Molecules and Cells

Minireview

Combinatorial Approach Using *Caenorhabditis elegans* and Mammalian Systems for Aging Research

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Aging is associated with functional and structural declines in organisms over time. Organisms as diverse as the nematode *Caenorhabditis elegans* and mammals share signaling pathways that regulate aging and lifespan. In this review, we discuss recent combinatorial approach to aging research employing *C. elegans* and mammalian systems that have contributed to our understanding of evolutionarily conserved aging-regulating pathways. The topics covered here include insulin/IGF-1, mechanistic target of rapamycin (mTOR), and sirtuin signaling pathways; dietary restriction; autophagy; mitochondria; and the nervous system. A combinatorial approach employing high-throughput, rapid *C. elegans* systems, and human model mammalian systems is likely to continue providing mechanistic insights into aging biology and will help develop therapeutics against age-associated disorders.

Keywords: aging, *Caenorhabditis elegans*, combinatorial approach, lifespan, mammal

INTRODUCTION

Aging is associated with the gradual structural and functional decline of organisms. Diverse species, such as yeast, the nematode *Caenorhabditis elegans*, fruit flies, and mice, share biological processes that influence aging. Longevity and healthy aging, respectively measured by lifespan and health span, are the major phenotypes measured for aging research (Brooks-Wilson, 2013). Interventions that improve health span have been reported focusing on various aspects of health parameters, including immunosenescence (Lee et al., 2021), stress resistance (Park et al., 2017), motility (Hahm et al., 2015), and cognitive function (Lu et al., 2014) in various organisms. For example, inhibition of insulin/IGF-1 signaling extends lifespan and health span by downregulating a phosphoinositide-3 kinase cascade, shown first in *C. elegans*, and subsequently in flies, mice, and very likely humans (An et al., 2017; Kenyon, 2010). Additionally, reducing the mechanistic target of rapamycin (mTOR) signaling increases lifespan first shown in yeast, and then mice, flies, and *C. elegans* (Johnson et al., 2013; 2015; Lee et al., 2015). Furthermore, dietary restriction delays aging in almost all tested species, including primates (Gems and Partridge, 2013; Santos et al., 2016). These findings have been mostly made through consecutive publications by various research groups, and have greatly contributed to our knowledge in multiple processes underlying the regulation of aging and lifespan that are evolutionarily conserved.

C. elegans and mice are two major model organisms for investigating aging-regulating mechanisms (Pitt and Kaeberlein, 2015) (Fig. 1). Indeed, *C. elegans* is the first model

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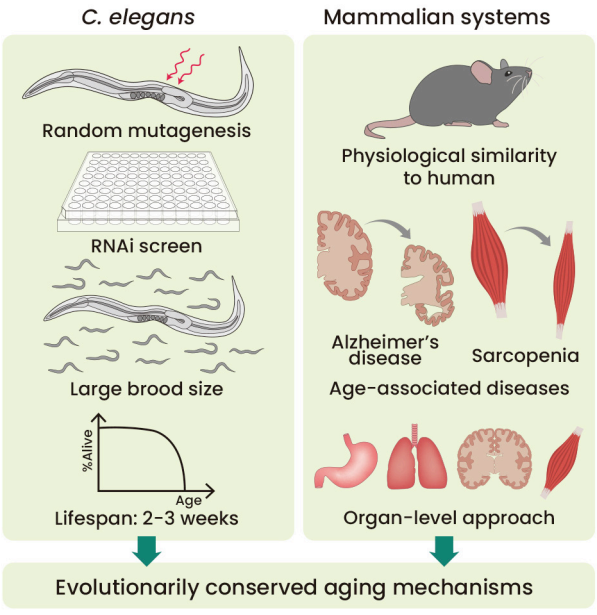

Fig. 1. Advantages of using a combinatorial approach, employing both *C. elegans* and mammalian systems, in aging research. *C. elegans* has various advantages as a model organism in aging research, e.g., genetic tractability for random mutagenesis and RNAi screen, a large brood size, and a relatively short wild-type lifespan of 2-3 weeks. Mammalian systems, such as mouse models, compensate the limitations of *C. elegans* because of their physiological similarity to humans, suitability as age-associated disease models (such as being models of Alzheimer's disease and sarcopenia), and amenability to organ-level approaches. By exploiting the advantages of these two complementary model systems, researchers can efficiently elucidate evolutionarily conserved aging mechanisms.

organism for which longevity mutants were discovered (Friedman and Johnson, 1988). *C. elegans* has been extensively used for aging research mainly because of relatively short wild-type lifespan (approximately 2-3 weeks) and large brood size (over 300 progeny/animal), making it suitable for population analysis. *C. elegans* is also genetically tractable to identify genes that modulate aging via random mutagenesis, RNAi screening, and CRISPR genome editing (Brenner, 1974; Dickinson and Goldstein, 2016; Kamath and Ahringer, 2003). However, *C. elegans* has limitations as a model for human aging. It lacks many essential anatomical and physiological characteristics found in mammals, such as a brain and an adaptive immune system (Tissenbaum, 2015). In addition, some aging-regulating pathways are specific for *C. elegans*; for example, sterol signaling comprising DAF-9/CYP27A1 and DAF-12/nuclear receptor, regulates lifespan in *C. elegans* (Antebi, 2013), but have not been reported to be conserved in mammalian systems. Thus, many researchers have used mammalian systems for aging research, with mice being the most popular model mammal (Folgueras et al., 2018). Mice have a short lifespan compared to longer-lived larger mammals, including humans, and are amenable to molecular genetics research of aging and age-related human disease (Mitchell et al., 2015). To exploit the strengths of both these systems, e.g., the short lifespan and genetic tractability of *C. elegans* and the physiological similarity to humans in mammalian systems, many recent studies on aging have employed them together.

In this review, we discuss previous research in which combinatorial approaches employing *C. elegans* and mammals were used to characterize the environmental and genetic factors that regulate aging. Specifically, we focus on individual research papers that identify conserved aging-regulating mechanisms by using both *C. elegans* and mammalian systems, rather than numerous consecutive publications that have been already covered by many outstanding previous reviews (Carmona and Michan, 2016; Fontana and Partridge, 2015; Gems and Partridge, 2013; Johnson et al., 2013; Kenyon, 2010; López-Otín et al., 2013; Pitt and Kaeberlein, 2015; Taormina et al., 2019). We describe findings regarding conserved aging-regulating factors and pathways, including insulin/IGF-1 signaling, mTOR, sirtuins, dietary restriction, autophagy, mitochondria, and neuronal regulation. Making advances in aging research using *C. elegans* and mammals will improve our understanding of conserved aging mechanisms, which will help devise strategies that prevent age-associated diseases in humans.

INSULIN/IGF-1 SIGNALING

Insulin/IGF-1 signaling is an evolutionarily conserved pathway that regulates aging in diverse organisms. Reduced insulin/IGF-1 signaling caused by genetic inhibition of *daf-2* (insulin/IGF-1 receptor) in *C. elegans* and *Igf1r* (IGF-1 receptor) or fat-specific insulin receptor in mice increases lifespan (Blüher et al., 2003; Holzenberger et al., 2003; Kenyon et al., 1993). In *C. elegans*, *daf-2* mutations promote longevity by activating downstream transcription factors, including DAF-16/forkhead box O (FOXO) (An et al., 2017; Kenyon, 2010).

Several studies have employed *C. elegans* and mammalian systems in various contexts to demonstrate the role of DAF-16/FOXO in longevity responses (Fig. 2A). Genetic inhibition of EFL-1/E2F1, a transcription factor for cell proliferation and development, promotes longevity in *C. elegans* and delays senescence by upregulating DAF-16/FOXO (Xie et al., 2014). This study efficiently used *C. elegans* for determining the effects of EFL-1 on organismal longevity, and mammalian cells for biochemical experiments showing that E2F1 inhibits FOXO via physical interaction. Mutations in *ercc-1/Ercc1*, which encodes a structure-specific endonuclease for DNA repair, increase stress resistance at a young age by activating DAF-16/FOXO in worms and mice, while accelerating aging by inhibiting DAF-16/FOXO in old age (Gurkar et al., 2018). The effect of *ercc-1/Ercc1* loss on DAF-16/FOXO arises through the tumor suppressor CEP-1/p53 in *C. elegans* and mice. These studies demonstrate the evolutionarily conserved roles of DAF-16/FOXO in regulating aging and lifespan using both *C. elegans* and mice.

mTOR SIGNALING

The mTOR signaling is a nutrient-sensing pathway that affects various cellular and physiological responses, including autophagy, metabolism, and protein synthesis (Johnson et al., 2015; Lee et al., 2015). Several combinatorial studies using both *C. elegans* and mice have demonstrated that reduced mTOR signaling promotes longevity (Houtkooper et al., 2013; Robida-Stubbs et al., 2012; Selman et al., 2009) (Fig. 2B). Genetic inhibition of ribosomal protein S6 kinase 1 (RSKS-1/S6K1), a key mTOR signaling component that regulates protein synthesis, increases the lifespan of both *C. elegans* and mice (Hansen et al., 2007; Pan et al., 2007; Selman et al., 2009). The activation of adenosine 5′-monophosphate (AMP)-activated protein kinase (AMPK), a key cellular energy sensor and longevity-promoting protein (Burkewitz et al., 2016), is required for the longevity caused by mutations in *C. elegans rsks-1* (Selman et al., 2009). Treatment with rapamycin, which inhibits mTOR, elicits mitochondrial–nuclear protein imbalance in both *C. elegans* and mice, which, in turn, contributes to longevity by activating the mitochondrial unfolded protein response (UPR^{mt}) (Houtkooper et al., 2013). In addition, the longevity-promoting effect of rapamycin in *C. elegans* requires SKN-1/Nrf, an oxidative-stress responsive transcription factor, and the activation of Nrf by rapamycin treatment is recapitulated in mouse livers (Robida-Stubbs et al., 2012). Overall, these studies have improved our knowledge in the role of mTOR in aging and confirmed that mTOR signaling is a universal means that promotes longevity across species.

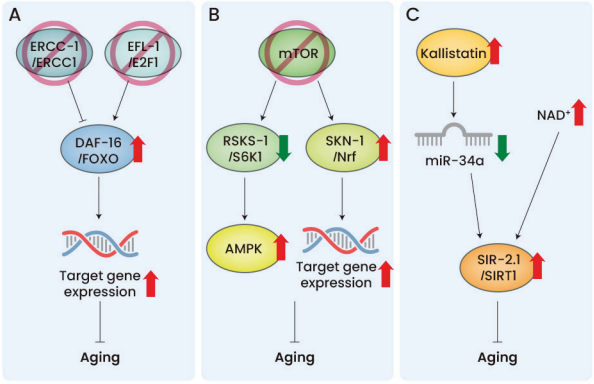

Fig. 2. Schematic models for combinatorial *C. elegans* and mammalian system studies regarding the role of insulin/IGF-1 signaling, mTOR pathway, and sirtuins. (A) DAF-16/forkhead box O (FOXO) is activated by genetic inhibition of EFL-1/E2F1 while being inhibited by mutations in *ercc-1/Ercc1*. Activated DAF-16/FOXO delays aging by increasing target gene expression. (B) Reduced mechanistic target of rapamycin (mTOR) signaling by genetic inhibition or treatment with rapamycin activates adenosine 5′-monophosphate (AMP)-activated protein kinase (AMPK) via downregulating RSKS-1/S6K1 and upregulates SKN-1/Nrf to induce protective genes, leading to longevity. (C) Kallistatin downregulates miR-34a leading to the activation of SIR-2.1/SIRT1 to delay aging. Increased nicotinamide adenine dinucleotide (NAD^+) levels also delay aging through upregulation of SIR-2.1/SIRT1.

SIRTUINS

Sirtuins are nicotinamide adenine dinucleotide (NAD^+)-dependent protein deacetylases that sense nutrient depletion (Covarrubias et al., 2021). Multiple sirtuins, including SIR-2.1/SIR2/SIRT1, delay aging in several organisms, while the longevity conferred by sirtuins requires the maintenance of NAD^+ (Lin et al., 2000) (Fig. 2C). NAD^+ levels decline with age, while genetic inhibition of NAD^+ synthase accelerates aging in *C. elegans* and mice (Hekimi and Guarente, 2003; Mouchiroud et al., 2013). Conversely, administration of NAD^+ precursors in *C. elegans* promotes longevity by activating SIR-2.1. In isolated mouse primary hepatocytes, replenishing NAD^+ increases mitochondrial function and biogenesis in a SIRT1-dependent manner. Kallistatin, a serine protease-inhibitor protein that functions as a vasodilator, delays aging in *C. elegans* and in cultured human cells by downregulating microRNA-34a (miR-34a), which leads to the activation of SIR-2.1/SIRT1 (Guo et al., 2017). Treatment with kallistatin also protects diabetic model mice defective in pancreatic beta cells from vascular aging, likely by upregulating SIRT1. These studies demonstrate the conserved prolongevity function of SIR-2.1/SIRT1 in both *C. elegans* and mammals. Currently, the conserved downstream longevity effectors of SIR-2.1/SIRT remain poorly understood; thus, identifying such factors will be an important avenue for future research employing *C. elegans* and mammalian systems in a combinatorial manner.

DIETARY RESTRICTION

After the first discovery demonstrating lifespan extension by dietary restriction in rats (McCay et al., 1935), the dietary restriction has been established as a universally conserved longevity-promoting regimen across species (Fontana and Partridge, 2015). In *C. elegans*, multiple dietary restriction methods, including dilution of dietary bacteria, food deprivation, and genetic mutations that impair feeding, have been shown to extend lifespan (Kapahi et al., 2017). In mice, dietary restriction improves overall health and promotes longevity (Fontana and Partridge, 2015).

Several studies using both *C. elegans* and mouse systems have indicated the crucial roles of RNA regulation in dietary restriction-mediated longevity (Fig. 3A). First, dietary restriction appears to promote longevity through mRNA processing and quality control in *C. elegans* and mice (Tabrez et al., 2017). In *C. elegans,* the prolongevity effect of dietary restriction is mediated by proper alternative splicing (Heintz et al., 2017) coupled with nonsense-mediated decay (Tabrez et al., 2017), which is crucial for the longevity conferred by various interventions (Kim et al., 2020; Son et al., 2017). In mice, dietary restriction enhances alternative splicing events in the brain, displaying higher alternative splicing levels than those found in other tissues (Tabrez et al., 2017). Consistent with these reports, dietary restriction upregulates age-dependently downregulated mRNA-processing genes in mice and humans

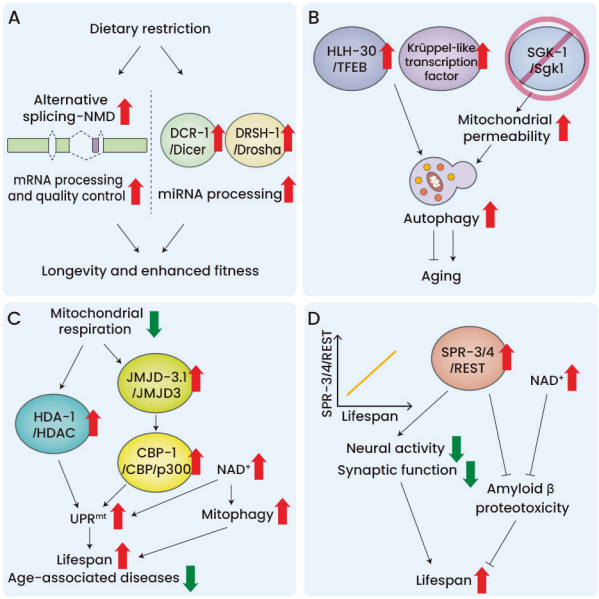

Fig. 3. Combinatorial *C. elegans* and mammalian studies regarding the conserved aging regulation by dietary restriction, autophagy, mitochondria, and neuronal system. (A) Dietary restriction upregulates age-dependently downregulated mRNA processing and quality control by improving proper alternative splicing coupled with nonsense-mediated mRNA decay (NMD). Dietary restriction also upregulates DCR-1/Dicer and DRSH-1/Drosha, ribonucleases responsible for miRNA processing, thereby improving longevity-associated miRNA processing. Enhanced mRNA processing and quality control, and miRNA processing contribute to longevity and enhanced fitness in *C. elegans* and mammals. (B) Autophagy activation regulates aging in a context-dependent manner. HLH-30/TFEB and Krüppel-like transcription factor delay aging by enhancing autophagy. In contrast, genetic inhibition of serum/glucocorticoid-regulated kinase 1 (SGK-1/Sgk1) accelerates aging by activating autophagy. (C) Reduced mitochondrial respiration induces mitochondrial unfolded protein response (UPR^{mt}) and promotes longevity through activation of histone deacetylase HDA-1/HDAC, histone lysine demethylase JMJD-3.1/JMJD3, and histone acetyltransferase CBP-1/CBP/p300. Increased levels of NAD^+ promote longevity and ameliorate age-associated disease models via enhancing mitophagy and UPR^{mt}. (D) Levels of SPR-3/4/REST positively correlate with lifespan in *C. elegans*, mice, and humans. SPR-3/4/REST downregulates neural activity and synaptic function to promote longevity. Increased NAD^+ and SPR-3/4/REST levels ameliorate the toxicity caused by amyloid β in *C. elegans* and mice.

(Lee et al., 2016; Swindell, 2009). Therefore, proper processing and quality control of mRNAs are essential for dietary restriction-mediated longevity.

Second, the regulation of miRNAs, through maintenance of the ribonucleases crucial for miRNA biogenesis also contributes to the longevity conferred by dietary restriction in *C. elegans* and mice (Guerra et al., 2019; Mori et al., 2012). miRNAs are generally downregulated with age in *C. elegans*, mice, and humans (Kim and Lee, 2019); this process is associated with decreased expression of ribonucleases responsible for miRNA processing, including *dcr-1/Dicer* and *drsh-1/Drosha* (Mori et al., 2012). In *C. elegans*, dietary restriction upregulates *dcr-1/Dicer*, which is required to promote longevity (Guerra et al., 2019; Mori et al., 2012). Consistently, in mouse adipose tissues, dietary restriction restores age-dependently downregulated miRNAs in a Dicer-dependent manner (Mori et al., 2012), leading to an antidiabetic response (Guerra et al., 2019). Thus, dietary restriction upregulates the miRNAs that mediate longevity in *C. elegans* and enhance fitness in mice. Overall, these studies suggest that proper regulation of RNAs contributes to dietary restriction-mediated longevity across species.

AUTOPHAGY

Autophagy is a process that degrades intracellular components and organelles to remove damaged molecules and to recycle nutrients (Chun and Kim, 2018). The major physiological function of autophagy is maintaining homeostasis, which helps protect organisms from various diseases, including cancer, neurodegenerative disease, and autoimmune disease (Levine and Kroemer, 2019; Yang and Klionsky, 2020). Autophagy also affects aging and lifespan in several organisms, including both *C. elegans* and mice (Gelino and Hansen, 2012; Wong et al., 2020).

Interestingly, recent studies employing both *C. elegans* and mice have shown that autophagy can be either beneficial or harmful for longevity (Fig. 3B). Two studies in which transcription factors were characterized have established the beneficial roles of autophagy in longevity. Activation of HLH-30/TFEB, the master transcriptional regulator of autophagy, promotes longevity caused by various interventions in *C. elegans,* including mutations in *daf-2*/insulin/IGF-1 receptor, dietary restriction, and germline deficiency (Lapierre et al., 2013). Mammalian *TFEB* is also upregulated in long-lived dietary restricted mice, implying that TFEB contributes to longevity in mammals. Another family of transcription factors, the Krüppel-like transcription factors, promote longevity by activating autophagy in *C. elegans* and maintain proper vascular functions during aging in mice by enhancing autophagy (Hsieh et al., 2017). In contrast, a recent study reported that upregulation of autophagy can negatively affect longevity (Zhou et al., 2019). Genetic inhibition of serum/glucocorticoid-regulated kinase 1 (SGK-1/Sgk1), increases mitochondrial permeability, leading to autophagy activation. This increased autophagy reduces lifespan in *C. elegans* and exacerbates hepatic ischemia/reperfusion injury in mice. Therefore, elevated autophagy can be either beneficial or harmful in a context-dependent manner.

MITOCHONDRIA

Mitochondria are responsible for cellular energy production and the regulation of lifespan and health span. Mild inhibition of mitochondrial respiration extends lifespan in *C. elegans*. Genetic inhibition of electron transport chain genes, including *clk-1* (coenzyme Q biosynthesis enzyme), *isp-1*

(Rieske iron-sulfur protein in complex III), *sod-2* (superoxide dismutase 2), and *sft-1* (cytochrome c oxidase assembly factor 1-like protein), promotes longevity (Lee et al., 2015; Maxwell et al., 2013; Wu et al., 2018). Likewise, mutations in each of *Mclk1*, *Risp*, *Sod2*, and *Surf1*, which are the respective orthologs of the aforementioned *C. elegans* genes, promote longevity in mice (Dell'Agnello et al., 2007; Hughes and Hekimi, 2011; Lapointe et al., 2009). Several studies using both *C. elegans* and mammalian systems have demonstrated that epigenetic regulators mediate the longevity caused through reduced mitochondrial functions by increasing UPR^{mt} (Fig. 3C). Histone deacetylase-1 (HDA-1) activates UPR^{mt} under mitochondrial stress conditions and subsequently extends lifespan in *C. elegans* (Shao et al., 2020). The expression levels of *HDAC*, a mammalian histone deacetylase gene, positively correlate with those of UPR^{mt} genes in primates. The histone lysine demethylases JMJD-1.2 and JMJD-3.1 promote longevity in mitochondrial mutant *C. elegans* by activating UPR^{mt} via downstream histone acetyltransferase CBP-1 (Li et al., 2021; Merkwirth et al., 2016). Consistently, *Phf8* and *Jmjd3* (*Kdm6b*) expression levels, i.e., the respective mouse homologs of *jmjd-1.2* and *jmjd-3.1*, as well as *CBP/p300*, the mammalian homolog of *cbp-1*, positively correlate with those of UPR^{mt} genes and mouse lifespan. These studies show that the conserved epigenetic regulation of mitochondrial stress responses contributes to longevity in animals with reduced mitochondrial electron transport chains.

Conversely, recent studies employing *C. elegans* and mammalian systems have also shown that increased mitochondrial function can contribute to longevity and ameliorate age-associated diseases. As discussed above, maintenance of the NAD^+ pool is crucial for longevity and increased mitochondrial function in *C. elegans* and mice. NAD^+ triggers mitochondrial–nuclear protein imbalance and UPR^{mt}, which leads to enhanced mitochondrial function in *C. elegans* and mice (Mouchiroud et al., 2013). Replenishing NAD^+ restores impaired mitophagy and lengthens lifespan in *C. elegans* and mouse ataxia models (Fang et al., 2016). Increasing NAD^+ levels also upregulates mitophagy and UPR^{mt} in *C. elegans* and mouse Alzheimer's disease models to alleviate amyloid β proteotoxicity (Sorrentino et al., 2017). In addition, mutations in *rgs-2/Rgs14* (regulator of G protein signaling 14), which upregulate mitochondrial functions, extend *C. elegans* lifespan and also promote longevity by increasing NAD^+ levels in mice (Vatner et al., 2018). Activation of sirtuins, longevity transcription factor DAF-16/FOXO, and UPR^{mt} through increased mitochondrial function promotes longevity in *C. elegans* and ameliorates age-associated diseases in mammals (Katsyuba et al., 2018). In addition to NAD^+, supplementation of D-glucosamine, which is used for treating osteoarthritis, increases lifespan by increasing mitochondrial biogenesis in *C. elegans* and mice (Weimer et al., 2014). Overall, the beneficial roles of NAD^+ and D-glucosamine, which promote longevity and ameliorate age-associated diseases in *C. elegans* and mammals, are at least partially mediated by increases in mitochondrial function or biogenesis. Because both decreased and enhanced mitochondrial function leads to longevity, future research should differentiate these opposing processes to improve our overall understanding of the role of mitochondria in aging.

THE NERVOUS SYSTEM

The nervous system coordinates physiological responses to internal and external stimuli across various tissues. Interestingly, neuronal activity regulates organismal lifespan and aging rates in many animals, including *C. elegans* and mammals (Jeong et al., 2012) (Fig. 3D). The expression level of *REST*, repressor element 1-silencing transcription factor, in the human brain positively correlates with lifespan (Lu et al., 2014). A subsequent study that employed *C. elegans*, mice, and humans to further characterize REST in aging, showed that *REST* downregulates genes associated with neural activity and synaptic function in long-lived humans (Zullo et al., 2019). Consistently, *spr-3* and *spr-4*, the *REST* orthologs, are required for the longevity conferred by genetic inhibition of insulin/IGF-1 signaling in *C. elegans*. Another report showed that the loss of TRPV1, a capsaicin receptor that acts in sensory neurons, extends the lifespan in *C. elegans* and mice by inactivating CREB/CRTC1, a nutrient-sensing cAMP-responsive element-binding protein, and its coactivator, which are negative regulators of longevity (Riera et al., 2014). A recent study demonstrated that loss of the epigenetic reader gene *baz-2/Baz2b* in neurons improves age-dependent decreases in mitochondrial function to promote longevity in *C. elegans* and enhances cognitive function in aged mice (Yuan et al., 2020). These studies highlight the contribution of combinatorial approaches using *C. elegans* and mammalian systems for understanding of lifespan regulation by neurons.

Neuronal degeneration and functional declines are associated with aging and age-associated disease in most animals, including *C. elegans* and mammals (Bishop et al., 2010; Chew et al., 2013). Several studies have shown the value of using transgenic *C. elegans* and mouse models of age-associated neurodegenerative disease to investigate pathophysiology. Upregulation of the aforementioned longevity-associated factors, including REST and NAD^+, ameliorates amyloid β proteotoxicity in both *C. elegans* and mice (Lu et al., 2014; Sorrentino et al., 2017). Pharmacological inhibition of steroid hormone sulfatase increases neuroprotection by reducing amyloid β aggregation in Alzheimer's disease model *C. elegans* and mice (Pérez-Jiménez et al., 2021). Age-dependent changes in the expression of synaptogenesis factors in *C. elegans* and mice appear to be critical for age-dependent synaptic loss (Maeder et al., 2018). Therefore, combined approaches employing both *C. elegans* and mammalian systems will help enhance our understanding of age-dependent neurodegeneration and relevant drugs. In addition, such approaches may help to elucidate the conserved mechanisms underlying neuronal regulation of aging.

CONCLUSIONS AND PERSPECTIVES

Here, we reviewed aging research literature in which a combinatorial approach, employing both *C. elegans* and mammalian systems, was applied. These studies have directly contributed to improving our understanding of many evolutionarily conserved aging-regulating factors and signaling

pathways, including insulin/IGF-1 signaling, mTOR, sirtuins, dietary restriction, autophagy, mitochondrial alterations, and the nervous system. In contrast to invertebrate models, such as *C. elegans*, aging research in which mammalian model organisms are employed presents a time challenge because of relatively long lifespan of mammals. Vertebrate model organisms with a very short lifespan, such as the African turquoise killifish, *Nothobranchius furzeri*, have recently been developed to overcome this time issue (Hu and Brunet, 2018). Nevertheless, *C. elegans* remains one of the most popular and powerful models for advancing the aging research field mainly because of its rapid aging and short lifespan. It will likely continue to serve as a workhorse for aging research, particularly when used in combination with mammalian systems.

Conventional research approaches, such as a research using *C. elegans* followed by consecutive studies using different systems, have contributed to the understanding of common aging mechanisms. However, such strategies have difficulties in defining conserved aging mechanisms without the publication of a subsequent mammalian research. Research using both *C. elegans* and mammalian systems together is advantageous, because it can show evolutionarily conserved physiological roles in organismal aging in a single paper. One difficultly for a group of researchers is to become experts in both systems. Therefore, in many cases, this combinatorial approach requires extensive collaboration among multiple laboratories. One alternative is using *C. elegans* for genetics of aging research and cultured mammalian cells for biochemical assays. For example, we recently showed that VRK-1, a nuclear protein kinase, promotes longevity in *C. elegans* and activates AMPK, a cellular energy sensor, by phosphorylation in mammalian cells (Park et al., 2020). Another alternative approach would be to use more diverse model systems in addition to *C. elegans* and mammals; however, only a handful of studies have employed such an approach. A study in which four different organisms were used demonstrated that nucleolar size negatively correlates with longevity in *C. elegans* and is reduced by interventions that extend lifespan in *Drosophila melanogaster*, mice, and very likely humans (Tiku et al., 2017). Regardless of the number of organisms used, studies that exploit the advantages of various organisms, including *C. elegans* and mammalian systems, in a combinatorial manner will continue to help elucidate underlying mechanisms in the evolution of aging and lead to developing new treatments for age-associated diseases in humans.

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AUTHOR CONTRIBUTIONS

G.Y.L., J.S., 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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