

THE GENETICS OF AGING

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■ **Abstract** The genetic analysis of life span has only begun in mammals, invertebrates, such as *Caenorhabditis elegans* and *Drosophila*, and yeast. Even at this primitive stage of the genetic analysis of aging, the physiological observations that rate of metabolism is intimately tied to life span is supported. In many examples from mice to worms to flies to yeast, genetic variants that affect life span also modify metabolism. Insulin signaling regulates life span coordinately with reproduction, metabolism, and free radical protective gene regulation in *C. elegans*. This may be related to the findings that caloric restriction also regulates mammalian aging, perhaps via the modulation of insulin-like signaling pathways. The nervous system has been implicated as a key tissue where insulin-like signaling and free radical protective pathways regulate life span in *C. elegans* and *Drosophila*. Genes that determine the life span could act in neuroendocrine cells in diverse animals. The involvement of insulin-like hormones suggests that the plasticity in life spans evident in animal phylogeny may be due to variation in the timing of release of hormones that control vitality and mortality as well as variation in the response to those hormones. Pedigree analysis of human aging may reveal variations in the orthologs of the insulin pathway genes and coupled pathways that regulate invertebrate aging. Thus, genetic approaches may identify a set of circuits that was established in ancestral metazoans to regulate their longevity.

INTRODUCTION

Life spans have a remarkable range between species, approaching 1,000,000-fold across all phyla and 10- to 50-fold within groups of the same grade of organization (13, 26, 28). In mammals, bowhead whales hold the record with a life span of more than 200 years, according to estimates from racemization of lens proteins (28, 33). Laboratory rodents are atypically short-lived at 2–3 years, by comparison with many other rodents of similar size that live 5–10 years (13, 26, 28). Even the simplest invertebrates and plants have similar major species differences. Immortality

of some cell lineages is possible, as evident in the endless chain of being that has emanated from the germlines of eukaryotes over the past billion years. Clearly, cells can produce viable progeny *ad infinitum*, but in the soma, a program of senescence has evolved that is highly plastic and enables the massive variation in animal life spans evident in phylogeny.

The regulatory tool kit that evolved a billion years ago in eukaryotes allowed the construction of organisms with virtually any life span. Specification of the life span may be sought at the level of the physiological architecture (31), which determines the degree of somatic maintenance through cellular protection, replacement, and regeneration as well as the risks associated with reproduction (55, 93).

Although no one can doubt that these species differences in life span are a result of genetic sequence differences, it is instructive that social insects use the same genome to build adults with very different life spans, e.g., honey bee queens live up to five years, whereas workers born in the summer live only a few months. Interestingly, the long-lived queen is extraordinarily reproductive, producing a brood that can number in the millions, while eating copiously (26). This challenges the general rule that reproduction and high metabolism are anticorrelated with longevity, as well as broad generalizations about caloric restriction. Evidently, there are programs that can decouple high metabolism from short life span.

Recent developments on the genetics of aging can be seen as several streams of effort. In general, humans show a relatively modest (<50%) heritability of life spans (results obtained from twin studies discussed below). The apoE polymorphisms are remarkable for their influence on both cardiovascular disease and Alzheimer disease. In contrast, rare mutant genes with high penetrance cause these same diseases but with early onset and a major shortening of the life span. Short-lived laboratory models (fruit flies, nematodes, mice) are yielding rapid advances, with the discovery of mutants that increase life spans in association with altered metabolism, which leads to questions on the physiological organization of aging processes. Although these early findings do not show that a conserved genetic program actually controls aging processes across animal phylogeny, it is striking how frequently findings of metabolic rate, insulin signaling, and free radicals have emerged from very different approaches to aging in nematodes and mammals, for example. These findings hint that the genetic control of life span was already developed in the common ancestor of modern animals so that subsequent evolution of life spans was mediated by quantitative changes in the control of metabolism through insulin and the production of free radicals.

GENETICS OF LIFE SPAN IN HUMANS

Most studies of human twins agree that the heritability of life span is less than 50% (45, 68). Of particular interest is an ongoing study of aging in Swedish twins that includes a large group of adopted twins who were reared separately. Ljungquist et al. (68) concluded that “a maximum of one-third the variance in integrated mortality risk is attributable to genetic factors and that almost all of the remaining variance

is due to nonshared, individually unique environmental factors.” Moreover, this heritability declined with age and was negligible after the age of 85 in men and 90 in women.

Nonetheless, some strong familial trends for great longevity are found (40, 44 88). For example, siblings of centenarians have a fourfold greater survival to >85 years of age than sibs of those who died by age 73 (88). In Iceland, familial effects on survival to the 95th percentile decrease monotonically with meiotic distance, either within the same generation (sibs and cousins) or between generations (children and grandchildren); the data do not distinguish between the models of a few genes enhancing longevity versus multiple genes with additive (independent) effects (40). Linkage analysis of the centenarian phenotype (L. Kunkel & T. Perls, personal communication) should soon identify probable long-lived offspring of centenarians. The presence of rare genes that favor longevity is, of course, not inconsistent with its modest heritability in the general population.

Only one gene, apolipoprotein E (apoE), is showing indications of having general effects on longevity. Centenarians, as first reported by Schachter et al. (95), tend to show a higher prevalence of the apoE e2 allele, relative to e4. More generally, the apoE e4 allele is remarkable, as its presence is the major susceptibility factor for elevated blood cholesterol, coronary artery disease, and Alzheimer disease (but not for cancer or diabetes). Although allele dose susceptibility varies between populations, and is not significant in some (19, 101, 104), no other public allele has yet approached this level of impact on the pathology of aging. By the age of 90, the risk of Alzheimer disease from apoE e4 reaches a plateau (72). Some e4/e4 centenarians are cognitively normal—we do not know if this is owing to protective effects of other genes or aspects of good luck. A new calculation of mortality risk in Danish centenarians showed that, relative to the apoE e3 allele, the mortality risk from e4 from age 40 to 100 was ~12% higher, whereas that of the rarer e2 allele was 8% lower (35). This differential mortality results in the progressive enrichment of e2 at the expense of e4, so that by 100 years, ~40% of survivors are apoE e2. Gerdes et al. (35) argue that apoE should be considered as a frailty gene rather than a longevity gene. Other frailty (or longevity) gene candidates include MHC haplotypes, methylenetetrahydrofolate reductase, and angiotensin converting enzyme (32, 44, 95).

We briefly comment on rare mutations that shorten life span through the early onset of diseases that are increasingly common during aging in the general population, e.g., familial forms of Alzheimer, breast cancer, coronary artery disease, type II diabetes, etc. The later onset forms of these diseases are associated with causes of death at later ages. A major question is what role the more common allelic variants of these same genes have in “normal aging”. Although examination of this huge emerging topic goes beyond the present discussion, we may consider the example of Werner’s syndrome, a rare autosomal recessive that causes adult onset progeria with a high incidence of cancer and atherosclerosis (70). The absence of Alzheimer-type dementia in Werner’s syndrome illustrates the “segmental” nature of this and other progerias (70). Thus, heritable shortening of life span should not be considered as a simple acceleration of general aging processes.

The Werner's lesion maps to a defective gene encoding a helicase and exonuclease, which also has several polymorphisms. In Japan, 1367Arg was associated with a lower risk of myocardial infarction (70), although it was not associated with longevity in Finland (14). In general, we know little of the genetic factors involved in frailty and morbidity at later ages, which are important to the gene-environment interactions implied in the major longevity increase seen during the twentieth century.

The heritability of menopause is of potential importance to longevity in women because estrogen deficits after menopause are strongly associated with osteoporotic fractures, which, in turn, increase mortality risk. (Less resolved and more controversial are associations of estrogen deficits/replacements with cardiovascular disease and Alzheimer disease.) From twin studies, the heritability of age at menopause is determined to be ~30%–60% (20, 102). Approximately 20% of monozygous twins differ in age at menopause by 5 or more years (102), which may be attributed to chance events during development that lead to differing numbers of ovarian oocytes (29). Genetic effects on menopause could include influences on the initial oocyte pool and its rate of loss through atresia during aging, as well as on hypothalamic controls that may be responsive to environmental effects, such as stress and nutrition (31, 117). The age of menarche and menopause are not correlated in twins (102), suggesting that these are governed by distinct neural and endocrine mechanisms.

Another association of ovarian functions with longevity is that female centenarians (1986 birth cohort) had a fourfold greater likelihood of having children after the age of 40 versus those who died by the age of 73 (87). On the other hand, premature ovarian failure before 40 sharply increases mortality (52) and osteoporotic fractures (29). Environmental factors that are implied in the major increases of life span during the twentieth century have not modified the age at menopause as far as is known, e.g., the 30-year increase in the life expectancy of women in developed countries during the twentieth century was not associated with increased fertility after age 50 (9).

Consistent with the findings that indicate neuroendocrine regulation of reproductive life span, one of the unifying features noted by Perls in his pedigrees of centenarians is an optimistic attitude (88a). Although it is hardly a quantitative trait at this point, this observation suggests that mood may be co-regulated with life span. For example, a neuroendocrine signal that triggers the slowing of the life span may also regulate feelings of satiety and satisfaction. On the other hand, optimism may also be the result of surviving longer than anyone else. Still, longevity determination may occur at a point above simple metabolic and reproduction regulation, and closer to the pacemakers of general mood.

OTHER MAMMALS

Whereas most mutations in mammals shorten the life span, a few in mice have the opposite effect. The best documented are those associated with dwarfism at three different loci that increased life span by 30%–75%, depending on strain and

gender. The Ames dwarf (*df/df* at *Prop-1*) and Snell dwarf (*dw/dw* at *Pit-1*) have growth deficiencies owing to the absence of pituitary growth hormone (GH) and thyroid stimulating hormone (TSH) (6, 11, 75). Dwarfism can also result from dysfunctional GH receptors (*GHR/BP*) (16). GH deficiency causes chronically lower blood levels of glucose and insulin-like growth factor-1 (IGF-1), as in caloric restriction (6, 16). However, unlike caloric restricted mice, pituitary dwarfs become obese during middle age (77). As discussed below, one of the *C. elegans* mutants with increased longevity has sequence similarities to the insulin receptor, suggesting some common features of life span regulation between mammals and invertebrates.

Inverse correlations of size and longevity of about 0.6–0.7 were found post hoc in two other paradigms of size selection. Mice selected for different postnatal growth rates proved to have 2.7-fold differences in life spans (11–31 months), a range that was larger than the twofold difference in adult size (76). Similarly, domestic dogs have a 35-fold range in body sizes (chihuahua to wolfhound) that differ by up to 4 years in life span (75). Although some breeds differ in IGF-1 production, the hormones that regulate this variation are not fully characterized (22a). Because artificial selection for body or organ size often exceeds the initial extreme value of a quantitative trait (24), greater size selection may further extend longevity. Curiously, these intraspecies differences are opposite to the classic allometric relationships in which body size is positively associated with greater longevity in several vertebrate orders (26).

Several mutations in genes that modulate apoptosis also influence life span. Ablations of *p66shc* showed gene dose effects on resistance to oxidative stress and also increased life span by up to 30% (74). (*p66shc* is a splice variant of *p52shc/p46shc*, which binds Grb2 and may be involved in Ras activation.) Another longevity gene candidate is Werner's syndrome *WRN*. The *WRN* protein enhances transcription of *p53*, which activates an apoptotic pathway in response to DNA damage and which is attenuated in Werner's cells (8). A helicase-null mouse appears normal, with no signs of progeria during life spans of at least two years, although it is unclear if this is the Werner's gene (69).

To identify other gene candidates in the laboratory mouse genome, several groups are studying gene influences on life span in common mouse strains by analysis of quantitative trait loci (QTL analysis). De Haan & Van Zant (18) have found a region of chromosome 11 with QTLs that influence life span, which overlapped with a QTL associated with the rate of cell cycling in hematopoietic progenitor cells. The short-lived DBA/2 (mean of 592 days) had a three-fold higher cell cycling rate than the somewhat longer-lived C57BL/6 (765 days), which those authors discuss as a possible in vivo example of the Hayflick limit of in vitro replicative potential in relation to organismal longevity. Concurrent with these studies of recombinant inbred mice, Miller and colleagues (76) are examining two of these strains in fourway crosses: (BALB/c × C57BL/6)F1 dams and (C3H × DBA/2)F1 sires. The progeny of this cross are genetically equivalent to sibs in that each has a random sample of half of its alleles with the others. Several segregating loci distinct from those above show large effects on life span, diseases,

and biomarkers of aging. Preliminary analysis indicates complex pleiotropic effects with gender differences on different spontaneous diseases of aging (77). Of course, the genetic diversity available in inbred mouse strains may underrepresent the rates in natural populations (see studies on *Drosophila* below).

INSULIN CONTROL OF *C. ELEGANS* METABOLISM, DEVELOPMENT, AND LONGEVITY

During the last decade, major progress on the genetics of life span has been realized through the study of long-lived mutants identified in the nematode *C. elegans*. The biochemical functions of many of these genes is now known, and because they are related to processes (e.g., metabolism, free radical production) implicated in aging of vertebrates, they have potential general significance to aging. The clearest example of such a biochemical convergence is the finding that an insulin-like signaling pathway regulates longevity and metabolism in *C. elegans* (54). *daf-2*, *age-1*, and *pdk-1* mutants (see below) that constitute components of the *C. elegans* insulin signaling pathway live 2 to 3 times longer than wild type (21, 53, 63) (Figure 1).

Insulin-like signaling controls *C. elegans* lifespan

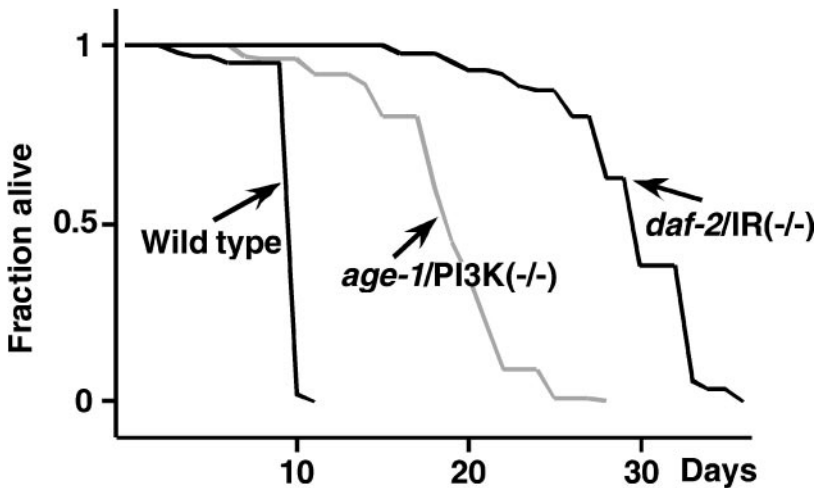


Figure 1 Regulation of life span by the *daf-2* insulin signaling pathway. *C. elegans* animals bearing reduction of function mutations in the insulin-like receptor gene *daf-2* or the downstream phosphatidylinositol kinase 3 gene *age-1* live much longer than wild type.

This insulin-like signaling pathway is part of a global endocrine system that controls whether the animals grow reproductively or arrest at the dauer diapause stage.

Dauer arrest is normally regulated by a combination of a high dauer pheromone (an unidentified fatty acid), high temperature, and low bacterial food (36). Genes that regulate the function of this neuroendocrine pathway were identified by two general classes of mutants: dauer defective and dauer constitutive mutants (36, 37, 96, 110). For example, *daf-2* dauer constitutive mutant animals form dauers in the absence of high pheromone levels. Conversely, *daf-16* dauer defective mutants do not form dauers under normal dauer pheromone induction conditions, and they suppress the dauer constitutive phenotype induced by *daf-2* mutations (37). Based on genetic epistasis and synergistic interactions, most of the dauer defective and dauer constitutive genes have been ordered into a genetic pathway (Figure 1). The most important conclusion from this genetic analysis is that the *daf* genes constitute multiple parallel signaling pathways that converge to regulate *C. elegans* diapause. The *daf-2/age-1/pdk-1/daf-18/akt-1/akt-2/daf-16* subpathway corresponds to an insulin-like signaling pathway (54, 79, 82, 83, 84, 84a) (Figure 2), and the *daf-7/daf-1/daf-4/daf-8/daf-14/daf-3* subpathway corresponds to a TGF-beta-like neuroendocrine signaling pathway (23, 34, 86, 89). Even though these pathways conspire to regulate metabolism and dauer arrest, only the *daf-2* insulin-like

Genetic pathways for dauer arrest

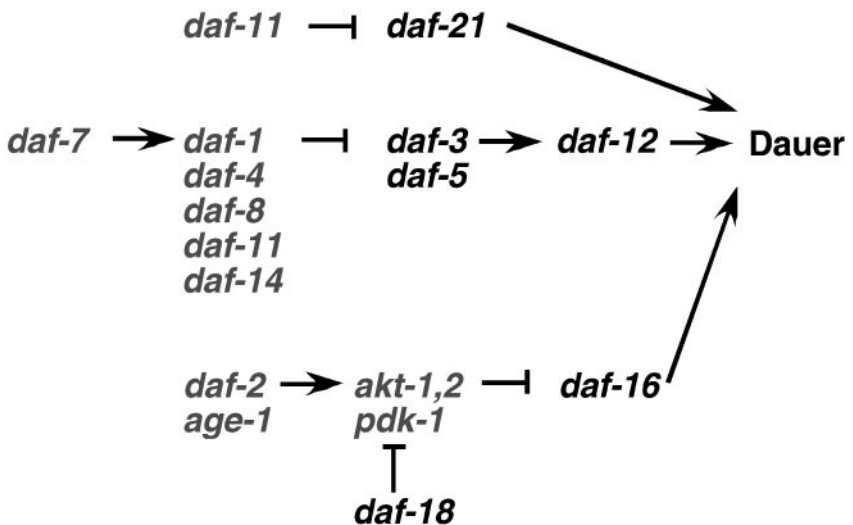


Figure 2 The genetic pathway that regulates dauer arrest and longevity in *C. elegans*.

pathway has effects on the longevity of reproductively growing adults. This is surprising because *daf-7* pathway dauers have increased longevity.

daf-2 encodes the worm ortholog of the insulin/IGF-I receptor gene and is necessary for reproductive development and metabolism (54). A large number of *C. elegans* insulin superfamily genes have been found (88b). Many members of the *C. elegans ins* gene family are organized into clusters, suggesting that these genes arose relatively recently by gene duplication. Clusters of linked insulin superfamily genes are also found in humans and *Drosophila* (see below). GFP fusions to the regulatory regions for 14 of the *ins* genes have shown that they are expressed primarily in subsets of sensory neurons, but also in tissues, such as the intestine and somatic gonad (88b). The INS-1 protein is the most closely related to human insulin. High gene dosage of *ins-1* acts antagonistically to DAF-2, enhancing dauer arrest in wild type or a weak *daf-2* mutant. Human insulin expressed using the *ins-1* regulatory region also antagonizes DAF-2 (88b). Although it is surprising that insulin, an agonist of its receptor, is an antagonist of the worm insulin receptor ortholog, it is important that these insulins engage this pathway. Because all of the INS proteins are predicted to adopt a similar tertiary structure, they may all bind to DAF-2, the only member of this receptor superfamily in the worm genome. Some INS proteins may be DAF-2 agonists, whereas others may be antagonists.

age-1 acts at the same point in the genetic pathway as *daf-2* and encodes the worm ortholog of mammalian phosphatidylinositol 3-kinase (PI 3-kinase) p110 catalytic subunit (37, 79). Reduction of function mutations in *age-1* cause a twofold increase in life span (79). PI 3-kinases generate a membrane-localized signaling molecule, phosphatidylinositol inositol phosphate P3 (PIP3), which binds to the pleckstrin homology domain of mammalian Akt/PKB, which are required for PIP3 activation (1). There are two Akt/PKB orthologs in *C. elegans*. Simultaneous inhibition of both *akt-1* and *akt-2* activities using the technique of RNA interference causes nearly 100% arrest at the dauer stage, whereas inactivation of either gene alone does not (84). One of the kinases that phosphorylates Akt/PKB and is required for its activation is 3-phosphoinositide-dependent kinase-1 (PDK1) (2). A loss of function mutation in *pdk-1* increases *C. elegans* life span almost twofold, similar to a mutation in *age-1* (84).

The dauer arrest, fat accumulation, and longevity phenotypes of *daf-2* and *age-1* mutants are suppressed by *daf-18* mutations. *daf-18* encodes the *C. elegans* ortholog of mammalian PTEN lipid phosphatase gene (83). By genetic epistasis experiments, *daf-18* was shown to act downstream of the AGE-1 PI3K, but upstream of AKT-1 and AKT-2, in this signaling cascade. The DAF-18 lipid phosphatase may normally decrease the level of PIP3 signals, perhaps to insulate signals that emanate from the DAF-2/AGE-1 signaling complex from other PIP3 signals in the cell or to resolve insulin-like signaling episodes. Genetic analysis has not revealed whether DAF-18/PTEN activity is regulated during insulin-like or other signaling.

Mutations in *daf-16* also completely suppress the dauer arrest and metabolic shift of animals bearing *daf-2*, *age-1*, or *pdk-1* mutations or RNAi inhibited *akt-1* and *akt-2* activity (37, 82, 84, 84a). *daf-16* mutations also suppress the increase

in longevity caused by decreased *daf-2*, *age-1*, or *pdk-1* signaling (21, 63, 84, 84a, 111a). Thus, DAF-16 is active in the absence of these upstream inputs and acts to increase life span. *daf-16* encodes two proteins with forkhead DNA binding domains. The mammalian orthologs to DAF-16 are human FKHR, FKHL1, and AFX (82). DAF-16 contains four consensus sites for phosphorylation by Akt/PKB, and three of these sites are conserved in the human DAF-16 homologs AFX, FKHR, and FKHL1. Mammalian FKHR, FKHL1, and AFX activities are regulated by AKT phosphorylation; these transcription factors are nuclearly localized only when insulin-like signaling (AKT activity) is low (22, 38, 41, 42, 80, 90, 98, 107, 112). Consistent with the activity of *C. elegans akt-1* and *akt-2* that lie upstream of and inhibit *daf-16* activity, mutation of those Akt sites in FKHL1 cause nuclear localization in the presence of insulin or IGF-I signaling (22, 41, 80, 90, 107). The nuclear localization of a functional DAF-16/GFP fusion protein is similarly controlled by upstream pathway activity (R. Lee & G. Ruvkun, personal communication) (Figure 3; Table 1).

The mammalian DAF-16 orthologs regulate the expression of target genes, such as the metabolic genes PEPCK and glucose 6 phosphatase (22, 38, 41, 42, 80, 90, 98, 107, 112). DAF-16 binds to this same insulin response sequence in vitro (81). *C. elegans* DAF-16 may regulate the *C. elegans* homologs of these and other metabolic genes.

The molecular genetic pathway suggests that DAF-2, AGE-1, DAF-18, AKT-1, AKT-2, and DAF-16 act in the same cells to regulate *C. elegans* metabolism and longevity. Under reproductive growth conditions, high DAF-2 receptor signaling activates AGE-1 and PDK-1 to, in turn, activate the AKT-1 and AKT-2 kinases, which negatively regulate DAF-16 activity. Phosphorylated DAF-16 is excluded from the nucleus and, therefore, does not activate the genes necessary for dauer

TABLE 1

Gene	Reduction of function phenotype	Ortholog
<i>daf-2</i>	Long life span, dauer arrest	Insulin/IGF receptor
<i>age-1</i>	Long life span, dauer arrest	Phosphatidylinositol 3-kinase
<i>pdk-1</i>	Long life span, dauer arrest	PDK1 kinase
<i>akt-1, akt-2</i>	? life span, dauer arrest	AKT/PKB kinase
<i>daf-18</i>	Short life span, dauer defective	PTEN lipid phosphatase
<i>daf-16</i>	Short life span, dauer defective	FKHR, FKHL1, AFX transcription factors
<i>daf-7</i>	Normal life span, dauer arrest	GDF-8 (myostatin), GDF-11
<i>daf-3</i>	Normal life span, dauer defective	DPC-4 Smad protein
<i>daf-11</i>	Normal life span, dauer arrest	Guanyl cyclase
Cilia mutants	Slightly longer life span, dauer defective	Kinesin, etc.

arrest and long life span or repress the genes that inhibit reproductive growth and short life span. Under dauer inducing conditions, these kinase cascades are inactive, and DAF-16 is active and nuclear. Active DAF-16 represses genes required for reproductive growth and short life span, and/or it activates genes necessary for dauer arrest and long life span.

The *C. elegans* insulin pathway regulates the expression of key free radical detoxifying enzymes, consistent with free radical theories of aging. *ctl-1* catalase and *sod-3* Mn superoxide dismutase genes are expressed at higher levels in a *daf-2* mutant than in a *daf-2; daf-16* double mutant (47, 62, 108). These enzymes convert the toxic superoxide radicals and peroxides to less reactive products. Supporting an important function for this regulation, a mutation in *ctl-1* reverses the longevity increase of a *daf-2* mutant (108) (Figure 4).

The *C. elegans* insulin-like signaling pathway regulates metabolism as well as free radical protection. These mutants accumulate much larger stores of fat (54). In addition, the rate of CO₂ production, a measure of metabolic rate, is reduced in a *daf-2* mutant to ~30% that of wild type (114). The fat accumulation and the decline in metabolic rate as well as the longevity increase are fully suppressed by a mutation in *daf-16* (82, 114). Similar but less severe declines in metabolism are observed

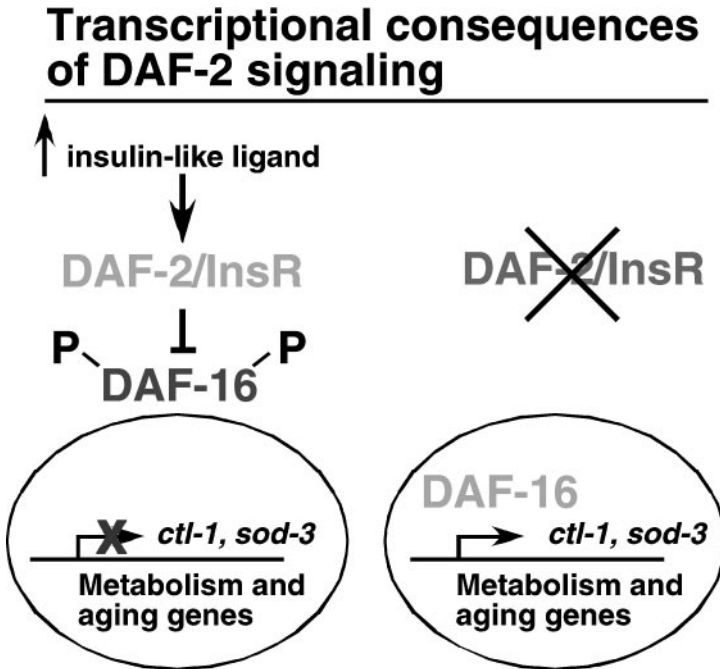


Figure 4 DAF-2 signaling to DAF-16 regulates the transcription of free radical protective enzyme genes. Regulation of free radical protective enzyme genes by the DAF-16 transcription factor.

with a weak *age-1* allele (114). The decline in metabolic rate of insulin-like signaling pathway mutants is supported by a 30% decline in the rate of egg laying and in total fecundity of *daf-2* and *age-1* mutants (111a, 114). Conflicting data regarding the metabolic control by *daf-2* pathway mutants also exists; however, because the other studies used a free radical surrogate measure of metabolism rate and because they were done under nonoptimal growth conditions, they must be viewed with caution (113).

THE CONNECTION TO CALORIC RESTRICTION

The increase in longevity associated with decreased DAF-2 signaling is analogous to mammalian longevity increases associated with caloric restriction (103). The involvement of an insulin signaling pathway in worm aging may be mechanistically related to the longevity increase caused by caloric restriction in mammals (26, 103). Insulin secretion by the pancreas is regulated by nutritional and autonomic neural inputs, and this endocrine signal of metabolic status is detected by target tissues to regulate the activities of metabolic enzymes that synthesize or breakdown glucose, amino acids, fat, etc. Like *daf-2* mutants, life span is dramatically increased in dwarf mice with defects in growth hormone signaling and with decreased IGF-I signaling (see above).

Mammalian orthologs of the *C. elegans* insulin signaling pathway may be components of a mammalian longevity determining pathway. Caloric restriction in mammals may cause a decline in insulin-like signaling that induces a partial diapause state (109), like that induced in weak *daf-2* and *age-1* mutants. The induction of diapause-like states, or changes in the mode and tempo of metabolism itself, may affect postreproductive longevity (109), as in *C. elegans*. This association of metabolic rate with longevity is also consistent with the correlation of free radical generation to aging (103).

Life span of *C. elegans* is also coupled to the rate of feeding (61, 105). Perhaps like caloric restriction, some *eat* mutants that ingest bacteria less efficiently than wild type live up to 50% longer. The increase in life span of the *eat* mutants is not fully suppressed by *daf-16* mutations, suggesting that the caloric restriction pathway engages some pathway besides the *daf-2* insulin-like signaling pathway (61).

THE AGING TRANSCRIPTOME

Given that the major output of worm insulin-like signaling is transcriptional and the apparent homology between life span extension by caloric restriction in mammals and *daf-2* pathway mutations in *C. elegans*, the monitoring of the changes in gene expression in calorically restricted animals seems especially attractive. This has been done in mouse skeletal muscle, which shows declines in mass and other

functions as animals age. Comparison of one muscle between 5-month-old and 30-month-old adult mice revealed a more than twofold change in 113 of 6347 genes (65). These genes could be classified into three major groups: stress response, energy metabolism, and neuronal signaling. When muscle gene expression was compared between calorically restricted animals, 30% of the 113 major aging response genes no longer showed the twofold or greater change during the aging period, suggesting that they are coupled to the aging process and its modulation by caloric restriction. Caloric restriction also caused an induction (1.8 fold or greater) of 51 and repression (1.6 fold or more) of 57 muscle genes in 30-month-old mice that were not induced in well-fed 30-month-old mice. These genes were also classified into energy metabolism, protein synthesis, and stress response pathways. Many genes not yet represented in EST databases are not present on current gene arrays.

Thus, approximately 1% of the genome that was tested (10% to 30% of total mouse genes, depending on how many genes there are in a mouse) is transcriptionally regulated in a particular tissue during aging. The induction of a stress response pathway could be a result of the accumulated damaged macromolecules, as predicted from the involvement of free radicals in aging processes. The induction of neuronal growth factor signaling pathways could be the response to a decline in muscle targets. The decrease in energy metabolism gene expression is consistent with the known decline in mitochondrial function during aging.

Global gene array searches based on whole organ RNA may be encyclopedic in their documentation of gene expression changes. However, cause and effect in aging can only be resolved by perturbing life spans by varying the expression or activity of these candidate genes. For example, the detection of mutations in these genes in pedigrees with life span variation, or engineering changes in life span by disrupting or misexpressing key regulatory genes, will probably prove valuable in identifying causal chains and, ultimately, possible primary causes of aging. In addition, without determining the exact cell types that regulate aging, the monitoring of gene expression in a particular tissue could miss the regulators. For example, analyses in *C. elegans* have revealed that aging of the entire animal is controlled hormonally from the nervous system (see below). Merely monitoring gene expression in aging muscle would not identify such triggers (though the response pathway could emerge if its transcription was modulated). Thus, the gene screens are the first stage in an exacting process, similar to that begun long ago by developmental biologists that has led to the present molecular understanding of cell fate determination and organogenesis.

NEURONAL CONTROL OF AGING

Insulin-like signaling may directly regulate metabolism and free radical production in aging skin and muscle or in signaling centers that then coordinately control the senescence of the entire organism. *pdk-1*, *daf-18*, *akt-1*, and *daf-16* are all expressed

throughout much of the animal, consistent with their function in either signaling cells or target tissues. Studies of *daf-2* genetic mosaic animals showed that *daf-2* can act nonautonomously to regulate life span but did not assign *daf-2* longevity control to particular cell types (3).

In animals that express normal insulin signaling only in the nervous system, life span is normal, whereas insulin signaling in muscles or gut does not confer normal life span. In these studies, *daf-2* pathway function was restricted to particular cell types by using distinct promoters to express *daf-2* or *age-1* in the neurons, intestine, or muscle cells of a *daf-2* or *age-1* mutant. The long life span of *daf-2* and *age-1* mutants is rescued by neuronal expression of *daf-2* or *age-1*, respectively, using a pan-neuronal promoter (118) (Figure 5). Restoration of *daf-2* pathway activity to muscles is not sufficient to rescue the long life span of *daf-2* or *age-1* mutants. Similarly, expression of *daf-2* or *age-1* in the intestine, the major site of fat storage, does not rescue life span as efficiently as neural expression of these genes.

These data argue that the key tissue where *daf-2* insulin-like signaling regulates aging is from the nervous system. Precedents for insulin signaling in the mammalian nervous system exist. Although the target tissue responses to insulin are better known, feeding and metabolic responses to insulin are also present in the mammalian brain (99). In addition, insulin receptor signaling defects in the neurosecretory beta cells of the mouse pancreas or only in the nervous system cause metabolic as well as reproductive defects, also suggestive of a role for insulin signaling in neuronal tissues (12, 59).

Neurons may be particularly sensitive to free radical damage during aging. In fact, overexpression of Cu/Zn superoxide dismutase (SOD) in just the motorneurons can extend *Drosophila* life span by 48% (85). And perhaps mechanistically related, motor neuron degeneration in amyotrophic lateral sclerosis is caused by mutations in Cu/Zn SOD (119). It is striking that aging in two different organisms can be controlled by neurons and is correlated with increased free radical protection in those neurons. If this model is correct, neuronal *daf-2* signaling regulates an organism's life span by controlling the integrity of specific neurons that secrete neuroendocrine signals, some of which may regulate the life span of target tissues in the organism (Figure 6). These results, together with those from *Drosophila*, suggest that oxidative damage to neurons may be a primary determinant of life span.

Given that insulin signaling in the *C. elegans* nervous system is implicated in the control of longevity, it is intriguing that several brain regions of aging mice show, via array technology, selective changes in mRNA (66). This study compared the neocortex (a recent mammalian invention) and the cerebellum in 5- and 30-month-old mice, which were fed to 90% and 60% of ad libitum intake. During aging, ~1% of the genes were increased (~twofold or more) and ~1% decreased (~twofold or more) in expression. These numbers are consistent with analysis of brain poly(A)mRNA population sequence diversity by hybridization kinetics, which did not detect age changes within a statistical upper limit of 5%

Models for neuronal control of lifespan

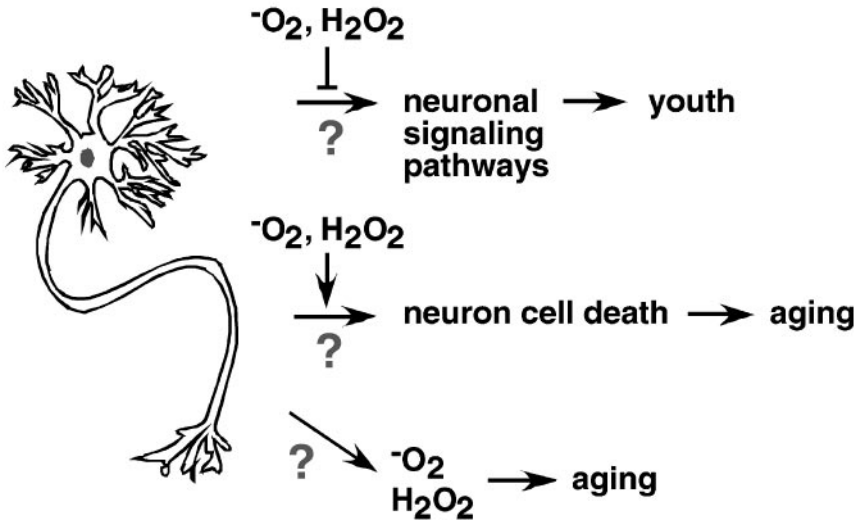


Figure 6 Models for neuronal regulation of life span. Three models for how free radicals produced by neurons could regulate whole animal aging: (a) The free radicals could compromise the production of hormonal signals produced by neurons. (b) The free radicals could cause cell death of neurons that normally send “youth” hormone signals. (c) The free radicals could be hormonal signals, analogous to NO, for example, that are received by target tissues, such as muscle and skin.

(15). Approximately 40% of the mRNAs showing increased expression were in the inflammatory and stress response pathways, whereas $\sim 15\%$ of the genes in the decreased expression class were growth factors. A subset of inflammatory mRNAs show parallel changes in both regions (30). The growth factor mRNA subset has tantalizing implications for age changes in neural plasticity, which is based on the elaboration of neurons and connections. Caloric restriction attenuated the aging responses in approximately half of those genes, with a complete attenuation of a few of them (66). In terms of the intersection between worm genetics and mammalian caloric restriction, it is noteworthy that IRS-3, a transduction component of insulin signaling, is downregulated ~ 1.7 -fold in calorically restricted mice—perhaps a decrease in insulin signaling caused by caloric restriction in turn causes a decrease in IRS-3 expression in an autocrine fashion.

For both *C. elegans* and mammals, particular neurons, rather than the entire nervous system, may prove to be key in aging. In the case of *C. elegans*, it is fairly simple to activate the insulin signaling pathway in particular neural types and then monitor life span, as was done for nervous system vs. muscle, etc. (118). Promoters

exist for restricting expression to particular classes of neurons (cholinergic, serotonergic, motor, etc.). If one class of neurons is then shown to be key for aging control, then discerning the homologous class of neuron from the mammalian brain might be possible. In such a case, gene array monitoring of the transcripts of purified or dissected neurons of this subtype over the life span of an animal might identify mRNAs critical for aging. For example, the hypothalamus is a plausible location for such regulation of the life span and life cycle because it regulates metabolism and the reproductive schedule (puberty, menopause), which are targets of selection in the evolution of life histories (31). In some examples of animals that die after first spawning (semelparous life history), reproduction and death can be postponed by neuroendocrine manipulations (27, 31). It is interesting that a worm thermoregulatory circuit bearing antagonistic warm and cold regulatory loci (single neurons) may be homologous to the vertebrate hypothalamus (46). Thus, there may be a future genetics of neuronal aging pacemakers.

But to understand aging in mammals and in worms, obtaining a set of molecular markers of the aging process, even if only in organs that may have a subsidiary physiological role, will be very helpful. These markers constitute molecular definitions of the aging process and can be used in classifying how mutants and physiological perturbations act to "reset" some, or even all, of the features of aging. Thus, gene array comparisons of *C. elegans* aging from any tissues are important, even if the trigger is in the nervous system.

Besides the *daf-2* insulin-like signaling pathway, dauer arrest is also regulated by the *daf-7/daf-1/daf-4/daf-8/daf-14/daf-3* TGF-beta signaling pathway. The signals in these two pathways are neither redundant nor sequential: Animals missing either of these two signals shift their metabolism and arrest at the dauer stage. Only mutations in the insulin-like pathway genes cause dramatic increases in longevity. This suggests that the transcriptional program for longevity does not depend on TGF-beta transcriptional cofactors in the same manner that the metabolic switch to fat storage depends on both pathways.

daf-7 encodes a TGF-beta neuroendocrine signal that is produced by the ASI neuron (89). *daf-7* expression in this neuron is inhibited by dauer-inducing pheromone (89,96). *daf-1* encodes a type I TGF-beta class ser/thr receptor kinase, *daf-4* encodes a type II TGF-beta class ser/thr receptor kinase, and *daf-8* and *daf-14* encode Smad proteins (34, 49), which couple TGF-beta signals from receptor kinases to the control of transcription. A *daf-3* null allele completely suppresses the dauer constitutive phenotype of mutations in *daf-1*, *daf-4*, *daf-7*, *daf-8*, and *daf-14* (110). Thus, mutations in *daf-3* bypass the need for any of the DAF-7 signal transduction pathway genes, suggesting that the major function of this signaling pathway is to antagonize DAF-3 gene activity. *daf-3* encodes a Smad protein that is most closely related to vertebrate DPC4, which is a cofactor for Smad1, Smad2, and Smad3 (86).

The DAF-16 Fork head and the DAF-3 Smad proteins are active in the absence of upstream signaling to induce arrest at the dauer stage and a shift to energy storage metabolism. It is interesting that the *daf-7* response pathway acts in neurons,

similar to the *daf-2* response pathway (49). DAF-16 may interact with DAF-3 on the promoters of genes that regulate metabolism and reproductive vs. dauer development.

DROSOPHILA INSULIN SIGNALING

Drosophila orthologs of the mammalian and nematode insulin receptor, insulin receptor substrate, *age-1* PI3 kinase, AKT/PKB, PTEN, and S6 kinase regulate cell, organ, and total body size (83a). Mutations in all of these genes except for PTEN cause a dramatic decrease in cell size, whereas a mutation in PTEN causes the opposite phenotype (83a). It is interesting that mutations in *Drosophila* IRS also affect metabolism, causing a dramatic increase in fat storage, similar to mutations in the *C. elegans* insulin signaling pathway. IRS mutant females also live longer than wild type, although the males do not (14a). Many reductions of function mutants in the *Drosophila* insulin pathway do not show an extension of life span, but a rare insulin receptor allele combination does cause a modest increase in life span (107a). Unlike in *C. elegans* and perhaps mammals, there may be a narrow window of decreased insulin signaling in *Drosophila* that can prolong life (14a). One interesting feature of the fly insulin receptor mutant is that the low fertility is coupled with a decrease in juvenile hormone (107a). Thus, as in the case of worm insulin regulation of life span, neuroendocrine outputs of the insulin pathway may be key. A *Drosophila daf-16* ortholog is known but has not been implicated in either the fly insulin pathway or in longevity (E. Hafen, personal communication).

Drosophila has seven insulin-like genes (DILPs) (10). Overexpression of DILP2, which has a C peptide and is most closely related to insulin, causes larger cells, larger organs, and larger animals (10). A deficiency that removes five of the DILPs (plus other genes) suppresses the large eye phenotype caused by overexpression of the wild type insulin receptor in the eye (10). The different DILPs are expressed in seven bilaterally paired cells in the brain. Neurosecretory cells that project to the ring gland where insulin should be released into the hemolymph (10) probably exist. These insulin-producing neurons may couple to the juvenile hormone-releasing cells of the fly corpus allata, a neuroendocrine organ.

One of the mammalian insulins, IGF-I and its receptor, is implicated in the control of body and organ size of this type. It is interesting that tissue specific knockout mutations of the mouse insulin receptor gene in pancreas and liver cause a decrease in postnatal growth of those organs, suggesting that both the insulin and IGF-I also feed into the regulation of cell size in mammals (59, 73). Cell size regulation was shown by genetic mosaic analyses of IRS, AKT, and S6 kinase to be autonomous in *Drosophila*.

There is no indication that the *C. elegans* insulin signaling pathway controls cell size. However, a TGF-beta signaling system that is distinct from the DAF-7 metabolic control pathway regulates body size in *C. elegans* (57). Size regulation by insulin-like signaling appears to have maintained in the fly and vertebrate lineages, but in *C. elegans*, it is served by another pathway. Thus, there is a distinction

between insulin signaling within the *C. elegans* nervous system to control aging and the autonomous regulation of cell size in *Drosophila*, and between the control of cell size by that pathway in vertebrates and one invertebrate, *Drosophila*, but not in another, *C. elegans*.

Aside from the insulin pathway, other mutations and inbred lines that increase or decrease *Drosophila* life span have been identified. For example, the *methuselah* mutation causes a 35% increase in fly life span as well as increased resistance to stress. *Methuselah* encodes a probable G protein-coupled (7 TM) receptor (67). Its ligand and mechanisms of coupling to stress resistance are unknown; an interesting possibility is that the Methuselah protein mediates the sensory inputs to the fly insulin pathway. Another locus that extends longevity is *Indy* (I'm not dead yet), which resembles a dicarboxylate transporter and is expressed in the gut and fat body (92). Moreover, *Indy* increases fecundity at later ages. Its implied role in intermediary metabolism suggests that the *Indy* mutation may induce a state similar to caloric restriction.

In one case, a gene identified by mutation recovered from a genetic screen in the laboratory, *methuselah*, may have variants in natural populations. In particular, the common ATATC haplotype has a sharp geographic (north-south) cline in U.S. populations, which, intriguingly, is associated with an 18% difference in life span (97). It would be interesting to examine these natural populations for differences in their reproductive schedule. Extensive studies show that life span can be rapidly selected as an indirect outcome of artificial selection for age at reproduction. Samples from natural populations of *Drosophila* contain genetic variants that can be rapidly selected, within 15 generations, for 50% or greater differences in life span on the basis of choosing individuals that are reproductive at early versus later ages (93). Selection was reversible, indicating that these life history variants depended on existing gene combinations not new mutations. Among the genes that differed in quantitative expression between young- and old-selected lines were heat shock proteins, e.g., *hsp 22* (60). An overarching conclusion from fly aging genetics is that stress resistance is coupled to longevity (94), as in *C. elegans*. Other gene candidates are being sought by QTL analysis and show complex interactions with gender and population density (17, 115).

SENSORY INPUT TO AGING IN *C. ELEGANS*

Dauer arrest in *C. elegans* is normally regulated by an uncharacterized dauer pheromone that is detected by sensory neurons (36). The dauer pheromone causes downregulation of *daf-7*, the TGF-beta ligand of the pathway that acts in parallel to the *daf-2* pathway (89, 96). It might also regulate the expression or release of any of the 37 worm insulins that have been detected, but this has not been established. The detection of the dauer pheromone depends on ciliated sensory endings; cGMP signaling in those sensory neurons has been implicated, suggesting that the pheromone receptor may be a 7 transmembrane receptor that couples to cGMP phosphodiesterase, as in mammalian odor sensation (7). Although no

evidence currently exists of any coupling of sensory input to *daf-2* insulin-like signaling, mutations that uncouple sensory neurons from the environment cause an increase in longevity that is suppressed by *daf-16* (3). Thus, it is likely that sensory input is needed to activate the *daf-2* receptor, presumably via the activation of insulin agonists or inactivation of insulin antagonists by food or other sensory inputs. Particular sensory neurons produce a dauer inhibitory signal because laser ablation of those neurons causes dauer arrest (5). This cell ablation induced dauer arrest is dependent on *daf-16* gene activity, supportive of a coupling to the *C. elegans* insulin-like signaling pathway (5). Moreover, some neurons activate dauer arrest rather than reproductive development (96).

The insulin hormone may be produced by the sensory neurons or neurons connected to those sensory neurons. Serotonin may be involved in the signaling pathway. A mutation in *C. elegans* tryptophan hydroxylase (TPH), which catalyzes the rate-limiting first step in serotonin synthesis, causes several behavioral defects that are associated with starvation: With abundant food, the mutant feeds more slowly, retains more eggs, accumulates larger stores of fat, and some animals arrest at the dauer stage (105). The metabolic phenotypes are a result of serotonin inputs to both the TGF-beta and the insulin-like pathways. The expression of a *daf-7::GFP* fusion gene is decreased in the *tph-1*(null) mutant, and dauer arrest of a temperature sensitive *daf-7*(mutant) at low temperature is enhanced. In addition, high gene dosage of *tph-1* suppresses dauer arrest of *daf-7(e1372)* at high temperature. This suggests that temperature may modulate serotonin levels to influence dauer arrest. There is also serotonergic input to the parallel insulin-like signaling pathway. A *daf-16* mutation suppresses the dauer arrest and fat accumulation *tph-1(mg280)* phenotypes (105). Moreover, like *daf-2* insulin-receptor mutants (but unlike *daf-7* pathway mutations), *tph-1*(mutant) hermaphrodites have an extended reproductive life span that is dependent on *daf-16* gene activity. Consistent with the effect on reproductive life span, *tph-1* animals have 25% longer life spans that is suppressed by *daf-16* (K. Ashrafi & G. Ruvkun, personal communication). Bacterial food and low temperature may normally upregulate serotonin signaling to in turn upregulate DAF-7 and insulin-like neuroendocrine signals. In the *tph-1* mutant, these hormones may be decoupled from such food signaling. Serotonin signaling has also been implicated in the control of mammalian feeding and metabolism.

Temperature is a potent regulator of dauer arrest (36). All mutations in the DAF-7 TGF-beta pathway, including *daf-7* null mutations, are temperature sensitive, whereas null mutations in the insulin pathway cause dauer arrest at all temperatures; they are not temperature sensitive (46). There is explicit temperature sensory input to this endocrine pathway. This pathway includes the AFD thermosensory neurons, which connect to the interneurons AIY and AIZ, which may, in turn, connect to secretory as well as motor control neurons. A mutation that decouples AIY from this thermoregulation of dauer arrest renders dauer arrest nontemperature sensitive. The antagonistic high and low temperature processing pathways of the *C. elegans* thermoregulatory pathway is similar to the organization of the vertebrate hypothalamus, which contains distinct warm and cold temperature

processing units (46). The coupling of thermosensory input to the *daf-2* insulin-like signaling pathway may be homologous to the hypothalamic modulation of autonomic input to the pancreatic beta cells. Because the life span of *C. elegans* and other cold blooded animals is highly dependent on temperature, it is possible that these thermoregulatory circuits feed into life span regulation.

DIAPAUSE, HIBERNATION, SLEEP, AND LONGEVITY

The *C. elegans* insulin-like signaling pathway genes control longevity as part of a global endocrine system that controls whether the animals grow directly to reproductive adults or arrest at the dauer diapause stage. The connection between longevity and diapause control may not be parochial to *C. elegans*. Diapause arrest is an essential feature of many vertebrate and invertebrate life cycles, especially in regions with seasonal temperature and humidity extremes (109). Animals in diapause arrest slow their metabolism and their rates of aging and can survive for periods much longer than their reproductive life span.

Caloric restriction may cause a decline in mammalian insulin and insulin-like signaling, which, in turn, causes a repertoire of diapause-like responses, such as the expression of an ancient set of aging protective genes that confer decreased aging during the diapause period. Because these diapauses are pre-reproductive, they would be selected. Induction of diapause-like states postreproductively could also induce longevity, but the program would, in this case, have been selected for its prereproductive action.

From the connection between diapause and life span in worms and the similarity between insulin-like regulation of worm life span and caloric restriction, one may predict that humans who live much longer than normal may share some features of the diapause state. This could be reflected in a lower overall rate of metabolism, either in the whole animal or in key cells, such as particular neurons. It could also be reflected in a lower body temperature, either all of the time or during specific times, for example, sleep. It would be very interesting to analyze brain activities of the progeny of centenarians, some of whom are destined to live longer than normal. One might find a deeper sleep or altered pattern of brain electrical activities in those who inherited a longevity phenotype.

Reproductive senescence is delayed in weak *daf-2* mutants, and this is also dependent on *daf-16* gene activity (32a, 105). The coregulation of reproductive senescence and longevity of the entire animal is reminiscent of their co-regulation in humans as well: Female centenarians tend to continue to bear children much later (after 45 years old) than noncentenarian humans (87), as discussed above. There is a strong correlation between late fertility and longevity: (a) both serotonin and *daf-2* mutants cause late reproduction, (b) the correlation between late reproduction and longevity in many species, and (c) the regulation of fertility by insulin signaling in mammals.

Just as environmental extremes can select for variation in the genetic pathways that regulate *C. elegans* dauer formation, famines and droughts in human history

may have selected for analogous variants in the human homologues of the *daf* genes. In fact, heterozygous mice carrying either the *db* or the *ob* recessive diabetes genes survive fasting ~20% longer than wild-type controls (87). The high frequency of type II diabetes in many human populations may be the legacy of such selections.

CLK MUTANTS AND METABOLISM

Mutations in the *C. elegans* coenzyme Q biosynthetic gene *clk-1* cause a modest increase in life span, in addition to a twofold slowing of the development rate (25). Because coenzyme Q is an essential component of the electron transport chain of mitochondria as well as bacteria, it is not surprising that the rate of living is affected in this mutant. What is surprising is that a null mutant in this gene is actually viable. It turns out that the *C. elegans* bearing this mutation is viable on wild-type *E. coli* but not on an *E. coli* mutant that produces no coenzyme Q (51). Thus, the *clk-1* null mutant uses coenzyme Q from *E. coli*. Presumably, the level or regulation of the *E. coli* derived coenzyme Q is not normal, leading to the modest increase in life span. These data weakly support the view that metabolic rate and aging are connected but do not implicate *clk-1* in any explicit regulation of aging.

FREE RADICALS AND AGING

The free radical theory of aging has been debated for years (103). It is supported by the general correlation between metabolic rate and longevity, by both comparing species as well as comparing life histories within a species. The increase in life span associated with declines in metabolic rates in insulin pathway mutants as well as *clk-1* coenzyme Q biosynthetic pathway mutants also support this model. Also supportive is the increase in life span that results from overexpression of free radical detoxifying enzymes, such as CuZn SOD, and free radical scavenging chemicals, such as vitamin E and others (103).

Recent genetic analysis suggests that free radical production can be increased by decoupling electron transport of ubiquinone to O₂. A missense mutation in *C. elegans* cytochrome b560 causes a decrease in life span and oxygen hypersensitivity (as well as radiation hypersensitivity, a reasonable pleiotropy because radiation induces free radicals, which damage DNA). Paradoxically, this is the opposite phenotype from the *clk-1* defect in coenzyme Q biosynthesis. A model that explains this is that the cytochrome missense mutation causes a toxic build up of ubisemiquinone (a free radical that can generate superoxide) because the normal pathway for further reduction of singly reduced coenzyme Q is compromised (50).

The best evidence for the involvement of free radicals in longevity has come from the treatment of *C. elegans* with free radical detoxifying mimetics, which cause a 44% increase in life span of wild type and 60% increase in the life span

of the above mutants that are expected to produce more free radicals than normal (71).

Similarly, increased expression of free radical scavenging enzymes, such as superoxide dismutase, increase invertebrate and vertebrate life span in some cases (85, 91) but not in others (48). Mouse knockout mutations in both mitochondrial Mn superoxide dismutase (64) and cytosolic CuZn SOD (100) show effects on whole animal and neural mortality. Why the conflicting results have been observed is not clear, but the effects of distinct genetic backgrounds with differences in the rate of production or detoxification are a likely variable. Some free radical generation may also be necessary to induce stress response pathways that are protective. These studies demonstrate that neurons are highly sensitive to free radical damage, consistent with the view from *C. elegans* that insulin signaling in the nervous system is the key to longevity control. In fact, expression of free radical scavenging enzymes in the nervous system is highly protective (58, 106, 116). Expression of catalase and SOD in the hypothalamic region, an ancient locus of the brain, is under GH/IGFI control, as expression of these genes is regulated by insulin signaling in the worm (43).

YEAST

Unlike metazoans, yeast do not have a segregated soma and germline. Despite this, an asymmetry in cell division does mark the parent cell, and the life span of those cells is subject to genetic control. This control of mother cell longevity, as measured by further cell division, is probably quite distinct from the postmitotic cell longevity of, for example, the animal brain, as recently reviewed by Guarente & Kenyon (39). In summary, even though the longevity control of a unicellular organism would be expected to be distinct from an organism with a separate mortal soma and immortal germline, points of tantalizing similarity emerge: A chromatin remodeling pathway has been implicated in yeast aging control that may also regulate the life span of *C. elegans* (111), and the activity of the control protein in this pathway Sir2 is regulated NAD, a surrogate measure of metabolism. Although yeast do not have an insulin signaling pathway, they appear to have a precursor to that metabolic control pathway, the yeast adenylate cyclase and SCH9, which function in glucose/nutrient signaling pathways (23a). SCH9 is homologous to the serine threonine kinase Akt/PKB of insulin signaling pathways in *Caenorhabditis*, *Drosophila*, and mammals. Mutations in the adenylate cyclase and SCH9 genes can extend the longevity of nondividing cells by up to threefold (23a).

CONCLUSIONS

Our purpose in this review is to outline the prospects of unifying mechanism in the genetics of aging. In case after case, from mice to worms to flies to yeast, genetic variants that modify metabolism also modify life span. These effects, collectively,

are as general as that of caloric restriction, which also increases longevity and resistance to stress in many situations. The evolutionary theory of aging proposes that the life span is indirectly selected on the basis of the reproductive schedule. In turn, the reproductive schedule is coordinated by neural and endocrine mechanisms in multicellular organisms. Therefore, to consider that genes determining the life span could be expressed in neuronal and endocrine cells in diverse animals is no longer far-fetched. Consistent with this hypothesis are experiments in *Drosophila* and *C. elegans* in which life span was manipulated by the expression of genes in specific neurons. Genetic approaches may, thus, be able to identify a set of circuits that regulate longevity that were established in ancestral metazoans.

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An insulin-like receptor to PI-3 kinase, PTEN, PDK-1, AKT-1, AKT-2, to DAF-16 signaling cascade controls *C. elegans* aging and metabolism

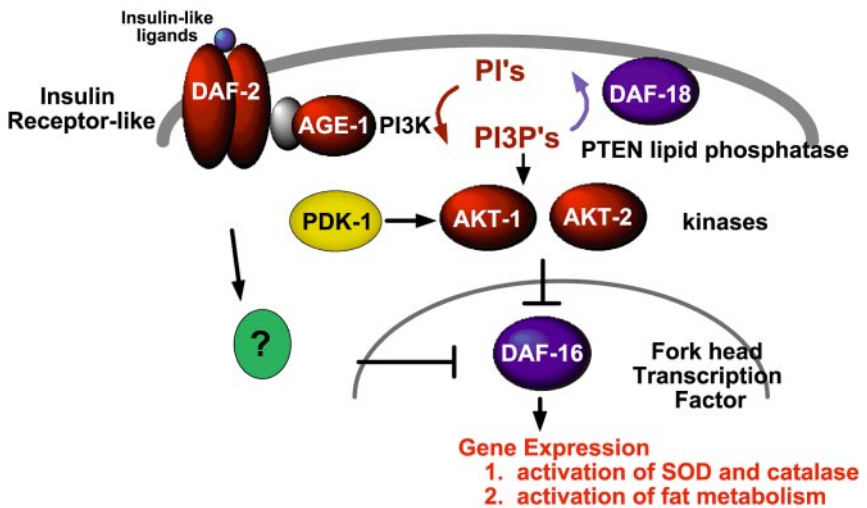


Figure 3 A molecular model for insulin like regulation of *C. elegans* longevity. High insulin signaling activates the DAF-2 receptor which in turn activates the AGE-1 PI 3-kinase to in turn activate the kinases PDK-1, AKT-1, and AKT-2. The AKT-1 and AKT-2 kinases phosphorylate the DAF-16 transcription factor causing cytoplasmic localization to in turn cause a change in transcription of target genes.

Neuronal *daf-2* signaling regulates lifespan

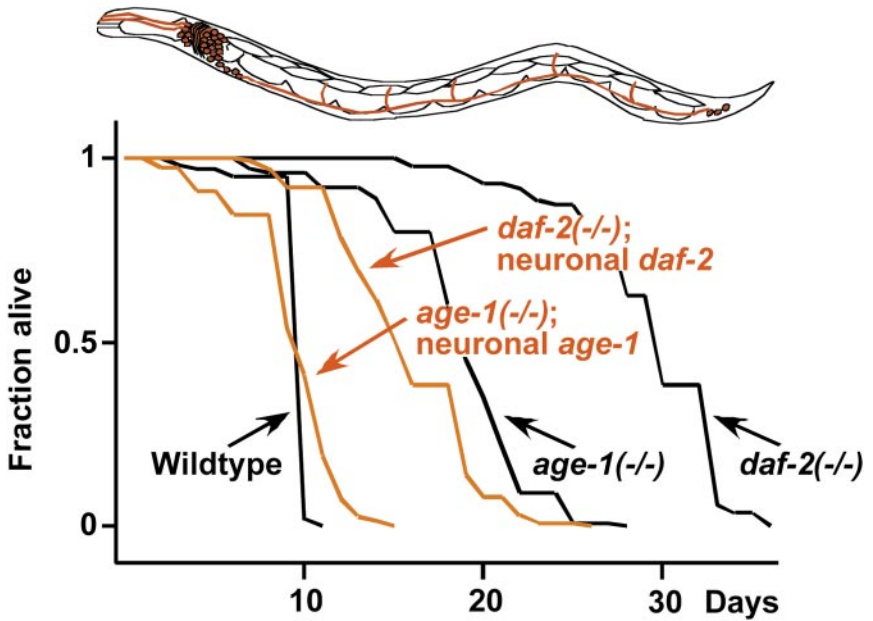


Figure 5 Neuronal regulation of life span by *C. elegans* insulin-like signaling. Neuronal expression of the *C. elegans* insulin pathway is sufficient to rescue the lifespan increase caused by deficits in insulin signaling.