the expression of various target genes that contribute to longevity, including chaperones, antioxidants, and antimicrobials. The representative longevity transcription factors downstream of IIS are DAF-16/Forkhead box O (FOXO), heat-shock transcription factor-1 (HSF-1), and skinhead-1 (SKN-1)/Nuclear factor-erythroid-related factor (Nrf).

DAF-16 DAF-16 is a FOXO transcription factor homologue [18, 19] that mediates a diverse array of cellular processes by regulating the expression of numerous genes, including those involved in ageing [20–25]. A variety of post-transcriptional regulators of this protein, including protein kinases and phosphatases, have been identified. Both AKT-1 and AKT-2 phosphorylate and inactivate DAF-16 by preventing nuclear translocation [26–30]. Phosphorylation of DAF-16 by serum/glucocorticoid-inducible kinase 1 (SGK-1)/SGK was also shown to obstruct the translocation into the nucleus [30]. However, subsequent studies using a *sgk-1* gain-of-function mutant or overexpression of *sgk-1* indicate that SGK-1 may activate DAF-16 [31, 32]. AMP (5′ adenosine monophosphate)-activated protein kinase (AAK-2) can also activate DAF-16 by phosphorylation and increases lifespan [33–36]. Similarly, CST-1/MST kinase and JNK-1/c-Jun N-terminal kinase phosphorylate and up-regulate DAF-16 to extend lifespan [37, 38]. Protein phosphatases also appear to regulate the activity of DAF-16 directly or indirectly. For example, SMK-1/suppressor of MEK null (SMEK), a homologue of the protein phosphatase 4 regulatory subunit, is required for the long lifespan of *daf-2* mutants in a *daf-16*-dependent manner [39]. PPTR-1/protein phosphatase 2A regulatory subunit (PP2A) decreases the phosphorylation of AKT-1 and leads to both activation of DAF-16 and increased longevity in *daf-2* mutants [40].

Other regulatory modes for DAF-16 include protein acetylation, protein stability control, protein-protein interactions, and transcriptional control of its isoforms. CBP-1/CREB-binding protein (CBP), which is an acetyl-transferase, contributes to the longevity of *daf-2* mutants [41], likely via acetylating and activating DAF-16 [42]. DAF-16 is also required for the long lifespan conferred by the overexpression of *sir-2.1*/NAD-dependent protein deacetylases [[43–45] but see also [46]]. Components of the ubiquitin proteasome system regulate the stability and activity of DAF-16. Specifically, an E3 ligase, RLE-1/RC3H1, ubiquitinates DAF-16, and consequently, *rle-1* mutants live long due to increased stability of DAF-16 [47]. MATH-33/deubiquitylase counteracts the RLE-1-dependent degradation of DAF-16 and extends lifespan [48]. In addition, components of the Skp1-Cul1-F-Box E3 ligase complex contribute to the longevity of *daf-2* mutants, perhaps by indirectly up-regulating DAF-16 [49]. Additionally, proteasome activation promotes long lifespan by increasing DAF-16 activity [50]. Scaffold proteins are also important for DAF-16 regulation. Genetic inhibition of the 14-3-3 scaffold protein, PAR-5 or

FTT-2, up-regulates DAF-16 by promoting its nuclear translocation [44, 51]. However, overexpression of these proteins paradoxically extends lifespan in a *daf-16*-dependent manner [52]. Another scaffold protein, SHC-1/Shc-like protein, promotes the nuclear localization of DAF-16 by acting upstream of JNK-1 [53]. In addition to these post-translational modes for regulation, the expression of different DAF-16 isoforms can be regulated at the transcription level [54, 55].

DAF-16 regulates the expression of its target genes by binding to specific DNA motifs: the DAF-16-binding element (DBE) and the DAF-16-associated element (DAE). The DBE was first identified using an iterative *in vitro* method, and the core sequence, TTGTTTAC, is located upstream of DAF-16 target genes [56]. DAE is a GATA sequence, CTTATCA, which is located within the promoters of many DAF-16 target genes [21, 57–59].

Several factors affect the downstream targets of DAF-16. For example, the PQM-1, a C2H2-type zinc finger and leucine zipper-containing transcriptional activator, increases the expression of DAF-16 targets by translocating in the opposite direction of DAF-16 in cells, and contributes to *daf-2* mutant longevity [59]. The ELT-2 and ELT-3/GATA factors, and MDT-15/mediator 15, also induce the expression of DAF-16 target genes [57, 60]. The XBP-1/bZIP transcription factor, along with DAF-16, enhances the expression of the DOX-1/Zn-finger protein [61]. Conversely, the ETS-4/ETS transcription factor alters the expression of a subset of DAF-16 target genes to promote longevity via a non-canonical IIS [62]. In addition, DAF-16 requires other cofactors to induce target gene expression; these include the HEL-1/RNA helicase [63], the PRMT-1/type I protein arginine methyltransferase [64], and the SWI/SNF/chromatin remodeler [65].

HSF-1 HSF-1 is a heat-shock transcription factor that induces transcription of chaperone genes and proteasome-related genes in response to various stresses, including heat [reviewed in [66]]. HSF-1 collaborates with DAF-16 to promote longevity that results from reduced IIS activity [67]. Inhibition of *hsf-1* decreases the long lifespan of *daf-2* and *age-1* mutants, and conversely overexpression of *hsf-1* is sufficient to increase lifespan [67, 68]. Neuron-, muscle-, or intestine-specific overexpression of *hsf-1* is also sufficient to extend lifespan [68, 69]. Experiments involving the temporal knockdown of *hsf-1* indicate that HSF-1 expression during larval stages is more crucial than during adulthood [70]; this result, however, is in contrast to the observation that DAF-16 is required during adulthood for *daf-2* mutant longevity [71]. HSF-1 regulates the expression of its target genes by binding to the heat-shock element (HSE), GAANNTTCNNGAA [72]. Together with DAF-16, HSF-1 regulates the expression of chaperone genes, including small heat-shock protein-encoding genes, which contribute to the longevity of *daf-2* mutants [21, 67, 68, 73, 74]. Moreover, truncated HSF-1 overexpression increases lifespan by improving actin cytoskeletal integrity, independently of typical molecular chaperone functions [75].

Several regulators of HSF-1 in IIS have been discovered. These include *daf-16*-dependent longevity-1 and -2 (DDL-1 and -2), which inhibit HSF-1 activity through the formation of a DDL-1-containing HSF-1-inhibitory complex (DHIC) [74].

Under reduced IIS conditions, DDL-1 is phosphorylated, and DHIC is dissociated to activate HSF-1 for lifespan extension [74]. DAF-41/co-chaperone p23 regulates lifespan via HSF-1, as well as DAF-16, at high temperature [76]. HSF-1 has also been shown to act as a hub protein that mediates crosstalk between IIS and target of rapamycin (TOR) signalling pathways [77]. Overall, HSF-1 is a key regulator for IIS-mediated longevity and appears to be as important as DAF-16.

SKN-1 Another crucial longevity-promoting transcription factor in IIS is SKN-1 [reviewed in [78]], an oxidative stress-responsive Nrf transcription factor [79]. Genetic inhibition of *skn-1* largely suppresses the long lifespan of *daf-2* mutants [80], and *skn-1* overexpression is sufficient to promote long lifespan [80]. Elimination of a putative AKT phosphorylation site enhances the nuclear translocation of SKN-1. Therefore, similar to DAF-16, dephosphorylated and nuclear-localized SKN-1 appears to promote longevity under conditions of reduced IIS [80]. SKN-1 regulates the expression of a number of genes involved in several stress responses [80–83] and protein translation [84, 85], many of which overlap with DAF-16 target genes [80, 84]. SKN-1 also up-regulates collagens to promote longevity by extracellular matrix (ECM) remodelling [86].

Various additional factors that affect the activity of DAF-16, HSF-1, and SKN-1, or the expression of their target genes, have been identified. Many of these additional factors work together to regulate the activity of the transcription factors in IIS-mediated longevity. Some of the molecular mechanisms by which these transcription factors are regulated have been revealed; however, most remain incompletely understood. Therefore, further research on these crucial transcription factors will be required to understand the fundamental mechanisms of IIS-mediated ageing regulation in *C. elegans*.

4.3 Sensory Neural Regulation of Longevity

C. elegans has a simple nervous system, comprised of 302 neurons, which have been mapped in detail [87] (see also Chaps. 2 and 8). Well-known functions of sensory neurons include the perception of environmental stimuli and the transmission of signals for proper physiological responses. Interestingly, sensory neurons in *C. elegans* also contribute to lifespan regulation [reviewed in [88]]. Chemosensory neurons appear to affect lifespan mostly by acting through IIS [89], whereas thermosensory neurons regulate lifespan via steroid signalling at high temperature [90]. Impairment of general chemosensory neuronal functions leads to the activation of DAF-16 and longevity via modulating the expression of insulin-like peptides (ILPs); chemosensory mutations also do not further extend the longevity of *daf-2* mutants [27, 89, 91–93]. Thus, it is likely that the inhibition of chemosensory neurons down-regulates IIS activity, and this may in turn activate DAF-16 to promote longevity (Fig. 4.2).

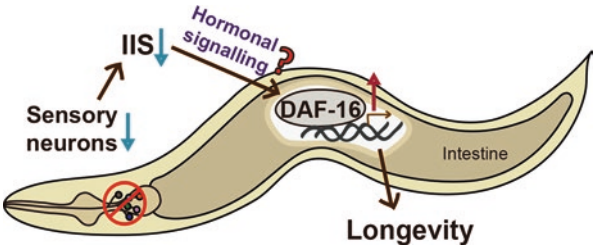

Fig. 4.2 Neuroendocrine regulation of IIS and longevity. Inhibition of sensory neural functions leads to down-regulation of IIS. This inhibition modulates the expression of hormonal insulin-like peptides that are secreted from sensory neurons, triggering the activation of DAF-16 in non-neuronal tissues, such as the intestine. Activated DAF-16 then translocates into the nucleus, where it induces the expression of target genes that confer organismal longevity

Inhibition of various components required for chemosensory neural function increases lifespan. These include the calcium-regulated neurosecretory factors, G-protein coupled receptors, G-proteins, cyclic nucleotide-gated channel subunits, and proteins that function in sensory signal transduction and synaptic transmission [89, 91, 92, 94–99]. Additionally, it has been shown that the induction of *mct-1*, a putative monocarboxylate transporter for small molecule trafficking, mediates the long lifespan of sensory mutants [100]. Further, a thermosensitive TRP channel, TRPA-1, increases lifespan by activating DAF-16 at lower temperatures in *C. elegans* [32, 101]. A recent study also demonstrated that food-derived chemosensory cues decrease lifespan via stimulating sensory neurons, which in turn increases the expression of an ILP/INS-6 that acts as an endocrine IIS-activating signal [93].

4.4 Endocrine Signalling and Tissue Specificity for IIS-Mediated Longevity Regulation

The discovery of the IIS-mediated longevity pathway in *C. elegans*, combined with the fact that mammalian IIS is regulated by insulin and IGF hormones, implies the presence of endocrine-mediated ageing regulation (Fig. 4.2). Extensive genetic and bioinformatic studies have identified 40 members of the ILP superfamily in *C. elegans*, including insulin (INS)-1 through INS-39, and DAF-28 [102–107]. *C. elegans* ILPs are structurally different from mammalian insulins, since most lack a connecting peptide (C-peptide), which is a typical feature of the mammalian counterparts. In addition, some *C. elegans* ILPs have a different inter-chain disulphide bond conformation between conserved cysteine residues [102, 105]. Interestingly, INS-6, which lacks the C-peptide, can bind to the human insulin receptor [108]. Thus, *C. elegans* ILPs may function as ligands for the DAF-2, despite the structural divergence.

Among the 40 ILPs that have been identified to date, only a few have been functionally characterized in depth, perhaps because of their redundancy and/or

complexity [93, 104–106, 109–117]. ILPs are known to modulate the activity of DAF-2 by acting as either agonists (e.g., INS-6 and DAF-28) or antagonists (e.g., INS-1) [21, 93, 105, 106, 111, 117–120]. However, some ILPs, such as INS-18 and INS-7, can serve as both agonists and antagonists of DAF-2 in a context-dependent manner [104, 105, 109, 112, 116, 121]. Recent studies have characterized the expression patterns and functions of all ILPs systematically [121, 122]. In contrast to the previous notion that ILPs function redundantly [[117, 122] also reviewed in [123]], these studies have suggested that ILPs can constitute combinatorial codes for the regulation of development and physiology in *C. elegans* [121]. Thus, ILPs appear to have distinct roles as individuals and to regulate various physiological outputs as members of an intricate ILP-regulatory network.

Various tissues in *C. elegans* express ILPs and appear to regulate IIS in an endocrine manner. ILPs are mainly expressed in neurons, although a few have also been shown to be expressed in other tissues, such as the intestine and the hypodermis [93, 105, 106, 109, 111, 115–117, 119, 120, 122, 124]. These expression patterns of ILPs imply that the nervous system of *C. elegans* may be a key regulatory centre for endocrine IIS. Consistent with this idea, neuronal IIS has a large impact on organismal physiology. For example, DAF-2, AGE-1, and DAF-18 regulate lifespan cell non-autonomously in the nervous system [125–127]. In addition, disruption of sensory neurons increases lifespan and up-regulates DAF-16 in the intestine and the hypodermis by decreasing the expression of INS-6 and DAF-28 [93]. Neuronal *daf-16* contributes to the long lifespan of *daf-2* mutants [128], again pointing to the important role of the nervous system in endocrine regulation of IIS-induced longevity.

Tissues other than neurons also play substantial roles in the endocrine IIS-regulated lifespan in *C. elegans*. The intestine of *C. elegans* is the major digestive organ [129] and serves as a signalling centre for nutritional status. Thus, IIS in the intestine may transmit signals regarding nutritional status to regulate organismal physiology. In fact, intestine-specific expression of *daf-16* substantially restores the longevity of *daf-2* mutants [128]. The intestine also regulates the expression of ILPs, in particular *ins-7*, to modulate IIS in distant tissues via a positive feedback loop [109]. In addition, intestinal *daf-16* prevents age-dependent deterioration of muscle [60]. Overall, this endocrine IIS system appears to coordinate the rates of ageing among different *C. elegans* tissues.

4.5 The Role of IIS in Stress Resistance and Age-Related Disease Models

In addition to lifespan, the *C. elegans* IIS pathway regulates various other physiological processes. For example, reduced IIS enhances resistance to a number of stresses, including heat [130, 131], oxidative stress [132–134], and osmotic stress [135], as well as hypoxia [136, 137]. Reduced IIS also allows *C. elegans* to

successfully cope with heavy metal toxicity [138], ultraviolet (UV) radiation [139], endoplasmic reticulum (ER) stress [61], and cytosolic proteotoxicity [67, 68, 140]. This signifies the importance of IIS pathway-regulated mechanisms for healthy ageing.

Stress resistance resulting from reduced IIS is mediated by a variety of factors, including longevity-promoting transcription factors DAF-16, HSF-1, and SKN-1 (see Sect. 4.2). For example, DAF-16 contributes to enhanced thermotolerance and resistance to hypertonicity, UV, heavy metals, and hypoxia conferred by reduced IIS [67, 130, 131, 135–139, 141–143]. Reduced IIS also protects against oxidative stress by triggering the activation of DAF-16 and SKN-1 [26–28, 39, 79, 80, 86, 132–134, 144]. The SMK-1 and EGL-27/GATA transcription factor promote UV resistance in *daf-2* mutants [39, 142, 145]. XBP-1, a key mediator of the ER unfolded protein response (UPR^{ER}), collaborates with DAF-16 to enhance UPR^{ER} in *daf-2* mutants [61]. Additionally, HSF-1, together with DAF-16, contributes to enhanced cytosolic protein homeostasis conferred by reduced IIS [67, 68]. The decreased levels of IIS also protect somatic cells from various stresses by equipping these cells with many characteristics of germline stem cells [146]. Overall, IIS-mediated stress resistance contributes to the proper management of stresses through a variety of factors, which are also essential for longevity.

Innate immunity ensures survival in the presence of pathogenic threats. *C. elegans* has an innate immune system that is regulated by evolutionarily conserved signalling pathways, one of which is the IIS pathway. Reduced IIS activity increases resistance to various fungal and bacterial pathogens via DAF-16 [147, 148], in parallel to the well-known immune regulator, p38 MAP kinase [147–151]. The transcription factors SKN-1 and HSF-1 also mediate the enhanced pathogen resistance under conditions of reduced IIS [152, 153]. *daf-2* mutants display mitigated internal bacterial colonization, enhanced bacterial clearance, and increased expression of antimicrobial genes [21, 151]. Moreover, *daf-2* mutants display enhanced efficiency in RNA interference (RNAi) [154], which is important for antiviral defence in *C. elegans* [155–157]. Thus, it will be interesting to test whether *daf-2* mutants are resistant to viral infections as well.

Importantly, reduced IIS has been shown to alleviate the pathological features of various disease models in *C. elegans*, including Huntington's disease [140, 158], Alzheimer's disease [159, 160], Parkinson's disease [161], and amyotrophic lateral sclerosis (ALS) [162] (Fig. 4.3). In a Huntington's disease model, reduced IIS ameliorates the polyglutamine (polyQ) aggregation mediated by CAG repeats in a DAF-16- and HSF-1-dependent manner [67, 140, 163, 164]. In a model for Alzheimer's disease, reduced IIS protects *C. elegans* from the toxicity caused by $A\beta_{1-42}$ expression via DAF-16, HSF-1, and autophagy [165, 166]. In a Parkinson's disease (PD) model, *C. elegans* expressing human α-synuclein in neurons displays both a motor deficit and progressive degeneration of dopaminergic neurons [161]; however, *daf-2* mutations result in complete retention of these dopaminergic neurons [164]. ALS originates from mutations in various genes, including superoxide dismutase 1 (*SOD1*) [167]. In a *C. elegans* model, *daf-2* mutations protect against the toxic mutant *SOD1*-induced motor neuron dysfunction by decreasing protein aggregation

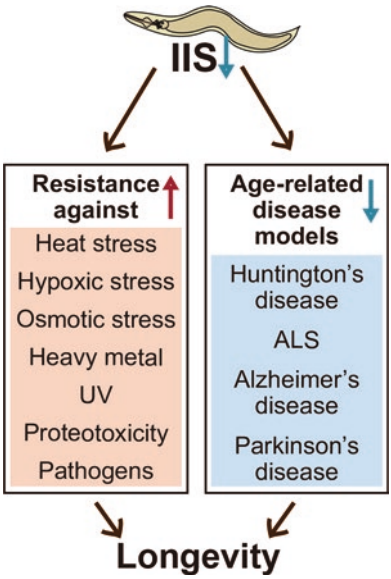

Fig. 4.3 The role of IIS in stress resistance and human disease models. Reduced IIS confers enhanced resistance against a variety of stresses, including heat, hypoxia, high osmolarity, heavy metals, UV radiation, proteotoxicity, and pathogens. Reduced IIS also ameliorates the impact of age-related human disease models in *C. elegans*, including those for Huntington's disease, amyloid lateral sclerosis (ALS), Alzheimer's disease, and Parkinson's disease. These features correlate with healthy ageing and longevity

[168]. Overall, it appears that the enhanced protein homeostasis conferred by reduced IIS underlies the protective mechanisms against these degenerative disease models in *C. elegans* [169, 170]. It is noteworthy that *daf-2* mutations delay age-dependent neuronal degeneration [171] and neurite branching [172]. Mutations in *daf-2* also enhance memory and learning capacity in early adulthood, and delay an age-dependent decline in short-term memory in a DAF-16-dependent manner [173]. These data strongly suggest that proper manipulation of the evolutionarily conserved IIS pathway in *C. elegans* may shed light on the molecular basis of age- and/or disease-induced defects. Further, this pathway may hold therapeutic potential for the treatment of various degenerative diseases.

4.6 Conclusions

In this chapter, we reviewed the functions of IIS and the mechanisms by which it influences *C. elegans* longevity. The entire IIS pathway appears to play a central role in linking environmental signals, such as food availability and stresses, to various physiological outputs, including ageing, reproduction, and development. Therefore, one possible reason why the IIS pathway has a huge impact on ageing is

because this system responds to changes in environmental conditions and alters physiological outputs accordingly. Thus, under favourable conditions, IIS may be activated to promote growth and reproduction, which may lead to normal or shortened lifespan. Conversely, under unfavourable conditions, such as food shortages, IIS is down-regulated and activates genetic programmes to promote organism-wise maintenance, rather than growth and reproduction; this may lead to a longer lifespan. Therefore, enhanced longevity may be associated with slow growth and reduced reproduction. Indeed mutations in many components of IIS result in developmental arrest (see Chap. 3) and reduced fecundity, as well as longevity. However, it is worth pointing out that the regulation of organismal development and ageing by IIS can be uncoupled by temporally modulating the signalling [71]. Further dissection of the pleiotropic aspects of IIS will be crucial for understanding the specific contribution of IIS to ageing regulation.

The establishment of the role of IIS in ageing has paved the way for discoveries showing that various IIS components, such as insulin receptor and IGF-1 receptor, as well as the AKT kinases and FOXO transcription factors, regulate mammalian longevity. These findings have further led to the identification of genetic variants of IGF-1 receptor and *FOXO3A* that are associated with human longevity [1, 174]. Therefore, the conservation between invertebrate models and mammals, including humans, will help us to understand the biology of human ageing. Ultimately, what we have learned from *C. elegans* IIS can potentially lead to therapies aimed at delaying the onset of ageing-associated diseases and achieving a healthier and longer life in humans.

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