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Translational Article

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Genetics, Life Span, Health Span, and the Aging Process in *Caenorhabditis elegans*

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As a tool for measuring the aging process, life span has been invaluable in dissecting the genes that modulate longevity. Studies over the past few decades have identified several hundred genes that can modify life span in model organisms such as yeast, worms, and flies. Yet, despite this vast amount of research, we still do not fully understand how the genes that affect life span influence how an organism ages. How does modulation of the genes that affect life span contribute to the aging process? Does life-span extension result in extension of healthy aging? Here, we will focus primarily on the insulin/IGF-1 signaling pathway in *Caenorhabditis elegans* because members of this pathway have been shown to be associated with extended life span across phylogeny, from worms to humans. I discuss how this connects to the aging process, age-associated disease, and the potential to increase healthy aging in addition to lengthening life span.

Key Words: Life span—Aging—Health span—*C. elegans*—Genetics.

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DEFINING the molecular cues that underlie the aging process has been the topic of many research studies over the last few decades. Many of the findings are derived from studies in model organisms including yeast, worms, and flies (1–4). The discovery of the first gene to modulate life span, *age-1* in *Caenorhabditis elegans*, led to an explosion of aging research in this system (5,6). Subsequently, these analyses resulted in the finding that the insulin/IGF-1 signaling (IIS) pathway is a central regulator of life span (1,3). Downstream of the insulin/IGF-1 receptor is a conserved signaling pathway that targets the forkhead box O transcription factor (FOXO), DAF-16. Importantly, since the cloning of DAF-16/FOXO in 1997, recent data across multiple human cohorts have shown an association with a FOXO variant and life-span extension (7–10).

Life Span As a Measurement for Aging Research

Over the last three decades, life span as a tool for aging research has been invaluable. Life span, defined as the time from birth until death, has been extensively used to study the aging process. This is in contrast to aging, which is a biological process that is not easily defined or measured. Definitions of aging can include what happens to an organism over time; the changes in tissues, cells, or organs over time; or an increased probability of death. The terms "life span" and "aging" tend to become intertwined, however, and, they are not equivalent.

Over the last decade, several hundred genes were found to modulate *C. elegans* life span. Analysis of these genes led to the finding that signaling pathways in addition to the IIS pathway also modulate life span, including target of rapamycin (TOR), sirtuins, Jun kinase (JNK), protein translation, and mitochondrial signaling (11–13). Mainly based on genetic epistasis analyses, these pathways have been shown to modulate life span either through intersection with the IIS pathway (eg, TOR signaling, dietary restriction [DR]) or as a separate pathway (DR and mitochondrial signaling) (13–15). In *C. elegans*, recent work has shown that six different DR regimens can extend life span (16). Genetic analysis of the different DR regimens shows that these methods seem to be regulated by either independent or additive pathways (16). In addition, TOR signaling is required for DR and intersects directly with the IIS (15,17). Therefore, primarily based on genetic analysis, the IIS and DR pathways function both in an overlapping and independent manner.

Many different types of genes (different functional classes) that modulate life span have been identified. However, thus far, mutants that show changes in life span also show changes in an additional process. For example, in addition to regulating life span, genes in the IIS pathway also modulate metabolism, development, and stress resistance (3,12). This is similar to longevity mutants in other organisms including flies in which mutations in the IIS pathway result in changes in size in addition to life span (18–20). Similarly, compared with wild-type mice, long-lived dwarf mice are smaller and have difficulty maintaining their body temperature (21). These findings suggest that regulation of life span may be molecularly linked to regulation of another biological process, such as growth or development. Does the fact that life-span mutants show pleiotropic (multiple related or unrelated phenotypic traits) effects indicate there are simply no "life-span genes?" Mutants that show changes in life span do not always show a similar secondary phenotype, although this has not been extensively studied. Alternatively, it is possible that the change in life span is simply a byproduct of another regulated process, such as reproduction or developmental stress that is coupled to life span. For example, perhaps the byproduct of a worm surviving some early developmental stress is life-span extension in the adult stage. Notably, inactivation of embryonic lethal genes in young adults can lead to life-span extension (22,23). It is also possible that the laboratory environment, in which *C. elegans* are grown with plenty of food, a constant temperature, and lack of crowding prevents identification of mutants that would help determine these answers. Consistent with this, *daf-16* mutants are sensitive to many external stresses (24,25). It is possible that *daf-16* mutants would not have ever been identified in the wild because they are stress sensitive. Alternatively, we may miss important genes because some life-extending genes would only display a longer life in the context of a "stressful" environment.

Therefore, using life span as a measurement gives us one insight into the process of how an animal ages. To date, many genes have been identified that have the ability to modulate life span when altered in dosage. But there are limited data on how these genes influence how an organism ages.

Dauer Formation and Life Span Are Interconnected

Much of our understanding of molecular pathways that regulate life span originated with studies in *C. elegans*. Klass (6,26) published a breakthrough study where mutants that had altered life span were identified. Subsequent studies by the Johnson lab showed that all of the mutants identified by Klass fell into a single genetic locus, named *age-1* (5,27). This finding was fundamentally important as it suggested that life span, similar to other processes in development, was under genetic control. It was not until a second age mutant, *daf-2* (28), was identified that aging studies in *C. elegans* seemed to become more popular and interesting to the public at large.

Age-1 and *daf-2* were originally isolated in a genetic screen that Don Riddle and coworkers conducted in *C. elegans* to identify mutations that altered development (29,30). Under normal growth conditions, worms progress from egg through four larval stages and then into a self-fertilizing hermaphrodite. However, under unfavorable growth conditions (particularly crowding) rather than form a reproducing adult hermaphrodite, worms enter an alternative larval stage and form a dauer larva at the third larval stage (L3). Dauers (for "enduring") are sealed from the environment and are basically a survival stage, where worms have a thick cuticle, do not feed, and show changes in their nervous system (similar to a spore-like state), and wait until growth conditions become more favorable, primarily for healthy progeny production (29,30).

Mutations in *age-1* (originally identified as *daf-23*) and *daf-2* were found to cause a temperature-sensitive dauer formation constitutive phenotype (*daf-c* [29,30]). A mutant that has a *daf-c* phenotype will form dauers even under favorable growth conditions, and most of these mutants are also temperature sensitive. Mutants that fail to form dauers even under unfavorable growth conditions were also isolated and were termed dauer formation defective, *daf-d*. In these original studies, *daf-16* was isolated as a *daf-d* mutant (29,30).

Interestingly, analysis of the genes that modulate dauer formation, which include both *daf-c* and *daf-d* mutants, placed *daf-2*, *daf-16*, *daf-18*, and *age-1*/*daf-23* in a separate linear pathway from the other genes involved in dauer formation (31,32). Further studies showed that these genes were in a pathway that modulated life span and stress resistance in addition to dauer formation and that was a separate genetic pathway from other genes that modulate dauer formation (31,33,34).

From 1996 to 2001, tremendous strides were made in our understanding of the molecular nature of the *daf-2*/*age-1*/*daf-16* pathway (3). Molecular cloning revealed that *daf-2* encoded for a receptor that bore sequence similarity to both insulin receptor and insulin-like growth factor-1 receptor (35), and *age-1* was the catalytic subunit of phosphatidylinositol 3-kinase (36). To date, analysis of the insulin-like mutants has shown that disruption of a single insulin gene cannot mimic loss of the *daf-2* receptor (37–39).

Following *daf-2* is a well-conserved phosphatidylinositol 3-kinase signaling cascade that ends in the direct modulation of DAF-16/FOXO (3,12,37–39). The IIS pathway regulates many biological processes including dauer/development, longevity, metabolism, and stress resistance. Therefore, DAF-16 is the target of the IIS pathway, and changes in the dosage or activity of DAF-16 result in changes in many biological functions including life span, dauer formation/development, stress resistance, and metabolism (3,12,37–39).

A large number of genes and signaling pathways that intersect with the IIS pathway or DAF-16 have been identified

within the last decade (12,17,37). These include signaling pathways that intersect with the IIS such as TOR signaling or pathways that intersect directly with *daf-16* such as JNK, 5' adenosine monophosphate-activated protein kinase (AMPK), and 14-3-3, sirtuins, protein translation, and mitochondrial signaling (37–39). In addition, recent studies using null mutants and methods to uncouple egg-laying problems from life span have led to a Transforming Growth Factor-β (TGF-β) signaling pathway intersecting with the IIS pathway for dauer formation and regulation of life span (40,41). Interestingly, microarray data from long-lived *daf-2* mutants and dauer larvae show significant overlap (42–44). Since worms do not age when in the dauer state (29), it is still possible in these long-lived mutants, that one is partially activating the dauer program. Therefore, in light of these findings, connections between the regulation of life span and the regulation of dauer formation need to be further examined.

Age-Associated Changes in *C. elegans*

Many mutants have been isolated that can extend life span in different model organisms. However, little attention has been paid to whether or not these mutations also extend healthy life or health span (defined herein as the healthy productive time before the onset of age-associated decline). With the large numbers of genes that can alter life span, one wonders which, if any, will lead to additional healthy time. Given the health costs associated with years of decrepitude, extending life span without also extending the duration of healthy time is not a goal of aging research. But how do we define health span in such disparate species as yeast, worms, flies, mice, monkeys, and humans? Will this be a similar measurement in the different model systems? What are the measurements that one can use to define health span in a laboratory setting? Can we model aspects of human health and human age-associated disease in *C. elegans*? To date, there have been limited studies on health span.

Studying health span in the laboratory creates several challenges. First, how does one define health span (45) or healthy aging? Second, do we study how a given laboratory animal ages and then define health (45)? Third, do we study aging traits that are common to both the lab animal and humans? To address any or all of these questions, one first needs to define normal patterns of age-related changes. To date, the most used measure for aging research is life span: a measurement of the length of time. However, is this the best measure? If one is studying the aging process in the laboratory to benefit humans, then surely measuring life span is just a small part of understanding the aging process to ultimately extend healthy aging.

Health-span studies are largely limited and have focused on age-associated decline characteristics that resemble human aging. *C. elegans* has been at the forefront of studies on healthy aging due to several factors including a short life cycle, transparency of the cuticle, and the ability to observe many changes under the dissecting microscope. Therefore, the normal aging process in wild-type worms can be easily dissected and observed. This has allowed documentation of age-related changes that occur at all levels—molecular, cellular, tissue, and organ. Examining the nature of the age-related changes in different tissues allows one to examine how a particular tissue changes with age and whether the changes are on the same time scale as changes in other tissues. These changes occur in muscular, neuronal, and reproductive tissues (46–50).

Age-related changes assays in *C. elegans* can often be scored longitudinally: The same worms can be monitored throughout their life span. In addition, some assays are done on populations of aged worms because the assay itself results in lethality. Important information on age-related changes can be garnered in both types of assays. For a subset of these age-related changes, analysis has been done on both wild-type worms and on longevity mutants as they age, including worms with mutations in the IIS pathway (*daf-2*, *age-1*, and *daf-16*), the mitochondrial timing gene *clk-1*, and the *eat-2* gene (which causes mutants to mimic DR). Here, we will focus on a subset of age-associated changes that have been studied in both wild-type worms and long-lived mutants (46,47,51,52). The reasoning is that if any of the age-related changes can be related to the causes of aging, then the physical, biochemical, and cellular change will occur either earlier than in wild-type worms (such as short-lived *daf-16* mutant) or later than in wild-type worms (such as long-lived mutant *daf-2*, *age-1*, *clk-1*, and *eat-2*).

A number of neuromuscular changes have been examined in wild-type worms as well as short-lived and long-lived mutants (46). It has been suggested that the *C. elegans* decline in movement with age is similar to human frailty in aged individuals, and similar to humans, worms show signs of sarcopenia (muscle wasting) as they age (53). These studies measure the rates of body movements and pharyngeal pumping (the pharynx is the organ through which a worm gets its food) chronologically. Body movements can be measured on solid media, where distance and movement patterns can be tracked, or in liquid media, where movements (thrashing/swimming) are measured. In both solid and liquid media, one can detect the ability of the worms to move in a coordinated fashion as they age. Worms move in a beautiful repetitive sinusoidal wave when they are young. As worms age, the shape of the wave on solid media changes and the patterns of body bends change in liquid media as well. These age-related changes correlate with deterioration of the body muscle wall, similar to mammalian sarcopenia and frailty. Importantly, compared with wild-type worms, long-lived mutants (eg, *daf-2*, *age-1*) have a delay in age-related deterioration of body movement and muscle deterioration (46).

Pharyngeal pumping has also been studied as a phenotype that can be easily measured and shows age-related changes. In *C. elegans*, the pharynx undergoes rhythmic contractions that facilitate feeding. This feeding rate can be

easily measured and counted under a dissecting microscope, and pharyngeal pumping shows age-related decline (46,51, 54–56). Therefore, these are age-related processes that are delayed in long-lived mutants (eg, *daf-2*, *age-1*).

Another age-related change is the accumulation of fluorescent compounds, which include lipofuscin and advanced glycosylation end products (AGEs). Lipofuscin is fluorescent and consists of the membrane-bound cellular waste. Because it cannot be degraded, it accumulates in postmitotic cells and therefore is often referred to as an "aging pigment" in both invertebrates and vertebrates (57,58). A second "age pigment" also conserved from worms to humans are AGEs, formed by a nonenzymatic addition of sugar to the free amino group of proteins which is followed by cross-linking by autocatalysis (59,60). Both of these pigments are modulated by the age of the organism from worms to humans (46). Therefore, in this case, similar effects of aging are seen across phylogeny. However, it is not known if the age pigments are a causative part of the aging process or simply a consequence.

In *C. elegans*, both age pigments have been shown to accumulate with age. In addition, several longevity mutants have been examined for effects on the accumulation of these pigments. The long-lived IIS mutants *age-1* and *daf-2* show lower levels of fluorescence in young animals and a slower rate of accumulation (46,58,61). Consistent with this, short-lived IIS *daf-16* mutants show the opposite effects including increased fluorescence and an accelerated accumulation of age pigments (46,57,58,61).

To further dissect the connections between age-related phenotypes, Gerstbrein and coworkers (58) examined the connections between body movement and age pigments as worms aged. Using spectrofluorimetry and microscopy, they performed a longitudinal study where they assessed both body movement and fluorescence. They found that in wild-type worms, there was a positive correlation between the decline in body movement and the accumulation of fluorescent material (58). Worms under conditions of dietary restriction as well as two long-lived mutants displayed both reduced levels of fluorescence and a slower rate of accumulation (58). For conditions that shorten life span, results have not been entirely correlated. The short-lived IIS mutant *daf-16* shows an increase in accumulation of age pigments, whereas the short-lived mitochondrial mutant *mev-1* has been shown to have either faster rate of accumulation of fluorescence (57) or decreased levels of age pigment (58). Therefore, accumulation of age pigments correlates with life span in wild-type and long-lived mutants.

The understanding of age-related changes in the nervous system of *C. elegans* is continuing to evolve. Interestingly, early studies on aging *C. elegans* where anatomy was examined by electron microscopy showed little change in the nervous system with age, especially when compared with muscle (53). In addition, genetic mosaic analysis suggested that the nervous system provided only a small contribution to the aging process (62). However, recent studies with newer and more specific examinations have shown evidence of neuronal aging in *C. elegans*. Pan and coworkers (63) examined both the touch receptor neurons and cholinergic neurons in aging worms. They observed changes in the electrical activity of the neurons, suggesting that neuronal integrity is compromised as worms age. They also found that the IIS pathway modulated neuronal integrity. Studies by Tank and coworkers (64) showed that as worms age, neurons frequently develop ectopic branches. They showed that these branches were seen in the mechanosensory neurons and suggest that the decreased mobility and decreased response to touch observed as worms age are due to these age-associated branching defects. Importantly, the age-associated ectopic branching phenotype was also modulated by the IIS pathway and the DAF-16 interactor JNK (64,65). Future studies on neuronal aging in *C. elegans* should examine long-lived mutants as a percentage of mean life span and determine if the neuronal changes observed correlate with associated phenotypic changes.

To further define the aging process in *C. elegans*, McGee and coworkers (47) performed extensive serial 3D reconstruction of aging worms, both wild-type and long-lived *daf-2* mutants. These studies identified a number of cellular age-related changes that were previously unidentified in the aging intestine, including changes in the size and shape of intestinal nuclei, loss of intestinal nuclei, and loss of microvilli. The loss of intestinal nuclei as worms age was highly stochastic at several levels, including within individuals and intestinal cells (47). When long-lived *daf-2* worms were examined, they showed a prolonged duration of time with a normal intestine, and the loss of intestinal integrity was delayed. However, it remains unclear how these age-associated changes in the *C. elegans* intestine contribute to any age-associated phenotypes in the worm or the death of the worm. Taken together, many *C. elegans* studies have shown that many age-related changes are accelerated in a short-lived mutant and delayed in a long-lived mutant.

Health-Span Studies in *C. elegans*

Recent studies have combined several of these changes to identify health-span regulators in *C. elegans*. Because there is no universal definition of health span nor is this term defined in the laboratory setting, Iwasa and coworkers (66) defined health span as "the period of midlife vigor that precedes significant functional decline." With this definition of health span, they performed a genetic screen using an automated recording device to screen for *C. elegans* mutants that showed a prolonged swimming ability (movement in liquid media) when compared with wild-type (66). This screen identified two genes termed high performance in advanced age locomotory 1 and 2 (*hpa-1* and *hpa-2*). Subsequent molecular and genetic studies showed that these genes were part of the epidermal growth factor

(EGF) signaling pathway. *hpa-1* and *hpa-2* mutants were further characterized for other age-associated phenotypes and showed decreased lipofuscin accumulation as well as decreased advanced glycation end-product accumulation. It is noteworthy that although these animals showed prolonged swimming ability, their median and mean life span were only slightly increased, and maximum life span was unaffected. *hpa-1* and *hpa-2* mutants also showed similar mortality rates over adult life when compared with wild type (where the rates of mortality rise exponentially with age). Therefore, these mutants live the same time as wild type but are active longer without changing their rate of aging. Analysis of additional components of the EGF signaling pathway components revealed that other EGF signaling genes also modulate healthy aging in worms, and these effects were largely independent of the IIS pathway. Therefore, by defining health span as locomotor swimming ability at advanced ages, the EGF signaling pathway was identified as a regulator of healthy aging in *C. elegans*.

The study by Iwasa and colleagues (66) showed for the first time that the ability to screen for healthy aging genes has the potential to identify new genes that modulate multiple age-associated parameters. It is somewhat surprising that the genes identified in this screen, *hpa-1* and *hpa-2*, seem to function independent of the IIS pathway (66) when multiple previous studies have suggested that the long-lived IIS mutants show a delay in many age-associated phenotypes including locomotion (46). How could this be?

One clue may come from important studies by Huang and coworkers (51) where different physiological processes were studied as worms age in wild type, IIS components (*daf-2*, *age-1*, and *daf-16*), *clk-1*, and *eat-2*. The life span was divided up into four different stages (I–IV) based on reproductive time, pharyngeal pumping time, and fast movement. Worms were evaluated as they aged and scored for the number of days spent in each stage. Data were then further analyzed by setting the mean life span equal to 100% and examining the fraction of the mean life span each strain occupied at the different stages. *daf-2* and *age-1* showed an expansion of the last stage when animals are no longer pumping and hardly moving. Therefore, examining all strains including wild type and mutants at their mean life span (51,66,67) gave additional insight into age-associated characteristics. In this way, one can also measure what proportion of life the animal is spending active. For example, if one defines health span as a disease-free state before the onset of age-associated decline, then all long-lived mutants have an increase in health span (Figure 1C). However, thus far, only one study (51) has measured a few parameters as a percentage of mean life span. Almost all studies examine age-associated changes in long-lived mutants chronologically: comparing mutants and wild type on the same day in the life span. It is also important to note that very few age-related assays have been done on older long-lived animals to examine their capabilities in their extended periods to determine if their extended life span results in extended health or decrepitude. Therefore, the connection between life span and health span needs to be further explored. It is unclear what the interplay between them will be: entirely separate (eg, EGF studies [66]), some genes in common, or completely overlapping (Figure 1).

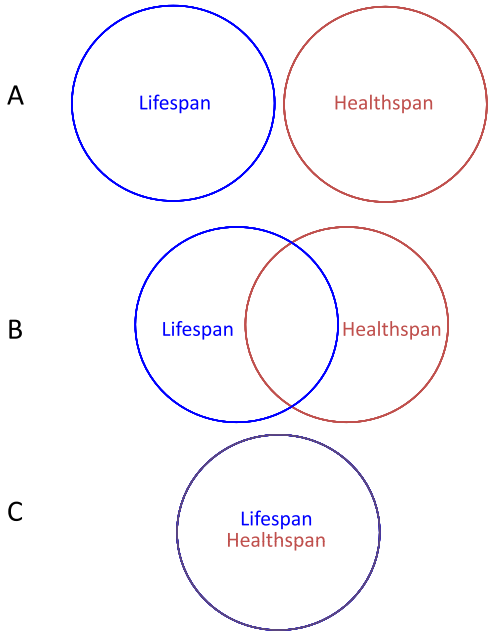

Figure 1. Determining the connection between life span and health span. In (**A**) all of the genes that regulate life span are different than the genes that modulate health span (eg, *pha-1* and *hpa-2*). In (**B**), there is partial overlap, and in (**C**), there is complete overlap. See text for details.

Modeling Human Age-Associated Disease

One possibility for extending life span and healthy aging is to prolong the time before the onset of age-associated disease. To examine this directly, several age-associated neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, and Huntington's disease have been explored using transgenic models in lower organisms. In *C. elegans*, this has allowed for dissecting connections between longevity and age-associated disease with a focus on whether or not life-span extension could change susceptibility to the neurodegenerative disease.

One of the attributes associated with Alzheimer's disease is the proteotoxicity and cell death that arises from the toxic aggregation of plaques consisting of beta-amyloid (Aβ) protein. In the *C. elegans* transgenic model for Alzheimer's disease, worms bear a transgene that expresses the human $A\beta_{1-42}$ driven by a muscle-specific promoter (known as Aβ worms) originally described by Link and colleagues (68). These worms develop plaque deposits in the muscle, and this leads to progressive paralysis. Two life-extending paradigms have been evaluated in the transgenic Aβ *C. elegans*. In the first study, transgenic worms were subject to one paradigm of DR, bacterial deprivation, where food is completely

removed and increases both median and maximum life span by 50% (69). Bacterial deprivation leads to a significant suppression in susceptibility to Aβ proteotoxicity (70). In the second study, reduction of IIS signaling also leads to protection against and Aβ aggregation–associated proteotoxicity (71). These data showed that when IIS is reduced during development and adulthood, animals are protected from paralysis mediated by $A\beta_{1-42}$. Interestingly, neither Aβ expression levels nor the Aβ total protein amounts are affected in these worms (71). More recently, Cohen and coworkers (72) directly assessed the connection between proteotoxicity and longevity with relation to IIS. Using temporal RNAi against the IIS revealed that decreasing levels of the IIS receptor *daf-2* either early or late in life protects from age onset proteotoxicity by invoking a mechanism that converts toxic aggregates into larger, less toxic high-molecular weight aggregates. Remarkably, further dissection of the temporal requirements of *daf-2* and *daf-16* revealed that these genes are required for protection against proteotoxicity well into adulthood, but this is independent of the life-span extension effects.

In Huntington's disease, individuals with the disease show an expansion of a CAG repeat. In the *C. elegans* model, transgenic worms bear a transgene with a tract of 35 conserved glutamine residues fused to YFP that is expressed in the body muscle, resulting in age-related proteotoxicity (73). Similar to the effects of Aβ, bacterial deprivation suppressed the proteotoxicity in this polyQ *C. elegans* strain (70), and reduction in IIS can also protect worms from polyQ-associated proteotoxicity (73,74). A third method of life-span extension is overexpression of the sirtuin *sir-2.1*, which has also been shown to be neuroprotective in this model (75).

Parkinson's disease is characterized by selective loss of dopaminergic neurons. In the *C. elegans* Parkinson's disease model, the *dat-1* promoter was fused to green fluorescence protein and specifically expressed in eight dopaminergic neurons. Worms were then treated with the neurotoxin 6-hydroxy dopamine, which resulted in dopaminergic neurodegeneration. Interestingly, under dietary restriction, these worms were protected from dopaminergic neurodegeneration. In addition, the sirtuin *sir-2.1* was necessary for achieving the neuroprotective effect of dietary restriction (76).

Therefore, dietary restriction and IIS signaling have protective effects against age-associated proteotoxicity in neurodegenerative disease. Importantly, one study in *C. elegans* suggests that this effect is independent of the longevity effect. Therefore, future studies are required to determine mechanistically how different signaling pathways can regulate these parameters independently of each other.

Longevity research has made tremendous strides since the first discovery of a gene that modifies life span (*age-1*) in 1983 (6). To date, several hundred genes have been found to be able to modulate life span. The hope and challenge for the future lies with dissecting the connections between life-span extension and healthy aging/health span. The key to determining these connections may lie in how we define, assess, and evaluate the data and the effects of the laboratory setting.

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