Cell, Vol. 120, 449–460, February 25, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.cell.2005.02.002

The Plasticity of Aging: Insights from Long-Lived Mutants

Review

Cynthia Kenyon*
Department of Biochemistry and Biophysics
University of California, San Francisco
San Francisco, California 94143

Mutations in genes affecting endocrine signaling, stress responses, metabolism, and telomeres can all increase the life spans of model organisms. These mutations have revealed evolutionarily conserved pathways for aging, some of which appear to extend life span in response to sensory cues, caloric restriction, or stress. Many mutations affecting longevity pathways delay age-related disease, and the molecular analysis of these pathways is leading to a mechanistic understanding of how these two processes—aging and disease susceptibility—are linked.

Introduction

Aging is a fundamental and fascinating process with great natural diversity. A rat lives three years, whereas a squirrel lives twenty-five. Some animals age and die rapidly after mating, while certain fish and turtles do not seem to age at all. Diversity in the aging process exists even within a single individual. The neurons of old, decrepit *C. elegans* show little signs of age (Herndon et al., 2002), and the germ lineage is immortal. In spite of this diversity, for many years aging was thought to be a haphazard process driven solely by entropy. However, we now know that aging, like many other biological processes, is subject to regulation by pathways that have been conserved during evolution. Changing single genes within these pathways can extend life span dramatically, causing the animal to age normally but just more slowly. Some of these long-lived mutants are breathtaking; in human terms, they look like forty-year-olds when they are actually eighty or even older. In this review, I describe some of these long-lived mutants and the remarkable insights they provide about the aging process.

Endocrine Regulation of Aging

Mutations Affecting Insulin/IGF-1 Signaling

Many mutations that extend life span perturb endocrine signaling. The best understood of these signaling pathways is the insulin/IGF-1 pathway, which influences life span in worms, flies, and mammals (Tatar et al., 2003) (Figure 1). This pathway was first linked to life span in *C. elegans*, where mutations in *daf-2,* a known regulatory gene (Riddle, 1997) encoding an insulin/IGF-1 receptor ortholog (Kimura et al., 1997), were found to double the life span of the animal (Kenyon et al., 1993). The life span extension caused by *daf-2* mutations required the activity of *daf-16* (Kenyon et al., 1993), which encodes a FOXO family transcription factor (Lin et al., 1997; Ogg et al., 1997). These findings demonstrated that aging in *C. elegans* is subject to regulation, and that it is regulated hormonally.

The DAF-2 receptor activates a conserved PI-3 kinase signaling pathway (Friedman and Johnson, 1988; Hertweck et al., 2004; Morris et al., 1996; Ogg and Ruvkun, 1998; Paradis et al., 1999; Paradis and Ruvkun, 1998; Wolkow et al., 2002) that affects life span, at least in part, by regulating the nuclear localization of DAF-16 (Henderson and Johnson, 2001; Lee et al., 2001; Lin et al., 2001). In addition to DAF-16, HSF-1, the *C. elegans* heat-shock transcription factor, is also completely required for *daf-2* mutations to extend life span (Hsu et al., 2003; Morley and Morimoto, 2004). Like DAF-16 (Henderson and Johnson, 2001; Lee et al., 2001; Lin et al., 2001), HSF-1 delays aging and extends life span (Garigan et al., 2002; Hsu et al., 2003; Morley and Morimoto, 2004). In *daf-2* mutants, HSF-1 promotes longevity by activating specific longevity genes, including genes that encode small heat-shock proteins (Hsu et al., 2003).

The longevity of *daf-2* mutants also requires the function of AAK-2, the catalytic subunit of the AMP-activated protein kinase (Apfeld et al., 2004). In mammals, AMP kinase regulates energy metabolism and food intake via phosphorylation of an array of substrates, including metabolic enzymes and transcription factors. The mechanism by which AAK-2 promotes longevity is not known, but its overexpression in worms increased life span ~13%, suggesting that AAK-2 has a causal role.

Insulin/IGF-1 receptor mutations can also increase the life span of *Drosophila*, by as much as 80% (Tatar et al., 2001). In addition, mutations in *chico,* a downstream insulin receptor substrate (IRS)-like signaling protein, increase life span by ~40% (Clancy et al., 2001; Tu et al., 2002a). It seems likely that this life span extension will be FOXO dependent: first, FOXO is required for the reduced cell division observed in *Drosophila* insulin/IGF-1 pathway mutants (Junger et al., 2003); second, FOXO overexpression extends the fly's life span (Giannakou et al., 2004; Hwangbo et al., 2004); and third, *Drosophila* genes with conserved FOXO binding sites have proven to influence life span (Lee et al., 2003).

Unlike worms and flies, which have a single insulin/IGF-1-like receptor, mice have separate receptors for insulin and IGF-1. IGF-1 receptor heterozygous knockout mice live ~30% longer than wild-type, and males live 16% longer (though the latter value was not statistically significant) (Holzenberger et al., 2003). In addition, mice that lack the insulin receptor in adipose tissue live ~18% longer than wild-type (Bluher et al., 2003). Thus the ability of insulin/IGF-1 signaling to influence life span appears to have been distributed to both receptors during evolution.

Mutations in upstream genes that regulate insulin and IGF-1 also extend life span, by ~50%. For example, growth hormone stimulates IGF-1 production, and growth hormone receptor mutants are long lived (Coschigano et al., 2003). The Ames and Snell dwarf mice, which have pituitary defects and consequently

*Correspondence: ckenyon@biochem.ucsf.edu

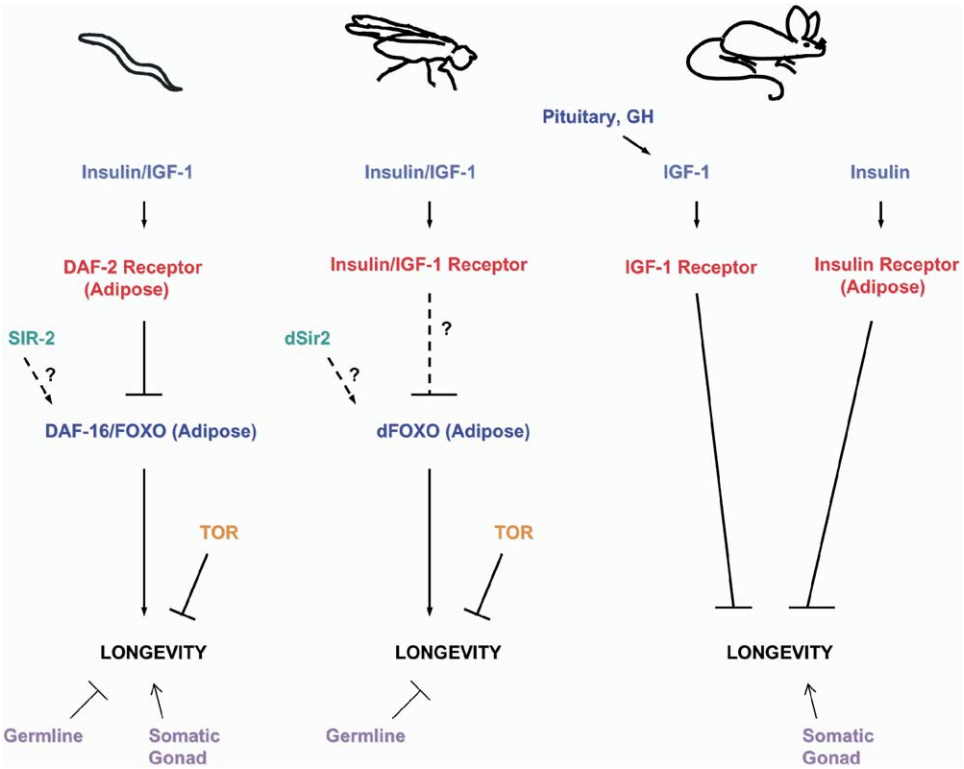

Figure 1. Genes, Pathways, and Tissues that Have Been Shown to Influence the Life Spans of More than One Animal Species
Dashed lines, plausible regulatory relationships (see text).

low levels of growth hormone and IGF-1, are also long lived (Brown-Borg et al., 1996; Flurkey et al., 2002). GH and IGF-1 promote growth, but the small size of these mice is unlikely to cause their life span extension because in worms, flies, and other mouse mutants, it is possible to change insulin/IGF-1 receptor activity in such a way that life span is increased with little or no change in body size (Clancy et al., 2001; Garigan et al., 2002; Holzenberger et al., 2003; McCulloch and Gems, 2003).

Whether FOXO proteins are responsible for life span extension in mice is not known; however, FOXO proteins function in mouse insulin and IGF-1 pathways that affect metabolism (Burgering and Kops, 2002). In addition, FOXO proteins have been implicated in the increased stress resistance of certain long-lived mice, such as mice lacking the adaptor protein p66shc (Nemoto and Finkel, 2002). Stress resistance is a general feature of insulin/IGF-1 pathway mutants, and in worms, this phenotype has been shown to be FOXO dependent (Clancy et al., 2001; Holzenberger et al., 2003; Larsen, 1993; Lin et al., 2001; Lithgow et al., 1995; Murakami and Johnson, 1996).

Even the life span of yeast appears to be regulated by insulin/IGF-1 signaling components. Mutations in the yeast AKT ortholog *SCH9* extend life span (Longo, 2003). *SCH9* is likely to be a bona fide AKT ortholog because as in flies and mice, it also controls growth; *sch9* mutant yeast are much smaller than wild-type. Likewise, overexpression of the histone deacetylase Sir2, which is part of the insulin/IGF-1 pathway in *C. elegans*, extends the life spans of both yeast and worms (Tissenbaum and Guarente, 2001).

Signaling Cascades and Tissue Interactions Affecting Life Span

Insulin/IGF-1 signaling appears to be only one step in a signaling cascade that affects life span. The first hint of this came from the finding that *C. elegans* genetic mosaics that lack the DAF-2 insulin/IGF-1 receptor in either cell at the two-cell stage are long lived (Apfeld and Kenyon, 1998). Thus cells that lack *daf-2* must be able to send a longevity signal to wild-type cells.

Which tissues produce these downstream signals? Adipose tissue is important since mice lacking the insulin receptor in adipose tissue are long lived. A signaling role for adipose tissue may be conserved since increasing FOXO activity specifically in adipose tissue extends the life spans of flies (Giannakou et al., 2004; Hwangbo et al., 2004), and in worms, increasing FOXO activity in the intestine, which also serves as the animal's adipose tissue, extends life span ~50% (Libina et al., 2003).

In *C. elegans*, activity of the insulin-response pathway in neurons also influences life span. For example, one mosaic animal that lacked *daf-2* only in a small set of neurons was very long lived (Apfeld and Kenyon, 1998). The relative importance of neurons is controversial. Wolkow et al. reported that expression of the DAF-2 receptor only in neurons shortened the life spans of *daf-2(–)* mutants to *daf-2(+)* control levels (Wolkow et al., 2000). However, Libina et al. produced the same

type of animals (*daf-2* activity only in neurons) using a *daf-2* RNAi feeding strategy (which does not affect neurons) and found that the animals lived twice as long as normal (Libina et al., 2003). Moreover, using tissue-specific gene expression, genetic mosaic analysis and RNAi, Libina et al. found that *daf-16/FOXO* activity in neurons accounted for only ~5%–20% of the life span extension seen in *daf-2* mutants.

The insulin/IGF-1 pathway controls at least two types of downstream signals. First, the pathway may feedback regulate the production of insulin-like signals. In *C. elegans,* increasing DAF-16/FOXO activity in the intestine increases DAF-16 activity in other tissues (Libina et al., 2003). DAF-16 is known to inhibit expression of the insulin-like gene *ins-7,* a putative DAF-2 agonist (Murphy et al., 2003), and reduced insulin levels could potentially mediate this effect. Likewise, in flies, increasing FOXO activity in adipose tissue inhibits insulin gene expression in neurons (Hwangbo et al., 2004). Positive, cell-nonautonomous feedback regulation may allow the effects of local perturbations in insulin/IGF-1 signaling levels to spread rapidly throughout the animal. However, it also complicates identification of tissues in which insulin/IGF-1 signaling is particularly important because experimentally changing the level of FOXO activity in one tissue can change its activity elsewhere.

In *C. elegans,* DAF-16/FOXO must also produce longevity signals that do not act through DAF-16/FOXO in responding cells since restricting DAF-16 activity to the intestine or neurons increases the life span of the whole animal (Libina et al., 2003). One candidate for such a signal is *scl-1* (Ookuma et al., 2003), a CRISP family member that is upregulated by DAF-16/FOXO and is required for the longevity of *daf-2* mutants. Another possible downstream signaling factor is the tyrosine kinase receptor *old-1,* which promotes longevity and is regulated by insulin/IGF-1 signaling (Murakami and Johnson, 2001). In flies, both juvenile hormone and ecdysone production are decreased in long-lived insulin receptor mutants (Tatar et al., 2003; Tu et al., 2002b), and juvenile-hormone analogs restore the life spans of these animals to normal without affecting wild-type longevity (Tatar et al., 2001). Moreover, ecdysone receptor mutant heterozygotes live 40%–50% longer than wild-type (Simon et al., 2003). Thus reducing insulin/IGF-1 signaling may extend life spans in flies, at least in part, by reducing the level of signaling by juvenile hormone and ecdysone.

Interestingly, in *C. elegans,* the heat-shock factor HSF-1 also acts in a cell-nonautonomous fashion to extend the life spans of *daf-2*-pathway mutants (Morley and Morimoto, 2004). Thus HSF-1 may be doing more than simply regulating cell-autonomous heat-shock genes.

A Regulatory Module for Longevity

How, ultimately, does insulin/IGF-1 activity influence life span? In *C. elegans,* many functionally significant downstream genes have now been identified. In one study, RNAi analysis was used to test the functional significance of the fifty genes whose expression was most strongly changed in *daf-2/daf-16* gene expression profiles (Murphy et al., 2003). In another, the functional significance of a set of genes with conserved FOXO binding sites was tested with RNAi (Lee et al., 2003). Many of these genes influenced life span, including antioxidant genes such as superoxide dismutase, metallothionine, catalase, and glutathionine S-transferase (some of which were known previously to be regulated by *daf-2* [Barsyte et al., 2001; Honda and Honda, 1999; Vanfleteren and De Vreese, 1995]), metabolic genes including apolipoprotein genes, glyoxylate-cycle genes, and genes involved in amino acid turnover, and chaperones, particularly small heat-shock protein genes, and antibacterial genes. Overexpression of at least some genes upregulated in the long-lived mutants, such as superoxide dismutase (Sun et al., 2002; Sun and Tower, 1999) and small heat-shock protein genes (Walker and Lithgow, 2003; Wang et al., 2004), can extend life span.

One set of downregulated genes, the apoliporotein genes, is intriguing since alleles predicted to reduce the function of genes involved in fat transport in humans are genetically linked to extreme longevity (Barzilai et al., 2003; Geesaman et al., 2003). The antibacterial genes are also interesting since bacterial proliferation contributes to the death of *C. elegans* (Garigan et al., 2002; Gems and Riddle, 2000) and *daf-2* mutants are resistant to pathogens (Garsin et al., 2003).

Autophagy, the turnover of cellular organelles, may also play an important role in the longevity of *daf-2* mutants. Autophagy is increased in *daf-2* mutants, and preventing autophagy shortens their life spans without affecting those of wild-type (Melendez et al., 2003).

The key concept to emerge from these studies is that the insulin/IGF-1 system acts as a "longevity module" in which master regulators like DAF-2 and DAF-16/FOXO control a wide variety of downstream genes with diverse functions that act together in a cumulative fashion to influence life span (Figure 2). Many of these downstream genes are likely to be conserved, with respect to both their function and regulation. For example, the longevity of yeast *sch9/akt* mutants appears to be due, at least in part, to upregulation of superoxide dismutase (Fabrizio et al., 2003). Likewise, mammalian FOXO proteins activate stress-response genes when insulin or IGF-1 levels are reduced (Kops et al., 2002; Nemoto and Finkel, 2002; Tran et al., 2002). Many of these downstream genes may also influence longevity in humans. In addition, their analysis in different kinds of animals may help us understand how different species can have such different life spans.

Evolution of a Regulatory System for Life Span

How could such a longevity module evolve? It may have been selected because of its effects on aging if, for example, species whose members have short life spans prospered from increased genetic diversity or decreased competition between parents and offspring. An alternative explanation is particularly appealing; namely that the insulin/IGF-1 longevity regulatory module arose during evolution not to influence longevity per se but to allow animals to endure harsh environmental conditions. Many DAF-2/DAF-16 downstream longevity genes not only extend life span but also protect the animal from harsh environmental stress, such as heat, UV, and oxidative-damaging agents. This would be logical since metabolic and environmental stresses may inflict similar types of damage on cellular components. The same chaperone, antioxidant, and other proteins that protect against one type of stress would protect

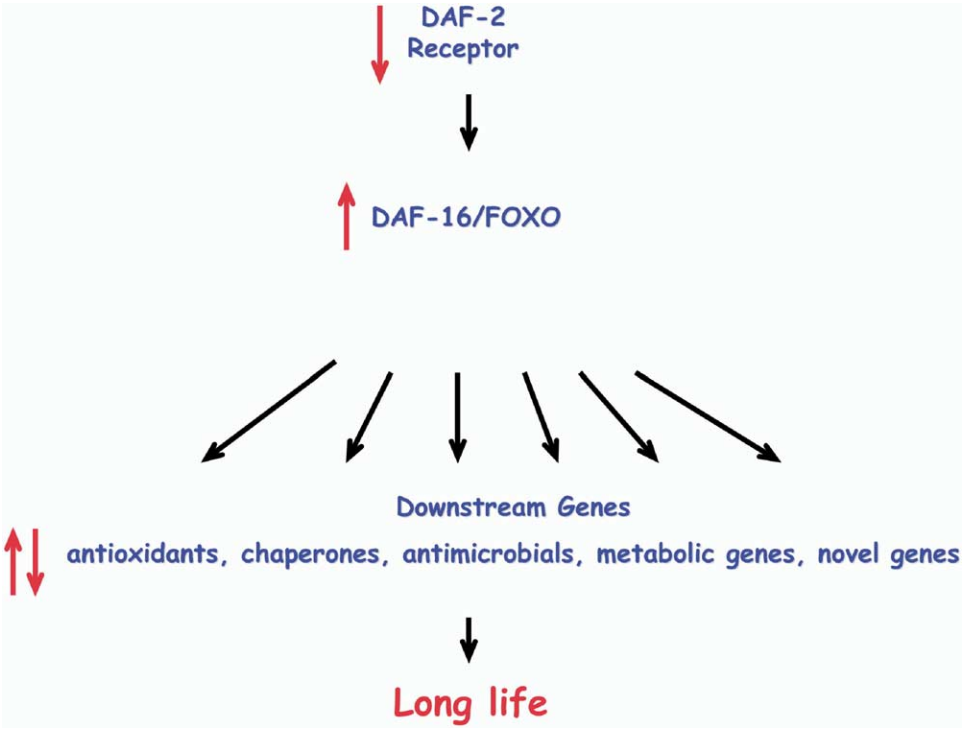

Figure 2. Longevity Regulatory Module
See text for details.

against the other. In fact, the stress resistance of vertebrate cells in culture correlates with the life spans of the species from which they were isolated (Kapahi et al., 1999).

Consistent with this idea, in *C. elegans*, the insulin/IGF-1 pathway not only regulates adult longevity but also entry of pre-pubescent juveniles into a growth-arrested larval state called dauer (Riddle, 1997). Harsh environmental conditions trigger dauer formation, in part, by downregulating insulin/IGF-1 signaling. Reduced insulin/IGF-1 signaling is likely to endow dauers with their longevity and stress resistance because many stress-response and other genes whose expression changes in long-lived adults also change in dauers (Jones et al., 2001; McElwee et al., 2004).

The ability of *C. elegans* to enter the dauer state has clear selective value because it delays reproduction under harsh environmental conditions. Thus the insulin/IGF-1 life span regulatory module may have evolved initially to allow the animal to survive harsh environmental conditions before reproducing. But at the same time (because the same genes protect against endogenous stress that accelerates aging) a means of regulating longevity itself was created.

Are the long-lived insulin/IGF-1 mutants dauers in disguise? Probably not. Dauers have low metabolism, a specialized morphology, and they do not reproduce. In contrast, whereas some *daf-2* mutations (class II) produce dauer-like traits in adults, others (class I mutations) do not; they have normal metabolism, reproduction, behavior, and body morphology (Gems et al., 1998; Van Voorhies and Ward, 1999). The same is true in mice, where long-lived insulin/IGF-1 mutants have normal rates of oxygen consumption and reproduce normally (Holzenberger et al., 2003). Thus the longevity module regulated by the insulin/IGF-1 system can be expressed independently of many diapause-like traits. Consistent with this, the insulin/IGF-1 system acts at different times to control dauer formation (a strictly larval state) and life span. Both DAF-2 and DAF-16 act in the young juvenile to influence dauer formation; however, they act exclusively in adults to affect aging (Dillin et al., 2002a) (The insulin/IGF-1 pathway also appears to regulate the life spans of flies during adulthood [Giannakou et al., 2004; Hwangbo et al., 2004]).

In nature, there are short-lived and long-lived insects, birds, and mammals, so large changes in life span must have evolved not once but many times. Moreover, large changes in life span can evolve rapidly, for example, in the case of guppies, over just a few years (Reznick et al., 2004). The existence of a "life span regulatory module" may render the process of aging "evolvable" since changes in one or a few regulatory genes (or perhaps individual downstream genes) could potentially produce large changes in life span. During evolution, such changes could have allowed animals to enter ecological niches that favor a longer life span. In fact, recent changes in this regulatory system may have produced different life spans among dogs. Small dogs live much longer than large dogs, possibly because they have lower levels of IGF-1 (Eigenmann et al., 1984). In model organisms, the effects of insulin/IGF-1 signaling on aging can be uncoupled from its effects on body size. Thus it will be interesting to learn whether long-lived species in general have lower levels of insulin IGF/1 pathway activity than short-lived species.

Insulin/IGF-1 Paradoxes

Insulin and IGF-1 are clearly beneficial. They are anabolic hormones that promote food storage and growth. Yet reducing the activities of these hormones also seems beneficial since it lengthens life span. How can we explain this apparent paradox? The finding that low insulin/IGF-1 signaling activates stress resistance genes provides a possible answer: most likely low signaling levels shift cells from states of growth, which may not equip them for long-term survival, to states of maintenance, which do equip them for long-term survival, thereby delaying aging.

It also seems paradoxical that reduced insulin/IGF-1 signaling extends life span but insulin resistance leads to type II diabetes. The basis for this is not known, but there are some interesting issues to consider. First, the specific perturbation is important: whereas loss of the insulin receptor in adipose tissue extends mouse life span, its loss in the liver cause diabetes (Michael et al., 2000). Presumably this is because adipose tissue lacking the insulin receptor produces longevity signals whereas liver lacking the insulin receptor does not. Second, in worms and flies, reducing the activity levels of insulin-like peptides, like reducing receptor activity, can extend life span (Broughton et al., 2005; Li et al., 2003; Murphy et al., 2003; Wessells et al., 2004). This finding does not seem paradoxical because in mammals, low-circulating insulin levels are generally associated with insulin sensitivity and longevity. (In fact, it would be interesting to learn whether low glycemic-index (low-carb) diets, which keep insulin levels low, increase life span.) Thus the real paradox is why, in mammals, low insulin levels are associated with good health, but low insulin responsiveness with bad health. It seems that insulin-resistant cells on the path to type II diabetes are in a fundamentally different regulatory state from normal cells exposed to low levels of circulating insulin. Insulin-resistant prediabetic cells signal the pancreas to overproduce insulin, thereby possibly creating an insulin gain-of-function situation. In contrast, insulin-sensitive cells in animals with low levels of circulating insulin do not trigger insulin secretion. Perhaps these cells instead produce downstream longevity signals.

Aging and Reproduction

A Price for Longevity?

Some evolutionary theories predict that longevity mutations will invariably be associated with reproductive or other tradeoffs. Obviously this cannot always be true, or magnificent creatures such as ourselves could never have evolved such long life spans. The tradeoff theory has recently suffered a setback: as predicted by evolutionary theory, guppies that have short life spans in nature due to predation grow faster and initiate reproduction sooner than guppies without natural predators. However, when their predators are removed, these same guppies go on to have more progeny and longer life spans than guppies without natural predators (Reznick et al., 2004). Likewise, some long-lived mutants, such as the *Drosophila indy* (amino acid transporter) mutant, have even more progeny than normal (Rogina et al., 2000). Thus, clearly, longevity need not be associated with reduced reproduction. Since they are not found in nature, it seems likely that long-lived mutants would be at a disadvantage under some environmental conditions. Consistent with this, long-lived *C. elegans age-1*/PI-3 kinase mutants compete well when placed in competition with wild-type under replete conditions, but they are outcompeted when subjected to periods of starvation (Walker et al., 2000). However, the survival of these same mutants is higher than wild-type at elevated temperature (Lithgow et al., 1995). Thus context is important, and it need not always favor wild-type.

Interestingly, the insulin/IGF-1 pathway regulates both aging and reproduction, but it regulates the two processes independently of one another. Treating worms with *daf-2* RNAi from the time of hatching extends life span and delays reproduction, but treating them as young adults extends life span to the same extent with little or no effect on reproduction (Dillin et al., 2002a). This is interesting because it hints at evolutionary flexibility: single mutations affecting this pathway could potentially affect both aging and reproduction or, alternatively, one but not the other.

Regulation of Life Span by the Reproductive System

Turning the situation on its head, the reproductive system can actually regulate aging. When the germline precursors of *C. elegans* are removed, life span is extended ~60% (Hsin and Kenyon, 1999). This is not due to sterility because removing the entire reproductive system (germine plus somatic gonad) has no effect on life span. Instead, the longevity effect is mediated hormonally. It is dependent on DAF-16/FOXO and a steroid signaling pathway involving the nuclear hormone receptor DAF-12 (Hsin and Kenyon, 1999) and DAF-9, a cytochrome P450 homolog thought to be involved in the synthesis of a DAF-12 ligand (Gerisch and Antebi, 2004). Removing the germ cells causes DAF-16 nuclear localization primarily in the intestine/adipose tissue (Lin et al., 2001), and its activity there is sufficient to account for the entire life span extension produced by removal of the germline (Libina et al., 2003). It is not clear how germ cells signal to the intestine, but the germline stem cells seem to be involved. Neither oocytes nor sperm are required for the germline to influence longevity, but preventing germline stem cell division in the adult extends life span (Arantes-Oliveira et al., 2002).

In wild-type animals, the somatic gonad must be present in order for germline ablation to extend life span. In *daf-2* mutants, however, germline ablation further extends life span whether or not the somatic gonad is present. Thus in the wild-type, killing the somatic gonad may restore to normal the life spans of germline-ablated animals by activating the insulin/IGF-1 pathway (Hsin and Kenyon, 1999).

Germline regulation of life span may provide a means of coordinating the rate of aging with the timing of reproduction. For example, if, during evolution, a mutation occurred that delayed germline development, reproduction would be delayed, but so would the rate of aging. As a consequence, the animal could still be in its prime when it reproduced.

An elegant experiment demonstrates that the reproductive system also influences life span in mammals

(Cargill et al., 2003). When the ovaries of either young or old mice are transferred into age-matched ovariectomized mice, life span is not affected. However, when young ovaries are transplanted into old recipients, life span is extended 40%–60%. Thus signals from the reproductive tissues must somehow influence life span. This transplantation kills the germ cells, so the long-lived mice contain a somatic gonad but no germ cells—a situation that leads to longevity in worms. Reproductive signaling could potentially influence fly life span as well. A mutation that kills oocytes extends life span, as does low-dose X-irradiation, which kills germ cells (Sgro and Partridge, 1999).

Regulation of Life Span by Environmental Cues

Stress-Induced Life Span Extension

There are many situations in biology in which low levels of stress trigger subsequent beneficial effects. This phenomenon, sometimes called "hormesis," influences aging since transient heat shock can extend the life spans of flies and worms (Apfeld et al., 2004; Hercus et al., 2003; Lithgow et al., 1995). The finding that overexpression of the heat-shock factor HSF-1 extends life span in *C. elegans* (Hsu et al., 2003; Morley and Morimoto, 2004) provides a possible explanation for this interesting phenomenon; namely, that stress activates HSF-1, which, in turn, increases life span by activating downstream life span-extending genes such as small heat-shock protein genes (Hsu et al., 2003).

C. elegans DAF-16/FOXO may be involved in hormesis as well. Heat shock triggers DAF-16 nuclear localization (Henderson and Johnson, 2001; Lin et al., 2001), and DAF-16 is required for the full expression of certain heat-shock genes, including the small heat-shock protein genes, following heat shock (Hsu et al., 2003). Interestingly, heat pulses produce large increases in life span, and large increases in the level of small heat-shock proteins, in long-lived *age-1*/PI-3 kinase mutations (Walker et al., 2001). This could occur because the disinhibition of DAF-16 in these mutants renders the longevity pathway more sensitive to further activation by stress. Mutations that increase insulin/IGF-1 signaling (PTEN mutations) prevent heat from triggering DAF-16 nuclear localization (Lin et al., 2001). This raises the possibility that stress increases life span, at least in part, by downregulating insulin/IGF-1 signaling.

Activating the stress-response Jun kinase (JNK) pathway increases life span in *Drosophila* by as much as 80% (Wang et al., 2003). This pathway would also be expected to promote stress-induced longevity. In addition, the AMP kinase subunit AAK-2 is completely required for transient heat stress—which lowers energy levels—to increase life span (Apfeld et al., 2004). AAK-2 may sense the effect of stress on energy levels to either support, or actually cause, the enhanced longevity of these animals.

Together these findings raise interesting questions about the effect of stress on human life span. Could it be that certain types of stress, such as fevers or hot flashes, might actually have a beneficial effect?

Regulation of Life Span by Sensory Perception

Many *C. elegans* sensory mutants, including animals with reduced activity of putative chemosensory receptors, are long lived (Alcedo and Kenyon, 2004; Apfeld and Kenyon, 1999). This life span extension is largely *daf-16* dependent (Alcedo and Kenyon, 2004; Apfeld and Kenyon, 1999) and is associated with DAF-16 nuclear localization (Lin et al., 2001). Many sensory neurons produce insulin-like peptides (Pierce et al., 2001), at least some of which have been shown to influence longevity (Li et al., 2003; Murphy et al., 2003; Pierce et al., 2001). Thus perception may affect life span by changing the activity of the insulin/IGF-1 pathway. Sensory control of life span is surprisingly complex. Some (but not all) gustatory and olfactory neurons influence life span, and some promote, whereas others inhibit, longevity (Alcedo and Kenyon, 2004). Sensory perception may or may not affect life span in higher animals, but it is interesting that the smell of food can increase insulin levels in humans (Lindemann, 2001).

The Longevity Response to Dietary Restriction

Genes in the Insulin/IGF-1 Pathway. Dietary restriction (DR) extends life span and postpones age-related disease in many animals, including yeast, worms, flies, rodents, and possibly primates. How does DR do this? Intuitively, one might expect reduced insulin/IGF-1 signaling to mediate this effect since in mammals, DR lowers insulin levels. This appears to be the case in flies since the life spans of animals subjected to DR are not further increased by mutations in the insulin/IGF-1 pathway (Clancy et al., 2002).

In *C. elegans,* reduced insulin/IGF-1 signaling does not mediate the response to DR because DAF-16/FOXO is required for low insulin/IGF-1 signaling to extend life span but not for DR to extend life span (Houthoofd et al., 2003; Lakowski and Hekimi, 1998). Instead, as described above, the insulin/IGF-1 pathway in worms appears to be regulated by sensory perception. Regulating the insulin/IGF-1 system by the perception of sensory cues rather than food consumption could provide a way for *C. elegans* to adjust its physiology rapidly in response to a change in the environment.

It is not clear whether the insulin/IGF-1 pathway mediates the response to DR in mice. DR nearly doubles the already long life spans of pituitary mutant mice (Bartke et al., 2001), suggesting that DR and insulin/IGF-1 signaling affect different pathways. However, one could argue that DR and the pituitary mutation do affect life span in the same way, but that neither the DR protocol employed, nor the pituitary mutation, induces the full DR longevity response on its own.

Dietary Restriction, Fat, and Longevity. Long-lived mice that lack the insulin receptor in adipose tissue are lean, even when fed a high-fat diet. The same is true of another long-lived mouse mutant, in which a C/EBP regulatory mutation has converted white adipose tissue to the more metabolically active brown adipose tissue (Chiu et al., 2004). The lean phenotypes of such mice have suggested the hypothesis that they are long lived because low fat levels trigger the longevity response to DR (Bluher et al., 2003). However, an interesting experiment indicates that this need not be the case: obese *ob/ob* (leptin-defective) mice subjected to DR are fatter than well-fed wild-type mice, but they live just as long as wild-type mice subjected to DR (Harrison et al., 1984). Moreover, long-lived pituitary mutant mice, as well as the worm and fly insulin/IGF-1-pathway mu-

tants, actually accumulate fat. (In worms, fat accumulation can be uncoupled from longevity [Ashrafi et al., 2003; Kenyon et al., 1993; Kimura et al., 1997; Wolkow et al., 2000].) Together, all of these findings suggest that if mutations in the mouse insulin/IGF-1 pathway mutation do trigger the DR longevity response, they trigger it independently of fat storage.

TOR Signaling. One pathway that might be predicted to be involved in the response to DR is the nutrient-sensing TOR (target of rapamycin) pathway. TOR is a protein kinase that phosphorylates ribosomal S6 kinase and translation initiation factor 4E binding protein 1, an inhibitor of the eukaryotic initiation factor 4E, in response to nutrients, which in turn promotes growth (Inoki et al., 2005). Mutations that decrease TOR activity extend the life spans of both flies (13%–30%) and worms (~30%) (Jia et al., 2004; Kapahi et al., 2004; Ramsey et al., 2002). Moreover, the life spans of the mutant flies cannot be further extended by DR. Thus it is plausible that the longevity response to DR involves downregulation of TOR.

SIR2. The molecular response to dietary restriction has been studied extensively in yeast. In response to reduced glucose levels, yeast cells undergo a metabolic shift from fermentation to oxidative phosphorylation. This shift is both necessary and sufficient to increase life span (Lin et al., 2002). The mechanism by which this shift extends life span is not clear, but activity of the histone deacetylase Sir2 is required. Sir2 is an interesting protein because its activity is NAD dependent. Thus Sir2 may sense, and respond to, the cell's metabolic state. Mutant yeast cells that do not generate life-shortening rDNA circles in response to DR no longer require Sir2 for DR to extend life span. Thus there exists a Sir2-independent DR pathway in yeast (Kaeberlein et al., 2004). The regulation and function of Sir2 in yeast is discussed in more detail in the review by Guarente and Picard (2005 [this issue of *Cell*]).

Sir2′s ability to extend life span has been conserved during evolution since overexpression of *SIR2* orthologs increases the life spans of both worms and flies (Rogina and Helfand, 2004; Tissenbaum and Guarente, 2001). In worms, SIR-2 seems unlikely to mediate the longevity response to DR since the life span extension produced by *sir-2* overexpression is *daf-16* dependent, whereas that produced by DR is not. In addition, SIR-2 activity potentiates dauer formation, suggesting that SIR-2 is part of the insulin/IGF-1 pathway. In flies, the insulin/IGF-1 pathway appears to mediate the response to DR, and consistent with this, in flies, *Sir2* activity is required for DR to extend life span. Another deacetylase, *rpd3,* may also function in this pathway in flies. Reduction of *rpd3* activity lengthens life span, and DR decreases *rpd3* expression. This, in turn, increases *Sir2* expression (Rogina et al., 2002).

In mammals, the SIR2 ortholog SirT1 has many intriguing roles in the regulation of metabolism and hormone signaling (see Guarente and Picard, 2005 [this issue of *Cell*]). For example, SirT1 deacetylates FOXO, which in turn shifts FOXO target selection toward stress-response genes (Brunet et al., 2004; Daitoku et al., 2004; Giannakou and Partridge, 2004; Motta et al., 2004). SirT1 may prove to affect the longevity response to DR in mammals since SirT1 expression is induced by DR in mice, and its activity is required for cultured mammalian cells to undergo additional divisions following DR (Cohen et al., 2004).

Interestingly, increased SirT1 activity promotes fat mobilization by inhibiting activity of the fat regulator PPAR-γ (Picard et al., 2004). It is possible that SirT1 will prove to increase mouse life span by decreasing fat levels since several mouse mutants that have reduced fat levels are long lived. However, as discussed above, some long-lived mouse mutants have increased fat levels. In addition, the antidiabetic thiazolidinediones, which activate PPAR-γ and increase fat levels, increase insulin sensitivity and have beneficial health effects (Savage, 2005). Thus it will be very interesting to learn how manipulating SirT1 activity influences the life span of the whole animal.

Metabolic Genes. Reduction of function of *Drosophila indy,* which encodes an amino acid transporter, doubles the mean life span of flies (Rogina et al., 2000). This mutation does not simply starve the animal because, unlike DR, it does not decrease and delay reproduction. However, *indy* appears to function in the longevity response to DR because its long life span cannot be further lengthened by DR (B. Rogina and S.L. Helfand, personal communication).

clk-1 and Ubiquinone. *C. elegans clk-1* mutants, which are unable to produce ubiquinone, an essential component of the electron transport chain, are long lived (Felkai et al., 1999). *clk-1* mutants contain a novel ubiquinone species called dimethoxy-Q9, which they produce from a precursor they acquire from bacteria (Jonassen et al., 2001; Larsen and Clarke, 2002). *clk-1* mutants have normal metabolism and ATP levels, so dimethoxy-Q9 presumably functions in electron transport. However, it is also distributed to the plasma membrane and other locations; so it is not clear whether or not the animal's long life is caused by changes in mitochondrial activity. The longevity of *clk-1* mutants is not further enhanced by "eat" mutations, which cause DR and life span extension, suggesting that *clk-1* functions in the DR pathway (Lakowski and Hekimi, 1998). The longevity phenotype of *clk-1* mutants can be rescued with maternal clk(+) product, so the gene may function in the adult to influence life span (Jonassen et al., 2001). Consistent with this, initiation of DR during adulthood extends life span in worms and other animals.

A Puzzle. On the whole, worm and fly mutants behave differently when subjected to DR. So far, a *Drosophila* longevity mutation that can further extend the life spans of animals subjected to DR has yet to be reported. In contrast, all but one of the worm mutations examined further extend the life spans of animals subjected to DR. Perhaps worms would behave more like flies if they were subjected to DR in a different way. Or perhaps genes that mediate the longevity response to DR in flies instead mediate a longevity response to sensory cues in worms, and the worm DR response is regulated in a different way.

Mitochondrial Mutations that Increase Life Span

Reactive oxygen species (ROS) damage cellular macromolecules and are thought to accelerate aging. Consistent with this, growth in low oxygen lengthens the life

span of *C. elegans,* and growth in high oxygen shortens life span (Honda et al., 1993). ROS are generated during respiration, and some respiratory-chain mutations shorten life span and accelerate age-related phenotypes, such as lipofuscin accumulation. These mutations, typified by the *C. elegans mev-1(kn1)* (succinate dehydrogenase cytochrome *b*) point mutation, appear to accelerate aging by increasing ROS levels (Ishii et al., 1998).

A number of mutations affecting respiration increase life span, and at least some may do this by decreasing ROS levels, though this has not been examined directly.

Two Respiratory-Chain Longevity Phenotypes in Yeast

As described above, a shift from fermentation to oxidative respiration in yeast extends life span (Lin et al., 2002). Conversely, yeast mutations that inhibit respiration have also been reported to increase life span. This life span extension may require genes that mediate the "retrograde response," a process by which inhibition of respiration stimulates nuclear gene expression (Kirchman et al., 1999).

Two Respiratory-Chain Longevity Phenotypes in Worms

As in yeast, in *C. elegans,* life span is increased by RNAi inhibition of respiratory-chain components (Dillin et al., 2002b; Lee et al., 2002). Surprisingly, respiratory-chain RNAi only increases life span if administered during development (Dillin et al., 2002b). If inhibiting respiration extended life span by reducing ROS levels, then one would expect it to influence life span in an ongoing way, throughout life. Instead, the animal's life span may be extended because it undergoes a regulated response to reduced respiration early in life that subsequently increases its life span.

Curiously, *qm50,* a point mutation in the *C. elegans* gene *isp-1,* which encodes a component of respiratory-chain complex III, extends life span but causes a different phenotype (Feng et al., 2001). First, this *isp-1* mutant has a normal body size. In addition, respiratory-chain RNAi further extends the life spans of *daf-2* mutants, but this *isp-1* mutation does not. Like *daf-2* mutants, this *isp-1* mutant is paraquat resistant and expresses increased levels of superoxide dismutase; however, its effect on life span is *daf-16* independent. Together these findings suggest that this *isp-1* mutation and *daf-2* mutations may ultimately influence the same downstream process.

In summary, these mitochondrial mutations, and the *clk-1* ubiquinone mutations described above, produce a fascinating array of unexpected effects on life span. Clearly we have much more to learn about how mitochondria influence life span.

Telomeres and Life Span

In many human tissues, telomeres progressively shorten with age. Forced expression of the telomere-extending enzyme telomerase can prevent human cells in culture from undergoing senescence (Bodnar et al., 1998). This finding supports the hypothesis that organismal aging, too, is caused by telomere shortening. Consistent with this, overexpressing a protein that lengthens telomeres extends the life span of *C. elegans* (Joeng et al., 2004). This is intriguing because the somatic cells of *C. elegans* are postmitotic, so they are not susceptible to replicative telomere shortening. The long-lived animals are stress resistant, suggesting unusually long telomeres may lengthen life span by inducing stress-response proteins.

Telomere shortening is unlikely to be a general cause of aging since mice age and die with long telomeres and loss of telomerase in mice has no effect on aging for several generations (Blasco et al., 1997). However, recent findings strengthen the case that telomeres may affect aging in humans. First, telomere length has been found to correlate with longevity and disease resistance in humans (Cawthon et al., 2003). In addition, loss of the Werner's gene, which encodes a DNA helicase, causes progeria (accelerated aging) in humans. Loss of the same gene in normal mice does not cause progeria, but loss of the gene in mice that lack telomerase does (Chang et al., 2004). This finding implies that telomere shortening makes humans susceptible to Werner's progeria syndrome, which in turn suggests that telomeres are involved in normal human aging. Interestingly, both with and without the Werner's mutation, aging telomerase-knockout mice exhibit phenotypes that are characteristic of old humans rather than old mice. Perhaps increasing telomere length will prove to increase human life span after all. And when we do get old, perhaps we will acquire signs of aging that are more like those of mice.

Age-Related Disease

Susceptibility to a wide variety of diseases increases with age. We are 100 times more likely to have a tumor at age 65 than at age 35. What, at the molecular level, links aging to age-related disease? It is not simply the passage of time. Mice exhibit high rates of cancer at 1.5 years (mean life span, 2 years), dogs at 10 years (life span ~12 years), and humans after decades. Interestingly, long-lived insulin/IGF-1 mutants are resistant to many age-related diseases, including Huntington's disease in a *C. elegans* disease model (Morley et al., 2002), sarcopenia in worms (Herndon et al., 2002), heart failure in *Drosophila* (Wessells et al., 2004), and cancer in rodents (Bielschowsky, 1961; Ramsey et al., 2002). Thus this pathway couples aging to age-related disease.

Protein Aggregation

In at least one case, Huntington's disease in the *C. elegans* model, a direct molecular link between normal aging and disease susceptibility has been identified (Hsu et al., 2003). Life span-extending insulin/IGF-1 mutations increase expression of small heat shock proteins, and these proteins not only increase life span but also delay the time of onset of Huntingtin-like protein aggregates. Small heat-shock proteins are chaperones that bind to damaged or misfolded proteins and prevent their aggregation (Haslbeck, 2002). By doing this, they could assist in the maintenance or turnover of normal cellular components, thereby forestalling aging and they could also delay disease-related protein aggregation.

Cancer

The incidence of cancer rises with age partly because more than one genetic alteration ("hit") is required. In addition, something correlated with biological (rather

than chronological) age must affect the likelihood of these hits. Mutations that upregulate insulin/IGF-1 signaling, such as PTEN tumor-suppressor mutations, increase the incidence of cancer. Insulin/IGF-1 signaling potentiates cancer, at least in part, by downregulating FOXO activity, which would otherwise activate genes that inhibit cell growth, such as p21Cip1 (Seoane et al., 2004), or trigger apoptosis, such as fas ligand and Bim (Brunet et al., 1999; Dijkers et al., 2000). In addition, PTEN mutations activate the growth-promoting TOR pathway, and this appears to promote certain types of cancer as well (Inoki et al., 2005). The genetic analysis of aging predicts that if insulin/IGF-1 signaling were more effective in one species than another, that species would have a shorter life span and a higher cancer susceptibility "set point." Whether the same FOXO target genes that affect cancer also affect aging is not clear. It is possible that FOXO activates the expression of one set of genes that inhibits cancer and another set (stress resistance genes perhaps) that influences aging.

Some mutations that increase cancer resistance, for example, certain gain-of-function p53 mutations (Tyner et al., 2002), decrease mouse life span and thereby break the correlation between longevity and cancer resistance. Either such mutations did not play an important role in the coevolution of longevity and cancer resistance, or their deleterious effects were offset by mutations in other genes.

Neuronal Degeneration

Increased activity of the mammalian SIR2 homolog SirT1 can promote axon regrowth after injury (Araki et al., 2004). The "Wallerian degeneration slow" (wlds) mutation delays Wallerian degeneration in response to axon injury because it causes overexpression of an NAD biosynthetic enzyme, which in turn activates SirT1. How SirT1 prevents degeneration is not known, but if the protective mechanism is a general one, then SirT1 may also protect cells from neurodegenerative disease, again potentially linking age-related disease to the aging process itself.

Prospects

Changes in many more genes, too numerous to describe here, also extend life span. Together all of these mutations make it immensely clear that the rate of aging is plastic and amenable to change. Moreover, truly astounding life span extensions are possible. For example, in *C. elegans,* removing the reproductive system and decreasing *daf-2* activity in the same animals extends life span by 6-fold. These animals have a normal appearance and remain vigorous for many months (Arantes-Oliveira et al., 2003). In human terms, they would be the equivalent of healthy, active 500-year-olds. A life span extension of this magnitude was once unthinkable, but still it pales in comparison to the thousand-fold life span extension that must have taken place as we humans evolved from animals living only a few weeks.

It may not be possible to slow the rate of aging in humans. But why has it taken so long for us to seriously try? Perhaps it is because there are no extremely long-lived, talented primates for us to emulate. It is unlikely that we would have invented airplanes without seeing birds fly. Now genetics has given us long-lived model organisms for inspiration instead.

References

Alcedo, J., and Kenyon, C. (2004). Regulation of *C. elegans* longevity by specific gustatory and olfactory neurons. Neuron *41*, 45–55.

Apfeld, J., and Kenyon, C. (1998). Cell nonautonomy of *C. elegans daf-2* function in the regulation of diapause and life span. Cell *95*, 199–210.

Apfeld, J., and Kenyon, C. (1999). Regulation of lifespan by sensory perception in *Caenorhabditis elegans*. Nature *402*, 804–809.

Apfeld, J., O'Connor, G., McDonagh, T., DiStefano, P.S., and Curtis, R. (2004). The AMP-activated protein kinase AAK-2 links energy levels and insulin-like signals to lifespan in C. elegans. Genes Dev. *18*, 3004–3009.

Araki, T., Sasaki, Y., and Milbrandt, J. (2004). Increased nuclear NAD biosynthesis and SIRT1 activation prevent axonal degeneration. Science *305*, 1010–1013.

Arantes-Oliveira, N., Apfeld, J., Dillin, A., and Kenyon, C. (2002). Regulation of life-span by germ-line stem cells in Caenorhabditis elegans. Science *295*, 502–505.

Arantes-Oliveira, N., Berman, J.R., and Kenyon, C. (2003). Healthy animals with extreme longevity. Science *302*, 611.

Ashrafi, K., Chang, F.Y., Watts, J.L., Fraser, A.G., Kamath, R.S., Ahringer, J., and Ruvkun, G. (2003). Genome-wide RNAi analysis of Caenorhabditis elegans fat regulatory genes. Nature *421*, 268–272.

Barsyte, D., Lovejoy, D.A., and Lithgow, G.J. (2001). Longevity and heavy metal resistance in daf-2 and age-1 long-lived mutants of Caenorhabditis elegans. FASEB J. *15*, 627–634.

Bartke, A., Wright, J.C., Mattison, J.A., Ingram, D.K., Miller, R.A., and Roth, G.S. (2001). Extending the lifespan of long-lived mice. Nature *414*, 412.

Barzilai, N., Atzmon, G., Schechter, C., Schaefer, E.J., Cupples, A.L., Lipton, R., Cheng, S., and Shuldiner, A.R. (2003). Unique lipoprotein phenotype and genotype associated with exceptional longevity. JAMA *290*, 2030–2040.

Bielschowsky, F.B. (1961). Carcinogenesis in the pituitary dwarf mouse. The response to dimethylbenzanthracene applied to the skin. Br. J. Cancer *15*, 257–262.

Blasco, M.A., Lee, H.W., Hande, M.P., Samper, E., Lansdorp, P.M., DePinho, R.A., and Greider, C.W. (1997). Telomere shortening and tumor formation by mouse cells lacking telomerase RNA. Cell *91*, 25–34.

Bluher, M., Kahn, B.B., and Kahn, C.R. (2003). Extended longevity in mice lacking the insulin receptor in adipose tissue. Science *299*, 572–574.

Bodnar, A.G., Ouellette, M., Frolkis, M., Holt, S.E., Chiu, C.P., Morin, G.B., Harley, C.B., Shay, J.W., Lichtsteiner, S., and Wright, W.E. (1998). Extension of life-span by introduction of telomerase into normal human cells. Science *279*, 349–352.

Broughton, S., Piper, M., Ikeya, T., Bass, T., Jacobson, J., Driege, Y., Martinez, P., Hafen, E., Withers, D., Leevers, S., and Partridge, L. (2005). Longer lifespan, altered metabolism and stress resistance in Drosophila from ablation of cells making insulin-like ligands. Proc. Natl. Acad. Sci. USA in press. Published online February 11, 2005.

Brown-Borg, H.M., Borg, K.E., Meliska, C.J., and Bartke, A. (1996). Dwarf mice and the ageing process. Nature *384*, 33.

Brunet, A., Bonni, A., Zigmond, M.J., Lin, M.Z., Juo, P., Hu, L.S., Anderson, M.J., Arden, K.C., Blenis, J., and Greenberg, M.E. (1999). Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. Cell *96*, 857–868.

Brunet, A., Sweeney, L.B., Sturgill, J.F., Chua, K.F., Greer, P.L., Lin, Y., Tran, H., Ross, S.E., Mostoslavsky, R., Cohen, H.Y., et al. (2004).

Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. Science *303*, 2011–2015.

Burgering, B.M., and Kops, G.J. (2002). Cell cycle and death control: long live Forkheads. Trends Biochem. Sci. *27*, 352–360.

Cargill, S.L., Carey, J.R., Muller, H.G., and Anderson, G. (2003). Age of ovary determines remaining life expectancy in old ovariectomized mice. Aging Cell *2*, 185–190.

Cawthon, R.M., Smith, K.R., O'Brien, E., Sivatchenko, A., and Kerber, R.A. (2003). Association between telomere length in blood and mortality in people aged 60 years or older. Lancet *361*, 393–395.

Chang, S., Multani, A.S., Cabrera, N.G., Naylor, M.L., Laud, P., Lombard, D., Pathak, S., Guarente, L., and DePinho, R.A. (2004). Essential role of limiting telomeres in the pathogenesis of Werner syndrome. Nat. Genet. *36*, 877–882.

Chiu, C.H., Lin, W.D., Huang, S.Y., and Lee, Y.H. (2004). Effect of a C/EBP gene replacement on mitochondrial biogenesis in fat cells. Genes Dev. *18*, 1970–1975.

Clancy, D.J., Gems, D., Harshman, L.G., Oldham, S., Stocker, H., Hafen, E., Leevers, S.J., and Partridge, L. (2001). Extension of lifespan by loss of CHICO, a *Drosophila* insulin receptor substrate protein. Science *292*, 104–106.

Clancy, D.J., Gems, D., Hafen, E., Leevers, S.J., and Partridge, L. (2002). Dietary restriction in long-lived dwarf flies. Science *296*, 319.

Cohen, H.Y., Miller, C., Bitterman, K.J., Wall, N.R., Hekking, B., Kessler, B., Howitz, K.T., Gorospe, M., de Cabo, R., and Sinclair, D.A. (2004). Calorie restriction promotes mammalian cell survival by inducing the SIRT1 deacetylase. Science *305*, 390–392.

Coschigano, K.T., Holland, A.N., Riders, M.E., List, E.O., Flyvbjerg, A., and Kopchick, J.J. (2003). Deletion, but not antagonism, of the mouse growth hormone receptor results in severely decreased body weights, insulin, and insulin-like growth factor I levels and increased life span. Endocrinology *144*, 3799–3810.

Daitoku, H., Hatta, M., Matsuzaki, H., Aratani, S., Ohshima, T., Miyagishi, M., Nakajima, T., and Fukamizu, A. (2004). Silent information regulator 2 potentiates Foxo1-mediated transcription through its deacetylase activity. Proc. Natl. Acad. Sci. USA *101*, 10042–10047.

Dijkers, P.F., Medema, R.H., Lammers, J.W., Koenderman, L., and Coffer, P.J. (2000). Expression of the pro-apoptotic Bcl-2 family member Bim is regulated by the forkhead transcription factor FKHR-L1. Curr. Biol. *10*, 1201–1204.

Dillin, A., Crawford, D.K., and Kenyon, C. (2002a). Timing requirements for insulin/IGF-1 signaling in *C. elegans*. Science *298*, 830–834.

Dillin, A., Hsu, A.L., Arantes-Oliveira, N., Lehrer-Graiwer, J., Hsin, H., Fraser, A.G., Kamath, R.S., Ahringer, J., and Kenyon, C. (2002b). Rates of behavior and aging specified by mitochondrial function during development. Science *298*, 2398–2401.

Eigenmann, J.E., Patterson, D.F., and Froesch, E.R. (1984). Body size parallels insulin-like growth factor I levels but not growth hormone secretory capacity. Acta Endocrinol. (Copenh.) *106*, 448–453.

Fabrizio, P., Liou, L.L., Moy, V.N., Diaspro, A., SelverstoneValentine, J., Gralla, E.B., and Longo, V.D. (2003). SOD2 functions downstream of Sch9 to extend longevity in yeast. Genetics *163*, 35–46.

Felkai, S., Ewbank, J.J., Lemieux, J., Labbe, J.C., Brown, G.G., and Hekimi, S. (1999). CLK-1 controls respiration, behavior and aging in the nematode Caenorhabditis elegans. EMBO J. *18*, 1783–1792.

Feng, J., Bussiere, F., and Hekimi, S. (2001). Mitochondrial electron transport is a key determinant of life span in *Caenorhabditis elegans*. Dev. Cell *1*, 633–644.

Flurkey, K., Papaconstantinou, J., and Harrison, D.E. (2002). The Snell dwarf mutation Pit1(dw) can increase life span in mice. Mech. Ageing Dev. *123*, 121–130.

Friedman, D.B., and Johnson, T.E. (1988). A mutation in the age-1 gene in Caenorhabditis elegans lengthens life and reduces hermaphrodite fertility. Genetics *118*, 75–86.

Garigan, D., Hsu, A.L., Fraser, A.G., Kamath, R.S., Ahringer, J., and Kenyon, C. (2002). Genetic analysis of tissue aging in Caenorhabditis elegans: a role for heat-shock factor and bacterial proliferation. Genetics *161*, 1101–1112.

Garsin, D.A., Villanueva, J.M., Begun, J., Kim, D.H., Sifri, C.D., Calderwood, S.B., Ruvkun, G., and Ausubel, F.M. (2003). Long-lived C. elegans daf-2 mutants are resistant to bacterial pathogens. Science *300*, 1921.

Geesaman, B.J., Benson, E., Brewster, S.J., Kunkel, L.M., Blanche, H., Thomas, G., Perls, T.T., Daly, M.J., and Puca, A.A. (2003). Haplotype-based identification of a microsomal transfer protein marker associated with the human lifespan. Proc. Natl. Acad. Sci. USA *100*, 14115–14120.

Gems, D., and Riddle, D.L. (2000). Genetic, behavioral and environmental determinants of male longevity in Caenorhabditis elegans. Genetics *154*, 1597–1610.

Gems, D., Sutton, A.J., Sundermeyer, M.L., Albert, P.S., King, K.V., Edgley, M.L., Larsen, P.L., and Riddle, D. (1998). Two pleiotropic classes of *daf-2* mutation affect larval arrest, adult behavior, reproduction and longevity in *Caenorhabditis elegans*. Genetics *150*, 129–155.

Gerisch, B., and Antebi, A. (2004). Hormonal signals produced by DAF-9/cytochrome P450 regulate C. elegans dauer diapause in response to environmental cues. Development *131*, 1765–1776.

Giannakou, M.E., and Partridge, L. (2004). The interaction between FOXO and SIRT1: tipping the balance towards survival. Trends Cell Biol. *14*, 408–412.

Giannakou, M.E., Goss, M., Junger, M.A., Hafen, E., Leevers, S.J., and Partridge, L. (2004). Long-lived Drosophila with overexpressed dFOXO in adult fat body. Science *305*, 361.

Guarente, L., and Picard, F. (2005). Calorie restriction—the SIR2 connection. Cell *120*, this issue, 473–482.

Harrison, D.E., Archer, J.R., and Astle, C.M. (1984). Effects of food restriction on aging: separation of food intake and adiposity. Proc. Natl. Acad. Sci. USA *81*, 1835–1838.

Haslbeck, M. (2002). sHsps and their role in the chaperone network. Cell. Mol. Life Sci. *59*, 1649–1657.

Henderson, S.T., and Johnson, T.E. (2001). daf-16 integrates developmental and environmental inputs to mediate aging in the nematode *Caenorhabditis elegans*. Curr. Biol. *11*, 1975–1980.

Hercus, M.J., Loeschcke, V., and Rattan, S.I. (2003). Lifespan extension of Drosophila melanogaster through hormesis by repeated mild heat stress. Biogerontology *4*, 149–156.

Herndon, L.A., Schmeissner, P.J., Dudaronek, J.M., Brown, P.A., Listner, K.M., Sakano, Y., Paupard, M.C., Hall, D.H., and Driscoll, M. (2002). Stochastic and genetic factors influence tissue-specific decline in ageing C. elegans. Nature *419*, 808–814.

Hertweck, M., Gobel, C., and Baumeister, R. (2004). *C. elegans* SGK-1 is the critical component in the Akt/PKB kinase complex to control stress response and life span. Dev. Cell *6*, 577–588.

Holzenberger, M., Dupont, J., Ducos, B., Leneuve, P., Geloen, A., Even, P.C., Cervera, P., and Le Bouc, Y. (2003). IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice. Nature *421*, 182–187.

Honda, Y., and Honda, S. (1999). The daf-2 gene network for longevity regulates oxidative stress resistance and Mn-superoxide dismutase gene expression in Caenorhabditis elegans. FASEB J. *13*, 1385–1393.

Honda, S., Ishii, N., Suzuki, K., and Matsuo, M. (1993). Oxygen-dependent perturbation of life span and aging rate in the nematode. J. Gerontol. *48*, B57–B61.

Houthoofd, K., Braeckman, B.P., Johnson, T.E., and Vanfleteren, J.R. (2003). Life extension via dietary restriction is independent of the Ins/IGF-1 signalling pathway in Caenorhabditis elegans. Exp. Gerontol. *38*, 947–954.

Hsin, H., and Kenyon, C. (1999). Signals from the reproductive system regulate the lifespan of *C. elegans*. Nature *399*, 362–366.

Hsu, A.L., Murphy, C.T., and Kenyon, C. (2003). Regulation of aging and age-related disease by DAF-16 and heat-shock factor. Science *300*, 1142–1145.

Hwangbo, D.S., Gersham, B., Tu, M.P., Palmer, M., and Tatar, M.

(2004). Drosophila dFOXO controls lifespan and regulates insulin signalling in brain and fat body. Nature *429*, 562–566.

Inoki, K., Corradetti, M.N., and Guan, K.L. (2005). Dysregulation of the TSC-mTOR pathway in human disease. Nat. Genet. *37*, 19–24.

Ishii, N., Fujii, M., Hartman, P.S., Tsuda, M., Yasuda, K., Senoo-Matsuda, N., Yanase, S., Ayusawa, D., and Suzuki, K. (1998). A mutation in succinate dehydrogenase cytochrome b causes oxidative stress and ageing in nematodes. Nature *394*, 694–697.

Jia, K., Chen, D., and Riddle, D.L. (2004). The TOR pathway interacts with the insulin signaling pathway to regulate C. elegans larval development, metabolism and life span. Development *131*, 3897–3906.

Joeng, K.S., Song, E.J., Lee, K.J., and Lee, J. (2004). Long lifespan in worms with long telomeric DNA. Nat. Genet. *36*, 607–611.

Jonassen, T., Larsen, P.L., and Clarke, C.F. (2001). A dietary source of coenzyme Q is essential for growth of long-lived Caenorhabditis elegans clk-1 mutants. Proc. Natl. Acad. Sci. USA *98*, 421–426.

Jones, S.J., Riddle, D.L., Pouzyrev, A.T., Velculescu, V.E., Hillier, L., Eddy, S.R., Stricklin, S.L., Baillie, D.L., Waterston, R., and Marra, M.A. (2001). Changes in gene expression associated with developmental arrest and longevity in Caenorhabditis elegans. Genome Res. *11*, 1346–1352.

Junger, M.A., Rintelen, F., Stocker, H., Wasserman, J.D., Vegh, M., Radimerski, T., Greenberg, M.E., and Hafen, E. (2003). The Drosophila forkhead transcription factor FOXO mediates the reduction in cell number associated with reduced insulin signaling. J. Biol. *2*, 20.

Kaeberlein, M., Kirkland, K.T., Fields, S., and Kennedy, B.K. (2004). Sir2-independent life span extension by calorie restriction in yeast. PLoS Biol. *2*, e296. 10.1371/journal.pbio.0020296

Kapahi, P., Boulton, M.E., and Kirkwood, T.B. (1999). Positive correlation between mammalian life span and cellular resistance to stress. Free Radic. Biol. Med. *26*, 495–500.

Kapahi, P., Zid, B.M., Harper, T., Koslover, D., Sapin, V., and Benzer, S. (2004). Regulation of lifespan in *Drosophila* by modulation of genes in the TOR signaling pathway. Curr. Biol. *14*, 885–890.

Kenyon, C., Chang, J., Gensch, E., Rudner, A., and Tabtiang, R. (1993). A C. elegans mutant that lives twice as long as wild type. Nature *366*, 461–464.

Kimura, K.D., Tissenbaum, H.A., Liu, Y., and Ruvkun, G. (1997). daf-2, an insulin receptor-like gene that regulates longevity and diapause in Caenorhabditis elegans. Science *277*, 942–946.

Kirchman, P.A., Kim, S., Lai, C.Y., and Jazwinski, S.M. (1999). Interorganelle signaling is a determinant of longevity in Saccharomyces cerevisiae. Genetics *152*, 179–190.

Kops, G.J., Dansen, T.B., Polderman, P.E., Saarloos, I., Wirtz, K.W., Coffer, P.J., Huang, T.T., Bos, J.L., Medema, R.H., and Burgering, B.M. (2002). Forkhead transcription factor FOXO3a protects quiescent cells from oxidative stress. Nature *419*, 316–321.

Lakowski, B., and Hekimi, S. (1998). The genetics of caloric restriction in Caenorhabditis elegans. Proc. Natl. Acad. Sci. USA *95*, 13091–13096.

Larsen, P.L. (1993). Aging and resistance to oxidative damage in Caenorhabditis elegans. Proc. Natl. Acad. Sci. USA *90*, 8905–8909.

Larsen, P.L., and Clarke, C.F. (2002). Extension of life-span in Caenorhabditis elegans by a diet lacking coenzyme Q. Science *295*, 120–123.

Lee, R.Y., Hench, J., and Ruvkun, G. (2001). Regulation of *C. elegans* DAF-16 and its human ortholog FKHRL1 by the daf-2 insulin-like signaling pathway. Curr. Biol. *11*, 1950–1957.

Lee, S.S., Kennedy, S., Tolonen, A.C., and Ruvkun, G. (2003). DAF-16 target genes that control C. elegans life-span and metabolism. Science *300*, 644–647.

Lee, S.S., Lee, R.Y., Fraser, A.G., Kamath, R.S., Ahringer, J., and Ruvkun, G. (2002). A systematic RNAi screen identifies a critical role for mitochondria in C. elegans longevity. Nat. Genet. *33*, 40–48.

Li, W., Kennedy, S.G., and Ruvkun, G. (2003). daf-28 encodes a C. elegans insulin superfamily member that is regulated by environmental cues and acts in the DAF-2 signaling pathway. Genes Dev. *17*, 844–858.

Libina, N., Berman, J.R., and Kenyon, C. (2003). Tissue-specific activities of the *C. elegans* DAF-16 protein in the regulation of lifespan. Cell *115*, 489–502.

Lin, K., Dorman, J.B., Rodan, A., and Kenyon, C. (1997). *daf-16*: an HNF-3/forkhead family member that can function to double the lifespan of *Caenorhabditis elegans*. Science *278*, 1319–1322.

Lin, K., Hsin, H., Libina, N., and Kenyon, C. (2001). Regulation of the *Caenorhabditis elegans* longevity protein DAF-16 by insulin/IGF-1 and germline signaling. Nat. Genet. *28*, 139–145.

Lin, S.J., Kaeberlein, M., Andalis, A.A., Sturtz, L.A., Defossez, P.A., Culotta, V.C., Fink, G.R., and Guarente, L. (2002). Calorie restriction extends Saccharomyces cerevisiae lifespan by increasing respiration. Nature *418*, 344–348.

Lindemann, B. (2001). Receptors and transduction in taste. Nature *413*, 219–225.

Lithgow, G.J., White, T.M., Melov, S., and Johnson, T.E. (1995). Thermotolerance and extended life-span conferred by single-gene mutations and induced by thermal stress. Proc. Natl. Acad. Sci. USA *92*, 7540–7544.

Longo, V.D. (2003). The Ras and Sch9 pathways regulate stress resistance and longevity. Exp. Gerontol. *38*, 807–811.

McCulloch, D., and Gems, D. (2003). Body size, insulin/IGF signaling and aging in the nematode Caenorhabditis elegans. Exp. Gerontol. *38*, 129–136.

McElwee, J.J., Schuster, E., Blanc, E., Thomas, J.H., and Gems, D. (2004). Shared transcriptional signature in Caenorhabditis elegans Dauer larvae and long-lived daf-2 mutants implicates detoxification system in longevity assurance. J. Biol. Chem. *279*, 44533–44543.

Melendez, A., Talloczy, Z., Seaman, M., Eskelinen, E.L., Hall, D.H., and Levine, B. (2003). Autophagy genes are essential for dauer development and life-span extension in C. elegans. Science *301*, 1387–1391.

Michael, M.D., Kulkarni, R.N., Postic, C., Previs, S.F., Shulman, G.I., Magnuson, M.A., and Kahn, C.R. (2000). Loss of insulin signaling in hepatocytes leads to severe insulin resistance and progressive hepatic dysfunction. Mol. Cell *6*, 87–97.

Morley, J.F., and Morimoto, R.I. (2004). Regulation of longevity in Caenorhabditis elegans by heat shock factor and molecular chaperones. Mol. Biol. Cell *15*, 657–664.

Morley, J.F., Brignull, H.R., Weyers, J.J., and Morimoto, R.I. (2002). The threshold for polyglutamine-expansion protein aggregation and cellular toxicity is dynamic and influenced by aging in Caenorhabditis elegans. Proc. Natl. Acad. Sci. USA *99*, 10417–10422.

Morris, J.Z., Tissenbaum, H.A., and Ruvkun, G. (1996). A phosphatidylinositol-3-OH kinase family member regulating longevity and diapause in Caenorhabditis elegans. Nature *382*, 536–539.

Motta, M.C., Divecha, N., Lemieux, M., Kamel, C., Chen, D., Gu, W., Bultsma, Y., McBurney, M., and Guarente, L. (2004). Mammalian SIRT1 represses forkhead transcription factors. Cell *116*, 551–563.

Murakami, S., and Johnson, T.E. (1996). A genetic pathway conferring life extension and resistance to UV stress in Caenorhabditis elegans. Genetics *143*, 1207–1218.

Murakami, S., and Johnson, T.E. (2001). The OLD-1 positive regulator of longevity and stress resistance is under DAF-16 regulation in *Caenorhabditis elegans*. Curr. Biol. *11*, 1517–1523.

Murphy, C.T., McCarroll, S., Bargmann, C.I., Fraser, A., Kamath, R.S., Ahringer, J., Li, H., and Kenyon, C. (2003). Genes that act downstream of DAF-16 tp influence the lifespan of C. elegans. Nature *424*, 277–283.

Nemoto, S., and Finkel, T. (2002). Redox regulation of forkhead proteins through a p66shc-dependent signaling pathway. Science *295*, 2450–2452.

Ogg, S., and Ruvkun, G. (1998). The *C. elegans* PTEN homolog, DAF-18, acts in the insulin receptor-like metabolic signaling pathway. Mol. Cell *2*, 887–893.

Ogg, S., Paradis, S., Gottlieb, S., Patterson, G.I., Lee, L., Tissenbaum, H.A., and Ruvkun, G. (1997). The Fork head transcription

factor DAF-16 transduces insulin-like metabolic and longevity signals in C. elegans. Nature *389*, 994–999.

Ookuma, S., Fukuda, M., and Nishida, E. (2003). Identification of a DAF-16 transcriptional target gene, scl-1, that regulates longevity and stress resistance in *Caenorhabditis elegans*. Curr. Biol. *13*, 427–431.

Paradis, S., and Ruvkun, G. (1998). Caenorhabditis elegans Akt/PKB transduces insulin receptor-like signals from AGE-1 PI3 kinase to the DAF-16 transcription factor. Genes Dev. *12*, 2488–2498.

Paradis, S., Ailion, M., Toker, A., Thomas, J.H., and Ruvkun, G. (1999). A PDK1 homolog is necessary and sufficient to transduce AGE-1 PI3 kinase signals that regulate diapause in Caenorhabditis elegans. Genes Dev. *13*, 1438–1452.

Picard, F., Kurtev, M., Chung, N., Topark-Ngarm, A., Senawong, T., Machado De Oliveira, R., Leid, M., McBurney, M.W., and Guarente, L. (2004). Sirt1 promotes fat mobilization in white adipocytes by repressing PPAR-gamma. Nature *429*, 771–776.

Pierce, S.B., Costa, M., Wisotzkey, R., Devahdhar, S., Homburger, S.A., Buchman, R., and Ruvkun, G. (2001). Regulation of DAF-2 receptor signaling by human insulin and *ins-1*, a member of the unusually large and diverse *C. elegans* insulin gene family. Genes Dev. *15*, 672–686.

Ramsey, M.M., Ingram, R.L., Cashion, A.B., Ng, A.H., Cline, J.M., Parlow, A.F., and Sonntag, W.E. (2002). Growth hormone-deficient dwarf animals are resistant to dimethylbenzanthracine (DMBA)-induced mammary carcinogenesis. Endocrinology *143*, 4139–4142.

Reznick, D.N., Bryant, M.J., Roff, D., Ghalambor, C.K., and Ghalambor, D.E. (2004). Effect of extrinsic mortality on the evolution of senescence in guppies. Nature *431*, 1095–1099.

Riddle, D.L. (1997). C. elegans II (Plainview, NY: Cold Spring Harbor Laboratory Press).

Rogina, B., and Helfand, S.L. (2004). Sir2 mediates longevity in the fly through a pathway related to calorie restriction. Proc. Natl. Acad. Sci. USA *101*, 15998–16003.

Rogina, B., Reenan, R.A., Nilsen, S.P., and Helfand, S.L. (2000). Extended life-span conferred by cotransporter gene mutations in drosophila. Science *290*, 2137–2140.

Rogina, B., Helfand, S.L., and Frankel, S. (2002). Longevity regulation by Drosophila Rpd3 deacetylase and caloric restriction. Science *298*, 1745.

Savage, D.B. (2005). PPARgamma as a metabolic regulator: insights from genomics and pharmacology. Expert Rev. Mol. Med. *2005*, 1–16.

Seoane, J., Le, H.V., Shen, L., Anderson, S.A., and Massague, J. (2004). Integration of Smad and forkhead pathways in the control of neuroepithelial and glioblastoma cell proliferation. Cell *117*, 211–223.

Sgro, C.M., and Partridge, L. (1999). A delayed wave of death from reproduction in Drosophila. Science *286*, 2521–2524.

Simon, A.F., Shih, C., Mack, A., and Benzer, S. (2003). Steroid control of longevity in Drosophila melanogaster. Science *299*, 1407–1410.

Sun, J., and Tower, J. (1999). FLP recombinase-mediated induction of Cu/Zn-superoxide dismutase transgene expression can extend the life span of adult Drosophila melanogaster flies. Mol. Cell. Biol. *19*, 216–228.

Sun, J., Folk, D., Bradley, T.J., and Tower, J. (2002). Induced overexpression of mitochondrial Mn-superoxide dismutase extends the life span of adult Drosophila melanogaster. Genetics *161*, 661–672.

Tatar, M., Kopelman, A., Epstein, D., Tu, M.P., Yin, C.M., and Garofalo, R.S. (2001). A mutant Drosophila insulin receptor homolog that extends life-span and impairs neuroendocrine function. Science *292*, 107–110.

Tatar, M., Bartke, A., and Antebi, A. (2003). The endocrine regulation of aging by insulin-like signals. Science *299*, 1346–1351.

Tissenbaum, H.A., and Guarente, L. (2001). Increased dosage of a sir-2 gene extends lifespan in Caenorhabditis elegans. Nature *410*, 227–230.

Tran, H., Brunet, A., Grenier, J.M., Datta, S.R., Fornace, A.J., Jr., DiStefano, P.S., Chiang, L.W., and Greenberg, M.E. (2002). DNA repair pathway stimulated by the forkhead transcription factor FOXO3a through the Gadd45 protein. Science *296*, 530–534.

Tu, M.P., Epstein, D., and Tatar, M. (2002a). The demography of slow aging in male and female Drosophila mutant for the insulin-receptor substrate homologue chico. Aging Cell *1*, 75–80.

Tu, M.P., Yin, C.M., and Tatar, M. (2002b). Impaired ovarian ecdysone synthesis of Drosophila melanogaster insulin receptor mutants. Aging Cell *1*, 158–160.

Tyner, S.D., Venkatachalam, S., Choi, J., Jones, S., Ghebranious, N., Igelmann, H., Lu, X., Soron, G., Cooper, B., Brayton, C., et al. (2002). p53 mutant mice that display early ageing-associated phenotypes. Nature *415*, 45–53.

Van Voorhies, W.A., and Ward, S. (1999). Genetic and environmental conditions that increase longevity in Caenorhabditis elegans decrease metabolic rate. Proc. Natl. Acad. Sci. USA *96*, 11399–11403.

Vanfleteren, J.R., and De Vreese, A. (1995). The gerontogenes age-1 and daf-2 determine metabolic rate potential in aging Caenorhabditis elegans. FASEB J. *9*, 1355–1361.

Walker, G.A., and Lithgow, G.J. (2003). Lifespan extension in C. elegans by a molecular chaperone dependent upon insulin-like signals. Aging Cell *2*, 131–140.

Walker, D.W., McColl, G., Jenkins, N.L., Harris, J., and Lithgow, G.J. (2000). Evolution of lifespan in C. elegans. Nature *405*, 296–297.

Walker, G.A., White, T.M., McColl, G., Jenkins, N.L., Babich, S., Candido, E.P., Johnson, T.E., and Lithgow, G.J. (2001). Heat shock protein accumulation is upregulated in a long-lived mutant of Caenorhabditis elegans. J. Gerontol. A Biol. Sci. Med. Sci. *56*, B281–B287.

Wang, M.C., Bohmann, D., and Jasper, H. (2003). JNK signaling confers tolerance to oxidative stress and extends lifespan in *Drosophila*. Dev. Cell *5*, 811–816.

Wang, H.D., Kazemi-Esfarjani, P., and Benzer, S. (2004). Multiple-stress analysis for isolation of Drosophila longevity genes. Proc. Natl. Acad. Sci. USA *101*, 12610–12615.

Wessells, R.J., Fitzgerald, E., Cypser, J.R., Tatar, M., and Bodmer, R. (2004). Insulin regulation of heart function in aging fruit flies. Nat. Genet. *36*, 1275–1281.

Wolkow, C.A., Kimura, K.D., Lee, M.S., and Ruvkun, G. (2000). Regulation of C. elegans life-span by insulinlike signaling in the nervous system. Science *290*, 147–150.

Wolkow, C.A., Munoz, M.J., Riddle, D.L., and Ruvkun, G. (2002). Insulin receptor substrate and p55 orthologous adaptor proteins function in the Caenorhabditis elegans daf-2/insulin-like signaling pathway. J. Biol. Chem. *277*, 49591–49597.