Longevity and stress in *Caenorhabditis elegans*

Katherine I. Zhou¹, Zachary Pincus¹ and Frank J. Slack¹

¹ Department of Molecular, Cellular and Developmental Biology, PO Box 208103, Yale University, New Haven, CT 06520

Received: 8/24/11; Accepted: 8/26/11; Published: 8/28/11
Correspondence to Frank J. Slack, frank.slack@yale.edu

Copyright: © Slack et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Abstract: It has long been understood that many of the same manipulations that increase longevity in *Caenorhabditis elegans* also increase resistance to various acute stressors, and vice-versa; moreover these findings hold in more complex organisms as well. Nevertheless, the mechanistic relationship between these phenotypes remains unclear, and in many cases the overlap between stress resistance and longevity is inexact. Here we review the known connections between stress resistance and longevity, discuss instances in which these connections are absent, and summarize the theoretical explanations that have been posited for these phenomena.

INTRODUCTION

*Caenorhabditis elegans* is an informative and convenient model for aging and stress studies. It was the first multicellular organism to have its complete genome sequenced [1], and its small size, transparency, and short life cycle further simplify studies of aging, genetics, and whole-body stress [2-4]. Often, manipulations that increase *C. elegans* longevity also increase stress resistance; conversely, mobilizing stress responses in the nematode can prolong lifespan. Many of the classic longevity mutations in *C. elegans*, including those in the insulin/insulin-like growth factor (IGF-1) receptor signaling pathway [5-7] and those involved in determining the rate of mitochondrial respiration [8, 9] or of feeding [10], also confer resistance to various stressors such as high temperature [11, 12], UV irradiation [13], reactive oxygen species [14-17], or pathogens [18]. Other lifespan-extending manipulations, such as dietary restriction or germline ablation, also increase resistance to thermal and oxidative stress [19-21]. Further, low-level stressors on the order of 20-25% of the minimum toxic dose often enhance future stress resistance and increase longevity, through a process termed stress-induced hormesis [22-26]. While pre-treatment with oxidative or thermal stress has been shown to increase stress resistance and lifespan, not all forms of stress produce these effects.

In this work, we first review several well-studied stressors in *C. elegans* and downstream effectors of the responses to those stressors, with an eye toward the effects of those effectors on longevity. Next, we examine common genes, pathways, and physiological processes that when manipulated extend lifespan, and the relationship of these manipulations to stress responses. The longevity and stress-response phenotypes of the genes discussed are summarized in Table 1 for convenient reference. Finally, we attempt to integrate these findings with evolutionary perspectives on the genetic control of longevity.

STRESS RESPONSES IN *C. ELEGANS*

Oxidative stress

Endogenous or exogenous reactive oxygen species (ROS) damage to protein, lipid, and nucleic acid components of the cell is known generically as oxidative stress. Unavoidably, during mitochondrial respiration, 0.4–4% of the consumed oxygen is transformed into various reactive byproducts, especially superoxide (O²⁻) [28]. Superoxide reacts with iron–sulfur clusters to release iron, or is converted to hydrogen peroxide (H₂O₂), which reacts with free iron...
Table 1: Genes involved in lifespan and stress-resistance.

| Gene   | Homologues                                | Principal Pathway(s)                  | Effect of gene suppression on lifespan | Effect of gene suppression on stress tolerance |
|--------|-------------------------------------------|---------------------------------------|---------------------------------------|-----------------------------------------------|
| eat-2  | non-alpha-subunits of nicotinic acetylcholine receptor (nACHR) | pharyngeal pumping                    | increased [174]                       | increased thermotolerance [142, 174]           |
| pep-2  | oligopeptide transporter                   | peptide uptake                        | none [180]                           | increased resistance to thermal and oxidative stress [180] |
| daf-2  | mammalian insulin and insulin growth factor (IGF-1) receptors | IS                                     | increased [91]                       | increased resistance to thermal [12], oxidative [92], heavy-metal [72], and pathogenic stress [18] |
| age-1  | p11, catalytic subunit of human phosphatidylinositol 3-kinase (PI3K) | IS                                     | increased [12, 34]                    | increased resistance to thermal [12], oxidative stress [34], heavy-metal [72], and pathogenic stress [18] |
| akr-1  | mammalian S/T kinase Akt/PKB              | IS                                     | weakly increased [96, 97]             | weakly increases resistance to thermal and oxidative stress [81] |
| akr-2  | mammalian S/T kinase Akt/PKB              | IS                                     | weakly increased [96, 97]             | weakly increases resistance to thermal and oxidative stress [81] |
| sgk-1  | serum- and glucocorticoid-inducible kinase SGK | IS                                     | increased [81]                       | increased resistance to thermal and oxidative stress [81] |
| daf-18 | human tumor suppressor PTEN               | IS                                     | decreased [5, 98]                    | decreased resistance to oxidative stress [126] |
| vhl-1  | Eph receptor tyrosine kinase               | IS                                     | increased [101]                      | N.D.                                          |
| daf-16 | Forkhead box proteins class O (FOXO)      | IS                                     | decreased [88]                       | decreased thermotolerance[104]; no effect on resistance to UV, oxidative, or pathogenic stress [118, 103] |
| sir-2  | yeast Sir2, mammalian SIRT                | IS                                     | decreased [87]                       | decreased resistance to thermal, oxidative, and UV stress [87] |
| jnk-1  | mammalian c-Jun N-terminal kinase (JNK)   | IS                                     | decreased [119]                      | decreased resistance to thermal stress [119]   |
| lin-14 | (putative transcription factor)            | IS                                     | increased [89]                       | increased resistance to thermal stress [89]   |
| hsf-1  | heat-shock transcription factor            | heat-shock response, IS               | decreased [88]                       | decreased resistance to thermal [211], oxidative [212], and pathogenic stress [105] |
| smk-1  | mammalian suppressor of MEK1 null (SMEK)  | IS                                     | weakly decreased [90]                | decreased resistance to pathogenic stress [90] |
| hcf-1  | mammalian host cell factor 1 (HCF-1)       | IS                                     | increased [121]                      | increased resistance to oxidative and heavy-metal stress; no effect on resistance to thermal stress [121] |
| cep-1  | tumor suppressor p53                       | IS                                     | increased [125]                      | no effect on resistance to thermal, oxidative, UV, or pathogenic stress [125] |
| smg-1  | S/T kinase in nonsense mediated mRNA decay | IS                                     | increased [126]                      | increased resistance to oxidative stress [126] |
| skn-1  | transcription factor                       | IS                                     | decreased [133]                      | decreased resistance to oxidative stress [133] |
| let-363 | S/T kinase target of rapamycin (TOR)      | TOR                                   | increased [143]                      | increased thermotolerance [142]                |
| daf-15 | Regulated Associated Protein of TOR (Raptor) | TOR                                   | increased [140]                      | N.D.                                          |
| ife-2  | eukaryotic initiation factor 4E (eIF4E)    | mRNA translation                      | increased [144]                      | increased resistance to thermal [142] and oxidative stress [144] |
| bec-1  | yeast and mammalian autophagy genes Apg6/Vps30/p62clin1 | autophagy                             | weakly decreased [94]                | N.D.                                          |
| isp-1  | iron sulfur protein of complex III in ETC | mitochondrial respiration             | increased [8, 9]                     | increased resistance to oxidative stress [8, 9] |
| lrs-2  | mitochondrial leucyl-tRNA synthetase       | mitochondrial                        | increased [152]                      | decreased resistance to oxidative stress [152] |
| cik-1  | hydroxylase for ubiquinone synthesis       | mitochondrial respiration             | increased [157]                      | cik-1 mutation increased oxidative stress resistance in daf-2 mutants [92] |
| mev-1  | subunit of complex II                      | mitochondrial respiration             | decreased [104, 159]                 | decreased resistance to oxidative stress [160] |
| cch-1  | cytochrome c heme lyase                    | mitochondrial respiration             | increased [152]                      | no effect on resistance to oxidative stress [152] |
| sod-1  | cytosolic Cu/ZnSOD                        | antioxidant enzymes                  | weakly decreased [164]               | decreased resistance to oxidative stress [164, 165] |
| sod-2  | mitochondrial MnSOD                       | antioxidant enzymes                  | slightly increased [166]             | decreased resistance to oxidative stress [164, 166] |
| sod-3  | mitochondrial MnSOD                       | antioxidant enzymes                  | no effect [166]                      | no effect [165]                               |
| sod-4  | (predicted) extracellular Cu/ZnSOD         | antioxidant enzymes                  | no effect [166]                      | no effect [165]                               |
| sod-5  | cytosolic Cu/ZnSOD                        | antioxidant enzymes                  | no effect [166]                      | no effect [165]                               |
| cti-1  | cytoplasmic catalase                       | antioxidant enzymes                  | no effect [213]                      | N.D.                                          |
| cti-2  | peroxisomal catalase and peroxidase        | antioxidant enzymes                  | decreased [213]                      | N.D.                                          |
| mtl-1  | metallothionein                           | antioxidant enzymes                  | N.D.                                 | decreased resistance to cadmium stress [70, 214] |
| glp-1  | Notch family receptors (N-glycosylated transmembrane protein) | germline                             | increased [21]                       | increased resistance to pathogenic stress [215] |
| mes-1  | receptor tyrosine kinase                   | germline                             | increased [21]                       | N.D.                                          |

to make hydroxyl radicals (OH-); these free radicals can then oxidize macromolecules, impairing their function [28, 29]. To protect against these species, nearly all cells, both prokaryotic and eukaryotic, use the...
enzymes superoxide dismutase (SOD), catalase, and glutathione peroxidase, which convert superoxide and other ROS into less-reactive forms (often eventually water) [30-33].

*C. elegans* can be experimentally subjected to exogenous oxidative stress by exposure to compounds such as tert-butylhydroperoxide, arsenite, paraquat, and juglone. High concentrations of any of these greatly reduces *C. elegans* lifespan [14, 17, 34-37]. Conversely, antioxidant compounds can be used to experimentally reduce the risks posed by endogenous and exogenous oxidative stressors. Nematodes have been found to live longer when treated with vitamin E [38], α-lipoic acid [39], resveratrol [40], or the catalytic antioxidants Euk-8 and Euk-134 [41], and new studies continue to show the beneficial role of various antioxidants on longevity [42-45]. However, other researchers have been unable to reproduce the extension of lifespan by Euk-8 [46], and the results for vitamin E have also been inconsistent [36]. These inconsistencies may be due to differences in dosage or culture conditions – for instance, it has been suggested that early results showing that certain antioxidants extend *C. elegans* lifespan may have been caused by dietary restriction instead [36, 46, 47]. Even for antioxidants consistently found to extend lifespan, it is not always clear whether the mode of action is in fact antioxidant activity: since α-lipoic acid and the vitamin E derivative trolox also increase thermotolerance, it has been suggested that they increase longevity by inducing a stress response [36, 39, 47]. Furthermore, other antioxidant molecules, such as N-acetylcysteine and vitamin C, do not appear to extend lifespan [36]. Subjecting *C. elegans* to low amounts of oxidative stress can also induce longer lifespan and heightened stress resistance via hormesis: nematodes pre-treated with hyperbaric oxygen or juglone subsequently exhibit increased resistance to both oxidative stressors; in addition, animals pre-treated with hyperbaric oxygen have a 20% longer life expectancy [22]. Oxygen pre-treatment has also been shown to increase X-radiation resistance in *C. elegans* [48].

Various indicators have been used to assess the extent of oxidative damage during aging. One common indicator is protein carbonyl content [16, 38, 49]; another is an autofluorescent species known as lipofuscin. This “age pigment” accumulates over time in many different species, including *C. elegans* [50], and mammalian studies suggest that its accumulation may be due to oxidative stress [51-54]. Similarly, some studies have shown that mutant animals with increased mitochondrial ROS damage have faster accumulation of fluorescent pigments, including lipofuscin [55, 56]. Other studies did not replicate these observations, however, suggesting that mitochondrial oxidative stress is not solely responsible for the accumulation of endogenous fluorescent compounds such as lipofuscin and advanced glycation end-products [57]. These inconsistent results may be due to disparities between pigment-measurement protocols [19, 57].

**Thermal stress**

Laboratory *C. elegans* are typically grown at 15–25°C; temperatures of 30–35°C constitute stressors. In response to high-temperature conditions, prokaryotes and eukaryotes alike upregulate heat-shock proteins (HSPs). Many of these proteins function as molecular chaperones, helping unfolded and misfolded proteins assume the correct conformation. HSP-4 and HSP-16 are two such proteins that accumulate in response to thermal stress in *C. elegans* [58-60]. The *C. elegans* heat-shock factor (HSF) is a transcription factor thought to regulate the response to thermal stress, and *hsf-1*(RNAi) has been shown to accelerate aging [61]. When first-larval stage (L1) or early L2 *C. elegans* larvae are subjected to thermal stress or food limitation, they enter an alternative, stress-resistant, growth-arrested L3 state called dauer [62]. Dauers have lower metabolic rate, are resistant to oxidative stress, and express higher levels of antioxidant enzymes and HSPs [14, 34, 62, 63]. Short exposure of adult wild-type *C. elegans* to high temperatures does not induce entry into a state of diapause, but instead can cause significant decrease in fertility [11]. Moreover, several hours of exposure to high temperatures can kill nematodes. As such, *C. elegans* thermotolerance is often assessed by measuring fertility or survival rate [11]. Treatment with certain antioxidant compounds, such as α-lipoic acid or trolox, increases both thermotolerance and lifespan in *C. elegans* [39]. Though these antioxidant treatments do not affect total fertility, in some cases they cause delayed or temporarily reduced fertility [39].

Thermal stress also has demonstrated hormetic effects. *C. elegans* exposed to 35°C for up to two hours exhibit greater tolerance to subsequent thermal stress and also extended life span [12, 22, 22, 64, 65]. While a single heat shock early in life gives rise to a 20% longer lifespan, multiple mild heat shocks throughout the *C. elegans* lifetime increase its lifespan by about 50% [24, 58, 66]. Furthermore, a single shock seems to increase lifespan without slowing aging, whereas multiple heat shocks both increase lifespan and slow the process of aging [24].
Heavy-metal stress

Trace levels of nonessential heavy metals such as cadmium, arsenic, mercury, and lead, as well as supraoptimal levels of the essential heavy metals copper and zinc, are toxic to cells. The mechanism behind heavy-metal toxicity is not well understood, but it is thought that heavy-metal ions cause damage to the cell by inactivating and denaturing proteins, and by promoting the formation of reactive oxygen species [67-69]. The thiol tripeptide glutathione, a class of cysteine-rich proteins called metallothioneins, and other larger metal-binding proteins protect the cell from heavy-metal stress by chelating and sequestering metal ions [67, 69, 70]. Heavy-metal stress also activates the expression of cell repair mechanisms, including heat-shock proteins [70]. The KGB-1 and PMK-1 mitogen-activated protein kinase (MAPK) pathways also function in the heavy-metal stress response, and the MAPK phosphatase (MKP) VHP-1 modulates this response by downregulating these two pathways [71]. Other genes that contribute to the heavy-metal stress tolerance of C. elegans have been identified by DNA microarray and RNAi studies, including the metallothionein-encoding genes mtl-1 and mtl-2 [70], the nuclear localized metal responsive proteins NUMR-1 and NUMR-2 [69, 70], and hmt-1, which encodes an ATP-binding cassette (ABC) transporter likely involved in cadmium sequestration [67]. Heavy-metal stress response may be regulated by the transcription factors DAF-16 and SKN-1, since SKN-1 activates metal detoxification genes including hmt-1 when C. elegans is exposed to the metalloid sodium arsenite [37], and DAF-16 regulates the expression of mtl-1 [72]. Moreover, putative binding sites for DAF-16 and SKN-1 have been found upstream of numr-1 and numr-2 [69]. Since DAF-16 and SKN-1 also play roles in the increased lifespan of longevity mutants, these transcription factors form a potential link between heavy-metal stress resistance and C. elegans longevity pathways. Consistent with this possibility, the long-lived mutants daf-2 and age-1 are resistant to heavy-metal stress, and daf-2 animals have elevated levels of mtl-1 mRNA [72].

GENES AND PHYSIOLOGICAL PROCESSES THAT CONNECT LONGEVITY AND STRESS RESISTANCE IN C. ELEGANS

Insulin/IGF-1 Signaling

The insulin/IGF-1-like signaling (IIS) pathway is an evolutionarily conserved endocrine signaling pathway that regulates food storage and growth [73]. Across taxa, reductions in insulin signaling and/or responsiveness are used as organismal signals for various types of stress – for example, IIS is involved in the entry into dauer in C. elegans. IIS pathway activity also influences lifespan in multiple different organisms, including yeast, nematodes, flies, and mammals [74-76], and it has been proposed that the regulation of longevity via the IIS pathway may have evolved to enable animals to survive harsh, stressful conditions [75]. In C. elegans, the gene daf-2 encodes a homolog of the mammalian insulin and insulin growth factor (IGF-1) receptors [7]. When ligand-bound, DAF-2 triggers the IIS pathway [77] by activating AGE-1, a homolog of p11, the catalytic subunit of human phosphatidylinositol 3-kinase (PI3K) [6]. (Of note: allelic variation in PI3K appears to affect human longevity, as determined by comparing the representation of genotypes in younger and older age groups [76, 78]). Once activated by DAF-2, AGE-1 catalyzes the formation of the second messenger phosphatidylinositol-3,4,5-triphosphate (PIP3), which in turn binds and activates AKT-1 and AKT-2, homologs of the mammalian serine/threonine kinase Akt/PKB [6, 79, 80]. Activated AKT proteins form a complex with SGK-1, a homolog of the serum- and glucocorticoid-inducible kinase SGK. All three kinases in this complex can directly phosphorylate the FOXO-family transcription factor DAF-16, constraining it to the cytoplasm [81-83]. Absent IIS activity, dephosphorylated DAF-16 enters the nucleus and regulates the transcription of a variety of genes regulating lifespan, dauer formation, stress resistance, development, and metabolism, among others [84]. IIS activity is antagonized by DAF-18, a homolog of the human tumor suppressor PTEN. DAF-18 limits AKT-1 and AKT-2 activity by dephosphorylating PIP3 [5, 85].

In the nucleus, DAF-16 is further regulated by several different proteins, including the transcription factor HSF-1 and the SMEK ortholog SMK-1 [86-90].

Certain mutant daf-2 alleles confer two-fold increased longevity and resistance to various stresses, including heat shock, oxidative stress, heavy-metal stress, and infection [12, 18, 72, 91, 92]. These mutants express higher levels of antioxidant enzymes such as catalase and SOD [92]. Additionally, daf-2 mutants often constitutively enter the dauer state, and some alleles also confer other pleiotropic traits including reduced adult motility, abnormal adult morphology, and reduced brood size [93]. Mutants in daf-2 also have an increase in autophagy, a catabolic process through which proteins and organelles are delivered to lysosomes for degradation. The autophagy gene bec-1, the homolog of mammalian beclin 1, is required for the increased...
lifespan of daf-2 mutant animals [94], suggesting that 
autophagy is an essential lifespan-prolonging 
component of the stress responses typically held in 
check by IIS activity.

Many mutations in IIS pathway genes downstream of 
daf-2 also modulate C. elegans lifespan and stress resistance. age-1(hx546) animals, which have a 
reduction-of-function point mutation in the age-1 gene, 
exhibit a 40-110% extension in mean lifespan, and 
higher tolerance to thermal stress [12, 14, 15, 34, 95]. 
Furthermore, the relative activity of catalase and SOD 
in these animals, as compared to wild-type, increases 
with age in a manner that correlates with an age-
dependent increase in the mutant animals’ relative stress 
resistance [14, 34, 95]. Null mutants for age-1, in which 
the AGE-1 protein is truncated upstream of its kinase 
domain, have a mean lifespan about ten times that of 
wild-type animals [15]. Compared with the weaker age-
1(hx546) allele, these animals are significantly more 
tolerant to oxidative stress, but also slightly less tolerant 
to thermal stress [15]. It is also worth noting that classic 
lifespan-extending mutants, including daf-2 and age-1 
nematodes, exhibit heightened resistance to various 
bacterial pathogens [18].

Mutations in akt-1 and akt-2 have a surprisingly small 
effect on C. elegans lifespan. akt-1(mg306) mutants, 
which have a point mutation in the akt-1 gene, live only 
around 10% longer than wild-type [96, 97]. The mutants 
akt-1(ok525) and akt-2(ok393) lack most of the kinase 
domain necessary for Akt/PKB activity, yet do not 
exhibit extended lifespan or increased stress resistance, 
while akt-2(ok393) mutants subjected to akt-1(RNAi) 
have only a 19% lifespan extension as well as a weak 
increase in resistance against high temperatures and 
oxidative stress [79, 81]. On the other hand, sgk-
1(RNAi) significantly increases lifespan as well as 
resistance to heat and oxidative stress, suggesting that 
SGK-1 may be more important for the regulation of 
stress response and lifespan, whereas AKT-1 and AKT-
2 are more important in regulating dauer formation [81].

Mutations in daf-18, an inhibitor of the IIS pathway, 
decrease C. elegans lifespan [5, 98]. A mutation in daf-
18 partially suppresses the increased lifespan of daf-2 
animals [98], and daf-18 also necessary for the nuclear 
localization of daf-16 in daf-2 animals [99].

Interestingly, daf-18 mutants subjected to horanetic 
levels of thermal stress do not have longer lifespans 
[100]. Though other C. elegans genes have been found 
to be required for various forms of hormesis (the 
radiation-sensitive mutants rad-1 and rad-2 mutants do 
not exhibit increased resistance to X-ray irradiation 
subsequent to pre-treatment with oxidative stress [48]), 
daf-18 appears to be the only such gene that does not 
also directly disrupt stress response mechanisms. 
Further, it has been proposed that VAB-1, an Eph 
receptor tyrosine kinase, diminishes DAF-18 activity by 
phosphorylation [101]. Both vab-1 mutants and C. 
elegans overexpressing daf-18 live longer and are more 
sensitive to dauer conditions than wild-type [101], but 
the potential regulation of DAF-18 by VAB-1 remains 
to be investigated in the context of lifespan extension 
and stress tolerance.

In all cases mentioned above, the increased lifespan and 
stress resistance of IIS pathway mutants is dependent on 
daf-16 [15, 81, 93, 98]. Null mutants in daf-16 have 
shortened lifespan, and their germ cells are 
hypersensitive to ionizing radiation [88, 102]. Interestingly, daf-16 mutants exhibit similar levels of 
resistance to UV, oxidative stress, and pathogenic stress 
as compared to wild-type animals [18, 103], although 
daf-16 animals are less thermostolerant than wild-type 
[104], and C. elegans which overexpress daf-16 are 
more resistant to P. aeruginosa [105].

Since DAF-16 is thought to be the main target of the IIS 
pathway, it has long been of interest to identify the 
genes which it regulates. The stress-response genes sod-
3, which codes for a mitochondrial MnSOD, and 
mtl-1, which codes for a metallothionein homolog, were 
among the first clear direct DAF-16 targets [72, 92]. 
Since then, a series of studies has revealed many 
antioxidant, metabolic, heat-shock, and antibacterial 
genes to be targeted by DAF-16 [106-109]. Furthermore, a DNA microarray study aimed at finding 
DAF-16 targets also uncovered two putative DAF-16 
binding sites based on the over-representation of these 
sequences upstream of target genes [107]. One of these 
sequences has also been shown to be bound by DAF-16 
in vitro [110].

In addition to IIS, other longevity pathways also 
converge on DAF-16. One such pathway involves the 
sirtuins, a highly conserved family of NAD+-dependent 
protein deacetylases that includes SIR-2.1 in C. elegans, 
Sir2 in yeast, and the SIRT proteins in mammals. 
Among other functions, sirtuins deacetylate histones, 
thereby playing a role in the regulation of chromatin 
state [111-113]. The sirtuins are known to regulate 
stress response and cell senescence – for instance, Sir2α.
in yeast represses stress-induced, p53-mediated apoptosis [40, 114-116]. In *C. elegans*, SIR-2.1 activates DAF-16, and *sir-2.1* mutants are short-lived and stress sensitive, while overexpression of *sir-2.1* increases lifespan by 50% in a *daf-16* dependent manner [86, 87]. Following heat shock, SIR-2.1, which is localized to the nucleus, physically interacts with DAF-16, and this interaction depends on PAR-5 and FTT-2, which are homologs of 14-3-3 proteins, small acidic proteins that bind phosphoserine and phosphothreonine residues in particular sequence contexts [117]. *Sir-2.1* is however not required for the longevity of IIS pathway mutants, leading to the suggestion that SIR-2.1 and the 14-3-3-like proteins act in a stress-dependent manner to activate DAF-16 and increase lifespan, which is nevertheless independent of IIS [117].

Another pathway converging on DAF-16 involves a member of the JNK family. The mammalian mitogen-activated c-Jun N-terminal kinases (JNKs) can be activated by cytokines or environmental stress and are known to regulate processes including development and cell survival [118]. JNK-1, the *C. elegans* homolog of mammalian JNK, is thought to regulate lifespan in parallel to the IIS pathway, by regulating the subcellular localization of DAF-16 [119]. Whereas phosphorylation of DAF-16 by the AKT and SGK kinases constrains it to the cytoplasm, phosphorylation of DAF-16 by JNK-1 causes it to translocate to the nucleus [119]. Consistent with this observation, *jnk-1* mutants have shorter lifespan than wild-type, and *C. elegans* overexpressing *jnk-1* exhibit extended lifespan in a manner independent of IIS but dependent on *daf-16* [119]. The putative transcription factor LIN-14, which is required for normal lifespan (see below), has also been found to regulate DAF-16, though the mechanism of this regulation remains unknown [89].

Nuclear localization of DAF-16 is neither necessary nor sufficient for lifespan extension [99]: many additional regulators act on this transcription factor once it has translocated to the nucleus, and translocation is not increased in certain *daf-16*-dependent longevity mutants. When mutations are introduced at the AKT phosphorylation sites on DAF-16, the transcription factor localizes to the nucleus, but the animals expressing this mutant DAF-16 do not constitutively become dauer and do not have extended lifespan [83]. The transcription factor HSF-1, which regulates the heat-shock response in *C. elegans*, is thought to act together in the nucleus with DAF-16 to activate the transcription of certain genes, including small heat-shock proteins [88]. Similarly, the SMEK ortholog SMK-1 appears to regulate the transcriptional specificity of DAF-16 already localized to the nucleus [90]. The mammalian protein SMEK1 is phosphorylated in response to stress and is involved in the regulation of glucose metabolism under varying conditions, such as fasting [120]. It is thus conceivable that SMK-1 in *C. elegans* also modulates DAF-16 specificity as a response to certain stressors. In fact, the *smk-1* gene in *C. elegans* is required for *daf-2* mutants to exhibit extended lifespan, as well as resistance to pathogenic, UV, and oxidative stress. However, reduced activity of *smk-1* does not affect the thermotolerance of *daf-2* nematodes [90]. In addition, the *C. elegans* homolog of the highly conserved protein host cell factor 1 (HCF-1) is localized to the nucleus and physically associates with DAF-16 [121, 122]. Mammalian HCF-1 plays key roles in cell-cycle progression and has been shown to act primarily by binding to transcription factors and by assembling protein complexes [121, 122]. Mutations in the *C. elegans* gene *hcf-1* result in a 40% increase in lifespan and greater resistance to oxidative stress, but not to heat stress [121]. It has been proposed that HCF-1 negatively regulates DAF-16 by forming a complex with DAF-16 in the nucleus and thus limiting access of DAF-16 to its target promoters [121].

Conversely, some results have indicated that DAF-16 function may not be limited to the nucleus [123]. First, though the longevity of *age-1* mutants depends on *daf-16*, these animals do not have higher nuclear localization of DAF-16 [124]. Next, either knockout of or RNAi against *cep-1*, a transcription factor that regulates the germline response to DNA damage and is an ortholog of the tumor-suppressor p53, increases longevity in *C. elegans* in a *daf-16*-dependent manner, yet RNAi against *cep-1* does not alter DAF-16 nuclear localization [125]. Further, *cep-1* mutants, though long-lived, are not resistant to heat, oxidative, UV, or pathogenic stress [125]. A related result involves the conserved gene *smg-1*, which encodes a serine-threonine kinase that functions in nonsense-mediated mRNA decay. Inactivation of *smg-1* by mutation or RNAi confers increased lifespan and resistance to oxidative stress in a *daf-18*, *daf-16*, and *cep-1*-dependent manner, yet in these animals DAF-16 is not localized to the nucleus to any degree beyond that in controls [126]. Finally, nuclear localization of DAF-16 in wild-type *C. elegans* occurs in response to starvation, heat shock, and oxidation (by treatment with juglone), but very little or not at all in response to UV irradiation [124, 127].
The effect of the IIS pathway on *C. elegans* lifespan seems to involve communication between cells and between tissues. Some *daf-2* mosaic animals exhibit longer lifespans than wild-type, suggesting that *daf-2* acts cell nonautonomously to modulate *C. elegans* lifespan (if *daf-2* acted cell autonomously, then the *daf-2(+) tissues of the mosaic would be expected to die earlier and thus kill the animal, resulting in a normal rather than a lengthened lifespan) [128]. One potential explanation for the cell-nonautonomy of *daf-2* is that the IIS pathway activates the expression of the insulin/IGF-1 homolog INS-7, which may potentially activate the IIS pathway in other cells [107]. Other potential signaling molecules regulated by DAF-2 and DAF-16, such as the putative secreted protein encoded by *scl-1*, may also contribute to the cell non-autonomy of *daf-2* [107]. Counterintuitively, tissue-specific studies suggest that DAF-2 and DAF-16 act in different tissues to influence *C. elegans* lifespan: DAF-2 functions mostly in the nervous system to modulate *C. elegans* longevity [129], whereas rescue of *daf-16* in the intestine is the most effective in restoring the lifespan and thermotolerance of *daf-2;daf-16* mutants [130]. To explain this discrepancy in tissue-of-action, it has been proposed that *daf-2(+) neurons may produce a signal that decreases DAF-16 function in intestinal cells; however an assay using the *sod-3::gfp* reporter suggests that DAF-16 activity in these animals remains high in the intestine [130]. Experiments on this subject remain difficult to interpret.

A more recently identified target of the IIS pathway is SKN-1, a transcription factor that orchestrates the oxidative stress response in adult *C. elegans*, independent of DAF-16 activity, by activating the expression of genes coding for enzymes including catalases, SODs, and some glutathione S-transferases [131-133]. *skn-1* mutants are short-lived and exhibit sensitivity to oxidative stress, and overexpression of SKN-1 in intestinal cells increases lifespan and oxidative stress resistance [131-133]. Mutations in *skn-1* also suppress the longevity and oxidative stress resistance of *daf-2* mutants [131]. Recently, it has been shown that IIS directly inhibits SKN-1 via phosphorylation by AKT-1, AKT-2, and SGK-1, whereas in IIS mutants, SKN-1 accumulates in the nucleus of intestinal cells and activates expression of genes involved in the oxidative stress response [131, 132]. Consistent with SKN-1 regulation of stress resistance, RNAi of two genes upregulated by SKN-1, *nlp-7* and *cup-4*, leads to reduction in lifespan and oxidative stress resistance [134]. RNAi of *ent-1*, which is down-regulated by SKN-1, increases oxidative stress resistance without affecting longevity [134].

Many IIS pathway mutants reflect a correlation between longevity and stress resistance. Many of the genes downstream of *daf-2* and *daf-16* have clear roles in stress resistance, although it is not as clear how they influence aging. However, inconsistencies exist in the correlation between longevity and stress resistance. Moreover, current results give only an incomplete understanding of their association, and further study of the lifespan and stress response of various IIS pathway mutants is necessary.

**MicroRNA regulation of aging**

microRNAs (miRNAs) are small, noncoding RNA molecules that post-transcriptionally suppress gene expression by binding to their target mRNAs. They play important roles in many biological processes, including developmental timing, cancer, and aging. The miRNA...
lin-4 and its target lin-14, which act in the heterochronic pathway to regulate the timing of C. elegans larval development, have also been found to modulate aging; reduced activity of lin-4 accelerates tissue aging in C. elegans, whereas increased activity of lin-4 or reduced activity of lin-14 increases lifespan in a DAF-16- and HSF-1-dependent manner [89]. Further, this study found that the longevity of daf-2 mutants depends on lin-4; thus, the modulation of lifespan by lin-4 and lin-14 is intimately linked to the IIS pathway. In addition, mir-71, mir-239, and mir-246, which each increase significantly in expression during aging in wild-type C. elegans, have longevity and stress-resistance phenotypes [136]. Deletions of mir-71 and mir-246 decrease nematode lifespan, and deletion of mir-239 increases lifespan, while overexpression of these miRNAs has the opposite effects.

Furthermore, mir-71 mutant animals are more sensitive to thermal and oxidative stress, whereas mir-239 mutants exhibit increased resistance to these stressors. Deletion of mir-71 partly suppresses the extended lifespan of nematodes treated with daf-2(RNAi), and daf-16 fails to further shorten the lifespan of mir-71 mutants; in addition, several members of the IIS pathway are putative targets of mir-71, further suggesting that the mechanism behind the lifespan effect of mir-71 is related to the IIS pathway [136]. More recently, inter-individual fluctuations in the levels of reporters for each of these microRNAs were shown to quantitatively predict individual lifespan (within a genetically identical cohort) [137]. Of these, mir-71 reporter levels measured during early adulthood predict up to 47% of longevity variation; this predictive ability required daf-16, suggesting that fluctuations in wild-type IIS levels set lifespan in intact individuals. This confirms another recent finding that sod-3::GFP, a well-known reporter of IIS levels, also predicts individual longevity [138].

Target of rapamycin pathway

Target of rapamycin (TOR) is an evolutionarily conserved serine/threonine protein kinase that promotes cell growth and metabolism in response to nutrients [139]. Under stress, TOR activity goes down, resulting in lower levels of protein synthesis and increased autophagy. The homolog of TOR in C. elegans is let-363, and that of the TOR binding partner Regulated Associated Protein of TOR (Raptor) is daf-15 [140]. In nematodes, deficiency in let-363 or daf-15 results in developmental arrest, intestinal atrophy, and death as dauer-like larvae [140, 141]. Non-lethal loss of let-363 or daf-15 can be achieved with daf-15 heterozygotes, let-363(RNAi) starting in adulthood, or mutations for decreased let-363 activity, all of which result in lifespan extension [19, 140, 142]. In addition, let-363(RNAi) confers greater thermotolerance on nematodes [142]. The TOR pathway may also be linked to the IIS pathway, as DAF-16 has been found to control the expression of daf-15 [140]. Moreover, TOR RNAi does not further extend the lifespan of daf-2 mutants, whereas it does extend the lifespan of daf-16 animals [143]. These mixed results suggest that the TOR pathway interacts with IIS in some fashion, but perhaps independently or downstream of daf-16 [143].

The mechanism of TOR’s influence on lifespan is similarly unclear. One possibility is that the TOR pathway influences lifespan by regulating mRNA translation. Mammalian TOR phosphorylates the translation initiation factor 4E binding protein 1 (4E-BP1), which reduces its affinity for the eukaryotic initiation factor 4E (eIF4E), thus allowing eIF4E to function in mRNA translation. In this way, TOR activity promotes mRNA translation. Similarly, let-363 and daf-15 mutant C. elegans exhibit decreased protein synthesis and generally bear similarities to deficiencies in mRNA translation initiation factors [139-141]. This suggests that let-363 might function through a pathway analogous to that of mammalian TOR. However, there is no known homolog of 4E-BP1 in C. elegans [144]. Deletion of ife-2, the homolog of eIF4E, extends lifespan independently of let-363, and has also been shown to reduce protein synthesis and increase resistance to oxidative stress [144]. In addition, knockdown of TOR further increases lifespan in animals carrying an ife-2 deletion, indicating that ife-2 and the TOR pathway influence longevity through at least partly distinct mechanisms [144]. Even if let-363 promotes longevity by decreasing protein synthesis, this cannot be the only mechanism of lifespan extension for the TOR pathway, since decreasing translation initiation factor levels by eIF2ß or eIF4G RNAi, or by ife-2 mutation, does not extend lifespan in daf-16 mutants and actually shortens that of daf-2 mutants, in contrast to the results of let-363(RNAi) [142]. Another possibility is that let-363 extends lifespan by promoting autophagy: mutants with low TOR pathway activity have increased autophagy, and bec-1, which functions in the formation of autophagosomes, is required for the longevity of daf-15 heterozygotes [145]. This makes an interesting parallel with the role of autophagy in lifespan extension by mutations in the IIS pathway, as daf-2 mutants also have increased autophagy, and bec-1 is also required for the longevity of daf-2 mutants [94].
Once again, the TOR and IIS pathways appear to have some overlap, though the details of their mechanistic relationship remains ambiguous.

**Autophagy**

Autophagy is a suite of cellular recycling processes in which macromolecules and organelles in the cell are degraded into raw materials that can then be used for the synthesis of new macromolecules. During autophagy, large vesicles full of cellular materials to be recycled, called autophagosomes, are formed and fuse with lysosomes, effecting the degradation of their contents. This process is typically held in check by TOR signaling, which is decreased through diverse signaling pathways in response to different stressors [146]. The protein LGG-1 is the *C. elegans* ortholog of the vacuolar protein Atg8/MAP-LC3, which is incorporated into autophagosome membranes, and GFP-tagged LGG-1 is frequently used as an indicator of autophagy [94]. Using this indicator, autophagy has been shown to be upregulated in nematodes under dietary restriction (DR) [145, 147], IIS inhibition [94], or TOR inhibition [145], as well as in animals that have entered the dauer state [94]. Both the longevity and increased autophagy of DR animals require the FOXA transcription factor PHA-4, suggesting that autophagy is a transcriptionally regulated response to DR [145, 148]. On the other hand, though the FOXA transcription factor DAF-16 is required for the longevity of IIS mutants, it is not necessary for the increased autophagy of *daf*-2 animals [145]. Various autophagy genes have been shown to be required for the longevity of IIS and TOR mutants, as well as DR animals. RNAi knockdown of either of the autophagy genes *bec-1* and *atg-12 shortens the lifespan of *daf*-2 mutants, while having a much smaller effect on the lifespan of wild-type animals [94, 149]. Mutants heterozygous for *daf-15* also require *bec-1* for their increased longevity, and both *bec-1* and *vps-34*, which encodes a phosphatidylinositol 3-kinase essential for autophagy, are required for the longevity of *eat-2* mutants, which are a genetic model for DR [145].

Autophagy appears to be an essential part of the longevity pathways activated in IIS mutants, TOR mutants, and DR animals. Given that autophagy is upregulated in response to stress (see also: [150]), the connection between autophagy and longevity is likely related to stress response pathways.

**Mitochondrial respiration**

Mitochondrial respiration defects caused by mutation or RNAi can often extend *C. elegans* lifespan [8, 106, 109, 151-154], but the influence of these defects on stress resistance varies. For instance, *isp-1* encodes the iron sulfur protein of complex III in the electron-transport chain in *C. elegans*, and a mutation in this gene yields significantly increased lifespan and resistance against paraquat [8, 9]. However, though RNAi inactivation of components of the electron-transport chain often increases resistance to H$_2$O$_2$ and high temperatures, it also increases sensitivity to paraquat [152]. Similarly, RNAi against *echl-1*, the *C. elegans* Cytochrome c heme lyase – an enzyme that covalently attaches heme to cytochrome c, which is essential to the electron-transport chain [155] – confers longer lifespan, but not resistance to paraquat or H$_2$O$_2$ [123, 152].

The mechanisms behind the extended lifespan of nematodes with mitochondrial dysfunction are not well understood. Since ROS are produced as byproducts of mitochondrial respiration, one possible explanation is that mutations in mitochondrial respiration genes reduce ROS production. In support of this hypothesis, the long-lived *isp-1* mutant has decreased oxygen consumption. The leucyl-tRNA synthetase gene *lsr-2* is needed for the proper translation of genes encoded by the mitochondrial genome; *lsr-2* mutations, which are thought to result in low electron-transport chain activity, extend *C. elegans* lifespan [106, 109, 152]. However, several observations controvert the theory that reduced ROS accounts for the longevity of mitochondrial mutants. For one, lifespan extension by mutations in mitochondrial respiration genes does not appear to correlate with decreases in lifelong oxidative stress as measured by whole-animal protein carbonyl content [156]. Moreover, at least one mitochondrial respiration mutant, *clk-1*, does not exhibit reduced respiration and has normal or slightly elevated levels of ATP, although it does have increased lifespan and reduced behavioral rates [123, 151, 154, 157]. *The clk-1* gene encodes a hydroxylase necessary for the biosynthesis of ubiquinone, which shuttles electrons as part of the mitochondrial respiratory chain [158]. Furthermore, not all disabling mutations in mitochondrial respiration lengthen lifespan. Mutants of *mev-1*, which codes for a subunit of complex II, have reduced mitochondrial respiratory rates and a thermostolerance comparable to wild-type, but also have a 30% decrease in lifespan [104, 159]. However, *mev-1* mutants express only half as much SOD as wild-type and are hypersensitive to paraquat [160], which makes it difficult to clearly interpret lifespan results from this strain. Together, these results suggest that the longevity of *C. elegans* mitochondrial respiration mutants cannot be explained
solely by lower rates of oxygen consumption and ROS production.

Given the many observations inconsistent with the theory of reduced ROS, alternative hypotheses have been put forth to explain the longevity of mitochondrial respiration mutants. It has been suggested that some mitochondrial mutations may slightly increase ROS levels, resulting in lifespan extension by a hormetic effect [106, 161, 162]. Another proposal is that reduced respiration early in life may stimulate a regulated response that subsequently extends *C. elegans* lifespan [75, 123, 154]. Both ideas hold that reduced respiration may cause a type of stress, and the response of the nematode’s cells to this stress might have a life-extending effect. Consistent with this theory, inhibition of certain mitochondrial respiration genes by mutation or RNAi leads to increased expression of certain cell-protective and metabolic genes, and greater abundance of mitochondrial DNA [163]. Further, RNAi against the potential signaling genes *fstr-1* and *fstr-2* reduces lifespan, increases behavioral rates, and suppresses gene expression changes in *clk-1* mutants [163]. These results suggest that *fstr-1* and *fstr-2* may be part of a pathway that triggers a life-extending response to reduced respiration in *clk-1* mutants [163]. However, these genes are not required for the longevity of all other mitochondrial mutants [163].

In most cases, lifespan extension by mutations in mitochondrial respiration genes does not depend on *daf-2* and *daf-16*, and is therefore independent of the IIS pathway [8, 106, 109, 151, 152, 154]. However, *gms-50*, a point mutation in *isp-1*, does not extend the lifespan of *daf-2* mutants, and its effect on lifespan is independent of *daf-16* [8, 75].

**Antioxidant defenses**

As discussed previously, many longevity mutants are resistant to oxidative stress and have altered levels of antioxidant enzymes. Mutants for *age-1*, which have a 40–110% longer lifespan than wild-type, express elevated levels of catalase and SOD [14, 34, 95, 104], while *mev-1* mutants, with a 30% shorter lifespan than wild-type, are hypersensitive to paraquat and have only about half the normal amount of SOD [16, 104, 159]. Furthermore, studies have found that oxidative damage (measured by protein carbonyl content) was decreased in *age-1* mutants and increased in *mev-1* mutants relative to wild-type [16, 104]. The short-lived *daf-16* mutants also express lower levels of SODs, although these animals also have higher expression levels of *ctl-1* and *ctl-2* as compared to wild-type animals [104]. The long-lived *skn-1* mutant also expresses high levels of antioxidant enzymes [131-133], and in *clk-1* mutants there is a significant increase in detoxification enzymes including UDP-glycosyl transferases, glutathione S-transferases, and SOD-3 [163].

Like *age-1* mutants, *daf-2* mutants are long-lived, resistant to oxidative stress, and have increased SOD and catalase expression [14, 34, 72, 91]. However, the longevity of *daf-2* mutants is only partly suppressed by RNAi against *ctl-1* (encoding a cytoplasmic catalase), *ctl-2* (encoding a peroxisomal catalase and peroxidase), or *sod-3* (encoding an iron/manganese SOD predicted to be mitochondrial)[107]. Long lived strains with mutations in *isp-1*, *daf-2*, *daf-2;clk-1*, *daf-2;isp-1*, or *daf-2;isp-1;ctl-1* show increased levels of both SOD-1 and SOD-2 but exhibit no quantitative correlation between increased SOD levels and lifespan [164]. For example, *daf-2* and *daf-2;clk-1* mutants express the same levels of SODs, but *daf-2;clk-1* mutants have much longer lifespan [8, 157, 164].

Moreover, directly altering the expression of antioxidant enzymes does not have a consistent effect on lifespan. Wild-type *C. elegans* treated with *sod-1* (RNAi) are more sensitive to paraquat but have only slightly shortened lifespan [164]. Mutations in *sod-3*, *sod-4*, or *sod-5* do not affect lifespan, and loss of *sod-2* might even increase lifespan despite higher levels of oxidative damage [36, 165, 166]. Similarly, deletion of *ctl-1* does not shorten lifespan [36].

**STRESS, HORMESIS, AND LONGEVITY**

Pre-treatment of many organisms, including *C. elegans*, with mild degrees of stress can prompt extended lifespan (induced lifespan extension), as well as greater resistance to oxidative stress (induced resistance to oxidative stress) and high temperatures (induced thermotolerance), in a process generally known as “hormesis”. [22, 48, 64, 65, 167]. The hormetic effect of thermal stress is dependent on members of the IIS pathway, though, interestingly, induced thermotolerance and lifespan extension have separate genetic requirements – induced thermotolerance depends on *daf-18*, while the lifespan effect is dependent on *daf-12*, *daf-16*, and *daf-18* [22, 167].

Several models have been proposed to explain how different levels of stress influence lifespan [25, 26, 64,
Each is premised on the assumptions that during the aging process, random damage accumulates in the nematode. Exposure to stress leads to a response that includes a dramatic increase in the levels of HSP-4 and HSP-16 in *C. elegans*, and the levels of the heat-shock proteins correlate with the lifespan of the hormetically treated nematodes [58]. In the “stochastic” model, the accumulation of damage is proportional to the applied stress, but the production of HSPs is not; thus at certain doses, the HSPs produced by the cells of the nematode in response to stress are in fact more than necessary to repair the damage caused, and thus have long-term protective functions, increasing the nematode’s longevity and tolerance to future stressors [24, 25, 58, 65]. The hormetic effect can also be explained in terms of energy capacity. In the “biphasic” model for survival, an organism’s ability to restore its normal functioning after exposure to stress decreases linearly with age, and death occurs when its energy capacity becomes too low to overcome the damage caused by stress [64]. The model posits that a hormetic level of stress causes an organism to increase its energy capacity so that it can respond to the anticipated future stresses, resulting in slower aging and increased stress resistance [64]. It has also been proposed that an isogenic population of *C. elegans* is actually composed of subpopulations that each have a different mortality pattern (“discrete heterogeneity” model). Hormetic stress shifts nematodes from one subpopulation to another, having the overall effect of increasing mean lifespan [26]. These different models approach the problem of explaining hromesis from different angles. While they are not necessarily incompatible with one another, the mechanisms underlying the hormetic effect are nevertheless incompletely understood.

**DIETARY RESTRICTION**

Dietary restriction (DR), which has been shown to prolong longevity across diverse taxa [168, 169], may also be considered a type of stress, as DR often leads to increased activities of antioxidant enzymes, as well as greater resistance to thermal and oxidative stress [19]. If no food is available when a *C. elegans* larva hatches, the nematode’s growth is arrested as an L1 stage larva until food is provided, at which point the nematode grows normally and has a typical adult lifespan [170]. Similarly, dietary restriction and crowding of L1 or L2 larvae causes nematodes to enter the dauer stage [62]. DR can also lead to extended adult lifespan rather than prompting a state of arrested growth, but the degree of lifespan extension depends on the type of DR used. *C. elegans* can be subjected to DR by culturing in an axenic, completely-defined liquid medium or in lower concentrations of *E. coli* [106], by complete removal of bacterial food early in adulthood (“dietary deprivation”) [171-173], through mutants defective in feeding, such as the *eat* mutants which have limited pharyngeal pumping ability and thus limited food uptake, or via mutations in specific nutrient transporters [106]. Nematodes cultured in axenic medium have a two-fold increase in length of development and adult lifespan [174, 175]; adult *C. elegans* maintained on plates with lower amounts of bacteria have a 15–30% increase in lifespan [176]; and *C. elegans* with *eat* mutations or subjected to dietary deprivation early in adulthood, have lifespans lengthened by up to 50% [171, 172, 177]. If DR is a form of mild stress, lifespan extension through DR could be considered a type of hormesis [19, 23].

However, each method of DR suffers from potential confounding factors, making results difficult to interpret [19]. Since proliferating *E. coli* are associated in some fashion with early mortality in *C. elegans* [61], restricting the nematode’s diet may lengthen lifespan by exposing *C. elegans* to fewer (or no) live bacteria rather than by the effect of decreased caloric intake. Further, restricting the *C. elegans* diet by lowering the concentration of *E. coli* or by *eat* mutation not only reduces calorie consumption but also limits intake of essential nutrients provided by the *E. coli* [19]. Likewise, though axenic medium contains all the compounds essential to *C. elegans*, it may still cause malnutrition – for instance, it may be more difficult for the nematodes to metabolize the compounds in the axenic medium, or to use the available compounds to synthesize a nutrient that is more directly available from *E. coli* [19, 178].

The physiological outcomes from dietary restriction can be diverse depending on the specific type of DR used. Mutants for *eat-2*, as well as wild-type grown in axenic medium or in lower concentrations of bacteria, have higher activities of SOD and catalase [174, 179]. *C. elegans* cultured on plates with lower amounts of bacteria or subjected to dietary deprivation in early adulthood have increased resistance to paraquat [172, 176], but nematodes grown in liquid culture with lower concentrations of bacteria are not more resistant to paraquat and H$_2$O$_2$ [179]. Growing wild-type or *eat-2* mutant nematodes in axenic culture results in greater resistance to high temperature, while *eat-2* mutants grown on bacterial lawns do not have increased thermotolerance [174]. Nematodes subjected to dietary
deprivation in early adulthood also have greater thermotolerance [171, 172]. Interestingly, mutants in pep-2, which codes for a carrier responsible for the uptake of peptides, have greater tolerance to thermal and oxidative stress, but are not long-lived [19, 180]. It has been suggested that DR might extend lifespan by decreasing metabolic rate and ROS production [177]. However, studies of oxygen consumption and heat production have shown that nematodes cultured in axenic medium or in lower concentrations of bacteria do not have a reduced metabolism. Meanwhile, eat-2 mutants grown in liquid culture actually have an increased metabolic rate, and the metabolic rate of eat-2 mutants grown on solid medium has not been evaluated [174, 179].

Although DR is known to increase lifespan in many different species [168, 181], the mechanism behind DR-mediated longevity remains unknown. Given that C. elegans undergoing DR are both long-lived and resistant to thermal and oxidative stress, it is conceivable that DR results in the upregulation of stress defense systems that in turn increase lifespan as well as stress resistance, possibly through a hormetic effect [19, 181]. Higher activity of SOD and catalase has been found in eat-2 mutants, in nematodes grown in axenic medium, and in nematodes cultured in lower concentrations of bacteria [174, 179]. Additionally, the longevity of DR animals requires the action of SKN-1 in the two ASI neurons [182], which control entry into dauer and are involved in chemotaxis to lysine [183]. As the transcription factor SKN-1 is known to stimulate the oxidative stress response [132], it has been suggested that the ASI neurons might detect the effects of dietary restriction and consequently activate skn-1, as well as trigger hormonal signals that lead to longevity [182]. However, knockout of SOD enzymes in DR nematodes does not significantly affect lifespan, though this result does not rule out the possibility that other aspects of the oxidative stress response may play a role in DR-mediated lifespan extension [184].

Because of the close connection between diet and the insulin/IGF-1 signaling pathway, one might expect the longevity effect of DR to be mediated by the IIS pathway [19]. However, life extension and increased stress resistance resulting from DR are still observed in daf-16, age-1, and daf-2 nematodes [19]. These tests are not entirely conclusive because the age-1 and daf-2 alleles used for lifespan studies are reduction-of-function rather than null mutations. The daf-16 allele, however, is a null. These results imply that the longevity effect of DR in C. elegans is either not dependent or only partially dependent on the IIS pathway, contrary to the result in flies and rodents, in which IIS plays a role in DR [185-187]. Another pathway that may be thought to mediate the longevity effect of DR is the TOR pathway, which responds to nutrient level. Indeed, DR does not further extend lifespan of let-363 mutants, suggesting that DR lengthens lifespan by downregulating let-363 [180]. However, this cannot be the only mechanism of DR-induced lifespan extension, since lifespan extension in let-363 mutants is dependent on the IIS pathway [19]. Interestingly, subjecting dietary-restricted C. elegans to let-363(RNAi) also reduces the translation rate by an additional 49% without lengthening lifespan [142], indicating that TOR signaling and protein translation remains active even in DR, though perhaps at levels below those which manipulate longevity. The longevity effect of DR also correlates with the upregulation of autophagy: nematodes under DR have higher levels of autophagy [145, 147], and the autophagy genes bec-1 and vps-34 are required for the increased lifespan of eat-2 mutants [145]. It has been suggested that autophagy is an essential part of longevity pathways related to nutrition in that it provides the raw materials needed for the synthesis of proteins that confer longevity and stress resistance [145].

THEORIES OF AGING AND STRESS

Though longevity and stress resistance seem to be correlated, the mechanism behind such a correlation remains unclear. In this section we examine several theories of aging, which posit different, though non-exclusive, explanations for why longevity and stress resistance should be correlated.

Free radical theory of aging

The free radical theory of aging (FRTA), proposed by Denham Harman in the 1950’s, postulates that the aging of all living beings is caused by the common biochemical process of accumulation of oxidative damage over time [188, 189]. To protect against internal and external sources of ROS, the cell uses enzymes such as SOD, catalase, and glutathione peroxidase. According to FRTA, these anti-damage defenses are insufficient to eliminate all oxidative damage from the cell, or they might even lose efficiency over time, resulting in an accumulation of cell damage that constitutes aging [188].

Consistent with FRTA, longevity is often correlated with oxidative stress resistance and levels of antioxidant
enzymes [14, 16, 34, 104, 159]. However, some longevity mutants are not resistant to oxidative stress, and levels of catalase and SOD do not always correlate with longevity in a quantitative manner [125, 152, 164]. Since most ROS are produced by mitochondria, changes in mitochondrial function might be expected to modulate lifespan [123]. Many, but not all, mitochondrial respiration mutants exhibit extended lifespan [8, 9, 104, 152]. Additionally, the inactivation of genes in mitochondrial respiration pathways must occur during development in order to affect lifespan, whereas based on FRTA the suppression of these genes in adult nematodes should also influence lifespan [123, 154].

The best way to test FRTA is to manipulate the level of oxidative damage and observe the effects on aging [36, 106]. FRTA is supported by evidence that C. elegans lifespan can be extended by the introduction of antioxidant compounds, but these results are not always reproducible, and it has been shown that some antioxidant molecules do not lengthen lifespan [36, 38, 40, 41, 46]. Moreover, changes in expression of antioxidant enzymes often do not have the expected effect on lifespan [36, 164-166]. Skeptics also argue that FRTA does not take into account the beneficial role of free radicals in normal functioning and survival [190]. Further, FRTA suggests that species with longer lifespans should be those with more efficient mechanisms for countering free radicals, but there is as yet no evidence that this is the case [190].

Antagonistic pleiotropy

Absent social, altruistic/kin-selective, or “grandparenting” behaviors, post-reproductive traits typically cannot be selected for or against by evolution. As aging is largely a post-reproductive process, any genetically-controlled, stereotyped features of senescence may simply be by-products of adaptive traits that manifest earlier. Specifically, George Williams noticed that evolution favors genes that have a positive effect during development and the reproductive stage, regardless of any potential negative pleiotropic effects at post-reproductive ages [191, 192]. Therefore, alleles with positive effects until the reproductive phase, but negative effects at post-reproductive ages, are not selected against [191]. This observation is the foundation for Williams’ theory of antagonistic pleiotropy [191], which predicts that stress response mechanisms and other pathways that benefit the organism would be activated early in life at levels ensuring optimal reproductive fitness, but reductions in activity at post-reproductive ages (“gene expression drift”) would not be selected against.

Several examples of antagonistic pleiotropy have been found in mammals [193-195], and the GATA transcription factor ELT-3 has been proposed as an example in C. elegans [196]. ELT-3 functions in hypodermal development, but also decreases in expression during normal aging [196, 197]. Since elt-3 regulates the expression of sod-3 and is required for the longevity of daf-2 and eat-2 mutants, it is conceivable that elt-3 functions in a stress response pathway downstream of the IIS and DR-mediated longevity pathways [196, 198]. Expression of elt-3 is negatively regulated by the GATA transcription factors ELT-5 and ELT-6, and these three genes may constitute an example of drift in a developmental pathway leading to the downregulation of a stress response pathway [196, 198, 199]. The role of the miRNA lin-4 and its target lin-14 on longevity and stress response may also be an example of antagonistic pleiotropy, given that lin-4 and lin-14 have known functions in C. elegans larval development [89].

Disposable soma

Like antagonistic pleiotropy, the disposable soma theory centers on the relationship between longevity and fitness. This theory proposes that, since resources are limited, an organism must carefully allocate resources between reproduction and somatic maintenance, depending the animal’s particular environment [106, 192, 200]. As stress responses may be particularly resource-intensive, this theory predicts that these responses will only be activated to the degree that will optimize an organism’s reproductive fitness. Additional resource expenditure to optimize somatic fitness (i.e. lifespan) would not be selected for. As an example, the disposable soma theory also offers an explanation for lifespan extension by DR: when food is scarce, it is beneficial for the animal to use more energy for somatic maintenance and less for reproduction [106]. This might allow the animal to live long enough to be able to reproduce after conditions improve, in an environment more congenial to the success of its offspring [106, 201, 202]. More generally, stressful conditions of any type might shift the evolutionarily optimal resource allocation toward somatic maintenance, so that the animal might “live to reproduce another day”; any collateral lifespan extension this might induce beyond the reproductive period, however, would be evolutionarily neutral.
Since the disposable soma theory predicts a trade-off between reproduction and somatic maintenance, long-lived and stress resistant *C. elegans* might be expected to have reduced fertility. However, studies on the fertility of longevity mutants give mixed results [12, 93, 98]. The long-lived, stress-resistant *daf*-2 mutant exhibits normal fertility levels, although it has a significant reduction in early fertility [93, 203], and the fertility of the long-lived *age*-1 mutant is not significantly different from wild-type [12, 204, 205]. There is evidence that *pep*-2 mutants, which are resistant to thermal and oxidative stress but are not long-lived, have reduced fertility under certain dietary conditions, but the broader relationship between stress resistance and fertility remains unclear [206]. Additionally, increasing production of eggs in *C. elegans* does not lead to a shorter lifespan, and the effect of such an increase on stress resistance has not been determined [207].

Fertility, especially as measured in lab conditions, is only one component of reproductive fitness, however. Various mutations conferring longevity and stress resistance have clear impact on fitness: *daf*-2 mutants have significantly reduced early fertility and are outcompeted by wild-type within four generations [93, 203], while *age*-1 mutants have lower fitness than wild-type under nutritional stress [204].

In further support of the disposable soma theory, it has been observed that removal of the germline extends lifespan and increases *C. elegans* resistance to thermal and oxidative stress [20, 21]. This suggests that resources can be devoted to somatic maintenance when not used by the reproductive system, and that when the germline is present, it down-regulates somatic stress responses, perhaps in order to conserve resources. The reproductive system of *C. elegans* consists of two parts – the germ cells and the somatic reproductive tissues. Removal of the entire reproductive system by laser ablation does not extend nematode lifespan; however, if the germline is removed and the somatic gonad left in place, *C. elegans* have increased thermal and oxidative stress resistance, as well as a ~60% lifespan extension [20, 21]. Additionally, both *glp*-1 mutants, in which the germline does not proliferate, and *mes*-1 mutants, which lack germ cells, are long-lived [21]. Thus, the somatic gonad is necessary for lifespan extension by germline removal; however, certain stress-resistant phenotypes produced by DAF-16 activity in response to gonad loss do not require the somatic gonad [208]. This suggests that germline removal influences lifespan and stress resistance through at least partially separate mechanisms.

Lifespan extension by germline removal also requires *daf*-16 [20, 91, 130]. However, *daf*-16 is not necessary for germline removal to increase *C. elegans* resistance to paraquat and heat [130, 208]. This result again suggests that the longevity and stress resistance phenotypes of nematodes lacking a germline are conferred by distinct pathways. The signaling that links the germline to stress response mechanisms remains unclear. However, it has been observed that DAF-16 accumulates in the nuclei of intestinal cells in nematodes in which the germline has been removed [83, 208]. The effect of germline ablation on lifespan also depends on *kri*-1, the gene for an intestinal ankyrin-repeat protein; *daf*-9, the gene for a cytochrome P450 protein; and *daf*-12, which codes for a nuclear hormone receptor [99]. Interestingly, lifespan extension through the IIS pathway does not depend on these genes, suggesting that *kri*-1, *daf*-9, and *daf*-12 function specifically in incorporating information on the status of the germline into the regulation of lifespan. Similarly, the predicted transcription elongation factor *tcer*-1 is required for the lifespan effect of germline removal, but not for the effect of DR, decreased IIS, or *isp*-1 mutation [209]. Furthermore, the *tcer*-1 gene is required for the expression of many DAF-16 target genes upon germline removal, suggesting that *tcer*-1 specifically links *daf*-16 to longevity signals from reproductive tissues [209].

The longevity effect of germline removal is also related to the effects of dietary restriction. Lifespan extension by DR is not suppressed by removal of the entire reproductive system, but it is suppressed when only the germ cells are removed [210]. This suggests that germline removal and DR might activate converging pathways in lifespan extension.

**DISCUSSION**

Studies on *C. elegans* have revealed a close connection between stress and aging. Although most long-lived mutant nematodes also exhibit increased stress resistance, exceptions and inconsistencies remain. Genes in the insulin/IGF-1 signaling pathway and in the target of rapamycin pathway, as well as those coding for mitochondrial respiration proteins and antioxidant enzymes, seem to play critical roles in both modulating stress response and aging. However, the mechanisms behind many of these modulations, and of the precise relationship between stress responses and aging, remain unclear. In Table 1, we attempt to summarize the genes
that have been identified to play a role in aging and/or stress responses, highlighting cases where the two do not clearly overlap.

The role of free radicals in aging is supported by correlation between longevity, oxidative stress resistance, and elevated levels of antioxidant enzymes. However, this correlation is incomplete, and studies modulating levels of reactive oxygen species have obtained mixed results. The theory of antagonistic pleiotropy explains the modulation of longevity from an evolutionary point of view, and the few extant examples of antagonistic pleiotropy in C. elegans have possible connections to stress responses. The disposable soma theory is supported by the longevity of germline-ablated nematodes, and genes have been identified that seem to carry signals between the reproductive tissues and both aging and stress response mechanisms. These theories are of course not mutually exclusive, and, indeed, overlap to a large degree (and make similar predictions) regarding the relationship between stress responses, evolutionary fitness, and longevity. Both aging and stress responses are complex, multi-factorial processes that integrate internal and external signals over long time-spans, and it is likely that no simple theoretical explanation or set of experimental findings will fully predict or explain the many connections between the two.

ACKNOWLEDGEMENTS

ZP was supported by a JCC Postdoctoral Fellowship. This work was supported by NIH R01 AG033921.

REFERENCES

1. C. elegans Sequencing Consortium. Genome sequence of the nematode C. elegans: A platform for investigating biology. Science. 1998; 282:2012-2018.
2. Brenner S. The genetics of Caenorhabditis elegans. Genetics. 1974; 77:71-94.
3. Luo Y. Long-lived worms and aging. Redox Rep. 2004; 9:65-69.
4. Prahald V, Morimoto R. Integrating the stress response: Lessons for neurodegenerative diseases from C. elegans. Trends Cell Biol. 2008.
5. Dormann JB, Albinder B, Shroyer T, Kenyon C. The age-1 and daf-2 genes function in a common pathway to control the lifespan of Caenorhabditis elegans. Genetics. 1995; 141:1399-1406.
6. Morris JZ, Tissenbaum HA, Ruvkun G. A phosphatidylinositol-3-OH kinase family member regulating longevity and diapause in Caenorhabditis elegans. Nature. 1996; 382:536-539.
7. 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; 277:942-946.
8. Feng J, Bussière F, Hekimi S. Mitochondrial electron transport is a key determinant of life span in Caenorhabditis elegans. Dev Cell. 2001; 1:633-644.
9. Sedensky MM, Morgan PG. Mitochondrial respiration and reactive oxygen species in C. elegans. Exp Gerontol. 2006; 41:957-967.
10. Avery L. The genetics of feeding in Caenorhabditis elegans. Genetics. 1993; 133:897-917.
11. Lithgow GJ, White TM, Hinerfeld DA, Johnson TE. Thermotolerance of a long-lived mutant of Caenorhabditis elegans. J Gerontol. 1994; 49:B270-6.
12. Lithgow GJ, White TM, Melov S, Johnson TE. Thermotolerance and extended life-span conferred by single-gene mutations and induced by thermal stress. Proc Natl Acad Sci U S A. 1995; 92:7540-7544.
13. Murakami S, Johnson TE. A genetic pathway conferring life extension and resistance to UV stress in Caenorhabditis elegans. Genetics. 1996; 143:1207-1218.
14. Vanfleteren JR. Oxidative stress and aging in Caenorhabditis elegans. Biochim J. 1993; 292 (Pt 2):605-608.
15. Ayyadevara S, Allia R, Thaden JJ, Shmoookler Reis RJ. Remarkable longevity and stress resistance of nematode PI3K-null mutants. Aging Cell. 2008; 7:13-22.
16. Adachi H, Fujiwara Y, Ishii N. Effects of oxygen on protein carbonyl and aging in Caenorhabditis elegans mutants with long (age-1) and short (mev-1) life spans. J Gerontol A Biol Sci Med Sci. 1998; 53:B240-4.
17. de Castro E, Hegl de Castro S, Johnson TE. Isolation of long-lived mutants in Caenorhabditis elegans using selection for resistance to juglone. Free Radic Biol Med. 2004; 37:139-145.
18. Garsin DA, Villanueva JM, Begun J, Kim DH, Sifri CD, Calderwood SB, Ruvkun G, Ausubel FM. Long-lived C. elegans daf-2 mutants are resistant to bacterial pathogens. Science. 2003; 300:1921.
19. Houthof K, Johnson TE, Vanfleteren JR. Dietary restriction in the nematode Caenorhabditis elegans. J Gerontol A Biol Sci Med Sci. 2005; 60:1125-1131.
20. Hsin H, Kenyon C. Signals from the reproductive system regulate the lifespan of C. elegans. Nature. 1999; 399:362-366.
21. Arantes-Oliveira N, Apfeld J, Dillin A, Kenyon C. Regulation of life-span by germ-line stem cells in Caenorhabditis elegans. Science. 2002; 295:502-505.
22. Cypser JR, Johnson TE. Multiple stressors in Caenorhabditis elegans induce stress hormesis and extended longevity. J Gerontol A Biol Sci Med Sci. 2002; 57:B109-14.
23. Cypser JR, Tedesco P, Johnson TE. Hormesis and aging in Caenorhabditis elegans. Exp Gerontol. 2006; 41:935-939.
24. Wu D, Cypser J, Yashin A, Johnson T. Multiple mild heat-shocks decrease the gompertz component of mortality in Caenorhabditis elegans. Exp Gerontol. 2009; 44:607-612.
25. Butov A, Johnson T, Cypser J, Sannikov I, Volkov M, Sehl M, Yashin A. Hormesis and debilitation effects in stress experiments using the nematode worm Caenorhabditis elegans: The model of balance between cell damage and HSP levels. Exp Gerontol. 2001; 37:57-66.
26. Yashin AI, Cypser JW, Johnson TE, Michalski AI, Boyko SI, Novoseltsev VN. Heat shock changes the heterogeneity distribution in populations of Caenorhabditis elegans: Does it tell us anything about the biological mechanism of stress response? J Gerontol A Biol Sci Med Sci. 2002; 57:883-92.
27. Johnson TE, Hartman PS. Radiation effects on life span in Caenorhabditis elegans. J Gerontol. 1988; 43:B137-41.
28. Golden TR, Hinerfeld DA, Melov S. Oxidative stress and aging: Beyond correlation. Aging Cell. 2002; 1:117-123.
29. Yanase S, Onodera A, Tedesco P, Johnson TE, Ishii N. SOD-1 deletions in Caenorhabditis elegans alter the localization of intracellular reactive oxygen species and show molecular compensation. J Gerontol A Biol Sci Med Sci. 2009; 64:530-539.
30. McCord JM, Fridovich I. Superoxide dismutase. an enzymic function for erythrocuprein (hemocuprein). J Biol Chem. 1969; 244:6049-6055.
31. Walker TK, Totic J. The 'catalase test', with special reference to acetobacter species. Biochem J. 1943; 37:10-12.
32. Mills GC. The purification and properties of glutathione peroxidase of erythrocytes. J Biol Chem. 1959; 234:502-506.
33. Brenot A, King KY, Janowiak B, Griffith O, Caparon MG. Contribution of glutathione peroxidase to the virulence of streptococcus pyogenes. Infect Immun. 2004; 72:408-413.
34. Larsen PL. Aging and resistance to oxidative damage in Caenorhabditis elegans. Proc Natl Acad Sci U S A. 1993; 90:8905-8909.
35. Olahova M, Taylor SR, Khazaipoul S, Wang J, Morgan BA, Matsumoto K, Blackwell TK, Veal EA. A redox-sensitive peroxiredoxin that is important for longevity has tissue- and stress-specific roles in stress resistance. Proc Natl Acad Sci U S A. 2008; 105:19839-19844.
36. Gems D, Doonan R. Antioxidant defense and aging in C. elegans: Is the oxidative damage theory of aging wrong? Cell Cycle. 2009; 8:1681-1687.
37. Oliveira RP, Abate JP, Dilks K, Landis J, Ashraf J, Murphy CT, Blackwell TK. Condition-adapted stress and longevity gene regulation by Caenorhabditis elegans SKN-1/Nrf. Aging Cell. 2009; 8:524-541.
38. Adachi H, Ishii N. Effects of tocotrienols on life span and protein carbonylation in Caenorhabditis elegans. J Gerontol A Biol Sci Med Sci. 2000; 55:B280-5.
39. Benedetti MG, Foster AE, Vantipalli MC, White MP, Sampayo JN, Gill MS, Olsen A, Lithgow GJ. Compounds that confer thermal stress resistance and extended lifespan. Exp Gerontol. 2008; 43:882-891.
40. Howitz KT, Bitterman KJ, Cohen HY, Lamming DW, Lavu S, Wood JG, Zipkin RE, Chung P, Kiselewski A, Zhang LL, Scherer B, Sinclair DA. Small molecule activators of sirtuins extend saccharomyces cerevisiae lifespan. Nature. 2003; 425:191-196.
41. Melov S, Ravenscroft J, Malik S, Gill MS, Walker DW, Clayton PE, Wallace DC, Malfroy B, Doctrow SR, Lithgow GJ. Extension of life-span with superoxide dismutase/catalase mimetics. Science. 2000; 289:1567-1569.
42. Kampkotter A, Nkworkam CG, Zurawski RF, Timpel C, Chovolou Y, Watjen W, Kahl R. Investigations of protective effects of the flavonoids quercetin and rutin on stress resistance in the model organism Caenorhabditis elegans. Toxiconology. 2007; 234:113-123.
43. Kampkotter A, Gombitang Nkworkam C, Zurawski RF, Timpel C, Chovolou Y, Watjen W, Kahl R. Effects of the flavonoids kaempferol and fisetin on thermotolerance, oxidative stress and FoxO transcription factor DAF-16 in the model organism Caenorhabditis elegans. Arch Toxicol. 2007; 81:849-858.
44. Wiegrant FA, Surinova S, Ytsema E, Langelaar-Makkinje M, Wikman G, Post JA. Plant adaptogens increase lifespan and stress resistance in C. elegans. Biogerontology. 2009; 10:27-42.
45. Zhang L, Jie G, Zhang J, Zhao B. Significant longevity-extending effects of EGCG on Caenorhabditis elegans under stress. Free Radic Biol Med. 2009; 46:414-421.
46. Keaney M, Gems D. No increase in lifespan in Caenorhabditis elegans upon treatment with the superoxide dismutase mimetic EUK-8. Free Radic Biol Med. 2003; 34:277-282.
47. Pun PB, Gruber J, Tang SY, Schaffer S, Ong RL, Fong S, Ng LF, Cheah I, Halliwell B. Ageing in nematodes: Do antioxidants extend lifespan in Caenorhabditis elegans? Biogerontology. 2010; 11:17-30.
48. Yanase S, Hartman PS, Ito A, Ishii N. Oxidative stress pretreatment increases the X-radiation resistance of the nematode Caenorhabditis elegans. Mutat Res. 1999; 426:31-39.
49. Yasuda K, Adachi H, Fujiwara Y, Ishii N. Protein carbonyl accumulation in aging dauer formation-defective (daf) mutants of Caenorhabditis elegans. J Gerontol A Biol Sci Med Sci. 1999; 54:B47-51; discussion B52-3.
50. Clokey GV, Jacobson LA. The autofluorescent "lipofuscin granules" in the intestinal cells of Caenorhabditis elegans are secondary lysosomes. Mech Ageing Dev. 1986; 35:79-94.
51. Sohal RS, Marzabadi MR, Galaris D, Brunk UT. Effect of ambient oxygen concentration on lipofuscin accumulation in cultured rat heart myocytes—a novel in vitro model of lipofuscinogenesis. Free Radic Biol Med. 1989; 6:23-30.
52. Marzabadi MR, Sohal RS, Brunk UT. Mechanisms of lipofuscinogenesis: Effect of the inhibition of lysosomal proteinases and lipases under varying concentrations of ambient oxygen in cultured rat neonatal myocardial cells. APMIS. 1991; 99:416-426.
53. Marzabadi MR, Sohal RS, Brunk UT. Effect of alphatocopherol and some metal chelators on lipofuscin accumulation in cultured neonatal rat cardiac myocytes. Anal Cell Pathol. 1990; 2:333-346.
54. Yin D. Biochemical basis of lipofuscin, ceroid, and age pigment-like fluorophores. Free Radic Biol Med. 1996; 21:871-888.
55. Hosokawa H, Ishii N, Ishida H, Ichimori K, Nakazawa H, Suzuki K. Rapid accumulation of fluorescent material with aging in C. elegans. Tox Ind Health. 2001; 11:17-30.
56. Braeckman BP, Houthoofd K, De Vreese A, Vanfleteren JR. Assaying metabolic activity in ageing Caenorhabditis elegans. Mech Ageing Dev. 2002; 123:105-119.
57. Gerstbrein B, Stamatas G, Kollas N, Driscoll M. In vivo spectrofluorimetry reveals endogenous biomarkers that report healthspan and dietary restriction in Caenorhabditis elegans. Aging Cell. 2005; 4:127-137.
58. Olsen A, Vantipalli MC, Lithgow GJ. Lifespan extension of Caenorhabditis elegans following repeated mild hormetic heat treatments. Biogerontology. 2006; 7:221-230.
59. Walker GA, White TM, McColl G, Jenkins NL, Babich S, Candido EP, Johnson TE, Lithgow GJ. Heat shock protein accumulation is upregulated in a long-lived mutant of Caenorhabditis elegans. J Gerontol A Biol Sci Med Sci. 2001; 56:821-7.
oligomeric assembly and interaction with unfolded polypeptides. J Biol Chem. 1997; 272:24646-24656.

61. Garigan D, Hsu AL, Fraser AG, Kamath RS, Ahringer J, Kenyon C. Genetic analysis of tissue aging in Caenorhabditis elegans: A role for heat-shock factor and bacterial proliferation. Genetics. 2002; 161:1101-1112.

62. Klass M, Hirsh D. Non-ageing developmental variant of Caenorhabditis elegans. Nature. 1976; 260:523-525.

63. Dalley BK, Golomb M. Gene expression in the Caenorhabditis elegans dauer larva: Developmental regulation of Hsp90 and other genes. Dev Biol. 1992; 151:80-90.

64. Michalski AI, Johnson TE, Cypser JR, Yashin AI. Heating stress patterns in Caenorhabditis elegans longevity and survivorship. Biogerontology. 2001; 2:35-44.

65. Yashin AI, Cypser JR, Johnson TE, Michalski AI, Boyko SI, Novoseltsev VN. Ageing and survival after different doses of heat shock: The results of analysis of data from stress experiments with the nematode worm Caenorhabditis elegans. Mech Ageing Dev. 2001; 122:1477-1495.

66. Wu D, Cypser JR, Yashin AI, Johnson TE. The U-shaped response of initial mortality in Caenorhabditis elegans to mild heat shock: Does it explain recent trends in human mortality? J Gerontol A Biol Sci Med Sci. 2008; 63:660-668.

67. Vatamaniuk OK, Bucher EA, Sundaram MV, Rea PA. CehMT-1, a putative phychoelatin transporter, is required for cadmium tolerance in Caenorhabditis elegans. J Biol Chem. 2005; 280:23684-23690.

68. Rea PA, Vatamaniuk OK, Rigden DJ. Weeds, worms, and more: papain's long-lost cousin, phychoelatin synthase. Plant Physiol. 2004; 136:2463-2474.

69. Tvermoes BE, Boyd WA, Freedman JH. Molecular characterization of nem-1 and nem-2: Genes that increase both resistance to metal-induced stress and lifespan in Caenorhabditis elegans. J Cell Sci. 2010; 123:2124-2134.

70. Cui Y, McBride SJ, Boyd WA, Alper S, Freedman JH. Toxicogenomic analysis of Caenorhabditis elegans reveals novel genes and pathways involved in the resistance to cadmium toxicity. Genome Biol. 2007; 8:R122.

71. Mizuno T, Hisamoto N, Terada T, Kondo T, Adachi M, Nishida E, Kim DH, Ausubel FM, Matsumoto K. The Caenorhabditis elegans MAPK phosphatase VPH-1 mediates a novel JNK-like signaling pathway in stress response. EMBO J. 2004; 23:2226-2234.

72. Barsyte D, Lovejoy DA, Lithgow GJ. Longevity and heavy metal resistance in daf-2 and age-1 long-lived mutants of Caenorhabditis elegans. FASEB J. 2001; 15:627-634.

73. Broughton S, Partridge L. Insulin/IGF-like signalling, the central nervous system and aging. Biochem J. 2009; 418:1-12.

74. Partridge L, Gems D. Mechanisms of ageing: Public or private? Nat Rev Genet. 2002; 3:165-175.

75. Kenyon C. The plasticity of aging: Insights from long-lived mutants. Cell. 2005; 120:449-460.

76. Barbieri M, Bonafe M, Franceschi C, Paolissio G. Insulin/IGF-I-signaling pathway: An evolutionarily conserved mechanism of longevity from yeast to humans. Am J Physiol Endocrinol Metab. 2003; 285:E1064-71.

77. Cohen E, Dillin A. The insulin paradox: Aging, proteotoxicity and neurodegeneration. Nat Rev Neurosci. 2008; 9:759-767.

78. Bonafe M, Barbieri M, Marchegiani F, Olivieri F, Rago E, Giampieri C, Mugianesi E, Centurelli M, Franceschi C, Paolissio G. Polymorphic variants of insulin-like growth factor I (IGF-I) receptor and phosphoinositide 3-kinase genes affect IGF-I plasma levels and human longevity: Cues for an evolutionarily conserved mechanism of life span control. J Clin Endocrinol Metab. 2003; 88:3299-3304.

79. 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; 12:2488-2498.

80. Toker A, Cantley LC. Signalling through the lipid products of phosphoinositide-3-OH kinase. Nature. 1997; 387:673-676.

81. Hertweck M, Gobel C, Baumesser R. C. elegans SKG-1 is the critical component in the Akt/PKB kinase complex to control stress response and life span. Dev Cell. 2004; 6:577-588.

82. Lee RY, Hench J, Ruvkun G. Regulation of C. elegans DAF-16 and its human ortholog FKHL1 by the daf-2 insulin-like signaling pathway. Curr Biol. 2001; 11:1950-1957.

83. 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; 28:139-145.

84. Ogg S, Paradis S, Gottlieb S, Patterson GI, Lee L, Tissenbaum HA, Ruvkun G. The fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in C. elegans. Nature. 1997; 389:994-999.

85. Ogg S, Ruvkun G. The C. elegans PTEN homolog, DAF-18, acts in the insulin receptor-like metabolic signaling pathway. Mol Cell. 1998; 2:887-893.

86. Tissenbaum HA, Guarente L. Increased dosage of a sir-2 gene extends lifespan in Caenorhabditis elegans. Nature. 2001; 410:227-230.

87. Wang Y, Tissenbaum HA. Overlapping and distinct functions for a Caenorhabditis elegans SIR2 and DAF-16/FOXO. Mech Ageing Dev. 2006; 127:48-56.

88. Hsu AL, Murphy CT, Kenyon C. Regulation of aging and age-related disease by DAF-16 and heat-shock factor. Science. 2003; 300:1142-1145.

89. Boehm M, Slack FJ. A developmental timing microRNA and its target regulate life span in C. elegans. Science. 2005; 310:1954-1957.

90. Wolff S, Ma H, Burch D, Maciel GA, Hunter T, Dillin A. SMK-1, an essential regulator of DAF-16-mediated longevity. Cell. 2006; 124:1039-1053.

91. Kenyon C, Chang J, Gensch E, Rudner A, Tabtiang R. A C. elegans mutant that lives twice as long as wild type. Nature. 1993; 366:461-464.

92. Honda Y, Honda S. The daf-2 gene network for longevity regulates oxidative stress resistance and sm-superoxide dismutase gene expression in Caenorhabditis elegans. FASEB J. 1999; 13:1385-1393.

93. Gems D, Sutton AJ, Sundermeyer ML, Albert PS, King KV, Edgley ML, Larsen PL, Riddle DL. Two pleiotropic classes of daf-2 mutation affect larval arrest, adult behavior, reproduction and longevity in Caenorhabditis elegans. Genetics. 1998; 150:129-155.

94. Melendez A, Tallozcy 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; 301:1387-1391.

95. Friedman DB, Johnson TE. A mutation in the age-1 gene in Caenorhabditis elegans lengthens life and reduces hermaphrodite fertility. Genetics. 1988; 118:75-86.
96. Zhang Y, Xu J, Puscau C, Kim Y, Wang X, Alam H, Hu PJ. Caenorhabditis elegans EAK-3 inhibits dauer arrest via nonautonomous regulation of nuclear DAF-16/FoxO activity. Dev Biol. 2008; 315:290-302.
97. Hu PJ, Xu J, Ruvkun G. Two membrane-associated tyrosine phosphatase homologs potentiate C. elegans AKT-1/PKB signaling. PLoS Genet. 2006; 2:e99.
98. Larsen PL, Albert PS, Riddle DL. Genes that regulate both development and longevity in Caenorhabditis elegans. Genetics. 1995; 139:1567-1583.
99. Berman JR, Kenyon C. Germ-cell loss extends C. elegans life span through regulation of DAF-16 by kri-1 and lipophilic-hormone signaling. Cell. 2006; 124:1055-1068.
100. Galbade T, Hartman PS. Repeated temperature fluctuation extends the life span of Caenorhabditis elegans in adaf-16-dependent fashion. Mech Ageing Dev. 2008; 129:507-514.
101. Brisbin S, Liu J, Boudreau J, Peng J, Evangelista M, Chin-Sang I. A role for C. elegans eph RTK signaling in PTEN regulation. Dev Cell. 2009; 17:459-469.
102. Quevedo C, Kaplan DR, Derry WB. AKT-1 regulates DNA-damage-induced germline apoptosis in C. elegans. 2007; 17:286-292.
103. Hyun M, Lee J, Lee K, May A, Bohr VA, Ahn B. Longevity and resistance to stress correlate with DNA repair capacity in Caenorhabditis elegans. Nucleic Acids Res. 2008; 36:1380-1389.
104. Yanase S, Yasuda K, Ishii N. Adaptive responses to oxidative damage in three mutants of Caenorhabditis elegans (age-1, mev-1 and daf-16) that affect life span. Mech Ageing Dev. 2002; 123:1579-1587.
105. Singh V, Aballay A. Heat-shock transcription factor (HSF)-1 pathway required for Caenorhabditis elegans immunity. Proc Natl Acad Sci U S A. 2006; 103:13092-13097.
106. Houthoofd K, Vanfleteren JR. Public and private mechanisms of life extension in Caenorhabditis elegans. Mol Genet Genomics. 2007; 277:601-617.
107. Murphy CT, McCarrick SA, Bargmann CI, Fraser A, Kamath RS, Ahringer J, Li H, Kenyon C. Genes that act downstream of DAF-16 to influence the lifespan of Caenorhabditis elegans. Nature. 2003; 424:277-283.
108. McElwee J, Bubb K, Thomas JH. Transcriptional outputs of the Caenorhabditis elegans daf-2 forking protein DAF-16. Aging Cell. 2003; 2:111-121.
109. Lee SS, Kennedy S, Tolonen AC, Ruvkun G. DAF-16 target genes that control C. elegans life-span and metabolism. Science. 2003; 300:644-647.
110. Furuyama T, Nakazawa T, Nakano I, Mori N. Identification of the differential distribution patterns of mRNAs and consensus binding sequences for mouse DAF-16 homologues. Biochem J. 2000; 349:629-634.
111. Guarente L. Sir2 links chromatin silencing, metabolism, and aging. Genes Dev. 2000; 14:1021-1026.
112. Imai S, Armstrong CM, Kaeberlein M, Guarente L. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. Nature. 2000; 403:795-800.
113. Smith JS, Brachmann CB, Celic I, Kenna MA, Muhammad S, Starai VJ, Avalos JL, Escalante-Semerena JC, Grubmeyer C, Wolberger C, Boeke JD. A phylogenetically conserved NAD-dependent protein deacetylase activity in the Sir2 protein family. Proc Natl Acad Sci U S A. 2000; 97:6658-6663.
114. Luo J, Nikolaev AY, Imai S, Chen D, Su F, Shiloh A, Guarente L, Gu W. Negative control of p53 by Sir2alpha promotes cell survival under stress. Cell. 2001; 107:137-148.
115. Kruzlewski M, Szumieli I. Sirtuins (histone deacetylases III) in the cellular response to DNA damage—facts and hypotheses. DNA Repair (Amst). 2005; 4:1306-1313.
116. Zhang T, Kraus WL. SIRT1-dependent regulation of chromatin and transcription: Linking NAD(+) metabolism and signaling to the control of cellular functions. Biochim Biophys Acta. 2010; 1804:1666-1675.
117. Berdichevsky A, Guarente L. A stress response pathway involving sirtuins, forkheads and 14-3-3 proteins. Cell Cycle. 2006; 5:2588-2591.
118. Davis RJ. Signal transduction by the JNK group of MAP kinases. Cell. 2000; 103:239-252.
119. Oh SW, Mukhopadhyay A, Srzvikapa 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; 102:4494-4499.
120. Yoon YS, Lee MW, Ryu D, Kim JH, Ma H, Seo WY, Kim YN, Kim SS, Lee CH, Hunter T, Choi CS, Montminy MR, Koo SH. Suppressor of MEK null (SMEK)/protein phosphatase 4 catalytic subunit (PP4C) is a key regulator of hepatic gluconeogenesis. Proc Natl Acad Sci U S A. 2010; 107:17704-17709.
121. Li J, Ebata A, Dong Y, Rizki G, Iwata T, Lee SS. Caenorhabditis elegans HCF-1 functions in longevity maintenance as a DAF-16 regulator. PLoS Biol. 2008; 6:e233.
122. Wysocka J, Herr W. The herpes simplex virus VP16-induced complex: The makings of a regulatory switch. Trends Biochem Sci. 2003; 28:294-304.
123. Wolff S, Dillin A. The trifacta of aging in Caenorhabditis elegans. Exp Gerontol. 2006; 41:894-903.
124. Henderson ST, Johnson TE. Daf-16 integrates developmental and environmental inputs to mediate aging in the nematode Caenorhabditis elegans. Curr Biol. 2001; 11:1975-1980.
125. Arum O, Johnson TE. Reduced expression of the Caenorhabditis elegans p53 orthog cep-1 results in increased longevity. J Gerontol A Biol Sci Med Sci. 2007; 62:951-959.
126. Masse I, Molin L, Mouchiriou L, Vanhems P, Palladino F, Billaud M, Solari F. A novel role for the SMG-1 kinase in lifespan and oxidative stress resistance in Caenorhabditis elegans. 2008; 3:e3354.
127. Weinikove D, Halstead JR, Gems D, Divinec N. Long-term starvation and ageing induce AGE-1/PI 3-kinase-dependent translocation of DAF-16/FOXO to the cytoplasm. BMC Biol. 2006; 4:1.
128. Apfeld J, Kenyon C. Cell nonautonomy of C. elegans daf-2 function in the regulation of diapause and life span. Cell. 1998; 95:199-210.
129. Wolkow CA, Kimura KD, Lee MS, Ruvkun G. Regulation of C. elegans life-span by insulinlike signaling in the nervous system. Science. 2000; 290:147-150.
130. Libina N, Berman JR, Kenyon C. Tissue-specific activities of C. elegans DAF-16 in the regulation of lifespan. Cell. 2003; 115:489-502.
131. Tullet JMA, Hertweck M, An JH, Baker J, Hwang JY, Liu S, Oliveira RP, Baumeister R, Blackwell TK. Cell. 2008; 132:1025-1038.
132. An JH, Vranas K, Lucke M, Inoue H, Hisamoto N, Matsumoto K, Blackwell TK. Regulation of the *Caenorhabditis elegans* oxidative stress defense protein SKN-1 by glycogen synthase kinase-3. Proc Natl Acad Sci U S A. 2005; 102:16275-16280.

133. An JH, Blackwell TK. SKN-1 links *C. elegans* mesodermal specification to a conserved oxidative stress response. Genes Dev. 2003; 17:1882-1893.

134. Park S, Tedesco PM, Johnson TE. Oxidative stress and longevity in *Caenorhabditis elegans* as mediated by SKN-1. Aging Cell. 2009; 8:258-269.

135. Dillin A, Crawford DK, Kenyon C. Timing requirements for insulin/IGF-1 signaling in *C. elegans*. Science. 2002; 298:830-834.

136. de Lencastre A, Pincus Z, Zhou K, Kato M, Lee SS, Slack FJ. MicroRNAs both promote and antagonize longevity in *C. elegans*. Curr Biol. 2010; 20:2159-2168.

137. Pincus Z, Smith-Vikos T, Slack FJ. MicroRNA predictors of longevity in *C. elegans*. PLoS Genet. 2011; In press.

138. Sanchez-Blanco A, Kim SK. Variable pathogenicity determines individual lifespan in *Caenorhabditis elegans*. PLoS Genet. 2011; 7:e1002047.

139. Syntichaki P, Tavernarakis N. Signaling pathways regulating protein synthesis during ageing. Exp Gerontol. 2006; 41:1020-1025.

140. Jia K, Chen D, Riddle DL. The TOR pathway interacts with the insulin signaling pathway to regulate *C. elegans* larval development, metabolism and life span. Development. 2004; 131:3979-3996.

141. 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; 12:1448-1461.

142. Hansen M, Taubert S, Crawford D, Libina N, Lee S, Kenyon C. Lifespan extension by conditions that inhibit translation in *Caenorhabditis elegans*. Aging Cell. 2007; 6:95-110.

143. Vellai T, Takacs-Vellai K, Zhang Y, Kovacs AL, Orosz L, Muller F. Genetics: Influence of TOR kinase on lifespan in *C. elegans*. Nature. 2003; 426:620.

144. Syntichaki P, Troulinaki K, Tavernarakis N. elf4E4E function in somatic cells modulates ageing in *Caenorhabditis elegans*. Nature. 2007; 445:922-926.

145. Hansen M, Chandra A, Mitic LL, Onken B, Driscoll M, Kenyon C. A role for autophagy in the extension of lifespan by dietary restriction in *C. elegans*. PLoS Genet. 2008; 4:e24.

146. Rajawat YS, Hilioti Z, Bossis I. Aging: Central role for mitochondrial mutants of *C. elegans*. Development. 2003; 129:1882-1887.

147. Doonan R, McElwee JJ, Matthijssens F, Walker GA, Oliveira N, Lehrer RA, Testi R. Long-lived mitochondrial mutants as a model for human mitochondrial-associated diseases. Exp Gerontol. 2006; 41:974-991.

148. Cristina D, Cary M, Lunceford A, Clarke C, Kenyon C. A regulated response to impaired respiration slows behavioral rates and increases lifespan in *Caenorhabditis elegans*. PLoS Genet. 2009; 5:e1000450.

149. 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; 177:2063-2074.

150. van Raamsdonk JM, Hekimi S. Deletion of the mitochondrial superoxide dismutase sod-2 extends lifespan in *Caenorhabditis elegans*. PLoS Genet. 2009; 5:e1000361.

151. Cypser JR, Johnson TE. Hormesis in *Caenorhabditis elegans* dauer-defective mutants. Biogerontology. 2003; 4:203-214.

152. 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; 33:40-48.

153. Hamilton B, Dong Y, Shindo M, Liu W, Odell I, Ruvkun G, Lee SS. A systematic RNAi screen for longevity genes in *C. elegans*. Genes Dev. 2005; 19:1544-1555.

154. Dillin A, Hsu A, Arantes-Oliveira N, Lehrer-Graiwer J, Hsin H, Fraser AG, Kamath RS, Ahringer J, Kenyon C. Rates of behavior and aging specified by mitochondrial function during development. Science. 2002; 298:2398-2401.

155. DuMont ME, Cardillo TS, Hayes MK, Sherman F. Role of cytochrome c heme lysase in mitochondrial import and accumulation of cytochrome c in saccharomyces cerevisiae. Mol Cell Biol. 1991; 11:5487-5496.

156. Rea SL, Ventura N, Johnson TE. Relationship between mitochondrial electron transport chain dysfunction, development, and life extension in *Caenorhabditis elegans*. PLoS Biol. 2007; 5:e259.

157. Lakowski B, Hekimi S. Determination of life-span in *Caenorhabditis elegans* by four clock genes. Science. 1996; 272:1010-1013.

158. Miyadera H, Amino H, Hiraishi A, Taka H, Murayama K, Miyoshi H, Sakamoto K, Iishi N, Hekimi S, Kita K. Altered quinone biosynthesis in the long-lived clk-1 mutants of *Caenorhabditis elegans*. J Biol Chem. 2001; 276:7713-7716.

159. Iishi N, Senoo-Matsuda N, Miyake K, Yasuda K, Ishii T, Hartman PS, Furukawa S. Coenzyme Q10 can prolong *C. elegans* lifespan by lowering oxidative stress. Mech Ageing Dev. 2004; 125:41-46.

160. Iishi N, Takahashi K, Tomita S, Keino T, Honda S, Yoshino K, Suzuki K. A methyl viologen-sensitive mutant of the nematode *Caenorhabditis elegans*. Mutat Res. 1990; 237:165-171.

161. Antebi A. Genetics of aging in *Caenorhabditis elegans*. PLoS Genet. 2007; 3:1565-1571.

162. Ventura N, Rea SL, Testi R. Long-lived *C. elegans* mitochondrial mutants as a model for human mitochondrial-associated diseases. Exp Gerontol. 2006; 41:974-991.

163. Cristina D, Cary M, Lunceford A, Clarke C, Kenyon C. A regulated response to impaired respiration slows behavioral rates and increases lifespan in *Caenorhabditis elegans*. PLoS Genet. 2009; 5:e1000450.

164. 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; 177:2063-2074.

165. Doonan R, McElwee JJ, Matthijssens F, Walker GA, Houthoofd K, Back P, Matscheski A, Vanfleteren JR, Gems D. Against the oxidative damage theory of aging: Superoxide dismutases protect against oxidative stress but have little or no effect on life span in *Caenorhabditis elegans*. Genes Dev. 2008; 22:3236-3241.

166. Van Raamsdonk JM, Hekimi S. Deletion of the mitochondrial superoxide dismutase sod-2 extends lifespan in *Caenorhabditis elegans*. PLoS Genet. 2009; 5:e1000361.

167. Cypser JR, Johnson TE. Hormesis in *Caenorhabditis elegans* dauer-defective mutants. Biogerontology. 2003; 4:203-214.

168. Masoro, E.J. (2002). Caloric restriction: A key to longevity in *Caenorhabditis elegans*. Mechanisms of Ageing and Development. 125:41-51.

www.impactaging.com
169. Masoro EJ. Dietary restriction-induced life extension: A broadly based biological phenomenon. Biogerontology. 2006; 7:153-155.

170. Johnson TE, Mitchell DH, Kline S, Kemal R, Foy J. Arresting development arrests aging in the nematode Caenorhabditis elegans. Mech Ageing Dev. 1984; 28:23-40.

171. Kaeberlein TL, Smith ED, Tsuchiya M, Welton KL, Thomas JH, Fields S, Kennedy BK, Kaeberlein M. Lifespan extension in Caenorhabditis elegans by complete removal of food. Aging Cell. 2006; 5:487-494.

172. Lee GD, Wilson MA, Zhu M, Wolkow CA, de Cabo R, Ingram DK, Zou S. Dietary deprivation extends lifespan in Caenorhabditis elegans. Aging Cell. 2006; 5:515-524.

173. Smith ED, Kaeberlein TL, Lydum BT, Sager J, Welton KL, Kennedy BK, Kaeberlein M. Age- and calorie-independent lifespan extension from dietary restriction by bacterial deprivation in Caenorhabditis elegans. BMC Dev Biol. 2008; 8:49.

174. Houthooft K, Braeckman BP, Lenaerts I, Brys K, De Vreeze A, Van Eygen S, Vanfleteren JR. Axenic growth up-regulates mass-specific metabolic rate, stress resistance, and extends lifespan in Caenorhabditis elegans. Exp Gerontol. 2002; 37:1371-1378.

175. Croll NA, Smith JM, Zuckerman BM. The aging process of the nematode Caenorhabditis elegans in bacterial and axenic culture. Exp Aging Res. 1977; 3:175-189.

176. Greer EL, Dowlatshahi D, Banko MR, Villen J, Hoang K, Blanchard D, Gygi SP, Brunet A. An AMPK-FOXO pathway mediates longevity induced by a novel method of dietary restriction in C. elegans. Curr Biol. 2007; 17:1646-1656.

177. Lakowski B, Hekimi S. The genetics of caloric restriction in Caenorhabditis elegans. Proc Natl Acad Sci U S A. 1998; 95:13091-13096.

178. Buecher EJ, Hansen E, Yarwood EA. Ficoll activation of a protein essential for maturation of the free-living nematode caenorhabditis briggsae. Proc Soc Exp Biol Med. 1966; 121:390-393.

179. Houthooft K, Braeckman BP, Lenaerts I, Brys K, De Vreeze A, Van Eygen S, Vanfleteren JR. No reduction of metabolic rate in food restricted Caenorhabditis elegans. Exp Gerontol. 2002; 37:1359-1369.

180. Meissner B, Boll M, Daniel H, Baumeister R. Deletion of the intestinal peptide transporter affects insulin and TOR signaling in Caenorhabditis elegans. J Biol Chem. 2004; 279:36739-36745.

181. Houthooft K, Gems D, Johnson TE, Vanfleteren JR. Dietary restriction in the nematode Caenorhabditis elegans. Interdiscip Top Gerontol. 2007; 35:98-114.

182. Bishop NA, Guarante L. Two neurons mediate diet-restriction-induced longevity in C. elegans. Nature. 2007; 447:545-549.

183. Bargmann CI, Horvitz HR. Chemosensory neurons with overlapping functions direct chemotaxis to multiple chemicals in C. elegans. Neuron. 1991; 7:729-742.

184. Yen K, Patel HB, Lublin AL, Mobbs CV. SOD isoforms play no role in lifespan in ad lib or dietary restricted conditions, but mutational inactivation of SOD-1 reduces life extension by cold. Mech Ageing Dev. 2009; 130:173-178.

185. Clancy DJ, Gems D, Hafen E, Leevers SJ, Partridge L. Dietary restriction in long-lived dwarf flies. Science. 2002; 296:319.

186. Lambert A, Merry B. Effect of caloric restriction on mitochondrial reactive oxygen species production and bioenergetics: Reversal by insulin. Am J Physiol Regul Integr Comp Physiol. 2004; 286:R71-9.

187. Masoro EJ. Overview of caloric restriction and ageing. Mech Ageing Dev. 2005; 126:913-922.

188. Harman D. Free radical theory of aging: An update: Increasing the functional lifespan. Ann N Y Acad Sci. 2006; 1067:10-21.

189. Harman D. Aging: A theory based on free radical and radiation chemistry. J Gerontol. 1956; 11:298-300.

190. Rattan S. Increased molecular damage and heterogeneity as the basis of aging. Biol Chem. 2008; 389:267-272.

191. Williams GC. Pleiotropy, natural selection, and the evolution of senescence. Evolution. 1957; 11:398-411.

192. Kirkwood TB, Austad SN. Why do we age? Nature. 2000; 408:233-238.

193. Krtolica A, Parrinello S, Lockett S, Desprez PY, Campisi J. Senescent fibroblasts promote epithelial cell growth and tumorigenesis: A link between cancer and aging. Proc Natl Acad Sci U S A. 2001; 98:12072-12077.

194. Holowacz T, Zeng L, Lassar AB. Asymmetric localization of numb in the chick somite and the influence of myogenic signals. Dev Dyn. 2006; 235:633-645.

195. Brack AS, Conboy MJ, Roy S, Lee M, Kuo CJ, Keller C, Rando TA. Increased wnt signaling during aging alters muscle stem cell fate and increases fibrosis. Science. 2007; 317:807-810.

196. Budovskaya YV, Wu K, Southworth LK, Jiang M, Tedesco P, Johnson TE, Kim SK. An elt-3/elt-5/elt-6 GATA transcription circuit guides aging in C. elegans. Cell. 2008; 134:291-303.

197. Gilleard JS, Shafi Y, Barry JD, McGhee JD. ELT-3: A Caenorhabditis elegans GATA factor expressed in the embryonic epidermis during morphogenesis. 1999; 208:265-280.

198. Pincus Z, Slack FJ. Transcriptional (dys)regulation and aging in Caenorhabditis elegans. Genome Biol. 2008; 9:233.

199. Koh K, Rothman JH. ELT-5 and ELT-6 are required continuously to regulate epidermal seam cell differentiation and cell fusion in C. elegans. Development. 2001; 128:2867-2880.

200. Kirkwood TB, Holliday R. The evolution of ageing and longevity. Proc R Soc Lond B Biol Sci. 1979; 205:531-546.

201. Houthooft K, Vanfleteren JR. The longevity effect of dietary restriction in Caenorhabditis elegans. 2006; 41:1026-1031.

202. Kirkwood TB, Shanley DP. Food restriction, evolution and aging. Mech Ageing Dev. 2005; 126:1011-1016.

203. Jenkins NL, McColl G, Lithgow GJ. Fitness cost of extended lifespan in Caenorhabditis elegans. Proc Biol Sci. 2004; 271:2523-2526.

204. Walker DW, McColl G, Jenkins NL, Harris J, Lithgow GJ. Evolution of lifespan in C. elegans. Nature. 2000; 405:296-297.

205. Friedman DB, Johnson TE. Three mutants that extend both mean and maximum life span of the nematode, Caenorhabditis elegans, define the age-1 gene. J Gerontol. 1988; 43:B102-9.

206. Brooks KK, Liang B, Watts JL. The influence of bacterial diet on fat storage in C. elegans. PLoS One. 2009; 4:e7545.

207. Gems D, Riddle DL. Longevity in Caenorhabditis elegans reduced by mating but not gamete production. Nature. 1996; 379:723-725.

208. Yamawaki TM, Arantes-Oliveira N, Berman JR, Zhang P, Kenyon C. Distinct activities of the germline and somatic reproductive tissues in the regulation of Caenorhabditis elegans' longevity. Genetics. 2008; 178:513-526.
209. Ghazi A, Henis-Korenblit S, Kenyon C. A transcription elongation factor that links signals from the reproductive system to lifespan extension in *Caenorhabditis elegans*. PLoS Genet. 2009; 5:e1000639.

210. Crawford D, Libina N, Kenyon C. *Caenorhabditis elegans* integrates food and reproductive signals in lifespan determination. Aging Cell. 2007; 6:715-721.

211. Walker GA, Thompson FJ, Brawley A, Scanlon T, Devaney E. Heat shock factor functions at the convergence of the stress response and developmental pathways in *Caenorhabditis elegans*. 2003; 17:1960-1962.

212. Zhang M, Poplawski M, Yen K, Cheng H, Bloss E, Zhu X, Patel H, Mobbs CV. Role of CBP and SATB-1 in aging, dietary restriction, and insulin-like signaling. PLoS Biol. 2009; 7:e1000245.

213. Petriv OI, Rachubinski RA. Lack of peroxisomal catalase causes a progeric phenotype in *Caenorhabditis elegans*. J Biol Chem. 2004; 279:19996-20001.

214. Swain SC, Keusekotten K, Baumeister R, Sturzenbaum SR. *C. elegans* metallothioneins: New insights into the phenotypic effects of cadmium toxicosis. J Mol Biol. 2004; 341:951-959.

215. TeKippe M, Aballay A. *C. elegans* germline-deficient mutants respond to pathogen infection using shared and distinct mechanisms. PLoS One. 2010; 5:e11777.