Common aging mechanisms:

Energy metabolism and longevity in *Caenorhabditis elegans*

Marta Artal-Sanz and Nektarios Tavernarakis*

Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology, Heraklion, Crete, Greece

*Correspondence:

Nektarios Tavernarakis
Institute of Molecular Biology and Biotechnology
Foundation for Research and Technology - Hellas
N. Plastira 100, Vassilika Vouton, PO Box 1385
Heraklion GR 70013, Crete, GREECE
tel: +30 2810 391066
fax: +30 2810 391067
eMail: tavernarakis@imbb.forth.gr

Summary

Aging studies in diverse species ranging from yeast to man have culminated in the delineation of several common signaling pathways that influence the process of senescent decline and aging. While understanding of these interlinked signal transduction cascades is becoming ever more detailed and comprehensive, the cellular and biochemical processes they impinge upon to modulate the rate of senescent decline and aging has lagged considerably behind. This fundamental question is one of the most important challenges of modern aging research and has been the focus of recent research efforts. Emerging findings provide insight into the facets of cellular metabolism which can be fine-tuned by upstream signaling events to ultimately promote longevity. In this chapter, we survey the mechanisms regulating aging in the simple nematode worm *Caenorhabditis elegans*, aiming to highlight recent discoveries that shed light into the interface between aging signaling pathways and cellular energy metabolism. Our objective is to review the current understanding of the processes involved and discuss mechanisms that are likely conserved in higher organisms.

Keywords: Aging; Adipose tissue; *Caenorhabditis elegans*; Caloric restriction; Dauer larva; Germline; Hormone; Insulin; Lifespan; Longevity; Mitochondrion, Reproduction.

1. Introduction

Simple model organisms such as *Caenorhabditis elegans*, *Drosophila melanogaster* and *Saccharomyces cerevisiae* have provided significant insights which have led to remarkable progress in understanding the molecular pathways that modulate aging and senescence[1-4]. The free-living soil nematode *C. elegans* has pioneered research on the genetic regulation of aging in part because of its relatively short lifespan and the capacity for self-fertilization, which facilitates the generation of genetically homogenous populations. A multitude of single-gene mutations altering lifespan have been identified in *C. elegans* and other species, providing evidence that aging can be modulated by evolutionary conserved regulatory pathways[4]. These pathways normally control growth, reproduction, stress response and energy metabolism.

In the nematode, neuroendocrine signaling, nutritional sensing and mitochondrial functions, have been shown to play important roles in the determination of lifespan. In this review, we focus on the role of insulin signaling in aging and the mechanisms by which insulin signals are translated through downstream effector kinases and transcriptional factors to modulate lifespan. In addition, we discuss physiological conditions that affect aging, including dietary restriction, altered mitochondrial function and reduced protein translation, aiming to highlight links between different pathways that reveal an integrated network of interactions, which coordinates the aging process.

2. The Insulin Signaling Pathway

Aging in *C. elegans* is mainly controlled by the Insulin/Insulin Growth Factor 1 (IGF-1) signaling pathway (IIS). This neuroendocrine system also modulates lifespan in flies and mammals, indicating that this pathway is a universal longevity regulator[5-7]. The *C. elegans* IIS pathway was first genetically identified by its effects on the dauer larva formation (Daf) process. Dauer is an alternative nematode developmental stage induced by harsh environmental conditions such as starvation, high population density, or high temperature. Under normal conditions *C. elegans* develops to the reproductive adult through four larval stages (L1-L4) in three days. However, when conditions are adverse, larvae arrest development at the second molt to enter the dauer stage. Dauers do not feed, are resistant to stress and can survive up to several months. Dauer larvae are considered to be non-aging because post-dauer lifespan is not affected by the duration of the dauer stage[8]. The Insulin/IGF-1 pathway is central to reproductive growth and metabolism, as well as normal lifespan. In addition to insulin signaling, the TGF-β-like pathway also regulates the choice between reproductive growth and dauer entry[9] and has recently been implicated in the aging process[10]

The IIS pathway was for the first time linked to aging in *C. elegans*, when mutations in two genes, *age-1* and *daf-2* encoding components of the pathway, were found to dramatically extend the lifespan of the worm[6]. Numerous subsequent investigations have led to the discovery of many additional genes affecting longevity via the IIS pathway.

How does the Insulin/IGF-1 pathway coordinate physiological processes to influence the aging rate? *C. elegans* senses environmental cues through ciliated sensory neurons. The *C. elegans* genome contains 38 insulin-like ligands that might mediate input

to the DAF-2 insulin receptor in response to environmental cues, such as nutritional status or growth conditions[11-13]. Insulin-like peptides (ILP) can act as either agonists or antagonists on DAF-2 to regulate metabolism, reproductive growth and lifespan. ILPs are mainly expressed in neurons, although they are also found in intestine, epidermis, muscle and gonad. A likely mechanism for the neuroendocrine control of aging is that environmental cues control the production and release of ILPs from sensory cells thereby influencing the physiology of the organism. Microsurgery or mutations abrogating sensory neurons extend the lifespan of *C. elegans*[14, 15]. Thus, it is plausible that sensory perception affects lifespan, at least in part, by influencing the activity of the insulin signaling pathway. However, these manipulations appear to have complicated interactions with the Insulin/IGF-1 pathway[14]

DAF-2 is the single *C. elegans* transmembrane insulin receptor kinase,[16]. Upon binding of insulin-like peptides (ILPs) to DAF-2, the kinase domain of the receptor phosphorylates and activates AGE-1, a phosphatidylinositol 3-kinase (PI3K)[17]. Activated AGE-1 PI3K generates 3-phosphoinositides (PtdIns-3,4-P2 and PtdIns-3,4,5-P3), which are second messengers, required for activation of downstream effector kinases. Downstream kinases include PDK-1, SGK-1, AKT-1 and AKT-2 protein kinase B (PKB) proteins[18-20]. These protein kinases regulate the forkhead (FOXO) transcription factor DAF-16, which translocates to the nucleus depending on its phosphorylation level[21-23]. Phosphorylated DAF-16 remains inactive in the cytoplasm, while upon dephophorylation it enters the nucleus and exerts its effects on transcription. Thus, the insulin signaling pathway functions to block the nuclear localization of DAF-16. An antagonist of the DAF-2/AGE-1 signaling pathway is the DAF-18/TEN lipid phosphatase[24]. Reduced Insulin/IGF-1 signaling, increased DAF-18/PETEN activity or stress conditions such as starvation, heat or oxidative

stress, result in the nuclear localization of DAF-16/FOXO. In the nucleus, DAF-16 regulates expression of many genes; among others, genes involved in metabolism, immune defense, autophagy and stress resistance[25-28].

In addition to the IIS kinase cascade, other kinases modulate DAF-16 activity. The c-Jun N-terminal kinase (JNK-1), a member of the mitogen-activated protein kinase (MAPK) superfamily, is activated upon environmental stress and its overexpression in *C. elegans* results in life extension[29]. JNK-1 mediated lifespan extension is DAF-16-dependent. JNK-1 phosphorylates DAF-16 and induces its translocation into the nucleus. JNK-1 overexpression results in increased resistance to oxidative and termal stress and further increases the lifespan of *daf-2* mutants. This indicates that this kinase pathway acts in parallel to the PI3/AKT pathway. The MST (mammalian sterile 20-like) kinase also phosphorylates DAF-16 to regulate oxidative-stress responses and lifespan[30]. MST is required for mutant *daf-2* longevity and its overexpression extends lifespan in a DAF-16-dependent manner. The AMP-activated protein kinase (AMPK) senses AMP/ATP ratios and is activated upon reduced energy levels. Knock out of the *C. elegans* homologue AAK-2 suppresses mutant *daf-2* longevity while its overexpression extends lifespan[31]. Recent observations indicate that AAK-2 mediates, together with DAF-16 the lifespan extension conferred by a specific form of dietary restriction[32]. The authors found that AMPK activates DAF-16 dependent transcription and that it phosphorilates DAF-1/FOXO in vitro at previously unidentified sites[32]. Other kinases partially required for mutant *daf-2* longevity are the p38 MAP kinase and RAS signaling kinases[33, 34]. Apart from phosphorylation, DAF-16/FOXO is also modulated by ubiquitination. The conserved E3 ubiquiitin ligase RLE-1 regulates aging by polyubiquitination of DAF-16. RLE-1 deficient

worms have increased levels of DAF-16, are stress resistant and show a *daf-16*-dependent life extension[35].

Additional factors also regulate DAF-16/FOXO activity in the nucleus. Increased expression of the NAD^+-dependent protein deacetylase, sirtuin, increases the lifespan of yeast, worms and flies[36-38]. Deacetylation of FOXO by the mammalian homologue SIRT1 is a requirement for its nuclear localization[39]. In *C. elegans*, lifespan extension conferred by extra copies of *sir-2.1* depends on DAF-16 and on 14-3-3 scaffold proteins. In response to stress, SIRT-2.1, 14-3-3 and DAF-16 form a complex that activates the DAF-16 target gene superoxide dismutase (*sod-3*;[40, 41]. Furthermore, the heat-shock transcription factor HSF-1, which is induced upon heat stress, mediates mutant *daf-2* longevity. HSF-1 overexpression extends lifespan in a DAF-16-dependent manner[42, 43]. In addition, SMK-1, a conserved nuclear factor, mediates longevity and DAF-16 nuclear localisation in worms lacking the germline[44]. Another transcriptional regulator of DAF-16 in worms and mammals is β-catenin/BAR-1, a component of the Wnt signaling pathway. β-catenin physically interacts with DAF-16 and enhances the expression of *sod-3*[45]. However, it is not know whether BAR-1 is required for the long life of Insulin/IGF-1 signaling mutants.

DAF-16/FOXO is well established as an important mediator of the effects of the IIS pathway. IIS inhibits DAF-16-/FOXO under favorable conditions, and DAF-16 activity is required for the increased longevity and stress resistance that result from reduced IIS. However, DAF-16 is not the only transcription factor directly regulated by IIS. Recently, it has been demonstrated that IIS directly regulates SKN-1, a transcription factor that induces the expression of antioxidant and detoxifying enzymes[46]. SKN-1 is the worm homologue of Nrf2. The increased activity of Nrf2/SKN-1 had been shown to increase stress resistance

and to extend the lifespan of worms and flies[1, 47, 48]. In *C. elegans* SKN-1 is expressed in the chemosensory ASI neurons and in the intestine. The ASI neurons sense food availability and provide endocrine signals that regulate metabolism, and expression of SKN-1 in the ASI neurons, but not in the intestine, is required for the lifespan extension conferred by dietary restriction[1]. In the intestine and in response to stress, SKN-1 localizes to the nucleus and promotes the expression of protective genes[1, 47]. Tullet and colleagues show that defective IIS results in the accumulation of SKN-1 in intestinal nuclei and the upregulation of SKN-1 target genes[46]. The downstream IIS kinases AKT-1, -2, and SGK-1 phosphorylate SKN-1 at multiple sites sequestering SKN-1 in the cytoplasm. Mutations in any of the three kinases results in the induction of SKN-1-responsive genes. Inductions of some of these genes require DAF-16 whereas others do not. So there is a partial overlap on the gene expression programs of DAF-16 and SKN-1 upon reduction of IIS. SKN-1 is required for the longevity phenotype and the stress resistance of IIS deficient worms. SKN-1 delays ageing when expressed transgenically, and a mutant form that constitutively localizes to intestinal nuclei extends lifespan in a DAF-16-independent manner[46]. The intestine is the major fat storage tissue of the worm, and there, IIS coordinates at least two transcriptional networks (SKN-1 and DAF-16) that integrate energy metabolism and stress-responsive pathways in response to environmental conditions.

3. Caloric Restriction

Caloric restriction (CR), a significant reduction in calorie intake without essential nutrient deprivation, can slow the intrinsic rate of aging in yeast, nematodes, flies, rodents and probably primates[49]. The fascinating effects of dietary restriction include maintenance

of most physiological processes in a youthful state and a delay in the occurrence and/or progression of age-associated disease. Little is actually understood about the mechanism by which reduced caloric intake is translated into longevity. In rodents, the anti-aging action of dietary restriction is dependent upon the reduced intake of calories, rather than reduction of the body fat content or metabolic rate[50]

If the life-prolonging stimulus is reduced calories, what are the molecules that "sense" this signal and convert it to the many physiological changes in calorie-restricted cells? In yeast, caloric restriction is mimicked by limiting media glucose levels or by genetic mutation of components of the cyclic AMP-dependent protein kinase A pathway. Caloric restriction effects on yeast replicative capacity requires the activity of the SIR2 histone deacetlyase and NPT1, a gene required for production of NAD, the oxidized form of nicotinamide adenine dinucleotide. NAD availability plays an important role in signaling, and may affect several metabolic processes[3]. In *C. elegans*, mutations in genes regulating feeding (*eat* genes) result in lowered food intake due to defects in pharyngeal function. The consequent, imposed dietary restriction significantly lengthens animal lifespan[51]. Dietary restriction in worms can also be achieved by diluting their bacterial food. At optimum levels of dietary restriction, worms typically live 20–50% longer than fully fed animals.

How does caloric restriction retard aging? Low calorie intake is correlated with reduced oxidative damage. Thus, the beneficial effects of dietary restriction upon lifespan may depend upon its ability to ameliorate oxidative stress by reducing protein oxidation. In addition, dietary restriction has been associated with elevated protein turnover[52]. A recent study confirms earlier reports that protein synthesis and degradation rates decline with age in liver tissue, and this decline is retarded by CR[53]. These findings indicate that elevation of

protein turnover and the consequent maintenance of a healthy protein pool, free of oxidant damage, is one of the lifespan-extending capacities of caloric restriction. The molecules that mediate this regulation are of clear interest for anti-aging interventions.

Recent research in *C. elegans* has revealed that two evolutionarily conserved transcription factors (PHA-4 and SKN-1) are required for lifespan extension under dietary restriction[1, 54]. These regulators may coordinate physiological responses to dietary restriction. PHA-4, which was originally described for its role in specifying the pharynx in worm embryos, is a member of the forkhead family of transcription factors, and is very similar to mammalian FOXA proteins. In mammals, FOXA proteins have developmental roles, and regulate glucose metabolism later in life. *pha-4* mutant animals do not respond to dietary restriction. By contrast, mutants lacking DAF-16/FOXO still showed a normal response to dietary restriction, indicating that longevity induced by restricted food intake is DAF-16/FOXO independent. Thus, PHA-4/FOXA appears to be specific for dietary restriction-mediated longevity, whereas DAF-16/FOXO is involved in regulating longevity induced by insulin/IGF-1 signaling. A conserved nuclear factor SMK-1 is required for longevity in both pathways[54].

SKN-1 is related to mammalian NRF2 transcription factors. Similarly to PHA-4, the nematode SKN-1 functions early in embryonic development, where it specifies the formation of the intestine and related tissues. Lack of SKN-1 specifically abolishes dietary-restriction-induced longevity over a wide range of food concentrations without affecting lifespan extension through reduction of insulin/IGF signaling[1]. The *skn-1* gene is expressed in the intestine and a single pair of neurons known as the ASIs. SKN-1 function in the ASI neurons, and not in the intestine, is required for the effects of dietary restriction longevity.

Moreover, dietary restriction increases *skn-1* expression specifically in these two neurons. Therefore, dietary restriction activates a highly regulated process, rather than passive metabolic changes. Interestingly, ASIs are sensory cells that integrate cues from the environment and produce various hormonal signals that are relayed to the whole body. It is tempting to speculate that these signals coordinate organism-wide physiological responses to dietary restriction.

4. Mitochondrial Dynamics

Mitochondria are involved in key aging associated processes, such as cellular metabolism, ATP synthesis and the production and detoxification of reactive oxygen species (ROS). It is, therefore, not surprising that mitochondrial dysfunctions influence the rate of aging. Paradoxically, however, unpaired mitochondrial function often results in increased lifespan. The first identified mitochondrial long lived mutant carried lesions in the nuclear gene *clk-1*[55], which encodes a mitochondrial protein involved in ubiquinone biosynthesis. Thereafter, a mutation in the *isp-1* gene, encoding the Rieske iron-sulfur subunit of complex III of the electron transport chain (ETC), was also found to increase lifespan[56]. Other genetic mutations in mitochondrial proteins increasing lifespan include *gro-1* and *lrs-2*, a isopentenylphosphat:tRNA transferase and a leucine tRNA synthase, respectively[11, 57]. Loss-of-function mutations in ETC components, such as *nuo-1*, *atp-2* and *frh-1* increase nematode lifespan, while causing developmental arrest at the L3 larval stage[58, 59]. A genetic mutation in *tpk-1*, a thiamine pyrophosphokinase which affects TCA cycle components, also increases lifespan[60]. Genome-wide RNAi screens have identified many other mitochondrial genes, which reduction results in increased lifespan. These

include mostly components of the ETC and ATP synthase, TCA cycle enzymes and mitochondrial carrier proteins[25, 61-64].

What is the mechanism by which altered mitochondrial function translates into increased lifespan? Mitochondria are major producers of (ROS) as a result of electron misplacement along the ETC. The free radical theory of aging postulates that ROS cause aging by damaging DNA, lipids and proteins. In view of this theory, one possibility is that mitochondrial mutations might result in reduced rate of living and decreased ROS production[56]. In other cases, ETC dysfunction might result in increased electron leakage and ROS production, which will consequently activate an adaptive hormetic response and ultimately be beneficial for longevity. That is, in response to mild stress, defense mechanisms will be activated resulting in increased oxidative stress resistance and life extension[65, 66]. Although a handful of data support the oxidative damage theory of aging, recent data put the correlation between oxidative stress and aging into question. Life-extending mitochondrial RNAi interventions respond differently to oxidative stress challenges[25, 67], indicating lack of correlation between protein oxidation levels and life extension in mitochondrial mutants[68]. Moreover, a measurable increase in oxidative damage, due to reduced detoxification, does not shorten the lifespan of long-lived mitochondrial mutants[69].

Another feasible mechanism involved in lifespan extension is cellular signaling and the activation of alternative metabolic routes that will counter the mitochondrial defect and the energy deficit. Long lived yeast mitochondrial mutants activate a retrograde signaling pathway that results in the activation of specific transcription factors that will shift metabolism away from the Krebs cycle towards the glyoxylate cycle. This metabolic shift

has also been observed in dauer larvae and long-lived *daf-2* mutants[70, 71]. It is possible that a metabolic shift also contributes to the extended lifespan of *C. elegans* mitochondrial mutants. Reduced AMP/ATP ratios activate the AMP-activated protein kinase (AMPK). The *C. elegans* aak-2/AMPK is partially required for the life extension of *daf-2* and mitochondrial mutants[31, 72]. In addition, disruption of *aak-2* abolishes the lifespan extension conferred by impaired glycolysis[66]. Recent investigations suggest that cell cycle checkpoint control plays an important role in specifying longevity of mitochondrial mutants[68].

Many mitochondrial dysfunctions seem to exert their effect on lifespan independently of the IIS pathway, because according to some reports, they extend lifespan independently of DAF-16 and show a synergistic effect with *daf-2* mutations[25, 55, 56, 62]. However, some mitochondrial mutations require DAF-16 for lifespan extension and influence its nuclear localization[25, 61]. IIS is coupled to mechanisms that regulate metabolism and oxidative stress. For example, mitochondrial defects are associated with insulin resistance and diabetes. Therefore, it is possible that alteration of mitochondrial function affects longevity, in part, through components of the IIS pathway. To date, there is no clear mechanistic explanation for the observed increased longevity of mitochondrial mutants. Mitochondrial mutations result in pleiotrophic effects and possibly, different mutations will affect the aging rate differently and in a tissue specific manner. Certainly, mitochondrial dysfunction results in more intricate physiological responses than merely increasing or reducing oxidative damage. Unveiling the mechanisms implicated in mitochondrial-mediated life extension is crucial to understand how lifespan is regulated.

5. Concluding Remarks

Despite its apparent simplicity, *C. elegans* has a surprisingly sophisticated neuroendocrine system that regulates development, metabolism and lifespan. The nervous system performs the task of sensing and integrating environmental cues into coordinated physiological responses that will ensure maximal survival and reproductive fitness. In *C. elegans*, food availability, temperature and a secreted pheromone are some of the sensory inputs that regulate the decision of entering the metabolically active reproductive mode or shifting to the non-reproducing, non-feeding dauer larva, with large amounts of stored fat. Importantly, the regulation of lifespan by insulin/IGF-1 signaling is conserved across taxa and reduction of insulin signaling has been shown to extend lifespan in worms, flies and mammals. Similarly, the physiological processes involved in the aging process also appear to be conserved. For example, signals from the reproductive system also influence lifespan in mammals and dietary restriction has been shown to extend lifespan in a wide variety of organisms. Likewise, sensory perception could also regulate lifespan in higher organisms, since blocking the sense of taste reduces insulin secretion in mammals and the smell of food increases insulin levels in humans.

How physiological processes are coordinated by neuroendocrine signaling to meet the biological demands of an organism is still not completely understood. Protein synthesis has emerged as one candidate target process for insulin signaling. Lifespan extension caused by decreasing mRNA translation establishes a direct link between protein synthesis and aging. The biological relevance of this relationship is underscored by the tight integration between the insulin/IGF-1 pathway and the caloric restriction response with mechanisms governing mRNA translation regulation[73-75]. Thus, the effects of insulin/IGF-1

signaling and caloric restriction on aging could in part be mediated by appropriately modulating protein synthesis, among other processes, to promote longevity.

It is of fundamental importance to understand which cells or tissues emit or receive signals to coordinate the aging process at the level of the whole organism. *C. elegans* has been instrumental for the discovery of conserved molecular pathways regulating aging. Its relatively short lifespan and its amenability for genetic and molecular analysis makes it an ideal organism to pursue these studies further aiming to ultimately understand why and how animals age.

References

1. Bishop NA, Guarente L. Two neurons mediate diet-restriction-induced longevity in C. elegans. Nature 2007;447(7144):545-9.
2. Giannakou ME, Partridge L. Role of insulin-like signalling in Drosophila lifespan. Trends Biochem Sci 2007;32(4):180-8.
3. Guarente L, Kenyon C. Genetic pathways that regulate ageing in model organisms. Nature 2000;408(6809):255-62.
4. Kenyon C. The plasticity of aging: insights from long-lived mutants. Cell 2005;120(4):449-60.
5. Bluher M, Kahn BB, Kahn CR. Extended longevity in mice lacking the insulin receptor in adipose tissue. Science 2003;299(5606):572-4.
6. Kenyon C, Chang J, Gensch E, Rudner A, Tabtiang R. A C. elegans mutant that lives twice as long as wild type. Nature 1993;366(6454):461-4.
7. Tatar M, Kopelman A, Epstein D, Tu MP, Yin CM, Garofalo RS. A mutant Drosophila insulin receptor homolog that extends life-span and impairs neuroendocrine function. Science 2001;292(5514):107-10.
8. Klass M, Hirsh D. Non-ageing developmental variant of Caenorhabditis elegans. Nature 1976;260(5551):523-5.
9. Ren P, Lim CS, Johnsen R, Albert PS, Pilgrim D, Riddle DL. Control of C. elegans larval development by neuronal expression of a TGF-beta homolog. Science 1996;274(5291):1389-91.
10. Shaw WM, Luo S, Landis J, Ashraf J, Murphy CT. The C. elegans TGF-beta Dauer pathway regulates longevity via insulin signaling. Curr Biol 2007;17(19):1635-45.
11. Li W, Kennedy SG, Ruvkun G. 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 2003;17(7):844-58.
12. Pierce SB, Costa M, Wisotzkey R, et al. 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 2001;15(6):672-86.
13. Hua QX, Nakagawa SH, Wilken J, et al. A divergent INS protein in Caenorhabditis elegans structurally resembles human insulin and activates the human insulin receptor. Genes Dev 2003;17(7):826-31.
14. Alcedo J, Kenyon C. Regulation of C. elegans longevity by specific gustatory and olfactory neurons. Neuron 2004;41(1):45-55.
15. Apfeld J, Kenyon C. Regulation of lifespan by sensory perception in Caenorhabditis elegans. Nature 1999;402(6763):804-9.
16. Kimura KD, Tissenbaum HA, Liu Y, Ruvkun G. daf-2, an insulin receptor-like gene that regulates longevity and diapause in Caenorhabditis elegans. Science 1997;277(5328):942-6.
17. Morris JZ, Tissenbaum HA, Ruvkun G. A phosphatidylinositol-3-OH kinase family member regulating longevity and diapause in Caenorhabditis elegans. Nature 1996;382(6591):536-9.
18. Hertweck M, Gobel C, Baumeister R. C. elegans SGK-1 is the critical component in the Akt/PKB kinase complex to control stress response and life span. Dev Cell 2004;6(4):577-88.

19. Paradis S, Ailion M, Toker A, Thomas JH, Ruvkun G. A PDK1 homolog is necessary and sufficient to transduce AGE-1 PI3 kinase signals that regulate diapause in Caenorhabditis elegans. Genes Dev 1999;13(11):1438-52.
20. Paradis S, Ruvkun G. Caenorhabditis elegans Akt/PKB transduces insulin receptor-like signals from AGE-1 PI3 kinase to the DAF-16 transcription factor. Genes Dev 1998;12(16):2488-98.
21. Henderson ST, Johnson TE. daf-16 integrates developmental and environmental inputs to mediate aging in the nematode Caenorhabditis elegans. Curr Biol 2001;11(24):1975-80.
22. Lee RY, Hench J, Ruvkun G. Regulation of C. elegans DAF-16 and its human ortholog FKHRL1 by the daf-2 insulin-like signaling pathway. Curr Biol 2001;11(24):1950-7.
23. Lin K, Hsin H, Libina N, Kenyon C. Regulation of the Caenorhabditis elegans longevity protein DAF-16 by insulin/IGF-1 and germline signaling. Nat Genet 2001;28(2):139-45.
24. Ogg S, Ruvkun G. The C. elegans PTEN homolog, DAF-18, acts in the insulin receptor-like metabolic signaling pathway. Mol Cell 1998;2(6):887-93.
25. Lee SS, Lee RY, Fraser AG, Kamath RS, Ahringer J, Ruvkun G. A systematic RNAi screen identifies a critical role for mitochondria in C. elegans longevity. Nat Genet 2003;33(1):40-8.
26. Melendez A, Talloczy Z, Seaman M, Eskelinen EL, Hall DH, Levine B. Autophagy genes are essential for dauer development and life-span extension in C. elegans. Science 2003;301(5638):1387-91.
27. Murphy CT, McCarroll SA, Bargmann CI, et al. Genes that act downstream of DAF-16 to influence the lifespan of Caenorhabditis elegans. Nature 2003;424(6946):277-83.
28. Oh SW, Mukhopadhyay A, Dixit BL, Raha T, Green MR, Tissenbaum HA. Identification of direct DAF-16 targets controlling longevity, metabolism and diapause by chromatin immunoprecipitation. Nat Genet 2006;38(2):251-7.
29. Oh SW, Mukhopadhyay A, Svrzikapa N, Jiang F, Davis RJ, Tissenbaum HA. JNK regulates lifespan in Caenorhabditis elegans by modulating nuclear translocation of forkhead transcription factor/DAF-16. Proc Natl Acad Sci U S A 2005;102(12):4494-9.
30. Lehtinen MK, Yuan Z, Boag PR, et al. A conserved MST-FOXO signaling pathway mediates oxidative-stress responses and extends life span. Cell 2006;125(5):987-1001.
31. Apfeld J, O'Connor G, McDonagh T, DiStefano PS, Curtis R. The AMP-activated protein kinase AAK-2 links energy levels and insulin-like signals to lifespan in C. elegans. Genes Dev 2004;18(24):3004-9.
32. Greer EL, Dowlatshahi D, Banko MR, et al. An AMPK-FOXO pathway mediates longevity induced by a novel method of dietary restriction in C. elegans. Curr Biol 2007;17(19):1646-56.
33. Nanji M, Hopper NA, Gems D. LET-60 RAS modulates effects of insulin/IGF-1 signaling on development and aging in Caenorhabditis elegans. Aging Cell 2005;4(5):235-45.
34. Troemel ER, Chu SW, Reinke V, Lee SS, Ausubel FM, Kim DH. p38 MAPK regulates expression of immune response genes and contributes to longevity in C. elegans. PLoS Genet 2006;2(11):e183.
35. Li W, Gao B, Lee SM, Bennett K, Fang D. RLE-1, an E3 ubiquitin ligase, regulates C. elegans aging by catalyzing DAF-16 polyubiquitination. Dev Cell 2007;12(2):235-46.
36. Kaeberlein M, McVey M, Guarente L. The SIR2/3/4 complex and SIR2 alone promote longevity in Saccharomyces cerevisiae by two different mechanisms. Genes Dev 1999;13(19):2570-80.

37. Rogina B, Helfand SL. Sir2 mediates longevity in the fly through a pathway related to calorie restriction. Proc Natl Acad Sci U S A 2004;101(45):15998-6003.
38. Tissenbaum HA, Guarente L. Increased dosage of a sir-2 gene extends lifespan in Caenorhabditis elegans. Nature 2001;410(6825):227-30.
39. van der Horst A, Tertoolen LG, de Vries-Smits LM, Frye RA, Medema RH, Burgering BM. FOXO4 is acetylated upon peroxide stress and deacetylated by the longevity protein hSir2(SIRT1). J Biol Chem 2004;279(28):28873-9.
40. Berdichevsky A, Viswanathan M, Horvitz HR, Guarente L. C. elegans SIR-2.1 interacts with 14-3-3 proteins to activate DAF-16 and extend life span. Cell 2006;125(6):1165-77.
41. Wang Y, Oh SW, Deplancke B, Luo J, Walhout AJ, Tissenbaum HA. C. elegans 14-3-3 proteins regulate life span and interact with SIR-2.1 and DAF-16/FOXO. Mech Ageing Dev 2006;127(9):741-7.
42. Hsu AL, Murphy CT, Kenyon C. Regulation of aging and age-related disease by DAF-16 and heat-shock factor. Science 2003;300(5622):1142-5.
43. Morley JF, Morimoto RI. Regulation of longevity in Caenorhabditis elegans by heat shock factor and molecular chaperones. Mol Biol Cell 2004;15(2):657-64.
44. Wolff S, Ma H, Burch D, Maciel GA, Hunter T, Dillin A. SMK-1, an essential regulator of DAF-16-mediated longevity. Cell 2006;124(5):1039-53.
45. Essers MA, de Vries-Smits LM, Barker N, Polderman PE, Burgering BM, Korswagen HC. Functional interaction between beta-catenin and FOXO in oxidative stress signaling. Science 2005;308(5725):1181-4.
46. Tullet JM, Hertweck M, An JH, et al. Direct inhibition of the longevity-promoting factor SKN-1 by insulin-like signaling in C. elegans. Cell 2008;132(6):1025-38.
47. An JH, Blackwell TK. SKN-1 links C. elegans mesendodermal specification to a conserved oxidative stress response. Genes Dev 2003;17(15):1882-93.
48. Sykiotis GP, Bohmann D. Keap1/Nrf2 signaling regulates oxidative stress tolerance and lifespan in Drosophila. Dev Cell 2008;14(1):76-85.
49. Sohal RS, Weindruch R. Oxidative stress, caloric restriction, and aging. Science 1996;273(5271):59-63.
50. Masoro EJ. Caloric restriction and aging: an update. Exp Gerontol 2000;35(3):299-305.
51. Lakowski B, Hekimi S. The genetics of caloric restriction in Caenorhabditis elegans. Proc Natl Acad Sci U S A 1998;95(22):13091-6.
52. Jazwinski SM. Metabolic control and ageing. Trends Genet 2000;16(11):506-11.
53. Lambert AJ, Merry BJ. Use of primary cultures of rat hepatocytes for the study of ageing and caloric restriction. Exp Gerontol 2000;35(5):583-94.
54. Panowski SH, Wolff S, Aguilaniu H, Durieux J, Dillin A. PHA-4/Foxa mediates diet-restriction-induced longevity of C. elegans. Nature 2007;447(7144):550-5.
55. Wong A, Boutis P, Hekimi S. Mutations in the clk-1 gene of Caenorhabditis elegans affect developmental and behavioral timing. Genetics 1995;139(3):1247-59.
56. Feng J, Bussiere F, Hekimi S. Mitochondrial electron transport is a key determinant of life span in Caenorhabditis elegans. Dev Cell 2001;1(5):633-44.
57. Lemieux J, Lakowski B, Webb A, et al. Regulation of physiological rates in Caenorhabditis elegans by a tRNA-modifying enzyme in the mitochondria. Genetics 2001;159(1):147-57.
58. Tsang WY, Sayles LC, Grad LI, Pilgrim DB, Lemire BD. Mitochondrial respiratory chain deficiency in Caenorhabditis elegans results in developmental arrest and increased life span. J Biol Chem 2001;276(34):32240-6.

59. Ventura N, Rea S, Henderson ST, Condo I, Johnson TE, Testi R. Reduced expression of frataxin extends the lifespan of Caenorhabditis elegans. Aging Cell 2005;4(2):109-12.
60. de Jong L, Meng Y, Dent J, Hekimi S. Thiamine pyrophosphate biosynthesis and transport in the nematode Caenorhabditis elegans. Genetics 2004;168(2):845-54.
61. Curran SP, Ruvkun G. Lifespan regulation by evolutionarily conserved genes essential for viability. PLoS Genet 2007;3(4):e56.
62. Dillin A, Hsu AL, Arantes-Oliveira N, et al. Rates of behavior and aging specified by mitochondrial function during development. Science 2002;298(5602):2398-401.
63. Hamilton B, Dong Y, Shindo M, et al. A systematic RNAi screen for longevity genes in C. elegans. Genes Dev 2005;19(13):1544-55.
64. Hansen M, Hsu AL, Dillin A, Kenyon C. New genes tied to endocrine, metabolic, and dietary regulation of lifespan from a Caenorhabditis elegans genomic RNAi screen. PLoS Genet 2005;1(1):119-28.
65. Ventura N, Rea SL, Testi R. Long-lived C. elegans mitochondrial mutants as a model for human mitochondrial-associated diseases. Exp Gerontol 2006;41(10):974-91.
66. Schulz TJ, Zarse K, Voigt A, Urban N, Birringer M, Ristow M. Glucose restriction extends Caenorhabditis elegans life span by inducing mitochondrial respiration and increasing oxidative stress. Cell Metab 2007;6(4):280-93.
67. Kim Y, Sun H. Functional genomic approach to identify novel genes involved in the regulation of oxidative stress resistance and animal lifespan. Aging Cell 2007;6(4):489-503.
68. Rea SL, Ventura N, Johnson TE. Relationship between mitochondrial electron transport chain dysfunction, development, and life extension in Caenorhabditis elegans. PLoS Biol 2007;5(10):e259.
69. Yang W, Li J, Hekimi S. A Measurable Increase in Oxidative Damage Due to Reduction in Superoxide Detoxification Fails to Shorten the Life Span of Long-Lived Mitochondrial Mutants of Caenorhabditis elegans. Genetics 2007;177(4):2063-74.
70. Ruzanov P, Riddle DL, Marra MA, McKay SJ, Jones SM. Genes that may modulate longevity in C. elegans in both dauer larvae and long-lived daf-2 adults. Exp Gerontol 2007;42(8):825-39.
71. Vanfleteren JR, Braeckman BP. Mechanisms of life span determination in Caenorhabditis elegans. Neurobiol Aging 1999;20(5):487-502.
72. Curtis R, O'Connor G, DiStefano PS. Aging networks in Caenorhabditis elegans: AMP-activated protein kinase (aak-2) links multiple aging and metabolism pathways. Aging Cell 2006;5(2):119-26.
73. Britton JS, Lockwood WK, Li L, Cohen SM, Edgar BA. Drosophila's insulin/PI3-kinase pathway coordinates cellular metabolism with nutritional conditions. Dev Cell 2002;2(2):239-49.
74. Holz MK, Ballif BA, Gygi SP, Blenis J. mTOR and S6K1 mediate assembly of the translation preinitiation complex through dynamic protein interchange and ordered phosphorylation events. Cell 2005;123(4):569-80.
75. Long X, Spycher C, Han ZS, Rose AM, Muller F, Avruch J. TOR deficiency in C. elegans causes developmental arrest and intestinal atrophy by inhibition of mRNA translation. Curr Biol 2002;12(17):1448-61.