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Untangling longevity, dauer, and healthspan in *Caenorhabditis elegans* insulin/IGF-1-signalling

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Abstract

The groundbreaking discovery that lower levels of insulin/IGF-1 signaling (IIS) can induce lifespan extension was reported 24 years ago in the nematode *Caenorhabditis elegans*. In this organism, mutations in the insulin/IGF-1 receptor gene *daf-2* or other genes in this pathway can double lifespan. Subsequent work has revealed that reduced IIS (rIIS) extends lifespan across diverse species, possibly including humans. In *C. elegans*, IIS also regulates development into the diapause state known as dauer, a quiescent larval form that enables *C. elegans* to endure harsh environments through morphological adaptation, improved cellular repair, and slowed metabolism. Considerable progress has been made uncovering mechanisms that are affected by *C. elegans* rIIS. However, from the beginning it has remained unclear to what extent rIIS extends *C. elegans* lifespan by mobilizing dauer-associated mechanisms in adults. As we discuss, recent work has shed light on this question by determining that rIIS can extend *C. elegans* lifespan comparably through downstream processes that are either dauer-related or – independent. Importantly, these two lifespan extension programs can be distinguished genetically. It will now be critical to tease apart these programs, because each may involve different longevity-promoting mechanisms that may be relevant to higher organisms. A recent analysis of organismal “healthspan” has questioned the value of *C. elegans* rIIS as a paradigm for understanding healthy aging, as opposed to simply extending life. We discuss other work that argues strongly that *C. elegans* rIIS is indeed an invaluable model, and consider the likely possibility that dauer-related processes affect parameters associated with health under rIIS conditions. Together, these studies indicate that *C. elegans* and analyses of rIIS in this organism will continue to provide unexpected and exciting results, and new paradigms that will be valuable for understanding healthy aging in humans.

Introduction

Over the last two decades, growth and nutrient-sensing pathways have emerged as conserved modulators of lifespan. The first longevity assurance “pathway” identified was insulin/IGF-1 signaling (IIS), and reductions in its activity are now known to increase lifespan across diverse metazoa [1–3]. Moreover, several single nucleotide polymorphisms in IIS components have been associated with human longevity, suggesting that this pathway may be relevant to enhancing our lifespan [3,4]. The finding that reduced IIS (rIIS) increases

lifespan was first obtained in *C. elegans* [5,6]. Because of its simplicity, short lifespan of about three weeks, and genetic advantages, this organism has been uniquely valuable for identifying mechanisms that mediate longevity. *C. elegans* studies have revealed that rIIS controls processes involved in stress defense, immunity, proteostasis, metabolism, signaling, and extracellular matrix (ECM) remodeling, and have defined interactions across tissues that affect aging of the entire organism [1,7,8]. If *C. elegans* rIIS is indeed a broadly relevant model for extension of healthy lifespan, these paradigms may serve as templates for understanding how to promote healthy aging in humans.

In *C. elegans*, the human insulin and IGF-1 receptors are represented by a single ortholog, DAF-2 [1,2]. The *daf-2* gene was originally identified in screens for mutations that promote constitutive development into the larval stage known as dauer (*daf* stands for "dauer formation variant"). Dauer is a diapause state, an alternative larval stage into which *C. elegans* develops when encountering harsh environmental conditions: high temperature, scarce food, and overcrowding (Figure 1). Dauers are relatively immobile and highly resistant to stress, and can survive under adverse conditions for extended periods [9,10]. Dauer has been considered a "non-aging" stage, because worms that have been dauers for more than 60 days, when exiting dauer and developing into adults, show the same adult lifespan compared to animals that have never gone through dauer [1] (Figure 1). The idea that the course of aging can be altered in a regulated fashion first arose from the groundbreaking finding that when *daf-2* mutants are maintained under conditions in which they form adults instead of dauers, they live far longer than wild type [6]. This work revealed for the first time that lifespan could be modulated by single mutations in a genetically defined pathway, albeit one that had not yet been molecularly identified as IIS.

From the beginning, the simplest model to explain the remarkable longevity of *daf-2* mutants was that they may benefit from protective dauer-related mechanisms being "switched on" during their adult life [10–13]. This notion derives in part from evidence that dauers reentering the reproductive cycle appear to have "reset the clock" and do not show signs of having aged [2]. It is also consistent with the finding that under typical conditions the most frequently studied *daf-2* mutants exhibit delayed reproduction, and a proportionally early decline in spontaneous movement as they age [8,10,14], characteristics that seem to be mild versions of dauer-like traits. On the other hand, rIIS conditions have been described under which *C. elegans* lives for extremely long periods without seeming to become lethargic, suggesting that inhibition of this pathway can indeed extend normal healthy life without "side effects" [8,15].

Why is it important to understand in detail processes that are affected by *C. elegans* IIS, given that this pathway has now been linked to longevity across evolution [1,3]? Through the many experimental advantages of *C. elegans*, we have obtained a deeper and more extensive understanding of *C. elegans* rIIS than of any other scenario of lifespan extension in any organism. It remains to be determined to what extent these mechanisms reflect the diapause developmental pathway becoming active in adults, or homeostatic processes that normally operate independently of the dauer program. As we describe below, we now know that rIIS longevity involves both dauer-related and dauer-independent pathways that can be discriminated genetically [8]. To understand how these rIIS programs might be applicable to

higher organisms like ourselves, it will be important to elucidate which mechanisms are critical in each case. Moreover, it appears likely that a better understanding of the contributions of dauer to rIIS longevity will shed light on an important question that has been debated in recent publications: whether rIIS promotes healthy aging, as opposed to simply increasing lifespan.

IIS regulates dauer development and modulates adult lifespan

Our understanding of IIS as a longevity pathway was built upon extensive genetic analyses of dauer development. Initial studies revealed that temperature is a critical factor that regulates dauer entry, with higher temperatures predisposing to dauer formation (Figure 1) [9,10]. Screening for mutations that influence dauer entry then identified numerous hypomorphic alleles of *daf-2* that allow constitutive dauer formation at 25°C, but not 15°C [10,16,17]. Under all conditions analyzed, dauer development required the *daf-16* gene. This pathway was linked to aging by the finding that when *daf-2* mutants are analyzed at 20°C, a temperature at which very few animals enter dauer, their lifespan is dramatically extended in a *daf-16*-dependent manner [6]. Within a short time, genes in the *daf-2/daf-16* dauer-regulatory pathway were identified as components of a conserved IIS pathway (Figure 2), and their effects on lifespan were found in general to parallel their effects on dauer [1,2]. These genes included *age-1* (phosphatidylinositol (PI)-3 kinase, Figure 2), hypomorphic alleles of which had been identified previously based upon their extended lifespan [1,5]. Identification of DAF-16 as the *C. elegans* counterpart to the mammalian Forkhead box O (FOXO) transcription factors ultimately led to the understanding that, across evolution, DAF-16/FOXO is inhibited directly by IIS through phosphorylation and nuclear exclusion (Figure 2) [1,2].

Two lines of evidence have supported the notion that rIIS increases lifespan by allowing DAF-16 to act in adults to activate dauer-related processes that slow aging. Expression profiling has revealed higher levels of expression of dauer-associated genes in these long-lived animals [2,12,18]. In addition, dauer-related processes can become active in rIIS adults. Mutations in *daf-2* are categorized phenotypically as either class 1 or 2 (Figure 3) [10], with class 1 mutations most commonly found in the extracellular domain, and class 2 mutations in the intracellular kinase domain (Figure 2)[17]. Each class of *daf-2* mutant is comparably predisposed to dauer entry at high temperature (25°C), but while class 1 (e.g., *e1368*) mutants appear normal as adults, class 2 (e.g., *e1370*) adults exhibit several dauer-related traits at higher temperatures (22.5°C or above) [10]. These DAF-16-dependent traits include decreased mobility and body size, darkening of the body, gonad shrinkage, reproductive delay, and reduced progeny production (Figure 3) [10]. The *daf-2* allele that is most commonly analyzed in aging studies, *daf-2(e1370)*, is a class 2 mutation. *daf-2(e1370)* animals that develop at 15°C and are shifted to high temperature as adults exhibit typical class 2 traits [10]. However, if these animals are moved back to 15°C, their progeny production is markedly increased, and their morphology and movement are restored to wild-type. Remarkably, the last two traits can be repeatedly induced and eliminated in the same individuals by shifting them back and forth between 15°C and 25.5°C [10], indicating that these class 2 traits reflect regulated processes and not simply sickness or decrepitude.

Importantly, *daf-2(e1370)* and other typical class 2 mutants exhibit milder versions of these traits at 20°C, the temperature usually used for lifespan and phenotypic analyses. At 20°C fewer than 1% of *daf-2(e1370)* larvae form dauers, and adults initially appear essentially normal. However, all of these adults exhibit a prolonged reproductive period, and prematurely cease moving spontaneously [8,10]. Similarly, *age-1* mutants that live approximately 10 times as long as wild type exhibit reduced movement and a severe reproductive defect [19]. These telltale signs suggest that dauer-related processes are active in these rIIS adults under these conditions.

By contrast, other evidence points away from the idea that rIIS longevity might derive from adulthood activity of the dauer program. Knockdown of *daf-2* by RNA interference (RNAi) from day one of adulthood extends lifespan comparably to class 2 mutations without reducing adulthood mobility, or inducing any other class 2 traits. This may be because RNAi is less effective in neurons, a tissue that is important in regulating dauer entry (discussed in Ref. [8]). Consistent with this idea, *daf-2* RNAi induces developmental dauer entry when performed in strains with enhanced neuronal RNAi function [8]. On the other hand, when *daf-2* RNAi is performed in a class 1 *daf-2* mutant, adults appear normal for an extended period and enjoy an extremely long lifespan [8,15]. Apparently, robust rIIS lifespan extension need not necessarily be linked to dauer-related processes.

Dauer-related and dauer-independent programs of rIIS longevity

Which of the above two models for rIIS longevity is correct? The answer is that each can be, depending upon the conditions. Our laboratory determined that rIIS can extend *C. elegans* lifespan through either a “dauer-predisposed” program that is associated with mild dauer traits, or a “dauer-independent” program that is not [8]. Importantly, these two programs can be distinguished genetically by their dependence upon the transcription factor SKN-1/Nrf [4]. SKN-1, the ortholog of the mammalian Nrf (NF-E2-related factor) proteins, is activated by various stresses, and controls genes involved in oxidative and xenobiotic stress resistance, proteostasis, and metabolism [20]. SKN-1 orthologs have been implicated in lifespan extension or protection from premature aging in *Drosophila*, mice, and humans, and SKN-1 is required for *C. elegans* lifespan extension by most genetic, nutritional, or pharmacologic interventions, including dietary restriction (DR) and inhibition of the mechanistic target of rapamycin (mTOR) kinase [20,21]. Like DAF-16, SKN-1 is inhibited by rIIS in *C. elegans* (Figure 2) [22]. Interestingly, however, SKN-1 does not appear to be involved in the dauer program [8,22]. When lifespan is extended by rIIS under multiple conditions where dauer traits are present, including analysis of the canonical *daf-2(e1370)* mutant at 20°C, SKN-1 is largely dispensable for lifespan extension (Table 1)[8]. By contrast, under conditions where dauer traits are absent, including analysis of *daf-2(e1370)* at 15°C or of *daf-2* RNAi animals between 15°C and 25°C, SKN-1 is fully required for rIIS longevity. This indicates that a dauer-independent program of rIIS longevity is defined by its requirement for SKN-1[8].

Several lines of evidence support the above model. The difference between these two longevity programs does not correlate with the apparent degree of IIS reduction, as indicated by either the magnitude of lifespan extension (Table 1) or the extent of de-repression and nuclear occupancy of the DAF-16 and SKN-1 transcription factors under these respective

conditions [8]. Temperature is not the key determinant either, other than its effect on the dauer program itself. *daf-2* mutations are frequently referred to as “temperature-sensitive” (*ts*) simply because they were identified as promoting dauer entry at high (25°C) but not low (15°C) temperature (discussed above). However, as indicated by lifespan extension (Table 1) and de-repression of DAF-16 and SKN-1, the degree to which a class 2 mutation like *e1370* reduces IIS is not appreciably different between 15°C and higher temperatures [8]. Such mutations are therefore unlikely to be “*ts*” in the true sense, which refers to an effect on protein activity that derives from temperature-induced misfolding. The predisposition to dauer entry and dauer traits is determined instead by the importance of temperature in regulation of the dauer program *per se* (Figures 1 and 3). Supporting this idea, the “*ts*” phenotype of forming dauers exclusively at higher (25°C) but not lower (15°C) temperatures has been described for a number of null mutations in dauerregulatory genes (*daf-4(m72)*/TGF- β receptor [23], *daf-7(m62)*/TGF- β [23], *tax-4(p678)*/CNGA1 [24], *daf-36(k114 and dh303)*/Rieske-like oxygenase [25], *akt-1(mg306)*/AKT kinase [25]). Most strikingly, even wild-type animals can develop into dauer at a slightly higher temperature (27°C) [9,16]. Thus, while some *daf-2* mutations are indeed *ts* in the appropriate sense [26], this does not appear to be true across the board. The effect of temperature in inducing class 2 *daf-2* mutants to adopt dauer-predisposed versus dauer-independent *daf-2* longevity programs appears instead to reflect its impact on dauer regulation.

Analyses of downstream genes that are regulated by SKN-1 provided additional evidence that distinguishes these two rIIS longevity programs. Every published expression-profiling analysis of long-lived *C. elegans* has detected a striking upregulation of particular collagen and extracellular matrix (ECM) genes and, importantly, rIIS was found to induce ECM remodeling (discussed in Ref. [8]). In *daf-2* mutants and other well-studied contexts of long life, SKN-1 is required for this ECM gene expression. Interestingly, upregulation of these particular ECM genes is required for *daf-2* lifespan extension under dauer-independent but not dauer-predisposed conditions, possibly because dauer is associated with a specialized cuticle structure [8].

Unbiased approaches to understand the mechanisms of rIIS-mediated lifespan extension have extended beyond transcriptomic analyses to include proteomic and metabolomic analyses of both young and aging animals (reviewed in [1,2] and [8,27–29]). This work has yielded paradigms that have been highly influential in our understanding of how lifespan can be extended. However, aside from the work described above, these phenotypic studies have not analyzed rIIS animals specifically under dauer-independent conditions, and in many examples the work was performed under conditions in which dauer-related traits would be highly prominent (i.e. class 2 *daf-2* mutants at 25°C). In general, it is therefore unclear which findings might reflect dauer-related characteristics, or processes that are independent of this diapause developmental program. Another question arises from the stark difference in these two programs with respect to the requirement for SKN-1 (Table 1), which is so frequently important for longevity. Why is SKN-1 fully required in one case, and largely expendable in the other? What is DAF-16 doing differently in these two scenarios? It could add considerable clarity to our understanding of the IIS pathway, and possibly of longevity assurance overall, if downstream mechanisms involved in these rIIS longevity programs are further teased apart in the future.

A broader role for diapause-related mechanisms in aging?

Dauer-related mechanisms might seem unlikely to be relevant to longevity across evolution, but this is not necessarily the case. Besides IIS, the mechanisms that oppose entry of *C. elegans* into dauer include TGF- β and mTOR signaling, among others [9]. Inhibition of these pathways increases *C. elegans* lifespan, suggesting a link between nutrient-sensing, dauer regulation, and longevity [1,30]. Various organisms can enter a state of quiescence during which metabolism decreases, cellular maintenance improves, and aging may slow or even halt. Yeast arrest in a stationary phase upon nutrient deprivation, and in a cold environment fruit flies enter a state of dormancy, and bears, bats, hamsters and other mammals hibernate (reviewed in [2,31]). Unfavorable environmental conditions can induce mammalian embryos to enter a form of diapause regulated by endocrine cues [32,33]. An intriguing speculative possibility is if these diverse quiescent states might involve some common mechanisms. In this regard, it was striking that administration of a *C. elegans* dauer pheromone component to old male mice extended their lifespan, and attenuated age-associated pathologies [34]. Together, these findings suggest that dauer-related mechanisms that affect *C. elegans* aging might well be relevant to mammals. It is therefore likely to be of value to identify not only specific mechanisms that are associated with dauer-independent rIIS longevity, but dauer-associated mechanisms as well. It will be interesting to determine whether longevity-associated mechanisms that are linked specifically to dauer in *C. elegans* might respond to particular types of challenges in mammals, such as severe nutritional, environmental, or metabolic stress.

Do dauer processes affect “healthspan” parameters?

The idea that dauer-predisposed and dauer-independent programs of rIIS longevity exist may also have implications for our understanding of how IIS influences “healthspan”, the period of life spent in a healthy and functional state. A central goal of the aging field is to understand how to increase healthy lifespan, and not simply extend time spent in age-related decline. Assessment of healthspan has been of interest in the *C. elegans* aging field for many years (see Ref, [14]), and to this end new metrics or analytical methods have been described recently [35–38]. One of these studies [36] reached conclusions that fundamentally question the validity of the rIIS pathway as a model for extending healthy lifespan.

The field has not developed a precise consensus for how best to measure healthspan, but it is generally agreed that assessment of movement is informative. Bansal, et al. monitored *C. elegans* health by analyzing spontaneous movements and stress resistance longitudinally in genetic models of four interventions that increase lifespan: DR, reduced translation, mitochondrial impairment, and rIIS (the class 2 mutant *daf-2(e1370)* at 20°C) [36]. After normalizing these parameters to maximum lifespan, they concluded that conditions that increase lifespan do not necessarily increase healthspan proportionally. *daf-2(e1370)* was particularly deficient in maintaining its capacity for movement [36], an observation reported previously by Huang, et al. in a study that described several healthspan metrics [14]. Bansal, et al. concluded that although these *daf-2* mutants live markedly longer than wild type, they spend a greater fraction of their life in a state of frail health, or “gerospan” [36]. This idea represented a major challenge to the aging field, because of the implication that the most

prominent long-lived *C. elegans* model (rIIS) is not a good one for understanding healthy aging.

The conclusions reached by Bansal, et al. are surprising because in *C. elegans* rIIS has been determined to enhance many parameters relevant to human health, including learning and memory, other aspects of neuronal function, immunity, and oocyte quality (reviewed in [1,37]). More recently, rIIS was shown to reduce dramatically the incidence of age-associated vulval disintegrity, an indicator of failing health that has typically been excluded from lifespan analyses [35]. Importantly, rIIS has also been extensively documented to retard age-related physiological decay and pathologies in *Drosophila* and mice [1,3,39]. In *Drosophila*, rIIS ameliorates age-related locomotor decline, sleep-fragmentation and heart failure, while in mice rIIS protects against glucometabolic dysfunction, improves biomarkers of skin, bone, immune and locomotor aging, and ameliorates behavioral abnormalities, neuroinflammation and protein aggregation associated with neurodegeneration (reviewed in [39]). Thus, while the results of Bansal et al. [36] called into question the notion that lessons learned from *C. elegans* rIIS may ultimately be applicable to healthy aging in humans, studies in more complex organisms support the relevance of this pathway. Moreover, two recent *C. elegans* studies suggest that much of the movement-related “gerospan” described in rIIS animals indeed may not reflect a relative increase in poor health.

A recent longitudinal analysis of locomotor behaviors determined that, when compared to wild type, *daf-2(e1370)*(class 2), *daf-2(e1368)*(class 1), and *daf-2(RNAi)* animals exhibited an increased fraction of later life spent in immobility, a state termed “decrepitude” [38]. The proportion of life spent in decrepitude was greater in the class 2 *daf-2* mutant *e1370* than in *daf-2(e1368)* or *daf-2(RNAi)* animals. This study also comprehensively investigated the capacity of *daf-2* animals for resistance to bacterial infection, a major cause of *C. elegans* death [38]. Worms that were colonized with bacteria on day 9 were found to have a significantly reduced lifespan [38], an observation that was also reported independently [40]. Importantly, when wild type animals were rendered colonization-resistant by feeding with dead or non-pathogenic bacterial food, they lived longer overall and spent the same proportion of life in “decrepitude” as *daf-2(e1368)*, although for technical reasons this analysis was problematic in the class 2 mutant analyzed by Bansal, et al. (*daf-2(e1370)*) [38]. Thus, a substantial proportion of the extended *daf-2* “gerospan” described by Bansal, et al., [36] can be explained by a greater number of those animals surviving into old age because they are resistant to infection.

A study that analyzed *C. elegans* movement using a different method revealed another explanation for the apparent gerospan of *daf-2(e1370)* [37]. This study analyzed the maximum velocity (MV) of spontaneous movements in the absence of bacterial food [37], in contrast to the more traditional assays performed by Bansal, et al., in which food was present [36]. Importantly, MV in the absence of food scaled proportionally with lifespan in both wild type and *daf-2(e1370)* animals [37]. *daf-2(e1370)* mutants were found to move less in the presence of food because they express higher levels of the odorant receptor ODR-10, and may be hypersensitive to food signals. When *odr-10* was downregulated, *daf-2(e1370)* but not wild type animals increased their average movement speed [37]. This suggests that *daf-2(e1370)* moves less when examined in the presence of food not because it

is unhealthy, but simply because it is less driven to seek food [37]. It will now be interesting to assess the effects of *odr-10* on *daf-2(e1370)* movement over the course of its lifespan, as well as on movement-related “gerospan”. This study [37] also re-analyzed data from Bansal, et al. and Huang, et al. in a way that assessed health parameters over time rather than defining a period of health- or gerospan, and concluded that by this metric *daf-2* mutants indeed enjoy an enhanced quality of life.

Finally, as was described above, evidence suggests that the proportionally reduced motility seen in class 2 *daf-2* mutants like *daf-2(e1370)* may be a dauer-related trait, not unhealthy old age [8]. This decreased motility has been demonstrated to be both temperature-dependent and reversible [10], and our analysis suggested that it can be separated from lifespan extension [8]. For example, at day 15 of life *daf-2(e1370)* is considerably more active at 15°C (no dauer traits) than at 20°C, even though the degree of lifespan extension is comparable at both temperatures (Table 1). An intriguing speculation is that elevated *odr-10* expression [37] might be a dauer-associated trait, a finding that would tie together these two models. To address the potential effects of dauer on the apparent “gerospan” of *daf-2* mutants conclusively will require comprehensive longitudinal healthspan analyses under different rIIS conditions, particularly studies of class 2 mutants at different temperatures. Clearly more should be done to investigate parameters associated with *C. elegans* health, and in the long run this will be good for the field. However, it is important to emphasize that the bulk of current evidence indicates that *C. elegans* rIIS remains a powerful and broadly relevant model for studying healthy aging.

Conclusions

It has been 24 years since the relationship between the DAF-2/IIS pathway and lifespan was first reported [6], yet this pathway continues to yield surprises. The early question as to whether rIIS lifespan extension was mediated through dauer-specific mechanisms turned out to have two answers. Yes, in that the conditions under which this pathway has most frequently been analyzed have involved contributions from the dauer pathway, and lifespan extension that was largely *skn-1*-independent. The question can also be answered with No, however, in that rIIS can extend lifespan equally robustly under dauer-independent conditions in which the animals lack even mild dauer-related traits. The latter conditions involve a genetically distinct program that is distinguished by its dependence upon *skn-1* and certain ECM genes. These findings indicate that we have much to learn about how rIIS extends lifespan, since none of the many studies that have examined downstream rIIS effects and longevity-associated mechanisms have done so under dauer-independent conditions. Additionally, an understanding of these differences is likely to shed light on the recent discussions concerning *C. elegans* longevity and healthspan, and may provide further support for the idea that long-lived animals with reduced IIS enjoy an enhancement of health that is at least proportional to their extended lifespan. Distinguishing the two rIIS longevity programs should continue to provide unexpected insights into how aging can be delayed in *C. elegans*, and may yield new paradigms applicable to more complex organisms.

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Figure 1. *C. elegans* hermaphrodite life cycle

The reproductive life cycle includes four larval stages, each separated by a molt. Unfavorable conditions (high temperature, crowding, low food availability) favor development into dauer, a diapause state that is characterized by relative immobility, decreased body and organ size, and a distinct cuticle [9,10]. Dauer represents an alternative L3 stage, but the decision to enter dauer is made late during L1. Dauer larvae survive without food for many weeks (2–3 months[10,12]), and are highly resistant to environmental stress. When conditions improve, dauers reenter the reproductive life cycle by developing into L4 larvae. Mutations that reduce IIS predispose to dauer entry even under favorable conditions [9,10].

Figure 2. Simplified model of the IIS pathway that indicates some processes regulated by rIIS in *C. elegans*

Under well-fed conditions, Insulin/IGF-1 like peptides bind to the Insulin/IGF-1 receptor (DAF-2), initiating a downstream kinase cascade via AGE-1 (phosphoinositide 3-kinase) in which Phosphatidylinositol 2-phosphate phosphorylation (PIP2 to PIP3) activates PDK-1 (3-phosphoinositide-dependent kinase 1). This in turn activates AKT-1/2, which phosphorylates DAF-16 (FOXO transcription factor) and SKN-1 (Nrf1,2,3 transcription factor), thereby inhibiting their nuclear translocation and target gene transcription initiation. DAF-18 is the ortholog of the human PTEN phosphatase. SKN-1 is discussed later in this manuscript.

Processes that are regulated by both DAF-16 and SKN-1 are shown in purple. For details, see references [1,7,8,12].

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Figure 3. Phenotypes resulting from by *daf-2* mutation and RNAi

Knockdown of *daf-2* by RNAi results in stress-resistant and long-lived animals over a wide range of temperatures (15°C–25°C), but does not induce dauer development. The *daf-2* class 1 mutants will additionally form dauers when placed as larvae at higher temperatures (>20°C), but do not show dauer-associated phenotypes during adulthood. Only *daf-2* class 2 mutants will display adulthood dauer-associated phenotypes at higher temperatures (>20°C), in addition to being stress-resistant and long-lived at high or low temperatures.

rIIS longevity and dauer-related phenotypes

Table 1

Representative data from Ewald et al. are summarized [8]. Percent lifespan extension indicates the increase in mean lifespan compared to wild type. The extent to which lifespan extension was *skn-1*-dependent is indicated in parentheses. Across these and other experimental conditions, *skn-1*-independent lifespan extension correlated with the presence of dauer traits during adulthood. Additional evidence supporting the idea of distinct dauer-predisposed and dauer-independent rIIS longevity programs is described in the text, and can be found in Ewald et al. [8].

Genotype	Dauer		Lifespan extension (<i>skn-1</i> -dependency)		
	Developmental entry (25°C)	Adult dauer-traits (20°C)	15°C	20°C	25°C
class 1 mutants (<i>daf-2(e1368)</i>)	Yes	No	34% (full)	46% (partial)	ND
class 2 mutants (<i>daf-2(e1370)</i>)	Yes	Yes	50% (full)	53% (no)	ND
<i>daf-2</i> RNAi	No	No	56% (full)	62% (full)	64% (full)

ND = not determined