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Lessons from *C. elegans:* Signaling pathways for longevity

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Abstract

Recent research using model organisms such as the nematode *Caenorhabditis elegans* has highlighted a critical role for several conserved signaling pathways in longevity determination. Here, we review three major endocrine- and nutrient-sensing signaling pathways with influence on lifespan, the insulin/insulin-like growth factor (IGF), target of rapamycin (TOR), and germline signaling pathways. Although these pathways engage distinct sets of transcription factors, the three pathways appear to modulate aging in *C. elegans* through partially overlapping effector mechanisms, including lipid metabolism and autophagy. This review highlights the latest advances in our understanding of how the insulin/IGF-1, TOR, and germline signaling pathways utilize different transcription factors to modulate aging in *C. elegans* with special emphasis on the role of lipid metabolism and autophagy.

Keywords

insulin/IGF-1; TOR; germline; autophagy; lipid metabolism; aging

Aging research in *C. elegans*

Studies in *C. elegans* have provided a wealth of information about the molecular mechanisms that modulate aging. *C. elegans* possesses a number of traits that make it an ideal model organism for aging research; 2–3 week lifespan, conserved developmental programs, transparency, small size, genetic tractability, and a fully sequenced genome [1]. Moreover, gene inactivation by RNAi has been extremely useful in identifying many new longevity genes [2], and has promoted a rapidly growing body of work that highlight multiple evolutionary conserved longevity paradigms. These include the insulin/IGF-1, TOR, and germline signaling pathways, which control many critical biological processes, including development, reproduction, metabolism, somatic maintenance, and stress resistance. Here, we review the most recent literature describing the molecular mechanisms by which these three pathways modulate *C. elegans* aging.

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Insulin/IGF-1 signaling

The basics

Research on the genetics of aging in *C. elegans* began with the seminal finding that the organism's lifespan was doubled by mutations in the *age-1* or *daf-2* genes, orthologs of the mammalian phosphoinositide 3-kinase (PI3K) and insulin/IGF-1 receptor (InR), respectively [3,4]. Both genes play key roles in insulin/IGF-1 signaling, and several additional pathway components have since been shown to modulate aging in flies, mice, and possibly humans, implying that the effects of the pathway on aging are conserved (reviewed in [5]).

Another important implication of these pioneering studies was that aging can be modulated by peptide hormones [6]. *C. elegans* expresses 40 insulin-like peptides [7], of which at least two are agonists (DAF-28 and INS-7) [8,9,10] and at least one functions as an antagonist (INS-1) of DAF-2/InR [7]. Ligand binding to DAF-2/InR activates its tyrosine kinase activity and initiates a cascade of sequential phosphorylation events that activate several kinases: AGE-1/PI3K, pyruvate dehydrogenase lipoamide kinase isozyme (PDK)-1, AKT-1/2, and serine/threonine-protein kinase (SGK)-1 (Figure 1A). Ultimately, AKT and SGK-1 phosphorylate and inactivate the FOXO transcription factor DAF-16 by preventing its translocation to the nucleus, thereby blocking transcription of target genes [11].

As expected, reduced insulin/IGF-1 signaling (e.g., mutations in *daf-2*/InR) relieve this block and allow entry of DAF-16/FOXO into the nucleus, where it induces the expression of genes that increase lifespan and promote resistance to various stresses ([12], see below). Several additional mechanisms promote DAF-16/FOXO nuclear localization (Figure 1A). DAF-18/PTEN dephosphorylates and inhibits AGE-1/PI3K, thereby preventing DAF-16/FOXO phosphorylation by downstream kinases [13]. Nuclear translocation of DAF-16/FOXO can also be stimulated by alternate phosphorylation, for example via the kinase JNK-1 [14]. Nuclear maintenance of DAF-16/FOXO is promoted by PRMT-1, an arginine methyltransferease [15]. The stability of DAF-16/FOXO is also regulated by RLE-1, an E3 ubiquitin ligase that catalyzes ubiquitination of DAF-16/FOXO and targets it for degradation [16]. Collectively, these factors modulate DAF-16/FOXO activity to ensure the appropriate transcriptional response to specific environmental and hormonal cues (Figure 1A).

DAF-16 transcriptional coregulators

Once in the nucleus, several proteins function together with DAF-16/FOXO to modulate its activity in response to reduced insulin/IGF-1 signaling. Coregulators such as suppressor of MEK (SMK-1/SMEK) and host cell factor-1 (HCF-1) are critical for DAF-16/FOXO–mediated protection against microbial infection, DNA damage, and oxidative stress [17], whereas other transcription factors, such as heat-shock factor-1 (HSF-1), guide DAF-16/FOXO activity and cooperatively induce transcription of subsets of target genes, including heat-shock proteins involved in proteostasis [18]. The transcription factor SKiNhead (SKN)-1 (Nrf) regulates resistance to oxidative stress and expression of detoxification genes in response to reduced insulin/IGF-1 signaling, but extends lifespan independently of DAF-16/FOXO [19]. In *daf-2*/InR animals, HSF-1, SMK-1/SMEK and SKN-1/Nrf become nuclear localized independently of DAF-16/FOXO. Instead, HSF-1 activity is regulated via a repression complex [20], SMK-1/SMEK-1 is constitutively nuclear [17] and SKN-1/Nrf subcellular localization is controlled by phosphorylation via the p38 MAPK pathway [21,22]. In sum, multiple nuclear factors and parallel signaling pathways converge on DAF-16/FOXO to modulate longevity in long-lived insulin/IGF-1 pathway mutants (Figure 1A).

DAF-16 target genes

Gene expression profiling of *daf-2*/InR mutants has identified hundreds of DAF-16/FOXO–regulated genes involved in various cellular processes including metabolism, proteostasis, and stress responses such as oxidative stress, detoxification, and immunity [23,24,25,26]. Examples of genes with elevated expression in *daf-2*/InR mutants are members of the heat shock *hsp-16* protein family, cytochrome P450 enzymes, glutathione-S-transferases, catalases, and the superoxide dismutase *sod-3* (reviewed in [12]). In contrast, a number of genes show reduced expression in *daf-2*/InR mutants, and these include the insulin-like peptide *ins-7* and the vitellogenin yolk proteins [23]. However, although DAF-16/FOXO directly regulates the expression of many of these genes [27], RNAi-mediated inhibition of any single target only modestly reduce the long lifespan of *daf-2*/InR mutants, suggesting that DAF-16/FOXO–dependent longevity likely requires the expression of multiple target genes [23].

Tissue specificity and endocrine signaling

Insulin/IGF-1 signaling in specific tissues in the worm, primarily neuronal and intestinal cells, is critical for the pathway to affect longevity systemically (reviewed in [11]) (Figure 1A). In brief, extensive genetic analysis has suggested that reduced DAF-2/InR activity in neurons leads to cell-autonomous DAF-16/FOXO activation and decreased production of hormones, including insulin-like peptide INS-7 [10]. In turn, reduced systemic levels of INS-7 signaling upregulates DAF-16/FOXO activity in the intestine, which increases the production of additional hormones while inhibiting the production of insulin-like peptides. Consequently, somatic cells acquire germline stem cell-like, stress-resistant properties conducive to longevity [28].

In sum, hormonal signaling is likely to regulate a number of cellular processes in long-lived *daf-2*/InR mutants. Below, we will focus on two recently identified processes with emerging roles in *C. elegans* longevity, namely energy/lipid metabolism and autophagy.

Cellular effector mechanisms

Energy and lipid metabolism—Insulin/IGF-1 signaling is a master regulator of metabolism that coordinates food intake and cellular energy homeostasis by stimulating glucose uptake and by driving anabolic processes. In *C. elegans*, glucose supplementation reduces lifespan by stimulating insulin signaling, and inhibiting DAF-16/FOXO–mediated transcription of longevity genes [29], while glucose restriction relieves this block and leads to lifespan extension [30]. Accordingly, impairing insulin signaling induces a survival response that alters feeding behavior [31] and promotes recycling of endogenous macromolecules. In support of this notion, *daf-2*/InR mutants show a DAF-16/FOXO-mediated increase in expression of the lipase LIPL-4 [32]. This lipase is not only partially required for *daf-2*/InR mutants to live long but also, when overexpressed, is sufficient to extend the lifespan of wild-type animals [32]. Taken together, these initial observations suggest that lipid breakdown is beneficial to *daf-2*/InR mutants. However, *daf-2*/InR mutants have increased lipid stores [33,34], indicating a complex role for lipid metabolism in these animals that remains to be investigated in more detail.

Energy metabolism also plays a central role in lifespan determination, as illustrated by the regulatory function of the energy-sensing enzyme AMP-activated kinase (AMPK). AMPK is activated by high intracellular AMP to ATP ratios, a condition that is observed in *daf-2*/InR mutants and in wild-type animals exposed to starvation or heat stress [35]. Consistent with this, a catalytic subunit of AMPK, AAK-2, is necessary for the long lifespan of *daf-2*/InR mutants [35], and AAK-2 overexpression is sufficient for lifespan extension of wild-type

animals [35,36], an effect that is mediated at least partially through inhibition of the CREB-regulated transcriptional coactivatorCRTC- 1 [36].

Autophagy—Macroautophagy (hereafter referred to as autophagy) is a multistep process resulting in large-scale lysosomal degradation and recycling of vacuole-sequestered cytosolic cargos [37], and is emerging as a key player in the modulation of longevity in *C. elegans.* Autophagy is increased in *daf-2*/InR mutants and is essential for their lifespan extension [38,39], implying that these mutants recycle cargos that otherwise would be limiting to long-term survival. Consistent with this idea, increased autophagy and lysosomal degradation protects against protein aggregation [40]. This likely delays the eventual collapse of proteostasis observed during aging [41,42], because toxic protein aggregates are more efficiently removed.

Autophagy is at least partially regulated by AMPK activity in *daf-2*/InR mutants [43]. In mammals, AMPK stimulates autophagy by directly phosphorylating the autophagy initiating kinase, UNC-51 (ATG-1/ULK-1) [43]. Consistent with this, *unc-51*/ULK1, like *aak-2/* AMPK*,* is required for autophagy induction in *daf-2*/InR mutants [43]. Although recent work has suggested that autophagy can be transcriptionally regulated [44] (see section on Germline Signaling), DAF-16/FOXO appears to be unimportant for autophagy induction in *daf-2*/InR mutants [45]. Instead, DAF-16/FOXO could contribute to later stages of the autophagy process, as DAF-16/FOXO induces the expression of a lysosomal protein necessary for thermotolerance [46]. Future studies should investigate how autophagy is regulated to mediate the lifespan extension observed in *daf-2*/InR mutants.

Summary

Research over the last two decades has made the insulin/IGF-1 signaling pathway the most-studied longevity paradigm and has identified DAF-16/FOXO as a prominent transcription factor that, along with additional transcription factors and coregulators, controls the expression of multiple longevity genes. It has also become clear that endocrine signaling between tissues, such as the nervous system and the intestine, have systemic effects on lipid metabolism, autophagy and consequently longevity. Key issues for future research include understanding the insulin/IGF-1–dependent network of transcription factors and effector mechanisms, as well as the intracellular signaling events and feedback loops that coordinate insulin secretion and modulate aging.

TOR signaling

The basics

The TOR (Target-of-rapamycin) pathway controls growth and reproduction in response to the availability of amino acids and growth factors. As a nutrient sensor, TOR is a mediator of the metabolic response to dietary restriction, another conserved lifespan-extending paradigm with multiple links to the signaling pathways discussed here (reviewed in [47]). Consistent with this, inhibition of TOR activity extends lifespan in a variety of species [48]. TOR exists in two complexes, TORC1 and TORC2, which have different functions in mammalian cells; TORC1 integrates mitogen and nutrient signals to control cell proliferation and size, whereas TORC2 regulates cell shape [49]. TORC1 and TORC2 contain different co-activators, DAF-15/Raptor and RICT-1/Rictor, respectively [50] (Figure 1B), and regulate development, lipid storage, mRNA translation and autophagy in *C. elegans* [45,51,52,53,54,55]. TORC1 affects longevity in *C. elegans* at least in part via the GTPAses RAGA-1/RAGC-1 [53,56], RHEB-1/Rheb [57], and DAF-15/Raptor [54]. RNAi inhibition of *raga-1* in the intestine is sufficient for lifespan extension [53], suggesting a role for this tissue in TOR-mediated longevity. Moreover, adult inhibition of *rict-1*/Rictor

extends *C. elegans* lifespan [53]. The two transcription factors DAF-16/FOXO and SKN-1/Nrf are required for lifespan extension mediated by TORC1 inhibition, whereas SKN-1/Nrf is required for TORC1/*rict-1*–mediated lifespan extension [53]. TOR inhibition also requires the FOXA transcription factor PHA-4 to extend lifespan [58], but it has so far not been addressed whether this is primarily a TORC1 or TORC2 effect (Figure 1B).

Target genes

TOR regulates expression of the insulin-like peptide *ins-7* [57], raising the possibility that TOR may, like DAF-2/InR, modulate aging through a systemic effect on hormones. Moreover, TORC1 regulates the expression of many *daf-16*/FOXO- and *skn-1*/Nrf target genes including detoxification genes [53]. Below, we discuss additional transcriptionally regulated targets that may contribute to TOR-mediated longevity in *C. elegans* (Figure 1B). TOR may also affect longevity by non-transcriptional mechanisms, including TORC1-mediated phosphorylation of S6 kinase (S6K), a key regulator of mRNA translation. Accordingly, inhibition of S6K as well as other regulators of mRNA translation, like *tor* inhibition, extends lifespan in multiple organisms [48]. While it is unclear how reduced protein synthesis extends lifespan, S6K may modulate longevity in *C. elegans* via both transcriptional (e.g., via PHA-4/FOXA) as well as post-translational mechanisms (e.g., involving AMPK) [48].

Cellular effector mechanisms

Lipid metabolism—Similar to *daf-2*/InR mutants, inhibition of TOR upregulates expression of the lipase LIPL-4 and increases lipolysis [44]. Moreover, *lipl-4* expression in animals with reduced TOR levels is at least partly mediated by DAF-16/FOXO [44]. Whereas the contribution of LIPL-4 and lipid metabolism to TOR-mediated longevity remains unclear, TOR, *daf-15*/Raptor and *rict-1*/Rictor mutants all paradoxically accumulate more lipid droplets [51,54,55,59], as do *daf-2*/InR mutants.

Autophagy—As observed in other organisms, autophagy is induced by inhibition of TORC1 in *C. elegans* [45, 53]. This is partly due to increased expression of *unc-51*/ULK1, an upstream activator of the multistep autophagy process, via PHA-4/FOXA [44]. Notably, *pha-4*/FOXA itself is subject to transcriptional regulation by TOR [44,57]. Along these lines, autophagy genes [45,60] and *pha-4*/FOXA [58] are required for TOR/TORC1 mutants to live long, suggesting that TOR/TORC1 inhibition extends lifespan through autophagy. Because lifespan extension induced by rapamycin, a chemical that inhibits TOR, in flies also depends on autophagy genes [61], this longevity mechanism may well be conserved.

Overlap with insulin/IGF-1 signaling

TOR inhibition and reduced insulin/IGF-1 signaling engage overlapping transcriptional targets and effector mechanisms, and consistent with this, TOR inhibition does not further extend the long lifespan of *daf-2*/InR mutants [59,62]. On the other hand, DAF-16/FOXO negatively regulates the expression of the TORC1 coactivator *daf-15*/Raptor [51]. Moreover, as mentioned above, DAF-16/FOXO and SKN-1/Nrf are required for lifespan extension mediated by TORC1 inhibition [53]. It will be of interest to elucidate how signals from the insulin-signaling pathway (possibly via AKT as observed in mammals [50]) may modulate TOR in *C. elegans*, as well as to elucidate how DAF-16/FOXO and SKN-1/Nrf is regulated upon TOR inhibition.

Summary

Studies in several species demonstrate that TOR signaling affects aging in a conserved manner. Although the molecular mechanisms by which TOR affects *C. elegans* lifespan are

only starting to be elucidated, it is clear that insulin/IGF-1 and TOR signaling converge at the level of transcription factors (DAF-16/FOXO and SKN-1/Nrf), target genes (the lipase *lipl-4* and the insulin-like peptide *ins-7*), as well as potential effector mechanisms (autophagy and possibly lipid metabolism). Future investigations should systematically address the tissue-specific functions of TOR in longevity.

Germline signaling

The basics

In *C. elegans*, the germline integrates nutrient signaling and communicates with other tissues that modulate aging. Removal of germline cells by laser ablation of precursor cells extends the lifespan by 60%; however, this effect is nullified by concomitant removal of the somatic gonad, suggesting the existence of opposing signaling pathways originating from the germline and somatic gonad [63]. Germline-mediated regulation of lifespan has also been observed in flies, and signals from the reproductive system may affect lifespan in mice as well [64].

As in *daf-2*/InR mutants, *daf-16*/FOXO is required for the long lifespan of germline-less animals, yet in contrast to the insulin/IGF-1 pathway that affect DAF-16/FOXO in both neuronal and intestinal cells, germline ablation induces DAF-16/FOXO activation and translocation to the nucleus primarily in intestinal cells, a key site for modulation of longevity through germline signaling [64]. Several factors with functions in insulin/IGF-1 signaling are required for germline-less animals to live long, including DAF-18/PTEN [65], the co-factor SMK-1/SMEK-1 [17] and the transcription factor HSF-1 [66]. Other factors are specific to germline signaling, including the transcription elongation factor TCER-1/TCERG1 [67], and the ankyrin repeat-containing protein KRI-1/KRIT-1 [65]. KRI-1/KRIT-1 is required for nuclear localization of DAF-16/FOXO as well as upregulation of TCER-1/TCERG1 in the intestines of germline-less animals, but not in *daf-2*/InR animals. TCER-1/TCERG1 may be involved in lifespan modulation in germline-less animals through its interactions with PHI-62, a predicted RNA-binding protein that binds FTT-2/14-3-3, a known FOXO-interacting protein [68]. Moreover, the microRNA *mir-71,* which is produced in neurons, was recently found to regulate DAF-16/FOXO localization in the intestine [69], supporting a role for cell-nonautonomous regulation of lifespan in germline-less animals. These DAF-16/FOXO activators are all required for germline removal to extend lifespan (Figure 2).

A lipophilic-hormone/steroid signaling pathway also affects DAF-16/FOXO localization and is required for longevity in germline-less animals. This pathway includes three key components, DAF-9, DAF-36 and DAF-12. DAF-9 is an ortholog of the mammalian cytochrome P450 enzyme, CYP27 and is expressed in the hypodermis, the spermatheca, and two neuron-like cells. DAF-36 is a Rieske-like oxygenase primarily expressed in the intestine [11]. DAF-9/CYP27 and DAF-36/oxygenase are involved in the synthesis or modification of ligands for the nuclear hormone receptor DAF-12/FXR. Consistent with this idea, supplementation with the DAF-12 ligand delta-4 dafachronic acid restores lifespan extension in germline-less animals deficient in *daf-9*/CYP27 or *daf-36*/oxygenase [70]. DAF-12/FXR is likely to interact in a complex manner with DAF-16 DAF-9/CYP27, and the two proteins may directly bind each other [71]. Thus, in response to germline removal, steroid signaling mediates specific changes in the intestine that stimulate DAF-16/FOXO activity to extend lifespan (Figure 2).

Target genes

Conveniently, *C. elegans* lifespan extension induced by germline ablation can be mimicked genetically. For instance, animals carrying mutations in the Notch-1 receptor (*glp-1/*

NotchR), a mediator of germline development, have extended lifespans [72]. These mutants were employed in a recent microarray analysis to identify DAF-16/FOXO and DAF-12/FXR target genes [68]. This detailed analysis revealed a significant overlap in DAF-16/FOXO–regulated genes in the *daf-2*/InR mutants and in germline-less animals, and a small but overlapping set of genes were regulated by both *daf-16*/FOXO and *daf-12*/FXR in germline-less animals. Of note, some of these genes are involved in lipid metabolism, as described below.

Cellular effector mechanisms

Lipid metabolism—Germline-less animals display notable changes in lipid metabolism. These include increased lipolysis, resulting in large part from DAF-16/FOXO–mediated increases in the lipase *lipl-4* expression [32], and concomitant increases in lipid stores [73], similar to *daf-2*/InR and *tor* mutants. Moreover, other lipid genes such as the lipase *lips-17* and the fatty acyl reductase *fard-17* are upregulated and required for the long lifespan of germline-less *glp-1*/NotchR mutants, further supporting a role for lipid remodeling in longevity [68]. In addition, a recent study showed that the nuclear hormone receptor NHR-80/HNF-4 regulates oleic acid synthesis via the fatty acid desaturases *fat-5, -6* and *-7* and mutation of *fat-6* and *fat-7* together shortens the lifespan of germline-less animals, an effect that can be reversed by adding oleic acid to the media [74]. Taken together, these results are consistent with a key role for fatty acid desaturation in germline signaling. It will be interesting to determine if products of these lipid-modifying genes could serve as signaling molecules to coordinate metabolism and longevity regulation of the entire organism.

Autophagy—As observed for both *daf-2*/InR and *tor* mutants, germline-less *glp-1*/NotchR animals display an increase in autophagy, which is required for their long lifespan [44]. Autophagy induction in *glp-1*/NotchR animals is at least partly mediated by a PHA-4/FOXA–dependent increase in expression of autophagy genes [44]. Autophagy is predominantly induced in hypodermal and intestinal cells, suggesting that these tissues are particularly important for longevity. In particular, overexpression of the lipase LIPL-4 in the intestine [32], or under its endogenous promoter [44] is sufficient to extend lifespan, which occurs in part through PHA-4/FOXA–mediated induction of autophagy [44]. On the other hand, autophagy is required for the increased lipase activity in germline-less animals, strengthening the hypothesis that a functional link exists between autophagy and lipid breakdown. Further investigation will be necessary to determine how autophagy functions as a possible regulator of lipid metabolism in germline-less animals.

Overlap with insulin/IGF-1 and TOR signaling—Germline-less animals and *daf-2*/InR mutants have several mechanistic similarities, including neuron-to-intestine communication for longevity, increased autophagy, nuclear-localized DAF-16/FOXO in the intestine, and significant overlap in DAF-16/FOXO–regulated targets [68]. However, notable differences also exist; for example, KRI-1/KRIT1 and TCER-1/TCERG1 regulate germline-dependent but not *daf-2*/InR–mediated longevity. Moreover, germline removal in *daf-2*/InR mutants leads to extreme longevity, suggesting that parallel pathways determine longevity in *daf-2*/InR and germline-less animals [75].

The lifespan of germline-less *glp-1*/NotchR animals is not further extended by TOR inhibition [44], suggesting that overlap exists between TOR and germline signaling. This is supported by the observation that TOR levels are reduced in *glp-1*/NotchR animals [44], and both pathways share common transcriptional targets (e.g., the lipase *lipl-4*) and regulators (e.g., DAF-16/FOXO). However, since inhibition of *raga-1* (i.e., TORC1) does extend the

lifespan of germline-less animals [53], this overlap is likely to be complex and awaits further investigation.

Summary

The effects of germline signaling on aging in *C. elegans* have received much attention in recent years, and this pathway is now known to share common effector mechanisms with insulin/IGF-1 and TOR signaling, most notably lipid metabolism and autophagy. Among the three longevity pathways discussed here, germline signaling is associated with the largest number of transcription factors known to modulate longevity, namely *daf-16*/FOXO, *daf-12*/FXR, *hsf-1, nhr-80*/HNF4, and *pha-4*/FOXA. Future experiments should evaluate how the function of these factors is coordinated, both intracellularly and at the intercellular level, to extend lifespan in germline-less animals.

Concluding Remarks and Future Perspectives

The involvement of the insulin/IGF-1, TOR, and germline signaling pathways in lifespan determination of *C. elegans* is the subject of intense investigation. Each pathway regulates downstream effector mechanisms, including lipid metabolism and autophagy, through overlapping sets of transcription factors. All three pathways converge on DAF-16/FOXO, whereas SKN-1/Nrf1, PHA-4/FOXA and HSF-1 are employed in specific combinations. Notably, some transcriptional target genes are known to be regulated by both DAF-16/FOXO and HSF-1, whereas others are specifically controlled by one factor [18]. It will be crucial to identify the targets of each transcription factor, and to determine how they are coordinately regulated in the different longevity models.

At least two cellular processes are regulated in common by insulin/IGF-1, TOR, and germline signaling to affect longevity; namely, autophagy and lipolysis. The interplay between autophagy and lipolysis may be critical for longevity as LIPL-4–mediated lipase activity and autophagy function interdependently [44]. Moreover, concomitant changes in fatty acid desaturation may be necessary for somatic maintenance [76]. As such, increased activity in lipid remodeling pathways, such as fatty acid desaturation observed in germline signaling, may stimulate membrane biogenesis and maintain functional autophagy [77]. The significance of lipid breakdown in aging appears to be conserved, because in humans, the capacity to maintain dynamic lipid turnover in adipocytes correlates with better health status [78]. Paradoxically, mutants of all three longevity pathways have excessive lipid stores [76]. Future biochemical experiments in *C. elegans* may reveal how aging is modulated by different metabolic routes governing energy and lipid homeostasis.

As in the past decades, future research in *C. elegans* will likely help clarify how endocrine and metabolic signaling between different tissues modulate organismal aging. Such studies could uncover novel genetic factors with effects on survival, which may help develop therapeutic solutions against human age-related diseases and aging. The nematode is also emerging as a practical platform to test compounds and rapidly validate their bioactivity in different disease models [79,80]. In sum, research using the model organism *C. elegans* will continue to accelerate discoveries, and will likely provide a better molecular and cellular understanding relevant to treatment of age-related disorders and metabolic diseases in humans.

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Glossary of C. elegans gene orthologs

aak-2	alpha-subunit of AMP-activated kinase (AMPK)
age-1	phosphatidylinosytol 3-kinase
akt-1,2	serine/threonine kinase Akt/PKB
daf-2	insulin/IGF-1 receptor
daf-9	cytochrome P450 of CYP27 subfamily
daf-12	nuclear receptor FXR
daf-15	TORC1-binding partner Raptor
daf-16	transcription factor FOXO3A
daf-18	phosphatidylinositol 3,4,5-triphosphate 3-phosphatase, PTEN
daf-36	catalytic subunit of Rieske-like oxygenase
fat-5	palmitoyl-CoA- Δ9-Desaturase
fat-6-7	stearoyl-CoA- Δ9-Desaturase SCD
ftt-2	14-3-3 (FOXO-interacting) protein
glp-1	Notch receptor
hcf-1	Host cell factor 1
hsf-1	Heat shock factor 1
ins-7	one of 40 insulin-like peptides in *C. elegans* and encodes an insulin/IGF-1-like peptide that likely functions as an agonist for DAF-2
jnk-1	c-Jun N-terminal kinase
kri-1	ankyrin-repeat protein KRIT-1
lipl-4	lipase with high homology to human lysosomal acid lipase
MAPK	mitogen-activated protein kinase
mir-71	one of hundreds of microRNAs with a specific effect on longevity germline-less animals
nhr-80	nuclear hormone receptor HNF-4
pdk-1	3-phosphoinositide-dependent kinase 1
pha-4	transcription factor FOXA
phi-62	Putative RNAse-binding protein
prmt-1	PRotein arginine MethylTransferase
raga-1	Ras-related GTPase RagA
ragc-1	Ras-related GTPase RagC
rheb-1	Rheb GTPase
rict-1	TORC2-binding partner Rictor (also referred to as *lpo-6*)
rle-1	E3 ubiquitin ligase
rsks-1	p70 ribosomal S6 kinase
skn-1	transcription factor Nrf

smk-1	suppressor of MEK
tcer-1	TransCription Elongation Regulator homolog 1
TOR	Target-of-rapamycin (also referred to as *let-363*)
TORC1	TOR complex 1
TORC2	TOR complex 2
unc-51	ULK-1 (Unc-51-Like Kinase)

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Outstanding Questions

- How are energy and lipid homeostasis changed to ensure survival?
- What is the cargo(s) degraded by autophagy that leads to longer lifespan?
- How do TOR complexes differ in their ability to mediate longevity?
- What are the mechanisms by which multiple transcription factors coordinate transcription of distinct subset of genes in a longevity pathway?

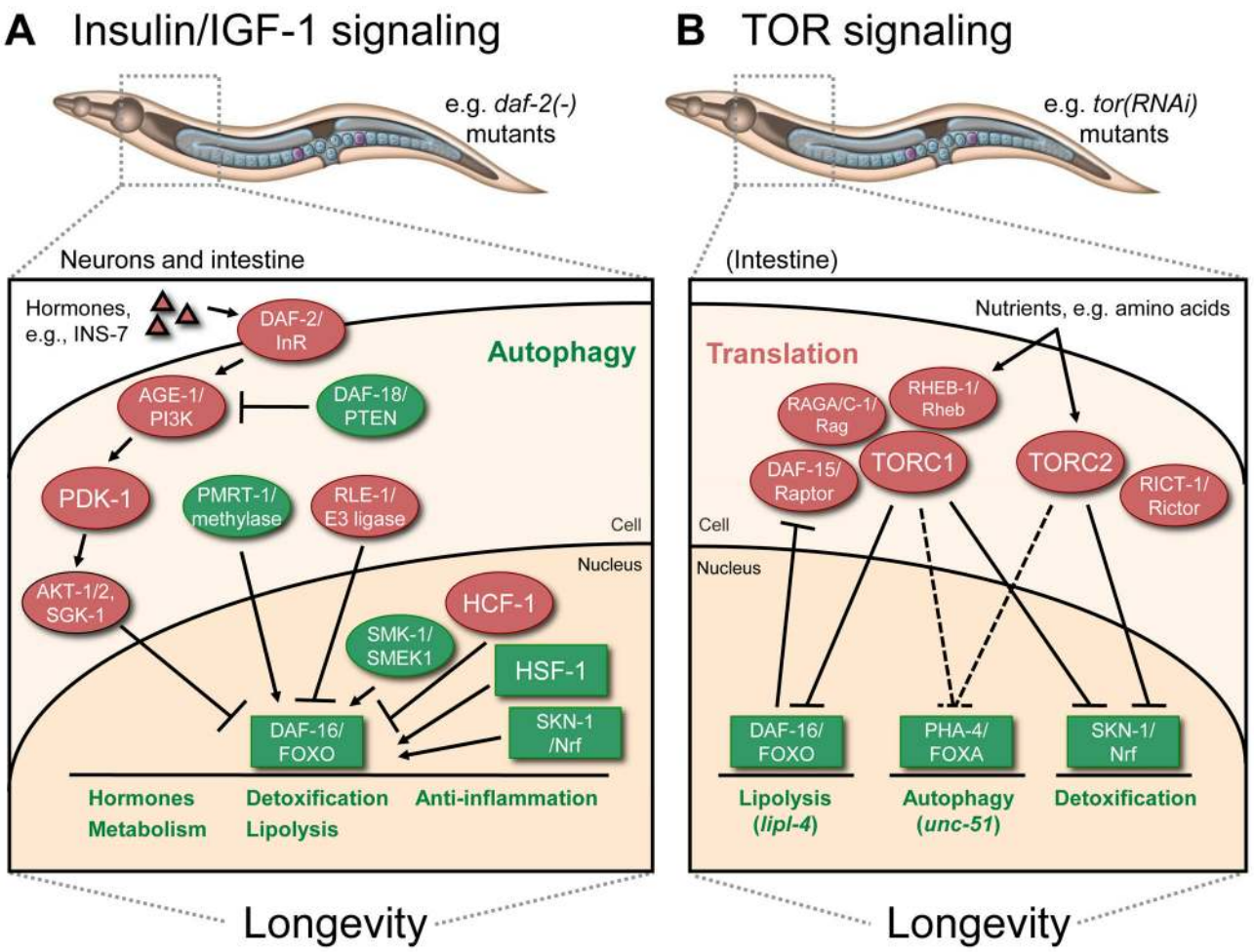

Figure 1. Overview of insulin/IGF-1 (A) and TOR (B) signaling in *C. elegans* aging

A. In *daf-2*/InR insulin-receptor mutants, insulin-like peptides (e.g., INS-7) secreted from neurons reach intestinal cells and trigger the canonical insulin-signaling pathway, which prevents DAF-16/FOXO from entering the nucleus. Other mechanisms of DAF-16/FOXO regulation include ubiquitination (RLE-1/E3 ligase) and arginine methylation (PMRT-1/ methylase). Nuclear-localized DAF-16/FOXO activity is enhanced by the action of SMK-1/ SMEK and HSF-1, and is inhibited by HCF-1. The transcription factor SKN-1/Nrf is also required for longevity in *daf-2*/InR mutants. Collectively, these factors transcriptionally regulate multiple output processes as noted. Autophagy is another cellular process required for *daf-2*/InR mutants to live long. It is not yet known whether autophagy is a transcriptionally regulated process in *daf-2*/InR mutants.

B. TOR responds to nutrients and functions in two different complexes, TORC1 and TORC2. In analogy with mammalian studies, TORC1 is thought to interact with DAF-15/ Raptor as well as Rag GTPases like RAGA-1, RAGC-1, and RHEB-1/Rheb. TORC1 and TORC1 specifically impair the activity of DAF-16/FOXO and SKN-1/Nrf, whereas it is not yet clear which of the two TOR complexes regulates PHA-4/FOXA (indicated by dashes lines). By using these transcription factors, TOR inhibits the expression of at least certain lipolysis-, autophagy-, and detoxification-associated genes. Listed as a cytoplasmic process, TOR signaling also likely modulates aging through a general suppression of translation. While the intestine has been linked to the longevity mediated by TORC1, the specific tissue

requirements for TOR-dependent effects on aging have not yet been systematically investigated.

Factors with longevity-promoting effects are in green and those with lifespan-limiting effects are in red. Transcription factors are in boxes. See text for details.

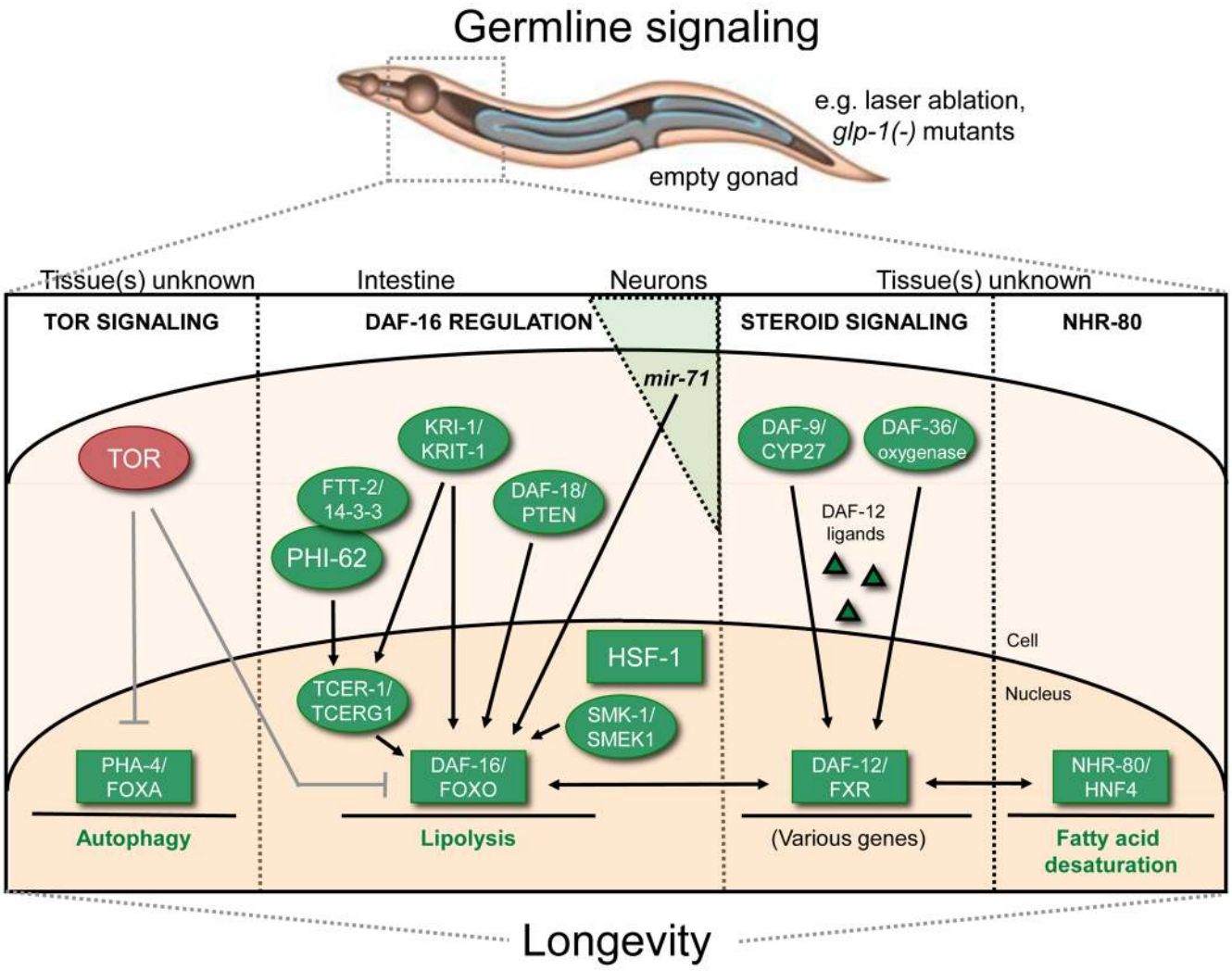

Figure 2. Overview of germline signaling in *C. elegans* aging

Lifespan extension via germline removal, for example by mutation of *glp-1*/Notch, depends on at least four signaling mechanisms: reduced TOR signaling (likely to regulate autophagy), DAF-16/FOXO regulation, increased steroid signaling via the DAF-36/DAF-9/DAF-12 pathway (which regulates various genes), and increased NHR-80/HNF-4 signaling (which enhances fatty-acid desaturation). Of these mechanisms, the most-studied mechanism is DAF-16/FOXO regulation. Germline-less animals require specific cofactors (not required in *daf-2*/InR mutants) for activation of DAF-16/FOXO in the nucleus of intestinal cells. DAF-12/FXR, TCER-1/TCERG1, KRI-1/KRIT-1 and PHI-62 all function specifically in germline-less animals, whereas DAF-18/PTEN, SMK-1/SMEK and HSF-1 are common between the insulin/IGF-1 and germline signaling pathways. Cell-nonautonomous regulation of DAF-16/FOXO is also mediated through a microRNA, *mir-71,* which is produced in neurons.

Factors with longevity-promoting effects are in green and those with lifespan-limiting effects are in red. TOR effects are listed in grey to indicate that these links are presently inferred. Transcription factors are in boxes. See text for details.