Molecules and Cells

Minireview

Recent Progress in Regulation of Aging by Insulin/IGF-1 Signaling in *Caenorhabditis elegans*

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Caenorhabditis elegans **has been used as a major model organism to identify genetic factors that regulate organismal aging and longevity. Insulin/insulin-like growth factor 1 (IGF-1) signaling (IIS) regulates aging in many species, ranging from nematodes to humans. *C. elegans* is a nonpathogenic genetic nematode model, which has been extensively utilized to identify molecular and cellular components that function in organismal aging and longevity. Here, we review the recent progress in the role of IIS in aging and longevity, which involves direct regulation of protein and RNA homeostasis, stress resistance, metabolism and the activities of the endocrine system. We also discuss recently identified genetic factors that interact with canonical IIS components to regulate aging and health span in *C. elegans*. We expect this review to provide valuable insights into understanding animal aging, which could eventually help develop anti-aging drugs for humans.**

Keywords: aging, *Caenorhabditis elegans*, health span, homeostasis, insulin/IGF-1 signaling, longevity

INTRODUCTION

Aging is accompanied by gradual time-dependent functional and structural changes at the molecular and cellular levels, usually leading to impaired health, age-related diseases and increased vulnerability to death in organisms (Lee et al., 2021a; 2015c; Lopez-Otin et al., 2013; Melzer et al., 2020; Son et al., 2019). The nematode *Caenorhabditis elegans* is a widely used model organism for aging research because of its relatively short lifespan (2-3 weeks) and genetic tractability. In addition, 83% of the *C. elegans* genes are homologous to human genes (Lai et al., 2000). Many studies have identified genes and pathways that affect aging and longevity using *C. elegans*. One of the most crucial breakthrough findings in the research field of aging is that missense mutations in *daf-2* double the lifespan of *C. elegans* (Kenyon et al., 1993). Subsequent studies have shown that *daf-2* encodes an insulin/insulin-like growth factor-1 (IGF-1) receptor homolog (Kimura et al., 1997) and that insulin/IGF-1 signaling (IIS) affects aging in diverse species, ranging from *C. elegans* to mammals, including humans (reviewed in Altintas et al., 2016; An et al., 2017; Kenyon, 2010). IIS is initiated by binding of agonistic or antagonistic insulin-like peptides (ILPs) to the DAF-2/insulin/IGF-1 receptor in *C. elegans*. The binding of an agonistic ILP to DAF-2 leads to the activation of phosphoinositide-3 kinase (PI3K) cascade composed of kinases, including AGE-1/PI3K, phosphoinositide-dependent kinase-1 (PDK-1)/3-phosphoinositide-dependent protein kinase 1, and AKT-1,2/protein kinase B (PKB). The activation of IIS subsequently inhibits multiple transcription factors, such as DAF-16/Forkhead box O (FOXO), heat shock factor-1 (HSF-1), and SKN-1/nuclear factor erythroid 2-related factor (NRF), thereby repressing their target genes that promote longevity. Conversely, reduction-of-function mutations in *daf-2* decrease the activity of the downstream kinase cascade, activating those transcription factors and inducing their target genes, and result in

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increased lifespan. The mechanisms by which reduced IIS promotes longevity have been attributed to diverse cellular and molecular aspects, including enhanced protein homeostasis, also known as proteostasis, and resistance to various stresses.

Here we review recent progress in studies regarding aging regulation by IIS using *C. elegans*, focusing on relevant works reported after our previous review paper covered a similar topic in 2016 (Altintas et al., 2016). We discuss findings

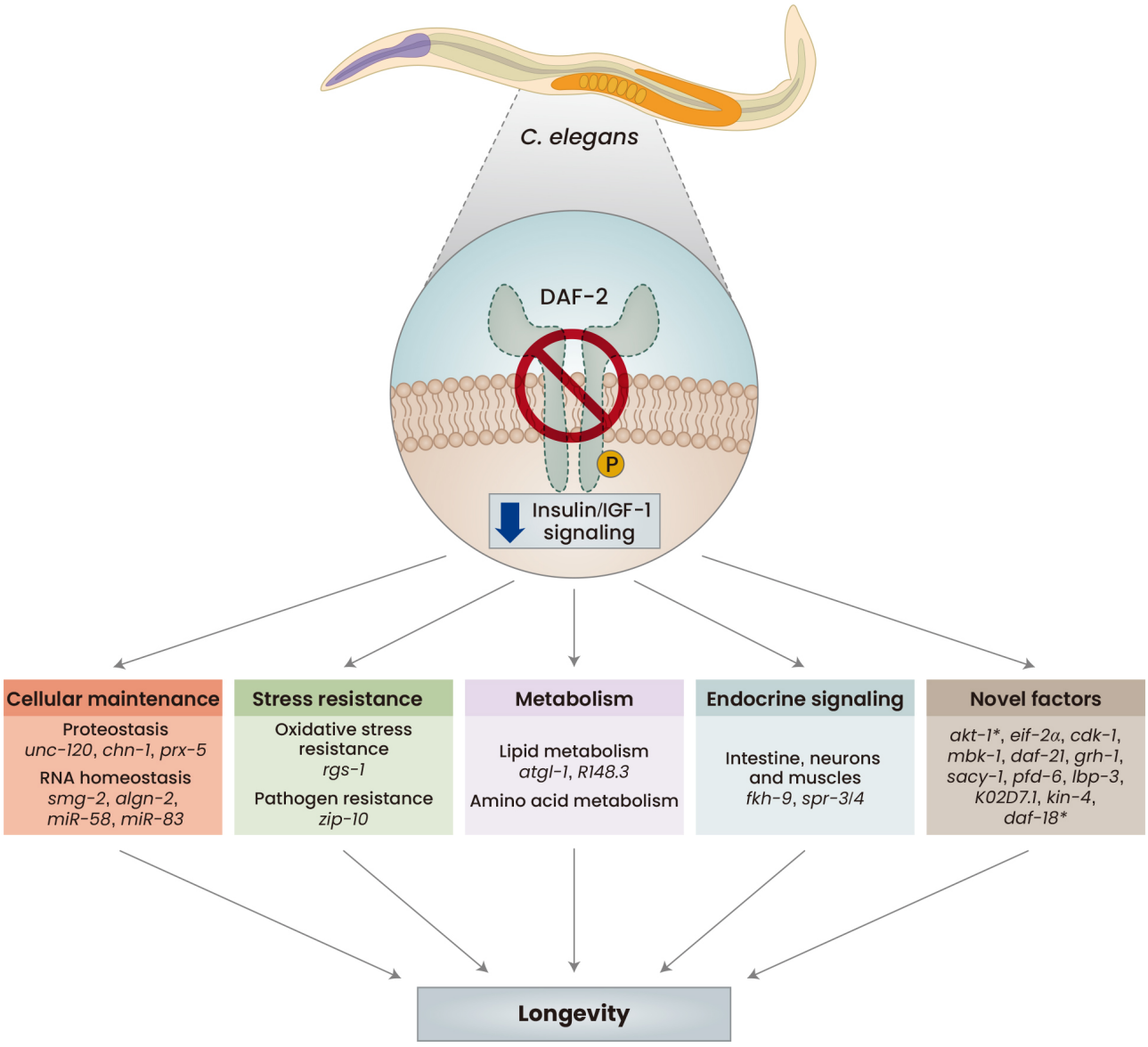

Fig. 1. Summary of recent progress in the roles of insulin/IGF-1 signaling (IIS) in *C. elegans* longevity. Reduced IIS promotes longevity by enhancing cellular maintenance, including proteostasis, via increasing autophagy and lysosome activities, RNA homeostasis, through regulating nonsense-mediated mRNA decay (NMD) and microRNAs (miRNAs), and oxidative stress and pathogen resistance. IIS also regulates aging by modulating lipid and amino acid metabolism, endocrine signaling among several tissues, including the intestine, neurons and muscles, and the activities of novel factors that interact with classical IIS components. These processes cooperatively contribute to longevity. The roles of the genes in aging regulation shown in this figure are discussed in the corresponding sections of the text more in detail. *unc-120*/serum response factor, *chn-1*/C-term of Hsp70-interacting protein (*CHIP*), *prx-5*/peroxisomal biogenesis factor 5 (*PEX5*), *smg-2*/*UPF1*, *algn-2*/alpha-1,3/1,6-mannosyltransferase (*ALG2*), *rgs-1*/regulator of G protein signaling 20 (*RGS20*), *zip-10*/bZIP transcription factor, adipose triglyceride lipase-1 (*atgl-1*), forkhead transcription factor-9 (*fkh-9*), *spr-3/4*/repressor element-1 silencing transcription factor (*REST*), initiation factor (EIF)-2α (*eif-2α*), cyclin-dependent kinase 1 (*cdk-1*), *mbk-1*/human dual specificity tyrosine phosphorylation regulated kinase 1A (*DYRK1A*), *daf-21/Hsp90*, prefoldin 6 (*pfd-6*), suppressor of ACY-4 sterility 1 (*sacy-1*), *lbp-3*/fatty acid-binding protein, *K02D7.1*/purine nucleoside phosphorylase, *kin-4*/microtubule-associated serine/threonine kinase, and *daf-18*/phosphatase and tensin homolog (*PTEN*). Asterisk (*), new alleles.

showing that IIS affects aging by regulating cellular maintenance systems, including proteostasis and RNA homeostasis, stress resistance, metabolism, and the endocrine system (Fig. 1). We also review novel genetic factors that interact with canonical IIS components that influence longevity in *C. elegans*. Overall, the current review will provide useful information for our better understanding of the conserved aging-regulating roles of IIS in humans, thereby devising therapeutic strategies for developing medications and treating age-related diseases.

MAIN BODY

Reduced IIS promotes longevity by enhancing cellular maintenance

The error catastrophe theory of aging proposes that aging results from errors in mRNA translation, increasing inaccurate protein synthesis (Orgel, 1963). This inaccurate protein synthesis accumulates misfolded or aggregated proteins, common features of age-associated diseases such as neurodegenerative diseases (Soto and Pritzkow, 2018). Therefore, to prevent harmful effects caused by the formation of erroneous proteins, organisms are equipped with protein quality control systems that monitor abnormal proteins, subsequently helping these proteins fold properly or degrading the proteins, maintaining proteostasis (Wolff et al., 2014).

Autophagy is a lysosome-dependent protein quality control system, which degrades abnormal cellular components and organelles (Levine and Kroemer, 2019; Shin, 2020). Autophagy is required for *C. elegans* longevity conferred by many regimens, including reduced IIS (Artan et al., 2022; Lee et al., 2015c; Nieto-Torres and Hansen, 2021). By performing autophagic flux assays in multiple tissues, autophagy activity has been shown to decrease with age in the intestine, the body wall muscles, the pharynx, and neurons (Chang et al., 2017). *daf-2* mutants display increased autophagic activities throughout aging in several tissues, including the intestine and muscles, suggesting that reduced IIS enhances proteostasis by upregulating autophagy in these tissues. Additionally, the myogenic transcription factor, UNC-120/serum response factor, activates gene expression in the muscles during aging (Mergoud Dit Lamarche et al., 2018). UNC-120 contributes to maintaining autophagic activities in the muscles of *daf-2* mutants. In addition to autophagy, the functional and anatomical features of lysosomes decline with age in *C. elegans* (Sun et al., 2020). *daf-2* mutations suppress age-associated increases in lysosome volume and decreases in lysosomal motility, acidity and degradation activity, in a DAF-16/FOXO- and SKN-1/NRF-dependent manner. Furthermore, lysosome activity is required for the clearance of protein aggregation and longevity in *daf-2* mutants. Thus, maintaining protein homeostasis by autophagy and lysosome activity during aging is pivotal for longevity conferred by reduced IIS.

Proper protein turnover contributes to proteostasis by removing damaged proteins. Several studies have reported the effect of IIS on age-associated proteome-wide changes in the protein abundance and turnover rates of *C. elegans* (Depuydt et al., 2016; Dhondt et al., 2016; Narayan et al., 2016; Visscher et al., 2016). Quantitative mass spectrometry indicates that the abundance of proteins involved in cellular stress responses increases in *daf-2* mutants (Narayan et al., 2016). In contrast, proteins acting in cellular metabolic pathways, including those regulating lipid and amino acid metabolism, tend to be downregulated. Among the proteins exhibiting age-dependent downregulation, the decreased level of PRX-5/peroxisomal biogenesis factor 5 (PEX5) results in dysfunction of peroxisomal protein import. Surprisingly, most age-dependently altered proteins do not scale with delayed biological aging caused by *daf-2* mutations. Thus, changes in the level or the activity of a small number of essential proteins appear to regulate longevity and biological aging by IIS. Interestingly, *daf-2* mutations decrease the turnover rates of approximately 56% of total proteins in a young age, in particular, the components of translation machinery, including ribosomal proteins and translation factors (Dhondt et al., 2016), likely by decreasing protein synthesis and degradation rates (Depuydt et al., 2016). In contrast, old *daf-2* mutant animals exhibit increased proteome-wide protein turnover rates (Visscher et al., 2016). Whether these opposite effects of *daf-2* mutations on protein turnover rates in young and old animals play causal roles in aging remains undetermined. Nevertheless, these results propose the intriguing possibility that altering proteome turnover rates during aging by reducing IIS can tune protein levels to promote homeostasis, consequently leading to longevity.

In addition to proteome-wide changes caused by *daf-2* mutations, the turnover rate of DAF-2 itself is regulated by the CHN-1/C-term of Hsp70-interacting protein (CHIP), an E4 ubiquitin chain elongation factor (Tawo et al., 2017). CHN-1/CHIP regulates the turnover of DAF-2 by targeting for monoubiquitylation, causing its degradation via the endocytic-lysosomal pathway. Hence, the genetic inhibition of *chn-1* stabilizes DAF-2 proteins, leading to a short lifespan. Additionally, CHN-1/CHIP contributes to protein homeostasis by ubiquitylating age-dependently accumulated damaged proteins, which limit the capacity of CHN-1/CHIP to degrade DAF-2. Thus, CHN-1/CHIP appears to integrate protein homeostasis and aging regulation by IIS in *C. elegans*. The activity of DAF-16/FOXO increases during aging, likely by impaired proteostasis, ameliorating aging-induced perturbation of transcriptome (Li et al., 2019). These findings suggest that IIS affects proteome-wide abundance by regulating gene expression and protein turnover to promote longevity. Future studies investigating the function of upstream processes of protein synthesis during organismal aging will be an exciting research avenue.

In addition to proteostasis, IIS regulates RNA homeostasis in *C. elegans*. For instance, nonsense-mediated mRNA decay (NMD) is a key process for maintaining RNA quality (Kim and Maquat, 2019; Lykke-Andersen and Jensen, 2015). NMD detects and degrades transcripts containing premature termination codons, which produce truncated proteins, and mRNAs with uORFs (upstream open reading frames) and long 3′ untranslated regions (3′ UTRs). The NMD machinery comprises multiple components, including SMG-2/UPF1, a key RNA helicase for proper NMD. We have previously shown that the genetic inhibition of *daf-2* enhances NMD, which is required for longevity (Son et al., 2017). Moreover, overexpression of *smg-1*, which encodes a protein kinase that activates SMG-

2/UPF1, significantly increases lifespan. In our subsequent study that performed genome-wide RNAi and mutagenesis screens to identify NMD modulators, we reported ALGN-2/alpha-1,3/1,6-mannosyltransferase (ALG2) as a positive regulator of NMD (Kim et al., 2020b). We showed that ALGN-2 is required for the longevity of *daf-2* mutants, and conversely, overexpression of *algn-2* increases lifespan. These findings suggest that enhanced RNA quality control by NMD is necessary and sufficient for the longevity conferred by reduced IIS. Future research to test whether other RNA surveillance mechanisms mediate longevity caused by reduced IIS will be interesting.

IIS regulates the expression of many microRNAs (miRNAs) that affect longevity (Kim and Lee, 2019; Kinser and Pincus, 2020). For example, *daf-2* mutations increase the miR-58 family of miRNA levels, in a DAF-16/FOXO-dependent fashion (Zhang et al., 2018). Moreover, the expression of miR-58 is required for the lifespan extension of *daf-2* mutants. The target mRNAs of miR-58 include heat shock protein-90 (*hsp-90/daf-21*), ILP-1 (*ins-1*), and yeast imitation SWI (ISW) homolog-1 (*isw-1*), of which post-transcriptional regulation by the miRNA may contribute to longevity. Another miRNA, miR-83 is downregulated in *daf-2* mutants, and a loss-of-function mutation in *miR-83* extends lifespan in a DAF-16/FOXO-dependent manner, suggesting that miR-83 modulates longevity by acting with IIS (Dzakah et al., 2018). In addition, intestinal miR-83 is transported to the body wall muscles to downregulate autophagy by suppressing *cup-5*, which encodes a lysosomal calcium channel (Zhou et al., 2019). Thus, miR-83 appears to act as an endocrine factor that tissue-nonautonomously influences aging by targeting a specific player in autophagy. Overall, these findings provide insights into the interaction between protein and RNA homeostasis and IIS-controlled aging.

Enhanced stress resistance by reduced IIS is closely associated with longevity

Organisms are equipped with stress-responsive systems to cope with external stressors, including heat shock, oxidative stress, and bacterial pathogens (Park et al., 2017; Rodriguez et al., 2013). Many mutations in genes that extend lifespan, including those that reduce IIS, are associated with enhanced resistance to multiple stresses (Zhou et al., 2011). For example, RGS-1, the *C. elegans* homolog of the regulator of G protein signaling 20 (RGS20), acts with IIS to modulate oxidative stress resistance (Wu et al., 2017). Loss-of-function mutations in *rgs-1* extend lifespan and enhance oxidative stress resistance in a DAF-16/FOXO-dependent manner. In long-lived *C. elegans* with mildly inhibited mitochondria (Hwang et al., 2012), increased reactive oxygen species (ROS) levels activate DAF-16/FOXO to extend lifespan (Senchuk et al., 2018). Along with previous studies demonstrating the longevity-promoting roles of mitochondrial ROS that act through AMPK (AMP-activated protein kinase) and HIF-1 (hypoxia-inducible factor-1) (Lee et al., 2010; Hwang and Lee, 2011; Hwang et al., 2014), these studies corroborated the positive role of ROS and resistance against oxidative stress in longevity conferred by reduced IIS. However, glycogen, which contributes to the lifespan-shortening effects of dietary glucose on *daf-2* mutants (Gusarov et al., 2017), paradoxically increases oxidative stress resistance (Zecic et al., 2022); this is in line with several recent studies that reported uncoupling between stress resistance and longevity (Amrit et al., 2019; Dues et al., 2017). As longevity often correlates with adverse physiological outputs such as impaired reproduction and growth (Kirkwood, 1977; Lee et al., 2016; Williams, 2001), glycogen may increase stress resistance while decreasing longevity as a tradeoff. These studies provide insights into the relationship between resistance to oxidative stress and reduced IIS-mediated longevity.

IIS also regulates resistance to pathogenic bacteria. For example, loss-of-function mutations in *daf-2* increase the resistance of *C. elegans* to bacterial colonization, the main cause of the death of aged animals, leading to extended survival in a decrepit state in old *C. elegans* (Podshivalova et al., 2017). Surprisingly, reduced IIS by *daf-2* mutations further enhances immunocompetence in aged *C. elegans*, compared with young animals, in a DAF-16/FOXO- and HSF-1-dependent manner (Lee et al., 2021b). Moreover, the upregulation of DAF-16/FOXO and HSF-1 decreases the expression of ZIP-10/bZIP transcription factor, which further decreases IIS by reducing the expression of an agonistic ILP, ILP-7 (INS-7). Thus, a feedforward loop consisting of DAF-2, DAF-16/FOXO, HSF-1, ZIP-10/bZIP, and INS-7 regulates immune aging in *C. elegans*, and this can be exploited for reversing immunosenescence. Overall, these findings suggest that longevity conferred by reduced IIS is generally coupled with stress resistance in *C. elegans*. Future studies for testing whether IIS affects stress resistance in mammals will provide insights into the conserved roles of IIS in stress resistance and longevity.

Proper modulation of metabolism contributes to longevity caused by reduced IIS

Excessive dietary glucose shortens the lifespan of *C. elegans* by affecting biological processes (reviewed in Lee et al., 2015a; 2017), including glycerol metabolism by downregulating DAF-16/FOXO (Lee et al., 2009), and by affecting lipid metabolism (Jung et al., 2020; Lee et al., 2015b). Other metabolic processes also contribute to the lifespan extension caused by reduced IIS. A combination of anti-aging drugs targeting multiple longevity pathways, including IIS, increases the level of monounsaturated fatty acids in a sterol regulatory element-binding protein (SREBP)-dependent manner (Admasu et al., 2018). The expression of adipose triglyceride lipase-1 (ATGL-1) increases in *daf-2* mutants in a DAF-16/FOXO-dependent manner, establishing it a common factor for the longevity conferred by both reduced IIS and dietary restriction (Zaarur et al., 2019). Similarly, *R148.3*, encoding a secreted protein in the body wall muscles and neurons, prevents abnormal accumulation of triglyceride and is critical for *daf-2* mutation-induced longevity (Roy-Bellavance et al., 2017). These findings are consistent with studies demonstrating the connection between lipid metabolism and longevity in *C. elegans* subjected to glucose-rich diets (Jung et al., 2020; Lee et al., 2015b). Transcriptomic changes caused by *daf-2* mutations and dietary restriction include the increased expression of lipid metabolism-related genes and decreased expression of macromolecule biosynthesis-related genes (Gao

et al., 2018). In addition, metabolomic analyses revealed that *daf-2* mutants and dietary restricted animals exhibit reduced total amounts of amino acids, adenine, and xanthine. Thus, reduced IIS affects amino acid and nucleotide metabolism, likely playing a key role in lifespan extension. Future research to identify biological factors that regulate these metabolic processes by IIS will provide important clues regarding the systematic connection between metabolic processes and longevity.

Endocrine signaling among tissues is a key feature for longevity conferred by reduced IIS

Studies have shown that the inter-tissue regulation of IIS between neurons and the intestine is pivotal for lifespan extension in *C. elegans* (Altintas et al., 2016; Kenyon, 2010), by transmitting environmental cues to physiological processes (Artan et al., 2016; Donato et al., 2017; Jeong et al., 2012; Kim et al., 2020a; Park et al., 2021b). For example, an agonistic ILP, INS-7, downregulates the activity of DAF-16/FOXO from the intestine to other tissues in a cell non-autonomous manner (Lee et al., 2009; Murphy et al., 2003; 2007). The expression of the intestinal *ins-7* is also cell-nonautonomously downregulated by DAF-16/FOXO, indicating the feedback regulation of DAF-16/FOXO to DAF-16/FOXO. In addition to intestinal factors, the nervous tissue plays a central role in regulating longevity by affecting IIS (Altintas et al., 2016; Kenyon, 2010). A recent study using CRISPR/Cas9-mediated genome editing confirmed that the genetic depletion of *daf-2* in neurons is sufficient to increase the activity of DAF-16/FOXO in the intestine and promotes longevity (Uno et al., 2021). Neuronal IIS also regulates mitochondrial function in the muscles in a DAF-16/FOXO-dependent manner, suggesting the role of neuroendocrine signaling by IIS in regulating muscle aging (Wang et al., 2019). Therefore, the inter-tissue regulation of IIS between neurons and other tissues may provide valuable information for developing therapeutics for diseases such as amyotrophic lateral sclerosis, which causes progressive loss of motor neurons, resulting in muscle defects. Previously, a study has identified FKH-9, a forkhead transcription factor, as a target of DAF-16/FOXO in neurons, by isolating adult *C. elegans* neurons and characterizing the neuronal transcriptome of *daf-2* mutants (Kaletsky et al., 2016). FKH-9 enhances axon regeneration, short-term associative memory and longevity in *daf-2* mutants. Thus, the transcriptional network that initiates from IIS and DAF-16/FOXO to neuronal FKH-9 appears to coordinate diverse behavioral and physiological processes in the whole body. Another study demonstrated that neuronal excitation increases with age in humans, mice, and *C. elegans*, and the transcription repressor, *C. elegans spr-3/-4*/repressor element-1 silencing transcription factor (REST), promotes longevity by downregulating the overall neural excitation (Zullo et al., 2019). Suppression of neural excitation by *spr-3/-4* upregulates DAF-16/FOXO, leading to longevity. Thus, neural excitation and DAF-16/FOXO activation is an evolutionarily conserved mechanism by which the nervous system affects aging. Together, these recent studies pave ways to address the question regarding how the modulation of IIS factors in one tissue such as neurons and the intestine can generate a pivotal impact on the aging rate and longevity of an entire organism.

Genetic factors that interact with canonical IIS components modulate aging and longevity

A recent study that conducted large-scale quantitative phosphoproteomic analyses of wild-type and *daf-2* mutant *C. elegans* identified phosphorylation sites of proteins that regulate longevity (Li et al., 2021b); these include novel phosphorylation sites in AKT-1, which is critical for its activity to inhibit DAF-16/FOXO, initiation factor (EIF)-2α, which contributes to inhibiting translation to promote longevity, and cyclin-dependent kinase 1 (CDK-1), which increases germ cell proliferation and limits longevity. Another study reported that *C. elegans* MBK-1, the ortholog of human dual specificity tyrosine phosphorylation regulated kinase 1A (DYRK1A) that phosphorylates FOXO1, contributes to longevity in *daf-2* mutants, presumably by regulating the phosphorylation of DAF-16/FOXO (Mack et al., 2017). Given that IIS is a kinase cascade signaling, these phospho-protein studies provide strong evidence for the role of protein phosphorylation in IIS-mediated longevity. DAF-21/Hsp90 is required for the nuclear localization of DAF-16/FOXO isoform A and the longevity of *daf-2* mutants (Somogyvari et al., 2018). Grainyhead-like transcription factor 1 (GRH-1) is necessary and sufficient for longevity conferred by reduced IIS by acting with DAF-16/FOXO (Grigolon et al., 2022). We reported that an RNA helicase, suppressor of ACY-4 sterility 1 (SACY-1), an ortholog of human DEAD-box helicase 41 (DDX41), is required for the longevity of *daf-2* mutants, acting in a DAF-16/FOXO-dependent manner (Seo et al., 2016). SACY-1 functions as a general factor for multiple longevity regimens, different from another RNA helicase HEL-1, which we showed to bind and to activate DAF-16/FOXO specifically for the longevity of *daf-2* mutants (Seo et al., 2015). In another report, we demonstrated that HSF-1 upregulates prefoldin 6 (PFD-6) that activates DAF-16/FOXO through physical interaction (Son et al., 2018), suggesting that PFD-6 mediates longevity response from HSF-1 to DAF-16/FOXO in animals with reduced IIS. Overall, these studies corroborated the central role of DAF-16/FOXO in the longevity caused by reduced IIS.

Global cysteine-reactivity profiling identified proteins whose activities change in the *daf-2* mutants compared with those in short-lived *daf-16; daf-2* double mutants (Martell et al., 2016). Specifically, the LBP-3/fatty acid-binding protein and K02D7.1/purine nucleoside phosphorylase exhibit decreased activities in *daf-2* mutants in a DAF-16/FOXO-dependent manner. These decreased activities appear to contribute to longevity, as knockdown of *lbp-3* or *K02D7.1* further increases the long lifespan of *daf-2* mutants. Thus, cysteine-reactivity profiling can identify novel mediators of *C. elegans* longevity, in addition to transcriptomic and proteomic analyses.

Our group reported novel regulatory mechanisms regarding DAF-18/phosphatase and tensin homolog (PTEN), which dephosphorylates phosphatidylinositol (3, 4, 5)-triphosphate (PIP_3) to phosphatidylinositol (4, 5)-biphosphate (PIP_2) for downregulating the downstream kinase cascade in IIS (An et al., 2019; Park et al., 2021a). We found that KIN-4, a microtubule-associated serine/threonine kinase, binds to DAF-18/PTEN through its PDZ domain and this physical interaction is

required for the longevity of *daf-2* mutants (An et al., 2019). Furthermore, we showed that a missense mutation in *daf-18/PTEN* that alters the cysteine 150 to tyrosine in DAF-18/PTEN decreases the lipid phosphatase activity, while partially retaining its protein phosphatase activity (Park et al., 2021a). This *daf-18/PTEN* mutant allele restores reduced motility in young *daf-2* mutants and extends the health span of the animals by maintaining the partial activity of DAF-16/FOXO, while preventing the harmful hyperactivation of SKN-1/NRF. Along with these findings, AID (auxin-inducible degradation)-mediated depletion of DAF-2 after reproduction is sufficient to promote longevity without the adverse effects of *daf-2* mutation on fertility and development (Venz et al., 2021). These studies suggest that the proper control of IIS components such as DAF-2 and DAF-18/PTEN can lead to increased health span by selectively promoting longevity and defying impaired fitness.

CONCLUSIONS AND PERSPECTIVES

After discovering the critical role of the IIS pathway in *C. elegans* longevity, researchers determined whether the mammalian homologs of IIS components influence human longevity. Minor alleles of the *IGF1R* and *FOXO3A*, *C. elegans daf-2* and *daf-16* homologs, respectively, are linked with human longevity (Kenyon, 2010; Tazearslan et al., 2012). In addition, growth hormone receptor deficiency in humans, which leads to reduced IGF1 levels, is associated with prevention against age-related diseases, including cancer and diabetes (Guevara-Aguirre et al., 2011). These findings suggest that the IIS pathway influences longevity and the pathophysiology of age-associated diseases in humans. A recent study reported that the chronic hyperinsulinemia induces cellular senescence in mature adipocytes (Li et al., 2021a), which may accelerate organismal aging (Kim and Kim, 2021). These findings indicate that the aging-regulatory roles of IIS are conserved across phyla, ranging from *C. elegans* to humans. Thus, research on aging using *C. elegans* is essential to identify novel anti-aging drug targets and/or to develop therapeutic interventions, thereby promoting healthy human longevity.

One of the most critical questions in the research field of aging is how to extend the healthy periods throughout life time, instead of simply extending the maximal lifespan. Thus, identifying factors that induce healthy organismal longevity is a key step for obtaining benefits from aging research. Recent studies suggest that elaborately modulating IIS can substantially improve healthy longevity. Future research to identify novel factors and mechanisms enhancing fitness and extending health span using *C. elegans* will help achieve healthy human longevity, the ultimate goal of aging research.

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H.L. and S.J.V.L. wrote the paper.

CONFLICT OF INTEREST
The authors have no potential conflicts of interest to disclose.

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