Review

Genetics of Aging in *Caenorhabditis elegans*

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ABSTRACT

A dissection of longevity in *Caenorhabditis elegans* reveals that animal life span is influenced by genes, environment, and stochastic factors. From molecules to physiology, a remarkable degree of evolutionary conservation is seen.

Introduction

Over the last 20 years, fundamental insights into the biology of aging have emerged through the study of model genetic organisms, where undoubtedly the tiny nematode *C. elegans* has led the way. Importantly, the discovery that the single gene mutants *age-1* and *daf-2* could extend the short three-week life span 1–2-fold revealed that longevity is under genetic control [1,2]. Long life was shown to depend on another locus, *daf-16*, defining an epistasis pathway for this process. Moreover, the molecular identification of all three as components of insulin/IGF-I signaling (IIS) [3–6] eventually led to the striking realization that a modest downregulation of IIS promotes stress resistance and longevity across taxa [7–10].

Molecular genetic studies suggest that in response to insulin-like peptides (ILPs) [11,12], activation of the DAF-2/insulin/IGF-1 receptor tyrosine kinase triggers a PI3/AKT/SGK kinase cascade that phosphorylates the DAF-16/FOXO transcription factor [13–16] (Figure 1). Consequently, DAF-16/FOXO is retained cytoplasmically and animals live normal life spans. In response to increased DAF-18/PTEN activity [14,17], stress (heat, oxidative, starvation) or reduced IIS, DAF-16/FOXO enters the nucleus [18–20], where it turns on survival genes, including those that manage oxidative stress, heat shock, innate immunity, metabolism, autophagy, and xenobiotic response, among others [21–25]. Indeed, over- or underexpression of such targets often impacts stress resistance and longevity [22,26]. Many of these points and more have been reviewed elsewhere [27,28].

Importantly, IIS is best understood as a signaling pathway selected in evolution to regulate organismal survival, not aging per se. Indeed, during larval development, DAF-16/FOXO specifies the dauer diapause, a long-lived stress-resistant larval stage specialized for survival, but can independently trigger longevity in adults [29]. Evidently, the primary role of IIS is to invest in somatic endurance to outlast hard times, and conversely specify growth and reproduction in good times, with secondary consequences on adult life span. Consistent with this, many longevity mutants have reduced Darwinian fitness under conditions favoring short-term rapid reproductive output [30].

Since the elucidation of this core pathway, scores of longevity genes have emerged from candidate approaches, unbiased genome-wide RNAi screens, and transcriptional profiles, some working through IIS, others not [22,31–35]. Because they cannot all be enumerated here, we have taken an admittedly "transduction-centric" view to highlight new developments. We start with upstream signaling events of sensory perception, walk through new signaling inputs, focus on transcriptional factors, and then discuss broad physiological processes (respiration, translation, checkpoint control, diet restriction), which may impact longevity through hormesis, the induction of enhanced robustness and survival by exposure to low levels of stress [36].

Sensory Input: The Senseless Liveth

Critical for making preemptive decisions that prepare the animal for times ahead, sensory perception can also dramatically influence life span. Mutants deficient in sensory neuron structure and function have extended longevity, which is largely but not entirely *daf-16* dependent [37]. Moreover, such mutants cause DAF-16/FOXO to relocate to the nucleus [19]. Components of sensory cell signal transduction, including G-protein coupled receptors, G-proteins, cGMP channel subunits, ciliary proteins, Tubby, and proteins implicated in synaptic transmission [37–42] are thought to regulate release of ILPs from sensory cells, thereby influencing organismal physiology. Cell ablation studies show that both gustatory and olfactory neurons contribute, with specific neurons promoting longevity and others suppressing it [38]. The exact sensory cues governing ILP synthesis and release are likely to include nutrients, and various repellants or attractants, but this area remains largely unexplored. Related findings have recently emerged in *Drosophila*, in which *or83b* mutants, broadly deficient in olfaction, live long [43]. Moreover, odorants from yeast paste reduce longevity induced under conditions of dietary restriction. Thus, perception as well as ingestion may impact animal life span in diverse taxa.

In addition to sensory cues, serotonergic inputs may work upstream of IIS. Notably, mutants in the serotonergic receptor *ser-1* are longer lived, in a *daf-16*/FOXO-dependent manner [44]. Surprisingly, mutants in *ser-4*, another serotonin receptor, are somewhat short lived, suggesting the receptors could act antagonistically, similar to the cells described above. Moreover, mutants deficient in serotonin production, such as *tph-1*, are stress resistant, but not long lived [44–46]; opposing effects of the receptors might explain this phenomenon.
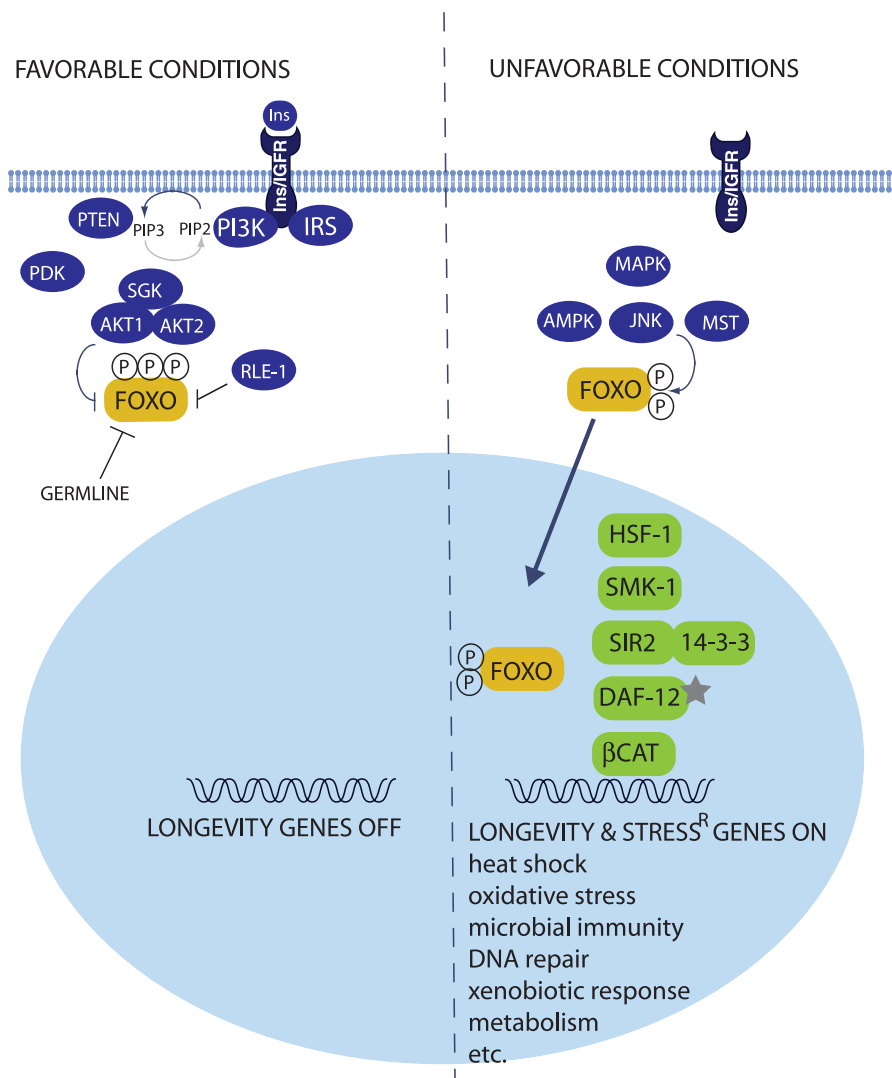
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FAVORABLE CONDITIONS

UNFAVORABLE CONDITIONS

GERMLINE

LONGEVITY GENES OFF

LONGEVITY & STRESS^R GENES ON
heat shock
oxidative stress
microbial immunity
DNA repair
xenobiotic response
metabolism
etc.

Figure 1. A Model for Insulin/IGF-1 Signal Transduction, Showing Associated Kinase Inputs and Nuclear Factors (See Text)

Because serotonergic signaling is phyletically ancient, it may have a conserved physiologic role in life span regulation, and be amenable to pharmacologic intervention.

Signal Transduction: Wiring Longevity

Aside from IIS, several other transduction pathways appear to impinge upon DAF-16/FOXO. Whereas the IIS kinase inputs described above inhibit DAF-16/FOXO, several kinases described below stimulate it (Figure 1). JUN kinase, a mediator of the stress response, results in life span extension, increased resistance to oxidative stress, and DAF-16/FOXO nuclear localization upon overexpression [47]. Longevity is *daf-16*/FOXO dependent, and FOXO is a JNK substrate. Because JNK overexpression further increases *daf-2*/IIR mutant longevity, it may work in parallel to PI3/AKT. Importantly, this role is evolutionarily conserved. In *D. melanogaster*, JNK activation specifically within insulin-producing neurons results in nuclear localization of dFOXO, suppression of insulin production, and extension of life span [48,49]. Intriguingly, this suggests that the organismal stress response can be coordinated centrally. MST is a *ste20*-like

kinase implicated in growth control. Like JNK-1, overexpression of the *C. elegans* homolog slows aging, extends life span in a *daf-16*-dependent manner, and further extends longevity of *daf-2*/RNAi treated animals [50]. Moreover, loss of function reduces *daf-2* longevity. Although JNK and MST phosphorylate different residues, it is unclear if they identify parallel pathways or the same pathway. The mammalian MST1 also activates FOXO, but instead stimulates apoptosis in primary neurons in response to oxidative stress, i.e., decreasing cellular survival [50]. Conceivably, the mammalian response may be tissue specific. The p38 MAP kinase pathway, implicated in the *C. elegans* stress response and innate immunity as well as RAS signaling, is required in part for *daf-2* longevity [51,52]. Finally, mutants of AMP-activated protein kinase (AMPK), a fuel sensor sensitive to AMP/ATP ratios, suppress *daf-2* longevity, while overexpression modestly extends life [53]. How these and other kinases interact to control FOXO activity is not well understood, and it will be fascinating to deduce the kinase inputs, decipher the signaling specificity, and elucidate the covalent code modulating FOXO activity.

Aside from phosphorylation, FOXO is also covalently modified by ubiquitination and acetylation (below). Recently, an evolutionarily conserved E3 ubiquitin ligase, called RLE-1, has been shown to ubiquitinate DAF-16/FOXO, leading to its destabilization [54]. Conversely, RLE-1 loss results in increased levels of FOXO protein, sustained nuclear localization, stress resistance, and longevity. As expected, the longevity of *rle-1* mutants is *daf-16* dependent. RLE-1 may be one of several ubiquitin ligases that controls FOXO stability.

Transcriptional Control: The Master Regulators of Survival

As the discussion above reveals, DAF-16/FOXO is a master regulator of survival that integrates multiple inputs. Likewise, several different transcription factors/cofactors work in concert with DAF-16/FOXO to modulate its output (Figure 1). The conserved nuclear factor, SMK-1, is responsible for mediating specific aspects of FOXO biology, namely IIS and germline longevity and pathogen, UV, and oxidative-stress resistance, but not heat resistance and dauer formation [55]. Conceivably, SMK-1 works as a coregulator or cofactor for DAF-16/FOXO, since it modulates its transcriptional output. Moreover, SMK-1 harbors LXXLL motifs typical of transcriptional coactivators, but its exact molecular activity remains to be determined. In response to thermal stress, HSF-1, a heat shock transcription factor, induces various heat shock chaperones, which clear misfolded proteins and protect cells. Loss of activity accelerates proteotoxicity and tissue aging, while overexpression increases heat resistance and extends life span in a *daf-16(+)*-dependent manner. Moreover, *hsf-1* is required for *daf-2* longevity [56,57] as well as the innate immune response, broadening the spectrum of its age-related functions [58].

Best known for its developmental role in *wnt* signal transduction, β-catenin also guards cells against oxidative stress. Mutants in the *C. elegans* homolog, *bar-1*, are more susceptible to oxidative stress and are short lived [59]. Interestingly, BAR-1 physically associates with DAF-16/FOXO, as do the mammalian counterparts. Moreover, β-catenin enhances superoxide dismutase expression in both systems. However, whether *bar-1* or other components of *wnt* signaling mediate *daf-2* longevity has not been directly tested.

Sirtuins are NAD+–dependent protein deacetylases whose increased dose augments longevity in yeast, worms, and flies [60–62]. In mammals, sirtuins deacetylate a number of targets, including p53, PGC-1alpha, and FOXO, to name a few [63–67], thereby modulating their transcriptional activity. Notably, extra copies of *C. elegans sir-2.1* enhance longevity and stress resistance, in a manner dependent on *daf-16*/FOXO and 14-3-3 scaffold proteins. Evidence suggests SIR-2.1 may work in parallel to IIS [61,68,69]. Indeed, in response to stress (but not reduced IIS), SIR-2.1,14-3-3 protein and DAF-16/FOXO form a nuclear complex that activates FOXO transcriptional targets such as *sod-3*.

SIR-2.1 may also have FOXO-independent outputs. Sirtuins are stimulated by polyphenolics such as resveratrol [70], one of the salutary compounds found in red wine. Interestingly, resveratrol extends the life span of yeast, worms, flies, and perhaps mice, in a sirtuin-dependent manner [71,72]. In *C. elegans*, resveratrol induces *abu-11* and other components of the endoplasmic reticulum (ER) stress response [73]. The ER stress response is stimulated as a result of misfolded or misglycosylated proteins in the secretory pathway. ABU-11 and related molecules are transmembrane proteins similar to scavenger receptors, and are proposed to target secretory components for lysosomal degradation [74]. Consistent with a rate-limiting role, *abu-11 RNAi* abrogates resveratrol-mediated longevity and overexpression extends life, while, surprisingly, *daf-16*/FOXO has little effect.

Nuclear hormone receptors (NHR) transcription factors regulate gene expression in response to lipophilic hormones, and are well poised to coordinate organismal physiology. *C. elegans* DAF-12/NHR, a relative of vertebrate vitamin D and LXR receptors, promotes longevity in several contexts. One is in the germline longevity pathway. When germline stem cells are removed, either by mutation or by laser microsurgery, animals live 50%–60% longer than wild type [75,76]. This is not due simply to sterility, since additional removal of somatic gonadal support cells produces infertile animals with normal life span. Conceivably, germline and somatic gonad produce opposing signals that coordinate organismal maturation, with secondary consequences on aging. Although distinct from IIS, germline longevity depends on functional DAF-16/FOXO as well as DAF-12/NHR [76]. Moreover, FOXO enters intestinal nuclei upon germline removal, indicating a signaling event [19]. Notably, mutants deficient in biosynthesis of DAF-12 ligands, as well as the conserved gene *kri-1*, also abolish germline longevity, and diminish FOXO nuclear accumulation [77–80]. Endogenous DAF-12 ligands include 3-keto bile acid-like steroids, called dafachronic acids [81], as well as the structurally related 25S-cholestenoic acid [82], which also serves as ligand for the mammalian homolog LXR [83]. As predicted from the genetics, ligand supplementation of hormone biosynthetic mutants restores DAF-16/FOXO nuclear localization and longevity of germlineless animals [80]. Recently, the steroid pregnenolone has been reportedly found in higher concentrations in germline-less animals compared to wild type [84]. Moreover, exogenous pregnenolone enhances longevity in a *daf-12(+)*-dependent fashion. However, pregnenolone is not a DAF-12/NHR ligand, suggesting an indirect effect. DAF-12 is also required for longevity in the dauer pathways. Biosynthetic mutants devoid of dafachronic acids, such as *daf-9*/cytochrome P450, manifest adult longevity and stress resistance that is *daf-12(+)* dependent [79,85,86]. Moreover, hormone supplementation restores normal life span and stress resistance [80]. Altogether, these findings provide crucial evidence for bile acid–like steroids modulating animal life span.

MicroRNAs: Timing Long Life?

The heterochronic loci control *C. elegans* larval developmental timing, specifying stage specific programs and the life plan, but curiously, a handful also impact adult life span. *lin-4* encodes an evolutionarily conserved microRNA, which downregulates the nuclear factor LIN-14 via translational inhibition of the 3′ UTR [87,88]. These genes work in tandem in a timing switch that advances early larval stage programs. With respect to adult longevity, *lin-4* mutants are short lived, while *lin-14* mutants are long lived, suggesting that *lin-4(+)* retards aging through downregulation of *lin-14(+)* [89]. Interestingly, longevity is also *daf-16*/FOXO dependent. Conceivably, long life arises from perturbations of a timer in

the adult. Alternatively, *lin-4/lin-14* may regulate IIS directly, since the insulin-like gene, *ins-33*, is a bona fide LIN-14 transcriptional target [90]. Intriguingly, numerous microRNAs undergo distinct changes in expression pattern during adult life, including those predicted to target known regulators of survival [91]. Perhaps they too function in adult aging.

Checkpoint Control

A critical facet of cellular function is the response to DNA damage, genotoxic stress, and other insults. Aging in higher animals may be influenced by the balance of cell survival versus death, a decision often governed by checkpoint proteins in dividing cells. However, adult *C. elegans* animals lack dividing cells except for the germline. Surprisingly, then, deficiencies in checkpoint control—including *cid-1* poly(A+) polymerase, *chk-1* kinase, *cdc-25* phosphatase, as well as *clk-2/* RAD5—result in longer adult life but reduced broods [92,93]. Moreover, checkpoint-deficient animals are typically thermotolerant and upregulate *sod-3/*MnSOD (in intestine) and the ER resident *hsp-4/*BiP (in vulva), showing molecular induction of a stress response. However, *chk-1 RNAi* longevity is *daf-16/*FOXO independent, and therefore likely works through another transcriptional regulator. A good candidate is *cep-1*, whose mammalian counterpart, p53, works downstream of *chk1*. In mammals, p53 loss increases tumorigenesis, while specific gain-of-function alleles reduce tumor incidence but accelerate aging, suggesting a trade-off between tumor surveillance and stem cell maintenance [94]. Intriguingly then, disabling checkpoint function in *C. elegans* mitotic germline cells or postmitotic somatic cells triggers states of enhanced organismal survival. Modulating checkpoints so that benefits are not outweighed by detriments remains a future challenge.

Mitochondrial-Induced Longevity

Mitochondrial function is central to cellular metabolism and apoptosis, while dysfunction causes numerous age-associated diseases, including diabetes, cardiomyopathy, and neurodegeneration. Surprisingly, then, a major class of *C. elegans* longevity mutants are deficient in mitochondrial function. The first described include *clk-1*, which is defective in ubiquinone biosynthesis, and *isp-1*, which lacks a Rieske FeS protein of complex III [93,95,96]. Genome-wide RNAi screens identified many other mitochondrial components, all of which extend life independent of *daf-16* and *daf-2* [33,34]. These mutants display increased developmental times, slowed behavior, and smaller broods. As expected, most diminish respiration, although it is controversial whether *clk-1* does or not [97,98]. Evidently, levels of mitochondrial gene activity must be optimized, since severe loss of function results in lethality or shortevity [99]. How might disabling mitochondria provoke extended survival? Conceivably, it produces a lower rate of living, and consequently decreased production of reactive oxygen species (ROS) [96]. Alternately, perturbation of electron transport may actually increase ROS, provoking a hormetic adaptive response [99]. Or it may stimulate mitochondrial turnover and thrifty metabolism. In any case, longevity likely depends in part on signaling, since molecules such as *aak-2/* AMPK are required [100]. Can this example illuminate aging in higher organisms? Perhaps yes, since heterozygous

knockouts of the mouse *clk-1* are longer lived, suggesting that partial loss of function can confer benefits [101].

Dietary Restriction–Induced Longevity

Dietary restriction (DR), the reduction of dietary intake without malnutrition, extends life span from yeast to rodents, and likely involves evolutionarily conserved mechanisms. In *C. elegans*, DR is induced by various regimens, including limiting the amount of bacterial food in liquid culture [102], liquid growth in a defined synthetic medium [103], or limiting dietary intake with feeding mutants such as *eat-2* [104]. Even shifting adults onto agar plates without bacterial food, but with residual nutrients from peptone and agar, robustly extends life [105,106]. Whether these regimens all pinpoint the same process is still unclear, and each one has its own merits and caveats.

Recently, some exciting progress has been made in identifying genes mediating DR. Namely, PHA-4/FOXA1 and SKN-1/NRF transcription factors have been shown to be required for DR induced longevity [107,108]. When these genes are inactivated in the adult, animals are no longer long lived under reduced dietary intake. The effect is specific, since these mutants have little effect on *daf-2/*IIR longevity; moreover, *daf-16/*FOXO mutants are still susceptible to DR. SKN-1 has an early role in pharynx and gut specification, and a later role in the response to oxidative stress in the gut [109,110]. Interestingly, however, SKN-1 activity specifically in two neurosensory cells, called the ASIs, mediates DR-induced longevity [108], implying sensory inputs into DR. Nonetheless, mutants deficient in sensory transduction still respond to DR. By inference, neuronal SKN-1 must regulate the organismal response to DR through a hormonal mechanism. Accordingly, DR globally stimulates respiration, presumably to maximize organismal energy efficiency. Similarly, PHA-4/FOXA1 has an early role in endo/mesoderm specification in worms and mammals, but also regulates mammalian glucose homeostasis later in life [111,112]. *C. elegans* PHA-4 is expressed in the adult gut, gonad, and nervous system, yet influences life span of the entire organism, also implying endocrine control. Consistent with this, the mammalian FOXA regulates pancreatic glucagon production [112]. Dissecting their respective signaling pathways promises to further open up DR to a molecular analysis.

Sirtuins and related molecules have been implicated as potential mediators of DR in yeast and flies, although this remains controversial [62,113,114]. Conflicting reports also leave this issue unresolved in worms [115,116]. Discrepancies might arise because of unknown differences in culture conditions. Evidence also suggests that TOR (target of rapamycin) kinase mediates DR in flies and yeast [117,118]. TOR kinase promotes growth and protein synthesis, while its reduction dampens translation and increases recycling of cellular components through autophagy. In worms, a reduction of TOR, the downstream S6 kinase, ribosomal initiation factors, as well as ribosomal protein subunits themselves, reduce translation and extend adult life span [115,119–123]. Consistent with a role in DR, TOR longevity is not further extended in *eat-2* mutants. Surprisingly, however, downstream components do extend life span beyond *eat-2*, suggesting that TOR outputs independent of translation could be critical for DR. Interestingly, deletion of ribosomal

subunits in *S. cerevisiae* also extends replicative life span [118]. It will be critical to understand whether longevity arises from benefits due to globally reduced translation itself or from the regulation of specific factors.

Aging and Age-Related Disease

With all these longevity genes, a critical question is how do worms age, and from what do they die? With age, there is a progressive decline in body movement and pharyngeal pumping [124]. Most striking, muscle integrity deteriorates dramatically [125], with derangement of muscle fibers and overt changes in nuclear morphology—phenotypes reminiscent of sarcopenia, a major contributor of age-related decline in people. Surprisingly, the worm nervous system appears resilient, with little obvious change in structure or reporter expression, although function has not been critically tested. Among other things, aging worms show an increase in the appearance of necrotic cells, crosslinked cuticle, lipid droplets [126], endomitotic DNA synthesis in the germline [127], AMP/ATP ratios [53], oxidized protein [128], and lipofuscin [129]. Worms are described to die of enteric bacterial infection, and antibiotics extend life [126]. Conceivably, infection may be secondary to a decline in enteric muscle function, which is necessary to expel bacteria.

Although genotype determines the mean life span of a population, individual longevity has a large stochastic component, with several-fold differences observed even with an isogenic population in a uniform environment. Nonetheless, specific markers serve as good predictors of individual life expectancy. For example, stochastic induction of a *hsp-16::gfp* reporter predicts survival, while premature appearance of lipofuscin predicts early death [129,130]. Significantly, long-lived genotypes also delay the onset of aging and age-related disease, e.g., *daf-2* mutants curtail many of the age-dependent changes described above, and ameliorate models of polyQ repeat protein disease, Aβ-proteotoxicity, and germline tumorigenesis [56,131,132]. Conversely, *daf-16* or *hsf-1* mutants often accelerate pathology. Conceivably, multiple age-related diseases could be mitigated at once by targeting IIS or other longevity pathways.

Conclusions

Animal life span is unexpectedly plastic, reflecting regulatory pathways responsive to environmental signals such as nutrients and stress. The future challenge will be to determine how these different pathways map onto and interact with each other, and decipher their molecular mechanisms. For some, basic questions such as where and when are they required, and whether they work cell autonomously or nonautonomously need to be addressed. Another major challenge will be to clarify how global processes (e.g., DR, mitochondrial longevity, checkpoint control, translation, and others) impact aging. What tradeoffs do they entail? Do they invoke signaling events or do they passively divert resources to somatic maintenance? Finally, what are the fundamental causes of aging and how can they be offset? Is it altered protein metabolism, organellar turnover, immune function, metabolic efficiency, ROS or xenobiotic detoxification, genome stability, or all of the above? Answers

to these and other questions will be key to understanding broadly conserved aspects of life span determination. ∎

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