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Models for Aging Research

The nematode *Caenorhabditis elegans* as a model for aging research

Hildegard I.D. Mack[1,*], Thomas Heimbucher[2], Coleen T. Murphy[2,*]

[1]Institute for Biomedical Aging Research, Leopold-Franzens-University Innsbruck, Rennweg 10, 6020 Innsbruck, Austria
[2]Department of Molecular Biology and Lewis-Sigler Institute for Integrative Genomics, Carl Icahn Laboratory 148, Washington Road, Princeton University, Princeton, NJ 08544, USA

The nematode *Caenorhabditis elegans* is a key model system for experimental research on the genetic regulation of aging, and has paved the way towards many important discoveries in this field. Importantly, in the course of its short lifespan of ~3 weeks, *C. elegans* displays many phenotypic, behavioral, and molecular changes that are widely shared among metazoans as they age. In this review, we summarize how aging research takes advantage of *C. elegans*' biology, and we describe the experimental toolbox available to study worm aging.

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Dr. Hildegard Mack – Institute for Biomedical Aging Research, University of Innsbruck, Austria.

Introduction

In the past three decades, the small nematode *Caenorhabditis elegans* has become a premier model system for the study of aging. Research conducted by many labs using *C. elegans* has contributed significantly to our fundamental understanding of organismal aging as a biological process that is modulated by cellular signaling pathways and gene expression programs [1]. The isolation of long-lived *C. elegans* mutants initiated a scientific discovery journey that has led to many insights into the mechanisms that regulate lifespan (Fig. 1**)**, with implications for human longevity (reviewed in Refs. [1–4]). This success story of *C. elegans* as a model system for basic research on the biology of aging emerged from the many favorable characteristics of this species, as well as from continuous advances in experimental technology. In this review, we first address the features that have made *C. elegans* a widely used experimental organism for aging research, and we provide examples for how this model system can be used to understand specific ''hallmarks'' of aging [5].

*Corresponding authors: Hildegard I.D. Mack (Hildegard.Mack@uibk.ac.at), T. Heimbucher (theimbucher@princeton.edu), C.T. Murphy (ctmurphy@princeton.edu)

C. elegans biology in the context of experimental aging research

Anatomy and lifecycle

C. elegans has many convenient features that make it particularly suitable as an experimental system for basic biological/biomedical research, particulary for aging research (Table 1**)**. Most notable is its comparatively short lifespan of just ~3 weeks [6–8]. Importantly, in the course of its life, *C. elegans* displays many readily observable and quantifiable changes that are commonly associated with aging, such as tissue degeneration, decreased movement, and the cessation of reproduction [9–11].

C. elegans develops from an egg to a fully fertile adult in ~3 days at 20 °C (Fig. 2**)**. The 959 somatic cells of *C. elegans* hermaphrodites (1031 for males) form multiple distinct tissues (cf. below) that can readily be observed by light and fluorescent microscopy of live animals due to their transpar-

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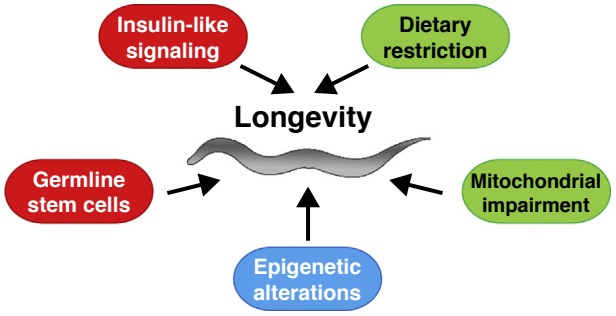

Fig. 1. Selected lifespan regulatory pathways in *C. elegans*. Pathways that promote longevity are highlighted in green, those that limit lifespan in red. Blue indicates varying longevity effects, depending on the precise factor that is targeted. These pathways are highly interconnected and engage partially overlapping sets of transcription factors and downstream mechanisms to modulate lifespan. See Refs. [1,3,4].

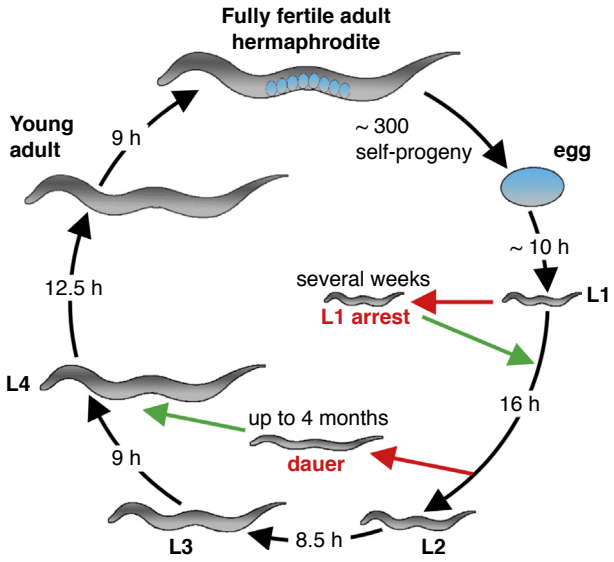

Fig. 2. The lifecycle of *C. elegans* at 20 °C. For a self-fertilizing hermaphrodite, the mean reproductive span is ~6 days, mean lifespan ~15 days, and maximum lifespan ~27 days. Self-progeny number is limited by the number of sperm produced by a hermaphrodite. In response to unfavorable conditions (red arrows), larvae arrest as L1 larvae (triggered by starvation) or as the alternative L3 dauer stage (triggered by starvation, high temperature, or overcrowding). L1 arrest or dauer formation allow survival for extended periods of time. Animals can exit these stages again when a favorable environment is restored (green arrows). Note that the duration of developmental stages is temperature-dependent. Adapted from www.wormatlas.org [17] with data from Refs. [10,18,19].

ence. Excellent microscopy images and schematic drawings that give a detailed overview of *C. elegans* anatomy are available at www.wormatlas.org. The balance between sufficient complexity and manageable size of the nervous system profoundly influenced the selection of *C. elegans* as an experimental model system [12]. The connections between the 302 hermaphrodite neurons, plus additional special circuits between the 385 male neurons have been mapped to near completeness, making *C. elegans* the organism with the best-defined connectome to date [13–16].

Table 1. At a glance: advantages of *C. elegans* as a model system for basic biomedical research.

Advantages of *C. elegans* as a model organism
• Non-hazardous, non-pathogenic to humans
• Easy and inexpensive culture and handling
• Small body size (~1 mm for young adults)
• Large brood size (~300 per self-fertilizing hermaphrodite)
• Short lifecycle/generation time (~3 days at 20 °C)
• Short maximum lifespan of ~3 weeks (at 20 °C)
• Several distinct tissues, including a nervous system
• Transparent body that facilitates microscopy analyses
• Displays multiple scorable behaviours (e.g. chemotaxis, egg-laying, foraging, habituation)
• Sexual dimorphism with self-fertilizing hermaphrodites (→ isogenic offspring) and males (→ new genotypes through crossing)
• Genetically tractable, with easy mutagenesis and mapping tools
• RNAi interference allows easy full-genome phenotype screens
• First animal fully sequenced; well-annotated genome
• Individual tissues with full transcriptomes available
• High degree of orthology between the *C. elegans* and human protein-coding genomes
• Comprehensive genetic toolbox
• Excellent online databases and resources on *C. elegans* biology, anatomy and genetics

See main text for references and details.

C. elegans has multiple distinct tissues (muscle, nervous system, reproductive system, intestine, and epidermis [also referred to as hypodermis]) that are implicated in both worm and mammalian aging. The *C. elegans* intestine is remarkable in that it performs functions comparable not just to the mammalian stomach and bowel, but also to liver, pancreas, and adipose tissue [20]. Moreover, the pseudocoelom has some functional similarity with a circulation system [21]. Worms utilize an innate immune system to fight pathogens that is active in the intestine and epidermis [22–24]. Muscle displays age-related decline that resembles human sarcopenia [25], and neurodegeneration causes loss of cognitive and behavioral abilities in aging worm [26–29]. Opposing signals from components of the reproductive system can promote or slow aging [30], while reproduction ceases with age.

Recently developed FACS-based methods for isolating individual cells and tissues has enabled the full transcriptome analysis of the adult neurons, intestine, muscle, and epidermis [31,32], and revealed that the hypodermis is a metabolic tissue. Lastly, thanks to the availability of tissue-specific transgenic expression and knockdown techniques (cf. below) studying tissue-to-tissue communication is possible in *C. elegans* (for example [33–35]).

Genetic and genomic features

C. elegans was the first multicellular organism whose genome was essentially completely deciphered by shotgun Sanger sequencing in 1998 [36], although refinements are continuously made as sequencing technology advances [37,38]. Table 2 summarizes the current annotation states of the *C. elegans* and human reference genomes. Remarkably, although the *C. elegans* genome is just 1/30th the size of the human genome, the number of protein coding genes is similar, and ~40–80% (depending on similarity thresholds) of *C. elegans* proteins have been estimated to have orthologs in humans [39,40].

The *C. elegans* karyotype comprises five pairs of autosomes (I–V), plus a pair of X chromosomes in hermaphrodites (XX). Males possess a single X chromosome (XO), and sex is determined by the X:autosome ratio. Hermaphroditic self-fertilization produces primarily hermaphroditic offspring, although males can arise through spontaneous X-chromosome non-disjunction during meiosis (~0.2%) [43], and this rate rises with maternal age [44]. Hermaphrodites and males each have particular experimental advantages: while hermaphrodites give rise to an isogenic population upon self-fertilization, mating hermaphrodites with males allows the creation of new allele combinations [12]. As many other types of studies in *C. elegans*, aging studies are usually performed using hermaphrodite populations, although male lifespan [45,46] and the impact of mating on both hermaphrodites [47,48] and males [46] has been studied.

Brief overview of the C. elegans *genetic toolbox*

C. elegans is amenable to both forward and reverse genetic approaches, including genome-wide screens. Forward genetic screens aim to identify the genetic basis of a given phenotype and are initiated by introducing random mutations, for example by treatment with chemicals, most commonly EMS [12], or by transposons [49,50]. With Next Generation Sequencing (NGS) becoming increasingly affordable and relevant computational tools being publicly available, forward genetic screening is becoming more and more feasible [51]. Reverse genetic studies examine the phenotypes resulting from changing the activity of a given gene and are routinely used in the *C. elegans* field, because powerful and easy-to-use tools for targeted gene knockdown by RNA interference have been available for >15 years. Feeding of bacteria carrying dsRNA-encoding plamids has become the predominant method of administering RNAi to *C. elegans*. Two feeding libraries that collectively cover >90% of the worm genome are commercially available [52,53]. Spatial restriction of RNAi-mediated gene knockdown can be achieved through the use of particular mutant strains that allow (enhanced) RNAi in specific tissue(s) [54–56]. To temporally restrict *C. elegans* gene knockdown, RNAi-bacteria are fed only during a specific time of the lifecycle. This strategy is particularly useful in separating developmental phenotypes from aging phenotypes [57]. Transgenes can readily be introduced into *C. elegans* through microinjection [58] or microparticle bombardment [59], and gene expression can be spatially and temporally restricted by using tissue-specific or inducible (e.g. heatshock) promoters [60]. CRISPR/Cas9-mediated targeted genome editing strategies were rapidly adapted to *C. elegans* [61–64]. With this technique, gain-of-function mutations, which commonly require specific amino acid changes, are more readily accessible than with random mutagenesis strategies. Reflecting the collaborative culture of the field, many *C. elegans* labs submit their mutant, transgenic, and edited strains to the *Caenorhabditis* Genetics Center (CGC) for community dissemination. Of note, even though *C. elegans* classically has been studied with genetic and genomic tools, other ''omics'' approaches, including metabolomics and proteomics, are also feasible and increasingly contribute to our understanding of *C. elegans* aging (e.g. [65–73]).

Table 2. Comparison between the *C. elegans* and human genomes.

	C. elegans	Humans
Complete genome		
Genome size (bp)	100,286,401	3,257,319,537
Total number of genes	48,446	58,721
Protein coding genes	20,203	19,940
Mitochondrial genome		
Genome size (bp)	13,794	16,569
Total number of genes	36	37
- *Respiratory chain proteins*	*12*	*13*
- *tRNA genes*	*22*	*22*
- *rRNA genes*	*2*	*2*

The complete *C. elegans* and human genomes comprise the nuclear and mitochondrial genomes. Statistics are according to Wormbase (WS266 https://wormbase.org/about/wormbase_release_WS266#0–10) and Gencode (release version 29 https://www.gencodegenes.org/human/stats.html; as of 10-19-2018). For annotation of the mitochondrial genome, see Refs. [41,42]. The respiratory chain protein encoded by the human, but not by the *C. elegans* mitochondrial genome is the complex V subunit MT-ATP8 [41].

Key parameters to consider during C. elegans *lifespan experiments*

C. elegans lifespan is influenced by many parameters that researchers can easily control (Table 3**)**. However, additional, yet-to-be-defined and stochastic factors also seem to play a role, since *C. elegans* lifespan measurements can vary between different labs and researchers [74]. Best practice includes careful worm husbandry (consistent temperature and food supply) and starting lifespan analyses at the time of the L4/adult molt, which is easily recognized and does not include variability that can arise from starting lifespan at the egg stage. Additionally, correct censoring (at the time of the event, not from the whole analysis) of worms that disappear or die from ''unnatural'' deaths can help with standardization. Recently developed automated ''lifespan machines'' open up the possibility of increasing throughput through *C. elegans* lifespan assays [75–77].

Table 3. At a glance: experimental parameters that can affect *C. elegans* lifespan.

Parental *C. elegans* generation	Experimental *C. elegans* generation
• Maternal age[§] • Early life starvation*	• Culture medium (plate vs. liquid) • Temperature • Food source (bacterial strain, live vs. dead/non-proliferating bacteria) • Larval population density (on plates, in liquid after bleaching*) • Visible light • Genetic background • Prevention of progeny development by treatment with FUdR[§] • Sterility-inducing mutations that affect germline maintenance

Parameters are grouped by the *C. elegans* generation in which they were examined for their influence on lifespan. * indicates that lifespan-related processes (stress resistance, expression of metabolic/signaling genes) were examined. Parameters that do not affect the lifespan of (otherwise) wild-type animals, but do or may do so in combination with other genetic mutations, are marked by[§]. Refs.: [81–88].

To circumevent the need for regular transfer to fresh plates (to avoid mixing of the experimental worm generation with its progeny), 5-Fluoro-2′-deoxyuridine (FUdR) or sterility-inducing mutations can be used during lifespan analysis [78]. Even though FUdR does not affect lifespan in wildtype worms across a wide range of concentrations, it does so in various mutant backgrounds, or under particular culture conditions (see [79], which includes a survey of the relevant literature). When using sterile mutants, the underlying mechanism carefully has to be considered as germline stem cells and somatic gonad both modulate *C. elegans* lifespan [30]. Indeed, many mutations that affect germline stem cell maintenance extend lifespan, while mutations that impair somatic gonad, sperm, or oocyte development do not [7,8,80].

Longevity vs. progeria

Most aging research in *C. elegans* has focused on ''normal'' aging and on processes that mediate lifespan *extension* (Fig. 3), which has greatly enhanced our understanding of longevity regulation. *C. elegans* also allows the possibility of deciphering mechanisms that underlie *accelerated* aging, or progerias. Genes mutated in human segmental premature aging syndromes, e.g. Werner, Bloom, and Cockayne syndromes, are required for genome maintenance and are conserved [89–92], as are many additional factors directly or indirectly involved in DNA repair [93], such that they can be experimentally probed in *C. elegans*. Mutations in mitochondrial DNA are observed in, and are sufficient to drive, diseases and aging in humans and mice [94–96]; short-lived *C. elegans* strains have been established in analogy to the ''Mito'' (heteroplasmic for a large mtDNA deletion [97]) and ''Mutator'' (*PolgA*/*polg-1* mutation [98]) mice used in these original studies and thus have the potential to accelerate research on progeric mechanisms.

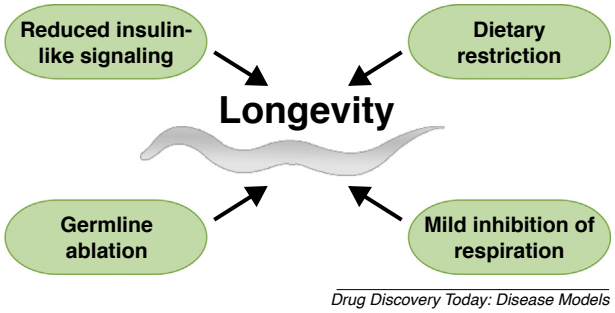

Fig. 3. Selected lifespan-extension paradigms in *C. elegans*. The paradigms depicted in the figure were among the first to be described in *C. elegans* and remained some of the most-widely studied. All paradigms can be triggered genetically or through appropriate interventions (pharmacologically, dietary, or in case of the germline paradigm, by laser-ablation of larval germline precursor cells) and lead to lifespan extension in otherwise wildtype worms.

Hallmarks of aging and their analysis in C. elegans

Aging is associated with distinct molecular and cellular changes that appear to be widely shared across evolution [5]. *C. elegans* allows the study of such changes and their underlying mechanisms in the context of a multicellular eukaryote. In the few decades since the first isolation of long-lived mutants [6–8], *C. elegans* has proven itself exceptionally useful for recognizing and better understanding diverse ''hallmarks'' of aging, such as genomic instability, epigenetic alterations, loss of proteostasis, mitochondrial dysfunction, deregulated nutrient and energy sensing, and altered intercellular communication [5], as described in greater detail below. Specifically, *C. elegans* allowed to experimentally demonstrate that changing the activity of factors and mechanisms involved in these hallmarks results in lifespan changes. Accordingly, longevity regulators in *C. elegans* include histone modifiers and chromatin remodelers [99–101], molecular chaperones and regulators of unfolded protein response (UPR) pathways such as endoplasmic reticulum UPR and mitochondrial UPR [34,102–107], the ubiquitin-proteasome system [108,109], factors involved in non-selective and selective forms of autophagy, including mitophagy [110–112], and components of nutrient/energy-sensing signaling pathways (e.g. IIS, AMPK, TOR signaling) [7,8,113,114]. Many insights from *C. elegans* research are likely to be applicable to mammalian and human aging, given that the molecular machineries shaping these hallmarks are highly conserved.

Genomic instability

Generally, DNA repair mechanisms are highly conserved across species. As is true for mammals, worms possess the molecular machinery for base- and nucleotide excision repair, mismatch-repair, trans-lesion synthesis, non-homologous end joining, homologous recombination, chromosome

number surveillance, and DNA damage and spindle assembly checkpoints [93]. Experimental tools available to study *C. elegans* (cf. above) facilitate not just the identification and characterization of cellular DNA damage response and repair factors, but also the study of systemic responses and of developmental stage- and tissue-specific aspects of genome maintenance [115]. Direct experimental manipulation of mitochondrial DNA is currently not feasible, but the effects of mtDNA mutation or depletion can be studied *in vivo* in *C. elegans* in specialized genetic backgrounds such as the *polg-1* mutation or a heteroplamic strain carrying a large mtDNA deletion (cf. above).

Epigenetic alterations

Epigenetic regulation, including changes in DNA methylation, histone posttranslational modifications, loss of core histones, and shifts in histone variant ratios, lead to reversible alterations in chromatin state and gene transcription [5,116]. The most prominent DNA methyl modification in mammals, 5-methylcytosine (5-mC), a proposed biomarker for human aging, is thought to be absent in *C. elegans*, and previously it was thought that worms had no methylation at all due to the lack of identified DNA methylases.

However, *C. elegans* [117], together with *D. melanogaster* [118] and *C. reinhardtii* [119] facilitated the discovery of another, ancient, form of DNA-modification in metazoans, namely of 6-methyladenine, and thus, sparked great interest in re-investigating this modification in mammals. Indeed, evidence is accumulating that 6-mA modified DNA and 6-mA regulatory enzymes are also present in mammals [120,121]. It will be intriguing to further investigate whether *C. elegans* is predestined to serve as a model for further studies on 6-mA regulation and function in eukaryotes. Of note, in *C. elegans*, the putative 6-mA methyltransferase DAMT-1 was implicated in the transgenerational regulation of fertility and longevity [117,122].

Multiple findings in *C. elegans* support the ''heterochromatin loss model of aging''. For example, repressive H3K9 and H3K27 marks decrease during *C. elegans* aging, and lifespan can be altered by knockdown or overexpression of various histone-modifying enzymes (reviewed in Refs. [4,116]). Remarkably, some histone modifiers control *C. elegans* lifespan transgenerationally [122,123]. Histones and histone-modifying enzymes are well conserved between *C. elegans* and humans, although histone modifiers also frequently target non-histone proteins, and some modifiers have not yet been fully characterized for their biochemical activity in worms [4,124]. Similarly, all families and subfamilies of mammalian ATP-dependent nucleosome remodelers (SWI/SNF, ISWI, CHD, INO80) possess orthologs of the catalytic and accessory subunits in *C. elegans* [125], and several of these factors have been implicated in lifespan regulation (reviewed in Ref. [4]).

Loss of proteostasis

Eukaryotic cells have developed several well-conserved mechanisms to maintain a functional proteome, including molecular chaperones and various organelle-specific unfolded protein response (UPR) pathways, the ubiquitin-proteasome system, and autophagy [126]. In addition to the endoplasmic reticulum UPR and non-selective macroautophagy (commonly referred to as ''autophagy''), mediators of the mitochondrial UPR and of mitophagy (selective autophagy of mitochondria), have been genetically dissected in *C. elegans* and implicated in lifespan regulation [34,102–112,127]. Moreover, evidence suggests that microautophagy and chaperone-mediated autophagy also occur in worms [128,129]. Key tools and assays to study proteostasis mechanisms are available in worms (reviewed in Refs. [130–132]). When combined with tissue-specific analysis techniques (cf. above), *C. elegans* further enables to study how proteostasis is maintained through cell-nonautonomous mechanisms and how it contributes to aging and neurodegenerative disease (e.g. [34,35]).

Deregulated nutrient sensing

Genetic or pharmacological perturbation of pathways involved in nutrient, energy, and growth factor sensing, such as TOR, AMPK, Sirtuin and IIS signaling, modulate lifespan and health from yeast to mammals [1,3]. These pathways are highly interconnected with each other and are highly conserved. While TOR, AMPK, and Sirtuin signaling mediators are present in yeast [133], pathways for inter-tissue communication, such as insulin/IGF-1 signaling (IIS) are specific for multicellular organisms. Reflecting its central role in metabolism, the IIS cascade is completely represented in *C. elegans* [134–141], where it controls dauer formation during development, and lifespan during adulthood; this latter function of IIS extends through mammals [1,2]. The fact that most IIS components are represented by a single gene in *C. elegans* has undoubtedly facilitated studies on its lifespan-regulatory function. Nutrient and growth factor signaling can further be modulated by dietary measures such as caloric restriction, which also confers health and longevity benefits in many species [142]. There are two basic strategies to restrict *C. elegans* diet: directly, by limiting the amount of nutrients (its bacterial food source), or genetically, by mutations that reduce pharyngeal pumping (*eat-2*). It is important to note that different dietary restriction regimens engage different genetic pathways in *C. elegans* lifespan extension [143].

Mitochondrial dysfunction

Multiple aspects of mitochondrial function have been implicated in longevity and aging across species, including mitochondrial DNA integrity, turnover, and protein homeostasis (cf. above), as well as mitochondrially-derived metabolites and reactive oxygen species (reviewed in Ref. [144]). While

excessive mitochondrial damage is obviously detrimental to an organism, studies in *C. elegans* were instrumental in experimentally demonstrating that *mild* impairment of mitochondrial function is beneficial for longevity [145–147]. Lifespan extension appears to result at least in part from the elevation of particular types of reactive oxygen species, which in this setting appear to primarily act as signaling molecules that trigger protective responses [148–150]. In *C. elegans*, mild inhibition of mitochondrial electron transport can easily be achieved by genetic mutation or RNAi-knockdown of respiratory chain components (e.g. *clk-1, cyc-1, isp-1, nuo-6*). Moreover, tools to morphologically and functionally characterize mitochondria are available in *C. elegans*, including fluorescently-tagged mitochondrial proteins and dye-staining protocols to determine mitochondrial morphology, membrane potential, and ROS levels (e.g. [151–154]). In summary, *C. elegans* is exceptionally useful in further elucidating how disturbance of various mitochondrial processes modulates aging.

Altered intercellular communication

Beyond IIS, additional endocrine and neuroendocrine factors have been identified that modulate lifespan or lifespan-related processes in *C. elegans* and that possess orthologous or at least chemically similar molecules in mammals. Examples include serotonin and bile-acid like steroids, such as dafachronic acids [35,155,156]. As discussed above, *C. elegans* researchers have developed powerful genetic tools to investigate the cell non-autonomous regulation of processes of interest *in vivo* in worms.

It is interesting to note that circulating factors that have been implicated in mammalian aging, such as GDF11 and oxytocin [157–159], also have relatives in *C. elegans*. Remarkably, in both of these cases, the orthologous *C. elegans* factor represents a common ancestor of two mammalian signaling mediators. Specifically, the TGF-β family member DAF-7 is the closest relative of GDF11 and GDF8 (but not of other mammalian BMP/TGF-β-family members, corresponding to *C. elegans* DBL-1), and has been implicated in dauer formation and lifespan regulation [160]. Similarly, the peptide hormone nematocin is related to oxytocin and vasopressin [161,162]. Thus, *C. elegans* can help to better understand the physiological roles of these factors and how they evolved, including but not limited to lifespan regulation.

Limitations

When comprehensively comparing *C. elegans* to mammalian aging, it is important to realize that despite sharing many common features, mammalian aging is characterized by additional hallmarks whose broad analysis in *C. elegans* is somewhat limited by the worm's biology. Specifically, while the fact that the worm reliably differentiates into 959 post-mitotic somatic cells enabled the discovery of apoptosis [163,164] and provides a good model of post-mitotic cells, it does not allow the study of somatic stem cell biology. Other aspects of stem cell biology however, can be investigated using *C. elegans* germline stem cells as a model [165]. Telomere length is not associated with lifespan in *C. elegans* [166], but mechanisms of *C. elegans* telomere maintenance may still have implications for cancer, a human-age associated disease [167]. No obvious cell senescence pathway has been reported in worms, limiting the ability of the use of *C. elegans* for the study of the senescence-associated secretory phenotype (SASP; [168]), although orthologous systems may function similarly. Additionally, while worms have an innate immune system [24], they lack an adaptive immune system, which hinders the study of important phenomena in mammalian aging such as ''immunosenescence'' and ''inflammaging.'' [169]. In summary, outside of some specific areas, *C. elegans* offers many other advantages that make it a powerful model system for aging research, particularly for pioneering or rapid discovery-based inquiry.

Lifespan and healthspan in C. elegans

The major goal of aging research is to increase healthspan, i.e., the disability-free fraction of life, rather than to simply extend lifespan. Thus, whether increased lifespan in *C. elegans* is actually associated with increased ''healthspan'' becomes an important question. As indicated above, the transition from a healthy to a frail state can be analyzed in *C. elegans* by several parameters such as body movement and pharyngeal pumping [10]. In addition, tissue integrity declines with age [9,25], which is accompanied by a shrinking of the worm body and an accumulation of fluorescent lipofuscin [6,76,170]. Recently, several new metrics and analytical tools have been described for monitoring the active, healthy period of life [76,77,171–173], reflecting great interest in the field to re-investigate the ''health span'' of longer-lived *C. elegans* mutants and wild-type individuals.

Several important insights already emerged from these studies. Specifically, not all metrics are equally informative: average velocity and thrashing rates are less predictive of an animal's lifespan than maximum velocity, which is highly predictive, while pharyngeal pumping is only a valid health (not healthspan) metric for young worms, with little correlation with future lifespan [171]. In addition, longer-lived wild-type individuals experience an extended ''twilight'' period of lowered physiological function [76,77]. It is important to note that several long-lived mutants, such as animals with reduced mitochondrial function, display lower overall health, as measured by various motility, reproduction, and pumping metrics [10,171,174]. These observations suggest that it cannot be assumed that all longevity-extending mechanisms also increase health [171,174]. However, *daf-2* IIS mutants are a notable exception, increasing all metrics of health other than total progeny number [10,171] and

providing support for the concept that lifespan and healthspan can be simultaneously extended.

Long-lived *daf-2* insulin receptor mutants appear to move less on bacterial plates [174]; however, Hahm et al. [171] showed that this behavior is controlled by high levels of the odorant receptor ODR-10 in this mutant. Reduction of *odr-10* expression levels by RNA interference caused *daf-2* worms to continue exploring continuously, distinguishing the *ability* to move from the ODR-10-driven ''motivation'' to move [171]. Moreover, *daf-2* mutants were found to be less susceptible to colonization by the bacterial food source [172], which appears to be the leading cause of early death in wild-type worm populations [86]. Thus, the presence of live bacteria is a confounding factor in motility assays, and therefore should be avoided in healthspan measurements.

Importantly, reduction of IIS in *C. elegans* improves many characteristics that are relevant for healthy human aging, including neuromuscular junction activity [175,176], axon regeneration [177], and the abilities to learn and remember [31,178]. *daf-2* mutants suppress neurodegenerative protein aggregation [179] and neuromorphological defects [180–182], are resistant to pathogenic infection [183], and they maintain high-quality oocytes with age [44,184]. Thus, *daf-2* mutants maintain many critical parameters of healthy aging. Transcriptional analyses of whole worms [185,186], oocytes [184], and neurons [31] in *daf-2*, wild-type, and *daf-16;daf-2* mutants have identified tissue-specific targets of the IIS pathway that allow *daf-2* worms to maintain different functions with age, which may shed light on how we might maintain our own health.

Future perspectives

In addition to the molecular analyses described above, there is considerable interest in developing drugs that postpone aging and frailty in humans [187]. Indeed, *C. elegans* already is used in candidate and high-throughput screening approaches to identify such drugs [188,189], and the *Caenorhabditis* Intervention Testing Program (CITP) has been established to complement a similar intervention testing program in mice (https://citp.squarespace.com). Moreover, the microbiome is increasingly recognized as an important contributor to healthy aging in humans, and recent studies indicate that *C. elegans* lifespan is also modulated by bacteria and their metabolites [190,191]. Beyond slowing aging, phenomena reminiscent of rejuvenation have been observed in *C. elegans* but require further mechanistic investigation [128,192–194]. In summary, *C. elegans* is a crucial experimental system for basic and translational research on aging.

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