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PRIMER

The role of insulin/IGF-like signaling in *C. elegans* longevity and aging

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Aging is characterized by general physiological decline over time. A hallmark of human senescence is the onset of various age-related afflictions including neurodegeneration, cardiovascular disease and cancer. Although environmental and stochastic factors undoubtedly contribute to the increased incidence of disease with age, recent studies suggest that intrinsic genetic determinants govern both life span and overall health. Current aging research aims at achieving the 'longevity dividend', in which life span extension in humans is accomplished with a concomitant increase in the quality of life (Olshansky et al., 2007). Significant progress has been made using model organisms, especially the nematode worm *Caenorhabditis elegans*, to delineate the genetic and biochemical pathways involved in aging to identify strategies for therapeutic intervention in humans. In this review, we discuss how *C. elegans* has contributed to our understanding of insulin signaling and aging.

C. elegans as a premier longevity and aging model

Aging studies in mammals are challenging owing to relatively long life spans and a limited set of genetic tools that are capable of assessing the genome-wide changes associated with disease. By contrast, *C. elegans* lives for only 2-3 weeks and is easily propagated in culture, facilitating rapid genetic analyses. Genetic resources for *C. elegans* include hundreds of publicly available mutant strains; full-genome RNA interference (RNAi) libraries, which allow efficient knockdown of gene expression by simply feeding worms with bacteria expressing double-stranded RNAs (dsRNAs); gene-deletion strains, which are being generated for each of the ~19,000 genes; and >2000 *promoter::gfp* strains, which allow characterization of cellular and sub-cellular gene expression patterns. Moreover, all of the 959 lineage-invariant somatic cells of *C. elegans* are post-mitotic, making the worm an attractive model for aging in non-dividing cells such as mammalian muscle cells and neurons that depend on maintenance instead of cell turnover for their longevity. The mitotically dividing *C. elegans* germline, which also ages (Garigan et al., 2002), provides an excellent model for the study of age-related reproductive decline (Hughes et al., 2007). Although signals from the germline influence life span (Hsin and Kenyon, 1999), and life span and reproductive span are linked (Luo et al., 2009), post-mitotic somatic cell integrity limits the overall life span of the animal (Herndon et al., 2002).

Importantly, *C. elegans* shares many similarities with mammals and humans, including aging and functional senescence (Fig. 1). Aging worms experience a decline in mobility (Herndon et al., 2002; Huang et al., 2004; Hsu et al., 2009), chemotaxis (Murakami and Murakami, 2005) and reproductive capacity (Hughes et al., 2007), and become increasingly susceptible to lethal infections (Garigan et al., 2002; Garsin et al., 2003). Exogenous expression of proteins such as amyloid beta (1-42) [Aβ(1-42)] and polyglutamine (polyQ) also make the worm a model for late-onset diseases such as Alzheimer's disease and Huntington's disease (Link, 1995; Faber et al., 1999). These features make *C. elegans* an excellent system to study conserved aging pathways and age-related diseases in mammals.

Insulin/IGF-1 signaling

Over 20 years ago it was discovered that mutations in *daf-2* and *age-1* double the life span of worms (Friedman and Johnson, 1988; Kenyon et al., 1993). Subsequent cloning of these genes uncovered the importance of the insulin/insulin-like growth factor-1 (IGF-1) signaling (IIS) pathway in aging regulation. *daf-2* encodes the only insulin/IGF-1 receptor expressed in worms, and *age-1* is the catalytic subunit of the downstream phosphoinositide 3-kinase (PI3K) (Morris et al., 1996; Kimura et al., 1997). In addition to the overall life span extension, worms bearing either of these mutations are highly resistant to oxidative stress (Honda and Honda, 1999), hypoxia (Scott et al., 2002), heat stress (Lithgow et al., 1995), heavy metals (Barsyte et al., 2001) and bacterial pathogens (Garsin et al., 2003). These findings support the notion that aging is a genetically regulated process and that mutations in longevity-linked genes delay the onset of stochastic decline (Herndon et al., 2002).

The IIS pathway also acts as a food and stress sensor during development. When food is abundant, worms develop rapidly and uninterrupted through four larval stages to reach adulthood. If worms develop in hot, food-limited or overcrowded conditions, they enter an alternative long-lived larval state called dauer in which reproductive maturity is delayed and stress resistance increases. Favorable conditions promote IIS-regulated exit from dauer, and development to reproductive adults (Cassada and Russell, 1975). In fact, *daf-2* mutants were originally characterized by a prominent dauer-constitutive phenotype (Riddle et al., 1981).

Both the long-lived and dauer phenotypes of *daf-2* worms are dependent on the

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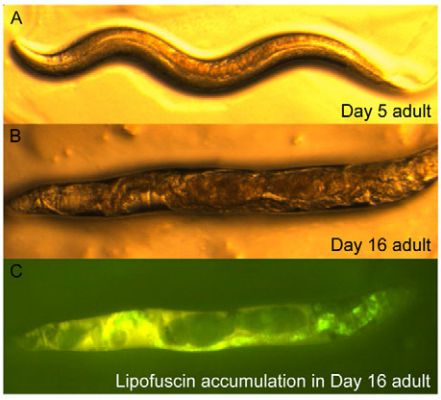

Fig. 1. Signs of aging in *C. elegans*. (A,B) The gross morphology of youthful day 5 adult worms (A) compared with aged day 16 worms (B) is strikingly different. Aging in old worms is accompanied by declines in mobility, and the ability to sense and respond to environmental stimuli. (C) Lipofuscin accumulation in a day 16 adult worm. Lipofuscin, meaning 'dark fat', accumulates in cells with age and serves as an autofluorescent aging biomarker in both worms and humans.

downstream forkhead transcription factor DAF-16 (Riddle et al., 1981; Kenyon et al., 1993). Briefly, signaling through DAF-2 activates PI3K, which leads to the phosphorylation of DAF-16 and its inactivation by nuclear exclusion. In the absence of IIS or in *daf-2* or *age-1* mutants, DAF-16 enters the nucleus and enacts a transcriptional program that doubles worm life span (Lin et al., 1997; Ogg et al., 1997; Lee et al., 2001). Loss of *daf-2* or *age-1* also slows the age-related declines described above (Herndon et al., 2002; Huang et al., 2004; Garigan et al., 2002; Garsin et al., 2003); thus, the IIS pathway regulates longevity through its modulation of aging processes. This inverse correlation between aging phenotypes and survival allows the study of aging through evaluation of life span.

IIS is an evolutionarily conserved pathway that regulates life span across many species. *Drosophila melanogaster*, like *C. elegans*, has a single insulin-like receptor (*dInR*) that, when mutated, extends life span in a manner that is dependent on its *daf-16* homolog, *dFOXO* (also known as *foxo*) (Tatar et al., 2001). Overexpression of *dFOXO* exclusively in the fat body (Hwangbo et al., 2004), or mutation of the insulin receptor substrate protein *chico*, similarly extends life span (Clancy et al., 2001). Mammals encode several *daf-2* homologs [IGF-1 receptor (IGF-1R), insulin receptor (IR)-A and IR-B] that display even greater complexity in their ability to form multiple homodimer and heterodimer pairs (Benyoucef et al., 2007). Despite these differences in insulin receptor expression, the functional consequences are similar, as reduced IIS extends life span in multiple mammalian species. Longevity in dogs is inversely proportional to body size (Greer et al., 2007). Interestingly, a single nucleotide polymorphism in IGF-1 is associated with small size, suggesting that IIS influences aging in dogs (Sutter et al., 2007). Heterozygous IGF-1R knockout mice are long-lived (Holzenberger et al., 2003), and adipose tissue-specific insulin receptor knockout (FIRKO) mice also exhibit increases in life span (Bluher et al., 2003). Further evidence comes from studies of the klotho (*Kl*) gene, which appears to regulate insulin sensitivity. Klotho knockout mice exhibit symptoms of enhanced aging, whereas overexpression of klotho results in repressed IIS and, consequently, life span extension (Kuro-o et al., 1997; Kurosu et al., 2005).

Recent studies suggest that IIS and aging are coordinated processes in humans as well. Variants of klotho in humans (*KL-VS*) are associated with age (Arking et al., 2002). Moreover, genetic variations in IIS pathway components are linked to long life (Pawlikowska et al., 2009). Mutations in a human *daf-16* homolog, *FOXO3a*, are linked to increased longevity (Willcox et al., 2008; Anselmi et al., 2009; Flachsbart et al., 2009). Long-lived men who are homozygous for the *FOXO3a GG* genotype also display greater insulin sensitivity (Willcox et al., 2008). In addition, IGF-1R mutations are highly represented in populations of centenarians (Suh et al., 2008). The highly conserved functional nature of IIS between worms, flies and mammals, including humans, demonstrates that findings from *C. elegans* can contribute greatly to our understanding of aging in higher organisms.

Transcriptional regulation of aging

Understanding the molecular nature of aging requires identification of the transcriptional and cellular changes that occur as a consequence of IIS and, in particular, DAF-16/FOXO activity. Genome-wide analyses of DAF-16 targets in *C. elegans* have contributed to our knowledge of how life extension is achieved in the worm. It is likely that some of these genes will also influence mammalian life span, with the goal being to identify targets for the treatment of aging and age-associated diseases.

Several approaches have been used to identify DAF-16 downstream targets, including bioinformatics, chromatin immunoprecipitation (chIP) and serial analysis of gene expression (SAGE) (Lee et al., 2003; Halaschek-Wiener et al., 2005; Oh et al., 2005). However, the greatest wealth of data was generated using unbiased microarray analyses. A comparison of *daf-2* and *daf-2/daf-16* RNAi-treated or mutant worms revealed that both activation and repression of many genes is required for longevity (Murphy et al., 2003). In fact, no single DAF-16 target that has been tested can recapitulate the long-lived phenotype of *daf-2* worms (Murphy et al., 2003), suggesting that many disparate cellular pathways are regulated in concert to extend life span (Murphy et al., 2003; McElwee et al., 2004).

Microarray analyses identified several genes that were expected to be involved in stress response, including *sod-3* (manganese superoxide dismutase), *mtl-1* (metallothionein), *ctl-1* (catalase) and *hsp-16.2* (heat shock protein), which were upregulated in *daf-2* mutants, as well as several novel categories of gene that had not been previously associated with life span extension (Murphy et al., 2003; McElwee et al., 2004). Pathogen resistance factors, cytochrome P450s, and various steroid, lipid and carbohydrate metabolism genes are all upregulated by DAF-16 (Murphy et al., 2003). Several other gene classes are conversely repressed by DAF-16, including some involved in neuronal signaling, apolipoprotein binding, RNA binding, DNA replication and protein turnover (Murphy et al., 2003). These genes antagonize life span and are accordingly downregulated in *daf-2* mutants. Many uncharacterized life span-enhancing or -shortening genes were also revealed in these studies, suggesting that additional pathways influencing longevity remain undiscovered (Murphy et al., 2003).

It is currently unknown whether the DAF-16/FOXO-dependent transcriptional changes that have been observed in worms are recapitulated in Drosophila and mammals. Although some longevity mechanisms will prove specific to *C. elegans*, several similarities in known FOXO targets across

Advantages of using *C. elegans* to study aging and age-related diseases

- Short life span and powerful genetic tools permit facile study of molecular factors and pathways underlying aging
- The insulin/insulin-like growth factor-1 signaling (IIS) pathway, a master regulator of aging in worms, is highly conserved in higher organisms including humans
- Worms exhibit many physical and behavioral traits that decline with age, facilitating the study of many late-onset diseases
- Genetic and small molecule drug screens in worm aging models will probably provide important clues towards understanding aging mechanisms and the discovery of novel disease treatment options

species suggest that the life span-extending pathways identified in worms will extend to many organisms.

Endocrine signaling and tissue specificity

C. elegans encodes ~40 insulin-like molecules (INS) that are expressed primarily in neurons, although a few are also expressed in the intestine (Pierce et al., 2001; Li et al., 2003; Murphy et al., 2003; Murphy et al., 2007). Worm insulins act as either DAF-2 agonists (such as INS-7 and DAF-28) or antagonists (INS-1 and INS-18), and it has been proposed that INS act in opposing pairs to regulate IIS in response to environmental signals (Murphy et al., 2007). Insulins are secreted from tissues and have both autocrine and paracrine effects that modulate DAF-2 signaling. Recent evidence also suggests that INSs have endocrine effects on IIS that influence the coordination of aging throughout the body (Murphy et al., 2007). For example, laser ablation of sensory taste neurons extends life span in a *daf-16*-dependent manner, indicating that environmental signals in one tissue influence the state of aging elsewhere (Alcedo and Kenyon, 2004).

In a current model of IIS endocrine signaling, environmental cues trigger the release of INS and other hormones from sensory neurons that subsequently regulate IIS in peripheral tissues. The central nervous system (CNS), intestine and gonad respond by producing secondary hormones that modulate DAF-16 activity in the entire worm. *daf-2* and *age-1* expression are required in the nervous system for non-cell-autonomous regulation of IIS. Mosaic worms that have lost *daf-2* activity solely in a subset of neurons are long-lived (Apfeld and Kenyon, 1998). Neuronal expression of wild-type *age-1* or *daf-2* in their respective mutant backgrounds is both necessary and sufficient to restore normal life span of long-lived mutants (Wolkow et al., 2000).

The tissue-specific requirement for *daf-16* is markedly different. Neuronal *daf-16* expression in a *daf-16;daf-2* background resulted in a small (5-20%) extension of life span, whereas *daf-16* rescue in the intestine, which serves as the worm's adipose tissue, partially recapitulated the long-lived phenotype (50-60%) (Libina et al., 2003). However, *daf-2* mutants expressing only non-intestinal *daf-16* also exhibited substantially extended life span (70-80% over background) (Libina et al., 2003). Furthermore, reconstitution of both neuronal and intestinal *daf-16* in *daf-16;age-1* mutants produces an additive effect on life span, suggesting that multiple sources of DAF-16 activity regulate aging (Iser et al., 2007).

Similarly, adipose tissue and the nervous system play a role in aging regulation in mice and flies. Fat-specific insulin receptor knockout (FIRKO) mice and flies expressing dFOXO in the fat body have increased longevity (Bluher et al., 2003; Hwangbo et al., 2004). Mice lacking neuronal IGF-1 receptors or insulin receptor substrate-2 (Irs2) are similarly long-lived (Taguchi et al., 2007; Kappeler et al., 2008).

Observations that *daf-16* expression in the worm intestine affects DAF-16 activity in distant tissues, and the discovery that DAF-16 regulates the expression of insulin-like peptides, led to the discovery of a positive feedback loop (Libina et al., 2003; Murphy et al., 2003). RNAi knockdown of the DAF-2 agonist *ins-7* extends life span (Murphy et al., 2003). In a process termed FOXO-to-FOXO signaling, DAF-16 represses expression of *ins-7* in the worm intestine. Lower INS-7 levels reduce DAF-2 activity in the entire worm, propagating DAF-2/DAF-16 signaling (Murphy et al., 2007). Endocrine regulation of aging is probably not unique to *C. elegans*. Several lines of evidence suggest that Drosophila neuronally derived insulin-like peptides (dILPs) act in peripheral tissues to influence IIS and aging (Hwangbo et al., 2004; Broughton et al., 2005; Bauer et al., 2007; Geminard et al., 2009). Moreover, mice with defects in growth hormone and reduced IGF-1 signaling are long-lived (Flurkey et al., 2001; Holzenberger et al., 2003).

C. elegans as a model for age-related diseases

The study of *C. elegans* provides a unique opportunity to model the complex genetics and biochemistry of several age-associated diseases in addition to the study of overall longevity. Systems have been developed for the study of Alzheimer's disease [human Aβ(1-42), Tau] (Link, 1995; Kraemer et al., 2003), Parkinson's disease (α-synuclein) (Lakso et al., 2003), Huntington's disease (polyQ) (Faber et al., 1999), amyotrophic lateral sclerosis (ALS) (*sod-1*) (Wang et al., 2009), Hutchinson-Gilford progeria syndrome (nuclear lamin) (Haithcock et al., 2005), cancer (ionization-induced apoptosis) (Bergamaschi et al., 2003) and diabetes (Schulz et al., 2007; Lee, 2009). Mutations in IIS appear to ameliorate the toxic effects of Aβ(1-42) and polyQ (Morley et al., 2002; Hsu et al., 2003; Florez-McClure et al., 2007). These findings demonstrate promise for identifying genes that influence disease outcomes. As additional disease models are established and specific genetic targets are identified, drug screens will facilitate the discovery of novel therapeutics. Such screens have successfully uncovered drugs that extend life span in worms (Petrascheck et al., 2007).

Conclusions

There is a continual search for genetic factors that influence both overall aging and the onset and progression of age-related diseases. The linkage of IIS to aging using the simple worm model *C. elegans* has laid the foundation for detailed molecular studies aimed at understanding and modulating longevity. Using disease models and drug screens, new findings in the worm can be tested in mammals with the goal of improving the extent and quality of life in humans. Such an achievement would have profound health, social and economic impacts on society.

COMPETING INTERESTS

The authors declare no competing financial interests.

REFERENCES

Alcedo, J. and Kenyon, C. (2004). Regulation of C. elegans longevity by specific gustatory and olfactory neurons. *Neuron* **41**, 45-55.

Anselmi, C. V., Malovini, A., Roncarati, R., Novelli, V., Villa, F., Condorelli, G., Bellazzi, R. and Puca, A. A. (2009). Association of the FOXO3A locus with extreme longevity in a southern Italian centenarian study. *Rejuvenation Res.* **12**, 95-104.

Apfeld, J. and Kenyon, C. (1998). Cell nonautonomy of C. elegans daf-2 function in the regulation of diapause and life span. *Cell* **95**, 199-210.

Arking, D. E., Krebsova, A., Macek, M., Sr, Macek, M., Jr, Arking, A., Mian, I. S., Fried, L., Hamosh, A., Dey, S., McIntosh, I. et al. (2002). Association of human aging with a functional variant of klotho. *Proc. Natl. Acad. Sci. USA* **99**, 856-861.

Barsyte, D., Lovejoy, D. A. and Lithgow, G. J. (2001). Longevity and heavy metal resistance in daf-2 and age-1 long-lived mutants of Caenorhabditis elegans. *FASEB J.* **15**, 627-634.

Bauer, J. H., Chang, C., Morris, S. N., Hozier, S., Andersen, S., Waitzman, J. S. and Helfand, S. L. (2007). Expression of dominant-negative Dmp53 in the adult fly brain inhibits insulin signaling. *Proc. Natl. Acad. Sci. USA* **104**, 13355-13360.

Benyoucef, S., Surinya, K. H., Hadaschik, D. and Siddle, K. (2007). Characterization of insulin/IGF hybrid receptors: contributions of the insulin receptor L2 and Fn1 domains and the alternatively spliced exon 11 sequence to ligand binding and receptor activation. *Biochem. J.* **403**, 603-613.

Bergamaschi, D., Samuels, Y., O'Neil, N. J., Trigiante, G., Crook, T., Hsieh, J. K., O'Connor, D. J., Zhong, S., Campargue, I., Tomlinson, M. L. et al. (2003). iASPP oncoprotein is a key inhibitor of p53 conserved from worm to human. *Nat. Genet.* **33**, 162-167.

Bluher, M., Kahn, B. B. and Kahn, C. R. (2003). Extended longevity in mice lacking the insulin receptor in adipose tissue. *Science* **299**, 572-574.

Broughton, S. J., Piper, M. D., Ikeya, T., Bass, T. M., Jacobson, J., Driege, Y., Martinez, P., Hafen, E., Withers, D. J., Leevers, S. J. et al. (2005). Longer lifespan, altered metabolism, and stress resistance in Drosophila from ablation of cells making insulin-like ligands. *Proc. Natl. Acad. Sci. USA* **102**, 3105-3110.

Cassada, R. C. and Russell, R. L. (1975). The dauerlarva, a post-embryonic developmental variant of the nematode Caenorhabditis elegans. *Dev. Biol.* **46**, 326-342.

Clancy, D. J., Gems, D., Harshman, L. G., Oldham, S., Stocker, H., Hafen, E., Leevers, S. J. and Partridge, L. (2001). Extension of life-span by loss of CHICO, a Drosophila insulin receptor substrate protein. *Science* **292**, 104-106.

Faber, P. W., Alter, J. R., MacDonald, M. E. and Hart, A. C. (1999). Polyglutamine-mediated dysfunction and apoptotic death of a Caenorhabditis elegans sensory neuron. *Proc. Natl. Acad. Sci. USA* **96**, 179-184.

Flachsbart, F., Caliebe, A., Kleindorp, R., Blanche, H., von Eller-Eberstein, H., Nikolaus, S., Schreiber, S. and Nebel, A. (2009). Association of FOXO3A variation with human longevity confirmed in German centenarians. *Proc. Natl. Acad. Sci. USA* **106**, 2700-2705.

Florez-McClure, M. L., Hohsfield, L. A., Fonte, G., Bealor, M. T. and Link, C. D. (2007). Decreased insulin-receptor signaling promotes the autophagic degradation of beta-amyloid peptide in C. elegans. *Autophagy* **3**, 569-580.

Flurkey, K., Papaconstantinou, J., Miller, R. A. and Harrison, D. E. (2001). Lifespan extension and delayed immune and collagen aging in mutant mice with defects in growth hormone production. *Proc. Natl. Acad. Sci. USA* **98**, 6736-6741.

Friedman, D. B. and Johnson, T. E. (1988). A mutation in the age-1 gene in Caenorhabditis elegans lengthens life and reduces hermaphrodite fertility. *Genetics* **118**, 75-86.

Garigan, D., Hsu, A. L., Fraser, A. G., Kamath, R. S., Ahringer, J. and Kenyon, C. (2002). Genetic analysis of tissue aging in Caenorhabditis elegans: a role for heat-shock factor and bacterial proliferation. *Genetics* **161**, 1101-1112.

Garsin, D. A., Villanueva, J. M., Begun, J., Kim, D. H., Sifri, C. D., Calderwood, S. B., Ruvkun, G. and Ausubel, F. M. (2003). Long-lived C. elegans daf-2 mutants are resistant to bacterial pathogens. *Science* **300**, 1921.

Geminard, C., Rulifson, E. J. and Leopold, P. (2009). Remote control of insulin secretion by fat cells in Drosophila. *Cell Metab.* **10**, 199-207.

Greer, K. A., Canterberry, S. C. and Murphy, K. E. (2007). Statistical analysis regarding the effects of height and weight on life span of the domestic dog. *Res. Vet. Sci.* **82**, 208-214.

Haithcock, E., Dayani, Y., Neufeld, E., Zahand, A. J., Feinstein, N., Mattout, A., Gruenbaum, Y. and Liu, J. (2005). Age-related changes of nuclear architecture in Caenorhabditis elegans. *Proc. Natl. Acad. Sci. USA* **102**, 16690-16695.

Halaschek-Wiener, J., Khattra, J. S., McKay, S., Pouzyrev, A., Stott, J. M., Yang, G. S., Holt, R. A., Jones, S. J., Marra, M. A., Brooks-Wilson, A. R. et al. (2005). Analysis of long-lived C. elegans daf-2 mutants using serial analysis of gene expression. *Genome Res.* **15**, 603-615.

Herndon, L. A., Schmeissner, P. J., Dudaronek, J. M., Brown, P. A., Listner, K. M., Sakano, Y., Paupard, M. C., Hall, D. H. and Driscoll, M. (2002). Stochastic and genetic factors influence tissue-specific decline in ageing C. elegans. *Nature* **419**, 808-814.

Holzenberger, M., Dupont, J., Ducos, B., Leneuve, P., Geloen, A., Even, P. C., Cervera, P. and Le Bouc, Y. (2003). IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice. *Nature* **421**, 182-187.

Honda, Y. and Honda, S. (1999). The daf-2 gene network for longevity regulates oxidative stress resistance and Mn-superoxide dismutase gene expression in Caenorhabditis elegans. *FASEB J.* **13**, 1385-1393.

Hsin, H. and Kenyon, C. (1999). Signals from the reproductive system regulate the lifespan of C. elegans. *Nature* **399**, 362-366.

Hsu, A. L., Murphy, C. T. and Kenyon, C. (2003). Regulation of aging and age-related disease by DAF-16 and heat-shock factor. *Science* **300**, 1142-1145.

Hsu, A. L., Feng, Z., Hsieh, M. Y. and Xu, X. Z. (2009). Identification by machine vision of the rate of motor activity decline as a lifespan predictor in C. elegans. *Neurobiol. Aging* **30**, 1498-1503.

Huang, C., Xiong, C. and Kornfeld, K. (2004). Measurements of age-related changes of physiological processes that predict lifespan of Caenorhabditis elegans. *Proc. Natl. Acad. Sci. USA* **101**, 8084-8089.

Hughes, S. E., Evason, K., Xiong, C. and Kornfeld, K. (2007). Genetic and pharmacological factors that influence reproductive aging in nematodes. *PLoS Genet.* **3**, e25.

Hwangbo, D. S., Gershman, B., Tu, M. P., Palmer, M. and Tatar, M. (2004). Drosophila dFOXO controls lifespan and regulates insulin signalling in brain and fat body. *Nature* **429**, 562-566.

Iser, W. B., Gami, M. S. and Wolkow, C. A. (2007). Insulin signaling in Caenorhabditis elegans regulates both endocrine-like and cell-autonomous outputs. *Dev. Biol.* **303**, 434-447.

Kappeler, L., De Magalhaes Filho, C., Dupont, J., Leneuve, P., Cervera, P., Perin, L., Loudes, C., Blaise, A., Klein, R., Epelbaum, J. et al. (2008). Brain IGF-1 receptors control mammalian growth and lifespan through a neuroendocrine mechanism. *PLoS Biol.* **6**, e254.

Kenyon, C., Chang, J., Gensch, E., Rudner, A. and Tabtiang, R. (1993). A C. elegans mutant that lives twice as long as wild type. *Nature* **366**, 461-464.

Kimura, K. D., Tissenbaum, H. A., Liu, Y. and Ruvkun, G. (1997). daf-2, an insulin receptor-like gene that regulates longevity and diapause in Caenorhabditis elegans. *Science* **277**, 942-946.

Kraemer, B. C., Zhang, B., Leverenz, J. B., Thomas, J. H., Trojanowski, J. Q. and Schellenberg, G. D. (2003). Neurodegeneration and defective neurotransmission in a Caenorhabditis elegans model of tauopathy. *Proc. Natl. Acad. Sci. USA* **100**, 9980-9985.

Kuro-o, M., Matsumura, Y., Aizawa, H., Kawaguchi, H., Suga, T., Utsugi, T., Ohyama, Y., Kurabayashi, M., Kaname, T., Kume, E. et al. (1997). Mutation of the mouse klotho gene leads to a syndrome resembling ageing. *Nature* **390**, 45-51.

Kurosu, H., Yamamoto, M., Clark, J. D., Pastor, J. V., Nandi, A., Gurnani, P., McGuinness, O. P., Chikuda, H., Yamaguchi, M., Kawaguchi, H. et al. (2005). Suppression of aging in mice by the hormone Klotho. *Science* **309**, 1829-1833.

Lakso, M., Vartiainen, S., Moilanen, A. M., Sirvio, J., Thomas, J. H., Nass, R., Blakely, R. D. and Wong, G. (2003). Dopaminergic neuronal loss and motor deficits in Caenorhabditis elegans overexpressing human alpha-synuclein. *J. Neurochem.* **86**, 165-172.

Lee, R. Y., Hench, J. and Ruvkun, G. (2001). Regulation of C. elegans DAF-16 and its human ortholog FKHRL1 by the daf-2 insulin-like signaling pathway. *Curr. Biol.* **11**, 1950-1957.

Lee, S.-J., Murphy, C. T. and Kenyon, C. (2009). Glucose shortens the life span of C. elegans by downregulating Aquaporin Gene Expression. *Cell Metab.* **10**, 379-391.

Lee, S. S., Kennedy, S., Tolonen, A. C. and Ruvkun, G. (2003). DAF-16 target genes that control C. elegans life-span and metabolism. *Science* **300**, 644-647.

Li, W., Kennedy, S. G. and Ruvkun, G. (2003). daf-28 encodes a C. elegans insulin superfamily member that is regulated by environmental cues and acts in the DAF-2 signaling pathway. *Genes Dev.* **17**, 844-858.

Libina, N., Berman, J. R. and Kenyon, C. (2003). Tissue-specific activities of C. elegans DAF-16 in the regulation of lifespan. *Cell* **115**, 489-502.

Lin, K., Dorman, J. B., Rodan, A. and Kenyon, C. (1997). daf-16: An HNF-3/forkhead family member that can function to double the life-span of Caenorhabditis elegans. *Science* **278**, 1319-1322.

Link, C. D. (1995). Expression of human beta-amyloid peptide in transgenic Caenorhabditis elegans. *Proc. Natl. Acad. Sci. USA* **92**, 9368-9372.

Lithgow, G. J., White, T. M., Melov, S. and Johnson, T. E. (1995). Thermotolerance and extended life-span conferred by single-gene mutations and induced by thermal stress. *Proc. Natl. Acad. Sci. USA* **92**, 7540-7544.

Luo, S., Shaw, W. M., Ashraf, J. and Murphy, C. T. (2009). TGF-beta Sma/Mab signaling mutations uncouple reproductive aging from somatic aging. *PLoS Genet.* **5**, e1000789.

McElwee, J. J., Schuster, E., Blanc, E., Thomas, J. H. and Gems, D. (2004). Shared transcriptional signature in Caenorhabditis elegans Dauer larvae and long-lived daf-2 mutants implicates detoxification system in longevity assurance. *J. Biol. Chem.* **279**, 44533-44543.

Morley, J. F., Brignull, H. R., Weyers, J. J. and Morimoto, R. I. (2002). The threshold for polyglutamine-expansion protein aggregation and cellular toxicity is dynamic and influenced by aging in Caenorhabditis elegans. *Proc. Natl. Acad. Sci. USA* **99**, 10417-10422.

Morris, J. Z., Tissenbaum, H. A. and Ruvkun, G. (1996). A phosphatidylinositol-3-OH kinase family member regulating longevity and diapause in Caenorhabditis elegans. *Nature* **382**, 536-539.

Murakami, S. and Murakami, H. (2005). The effects of aging and oxidative stress on learning behavior in C. elegans. *Neurobiol. Aging* **26**, 899-905.

Murphy, C. T., McCarroll, S. A., Bargmann, C. I., Fraser, A., Kamath, R. S., Ahringer, J., Li, H. and Kenyon, C. (2003). Genes that act downstream of DAF-16 to influence the lifespan of Caenorhabditis elegans. *Nature* **424**, 277-283.

Murphy, C. T., Lee, S. J. and Kenyon, C. (2007). Tissue entrainment by feedback regulation of insulin gene expression in the endoderm of Caenorhabditis elegans. *Proc. Natl. Acad. Sci. USA* **104**, 19046-19050.

Ogg, S., Paradis, S., Gottlieb, S., Patterson, G. I., Lee, L., Tissenbaum, H. A. and Ruvkun, G. (1997). The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in C. elegans. *Nature* **389**, 994-999.

Oh, S. W., Mukhopadhyay, A., Svrzikapa, N., Jiang, F., Davis, R. J. and Tissenbaum, H. A. (2005). JNK regulates lifespan in Caenorhabditis elegans by modulating nuclear translocation of forkhead transcription factor/DAF-16. *Proc. Natl. Acad. Sci. USA* **102**, 4494-4499.

Olshansky, S. J., Perry, D., Miller, R. A. and Butler, R. N. (2007). Pursuing the longevity dividend: scientific goals for an aging world. *Ann. NY Acad. Sci.* **1114**, 11-13.

Pawlikowska, L., Hu, D., Huntsman, S., Sung, A., Chu, C., Chen, J., Joyner, A. H., Schork, N. J., Hsueh, W. C., Reiner, A. P. et al. (2009). Association of common genetic variation in the insulin/IGF1 signaling pathway with human longevity. *Aging Cell* **8**, 460-472.

Petrascheck, M., Ye, X. and Buck, L. B. (2007). An antidepressant that extends lifespan in adult Caenorhabditis elegans. *Nature* **450**, 553-556.

Pierce, S. B., Costa, M., Wisotzkey, R., Devadhar, S., Homburger, S. A., Buchman, A. R., Ferguson, K. C., Heller, J., Platt, D. M., Pasquinelli, A. A. et al. (2001). Regulation of DAF-2 receptor signaling by human insulin and ins-1, a member of the unusually large and diverse C. elegans insulin gene family. *Genes Dev.* **15**, 672-686.

Riddle, D. L., Swanson, M. M. and Albert, P. S. (1981). Interacting genes in nematode dauer larva formation. *Nature* **290**, 668-671.

Schulz, T. J., Zarse, K., Voigt, A., Urban, N., Birringer, M. and Ristow, M. (2007). Glucose restriction extends Caenorhabditis elegans life span by inducing mitochondrial respiration and increasing oxidative stress. *Cell Metab.* **6**, 280-293.

Scott, B. A., Avidan, M. S. and Crowder, C. M. (2002). Regulation of hypoxic death in C. elegans by the insulin/IGF receptor homolog DAF-2. *Science* **296**, 2388-2391.

Suh, Y., Atzmon, G., Cho, M. O., Hwang, D., Liu, B., Leahy, D. J., Barzilai, N. and Cohen, P. (2008). Functionally significant insulin-like growth factor I receptor mutations in centenarians. *Proc. Natl. Acad. Sci. USA* **105**, 3438-3442.

Sutter, N. B., Bustamante, C. D., Chase, K., Gray, M. M., Zhao, K., Zhu, L., Padhukasahasram, B., Karlins, E., Davis, S., Jones, P. G. et al. (2007). A single IGF1 allele is a major determinant of small size in dogs. *Science* **316**, 112-115.

Taguchi, A., Wartschow, L. M. and White, M. F. (2007). Brain IRS2 signaling coordinates life span and nutrient homeostasis. *Science* **317**, 369-372.

Tatar, M., Kopelman, A., Epstein, D., Tu, M. P., Yin, C. M. and Garofalo, R. S. (2001). A mutant Drosophila insulin receptor homolog that extends life-span and impairs neuroendocrine function. *Science* **292**, 107-110.

Wang, J., Farr, G. W., Hall, D. H., Li, F., Furtak, K., Dreier, L. and Horwich, A. L. (2009). An ALS-linked mutant SOD1 produces a locomotor defect associated with aggregation and synaptic dysfunction when expressed in neurons of Caenorhabditis elegans. *PLoS Genet.* **5**, e1000350.

Willcox, B. J., Donlon, T. A., He, Q., Chen, R., Grove, J. S., Yano, K., Masaki, K. H., Willcox, D. C., Rodriguez, B. and Curb, J. D. (2008). FOXO3A genotype is strongly associated with human longevity. *Proc. Natl. Acad. Sci. USA* **105**, 13987-13992.

Wolkow, C. A., Kimura, K. D., Lee, M. S. and Ruvkun, G. (2000). Regulation of C. elegans life-span by insulinlike signaling in the nervous system. *Science* **290**, 147-150.