

Evolutionary Medicine: From Dwarf Model Systems to Healthy Centenarians?

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Restriction of the number of calories consumed extends longevity in many organisms. In rodents, caloric restriction decreases the levels of plasma glucose and insulin-like growth factor I (IGF-1) and postpones or attenuates cancer, immunosenescence, and inflammation without irreversible side effects. In organisms ranging from yeast to mice, mutations in glucose or IGF-I-like signaling pathways extend life-span but also cause glycogen or fat accumulation and dwarfism. This information suggests a new category of drugs that could prevent or postpone diseases of aging with few adverse effects.

Restriction of caloric intake (CR) extends longevity in organisms from yeast to mice (1, 2) and postpones or prevents a remarkable array of diseases and age-dependent deterioration, without causing irreversible developmental or reproductive defects. By contrast, most genetic manipulations that extend life-span cause major side effects (Fig. 1). By combining our knowledge of the molecular pathways that regulate longevity and CR, we can begin to develop a novel strategy to prevent diseases such as cancer, Alzheimer's, and vascular diseases. The molecular pathways involved in the regulation of chronological life-span were identified at about the same time in yeast and worms (3–6). Similar to the life-span of higher eukaryotes, the yeast chronological life-span is determined by measuring survival time (7, 8). However, aging in the unicellular yeast is also studied by measuring the number of buds generated by an individual mother cell (replicative life-span) (9, 10). The down-regulation of glucose-dependent signaling by mutations in the *RAS2*, *CYR1/PKA* (11), or *SCH9* (12), genes extends the yeast chronological life-span up to 300% and increases resistance to oxidative stress and heat shock (Fig. 2) (7, 13). The down-regulation of the *CYR1/PKA* pathways also extends the yeast replicative life-span by a mechanism dependent on the silencing protein Sir2 (14). Chronological life-span extension in yeast is mediated by stress-resistance transcription factors Msn2 and Msn4 and mitochondrial superoxide dismutase (SOD2) (7, 13). In the worm *Caenorhabditis elegans*, the down-regulation of the pathways that includes the DAF-2, AGE-1, and AKT-1/AKT-2 proteins extends survival up to 300%

(15–17) and increases thermotolerance and antioxidant defenses, through the stress resistance transcription factor DAF-16 (Fig. 2) (5, 18). These yeast and worm “longevity pathways” share several homologous proteins, including superoxide dismutases, catalase, heat shock proteins, and the serine threonine kinases *SCH9* (yeast) and AKT-1/AKT-2 (worm) (Fig. 2) (19). The conserved function of longevity genes is also supported by the role of a gene homologous to yeast *SIR2* in extending longevity in worms (20). Thus, chronological longevity in yeast and worms is extended by inactivation of pathways that promote growth and, by an increase in protection against oxidative damage, other forms of stress. Systems that repair and replace damaged DNA, proteins, and lipids are also likely to play a major role in extending survival.

Conserved genes also regulate longevity in fruit flies. Mutations that decrease the activity of the fly insulin/IGF-I-like pathway cause dwarfism but nearly double longevity (21, 22). These mutations also increase the expression of SOD and the storage of nutrients (Fig. 2) (22). The similarities between

the yeast, worm, and fly longevity regulatory pathways suggest that portions of these pathways have evolved from common ancestors. Because glucose and insulin/IGF-I-like signaling pathways are down-regulated in the absence of nutrients, mutations in these pathways may simulate starvation conditions.

A decrease in IGF-I signaling may also extend longevity in mice. Mice homozygous for mutations in the *Prop-1* (23) or *Pit-1* (24) genes are dwarfs but live 25 to 65% longer than wild-type (25, 26). *Prop-1* or *Pit-1* homozygotes are deficient in serum growth hormone (GH), thyroid stimulating hormone (TSH), and prolactin as well as for IGF-I, which is secreted by liver cells upon stimulation with GH. The plasma GH deficiency appears to mediate the effects of *Prop-1* and *Pit-1* mutations on longevity, because the mice that cannot release GH in response to growth hormone releasing hormone also live longer (27). Furthermore, dwarf mice with high plasma GH but a 90% lower IGF-I [GH receptor/binding protein (GHR/BP) null mice] (28) live longer than the wild-type mice (29). Taken together, these studies suggest that the reduction in plasma IGF-I is responsible for a major portion of the life-span increase in dwarf, GH-deficient, and GHR/BP null mice.

Mammals also exhibit an association between stress resistance and reduced IGF-I signaling. The activities of antioxidant enzymes such as superoxide dismutases and catalase are decreased in murine hepatocytes exposed to GH or IGF-I and in transgenic mice overex-



Fig. 1. Wild-type (left specimen) and long-lived dwarf (right specimen) yeast, flies, and mice with mutations that decrease glucose or insulin/IGF-I-like signaling. Yeast *sch9* null mutants form smaller colonies (left). *sch9* mutants are also smaller in size, grow at a slower rate, and survive three times longer than wild-type yeast. *chico* homozygous mutant female flies are dwarfs and exhibit an increase in life-span of up to 50% (center) (fly image provided by D. Gems) (21). *Chico* functions in the fly insulin/IGF-I-like signaling pathway. The GHR/BP mice are dwarfs deficient in IGF-I and exhibit a 50% increase in life-span (right) (mouse images provided by A. Bartke). Other yeast and worm mutants exhibit life-span extension of more than 100% but do not have detectable growth defects.

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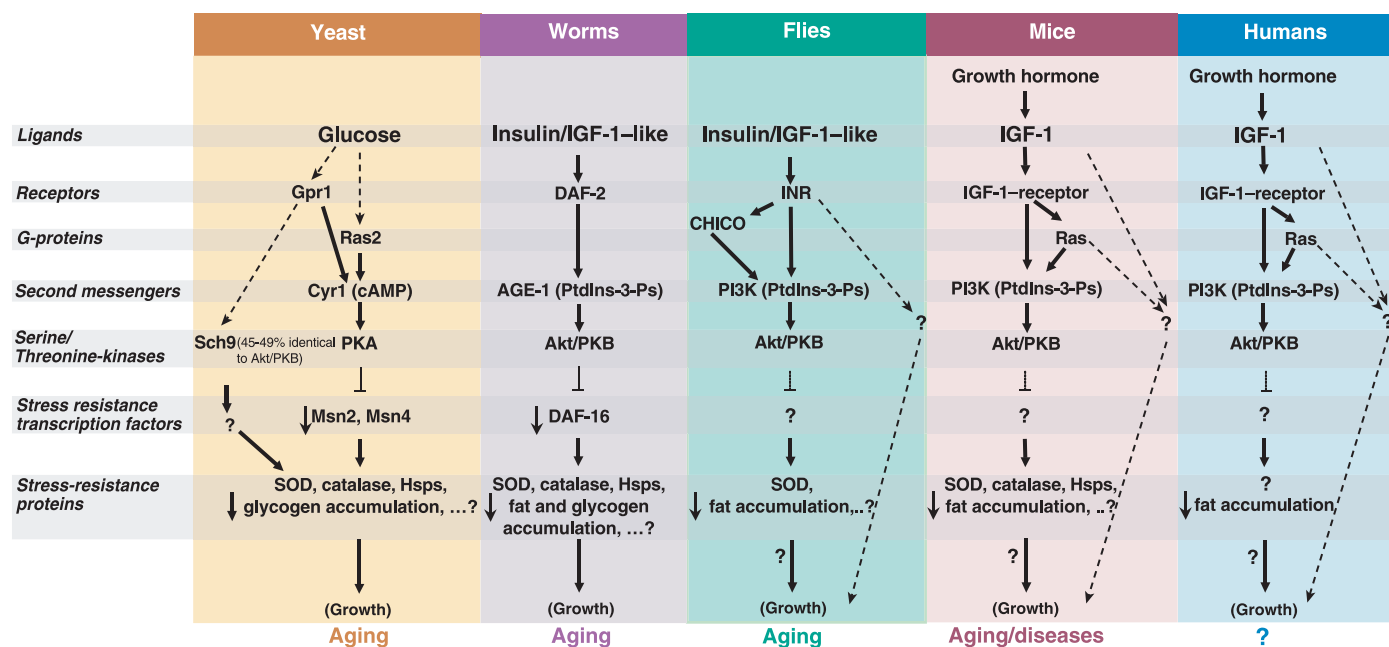


Fig. 2. Conserved regulation of longevity. In yeast, worms, and flies, the partially conserved glucose or insulin/IGF-I-like pathways down-regulate antioxidant enzymes and heat shock proteins, reduce the accumulation of glycogen or fat, and increase growth and mortality. Mutations that reduce the activity of these pathways appear to extend longevity by simulating caloric restriction or more severe forms of starvation. In yeast and worms, the induction of stress-resistance genes is required for

longevity extension. In mice, IGF-I activates signal transduction pathways analogous to the longevity regulatory pathways in lower eukaryotes and increases mortality. However, the intracellular mediators of life-span extension in GH- or IGF-I-deficient mice have not been identified. In humans, mutations or diseases that result in plasma GH or IGF-I deficiencies cause dwarfism, obesity, and other adverse effects, but their effect on longevity is unclear.

pressing GH (Fig. 2) (19). In rats, IGF-I attenuates cellular stress response and the expression of stress response proteins heat shock protein 72 (HSP72) and hemoxygenase (19). The storage of fat or glycogen is another aspect of the stress response. In yeast, the down-regulation of the Ras2/Cyr1/PKA pathway (where PKA is protein kinase A) results in the accumulation of glycogen, which is the major carbon source catabolized during periods of starvation. By contrast, in worms, flies, and mice, the down-regulation of the insulin/IGF-I-like pathways results in the accumulation of fat (Fig. 2). Dwarf mutations cause fat accumulation, which is reversed by administration of GH (19). IGF-I deficiency also increases fat accumulation in humans (see "Human GH/IGF-I Deficiency Diseases" section). In mammals fat is the major carbon source during long periods of starvation (hibernation), whereas glycogen provides glucose only during short periods of fasting. Therefore, the switch between glycogen storage in yeast and fat storage in metazoans is consistent with the role of longevity regulatory pathways in inducing accumulation of the carbon source that would maximize long-term survival during periods of starvation.

GH/IGF-I and Diseases in Rodents

The ability of GH and IGF-I to lower antioxidant defenses in hepatocytes, as described above, indicates that IGF-I can promote cellular damage and diseases in mammals. Thirty years ago, Silberberg showed that *Pit-1*

dwarf mice, which are deficient in plasma GH and IGF-I, had less osteoarthritis than wild-type mice (30). Since then, high levels of IGF-I have been associated with increased risk of several human diseases including breast, lung, colorectum, and prostate cancer (31). IGF-I appears to also promote cancer in mice, as tumors in *Pit-1* or *Prop-1* dwarf mice are either reduced or delayed (32, 33). Mice with elevated GH and IGF-I instead exhibit severe kidney lesions and a much shorter life-span (29, 30). Liver adenomas and carcinomas, as well as heart lesions, are common in older mice that overexpress GH (34, 35) (although the GH levels are supra-physiological) (36). The reduction of plasma GH and IGF-I may also have beneficial therapeutic effects in diabetic nephropathy (37).

The role of GH and IGF-I in age-dependent cognitive decline is unclear. Infusion of IGF-I into the brains of old rats for 4 weeks partially reverses the age-dependent decline in memory, but has no effect on sensorimotor skills (38). By contrast, *Prop-1* dwarf mice show improved cognitive function compared with age-matched normal mice (28). Further studies are needed on the role of plasma IGF-I in cognitive decline and neurodegenerative diseases.

Caloric Restriction, Aging, and Diseases

CR has remarkably broad effects on increasing the life-span and attenuating chronic diseases of aging in rodents that are unequalled

by any pharmacological intervention. McCay's pioneering work 60 years ago showed that CR increases life-span by about 35% and also results in a lower incidence of tumors, kidney disease, vascular calcification, and chronic pneumonia (39). CR acts similarly in most rodent genotypes, extending their life-span by slowing mortality rate increases (40) and decreasing strain-specific tumors and other diseases (41).

The small but persistent decreases in blood glucose, insulin, and IGF-I; the increased insulin sensitivity; and elevated glucocorticoids may be responsible for the beneficial effects of CR in mice (Table 1). These changes are homeostatic responses to reduced body stores of fat and the need for increased gluconeogenesis (42). Monkeys show similar effects of CR on glucose and insulin, as well as indications of reduced mortality (43). In a small group of healthy nonobese humans, CR also causes physiologic, hormonal, and biochemical changes resembling those caused by CR in rodents and monkeys (44). CR counteracts the general trend for laboratory rodents and primates to progressively add body fat during aging. Laboratory animals have much reduced daily activity as compared to their unlimited food (by not needing to forage) and therefore can be considered as models for sedentary humans who are at high risk for obesity and insulin resistance. CR has few adverse outcomes for confined animals or sedentary obese humans.

In rodents, CR increases protein synthesis in liver (45) and probably in skeletal muscle (46), and it decreases the load of oxidized proteins (1). These substantial shifts may be due to faster protein turnover (which decreases exposure to endogenous damage), decreased production of metabolic toxins, and increase of free-radical scavenging functions.

CR also modulates host defense functions. CR attenuates cellular immune age changes in more than 10 rodent genotypes (47). For example, lectin-induced proliferation of splenic lymphocytes, which decreases sharply during aging in mice fed ad libitum, is increased twofold by CR at all ages (48). CR also enhances the immune responses to influenza during aging (49), blunts the aging changes in T cell functions by maintaining interleukin 2 (IL-2) production (47), and delays or prevents changes in the proportion of CD4 and CD8 memory cells during aging (50).

On the other hand, CR strongly inhibits inflammatory responses of aging. Blood levels of IL-6 and tumor necrosis factor- α (TNF- α) (which are both involved in acute phase responses) commonly increase during aging in rodents and humans (51). Microarray analyses confirm that CR attenuates inflammatory gene expression throughout the body (46, 52, 53). CR has anti-inflammatory effects in brain, which normally shows prominent activation of microglia-monocytes during aging and in neurodegenerative diseases (52, 53). High calorie intake is associated with increased risk of Alzheimer's disease (54), consistent with a role for CR in protecting against inflammation and neurodegeneration. Alzheimer's disease shows remarkable benefits from a broad group of anti-inflammatory drugs, particularly the nonsteroidal anti-inflammatory drugs (NSAIDs) (55). Long-term use of NSAIDs may be responsible for a reduction in the risk of Alzheimer's diseases, up to 80% (50). NSAIDs also reduced the risk of breast, colon, and other cancers, possibly by inhibiting proliferation and decreasing angiogenesis (56).

Long-term reduction of glucose levels may contribute to the decrease in inflammation and diseases in CR mice. For example, in normal humans, acute hyperglycemia induces Mac-1, a monocyte adhesion molecule (57). In vitro, high physiological glucose induces IL-6 and TNF- α expression and secretion in monocytes (58). TNF- α is well known to inhibit insulin receptor signaling in adipocytes, hepatocytes, and skeletal muscle and is implicated in the insulin resistance of aging (59). Furthermore, IL-6 stimulates hepatic glucose release (60). Hyperglycemia also alters redox status in muscle cells in vitro, by decreasing glutathione levels and repressing γ -glutamylcystein synthetase, which is the rate-limiting enzyme of glutathione synthesis (61).

The similarities of the effects of CR and NSAIDs in diseases of aging is consistent with the role of inflammatory mechanisms in vascular disease, Alzheimer's disease, and many cancers. Thus, CR may extend life-span in mammals by attenuating the major inflammatory diseases of aging.

CR, Dwarf, and IGF-1-Deficient Mice

CR, dwarf, and IGF-I-deficient (GHR/BP) mice share a number of biochemical and phenotypic characteristics, including reduced plasma insulin, IGF-I, and glucose; reduced fertility and body size; and delayed sexual maturation (Table 1). Many of these characteristics are also shared with IGF-I-deficient humans. Mutations in the yeast Ras and worm Daf-2 pathways can extend longevity but can also induce dormant phases that are normally entered during periods of starvation. In fact, these pathways are inactivated under starvation conditions. Thus, the reduced levels of plasma IGF-I in dwarf mice may contribute to disease prevention and life-span extension by simulating CR or more severe starvation conditions (Table 1). Consistent with this notion is the role of IGF-I in reversing the

ences in the effects of fat accumulation on mortality. In dwarf mice, CR may further increase longevity by preventing fat accumulation and reducing mortality associated with obesity. The obesity caused by GH deficiency in humans may promote cardiovascular diseases and age-dependent mortality (65). Notably, dwarf mice have more degenerative cardiopulmonary lesions (32).

Human GH/IGF-I and Diseases

Diseases that result in either overproduction or reduction of plasma GH and/or IGF-I can be informative for developing therapies that prevent multiple age-related diseases. Human somatotroph adenomas of the pituitary gland can cause chronic secretion of excessive GH, resulting in acromegaly, which is associated with a major life-shortening from cardiovascular diseases and cancer (66). Treatment of acromegaly with somatostatin analogs decreases GH and IGF-I, resulting in clinical improvements (67). Although these studies imply a role for GH and IGF-I in diseases of aging, the abnormally high GH levels in acromegaly patients provide limited informa-

Table 1. Comparison of CR, dwarf mice, IGF-I-deficient mice (GHR/BP), and IGF-I-deficient humans (Laron syndrome) [modified from Bartke and Turyn (80)]. N/A, no available data.

| | CR mice | Dwarf mice | IGF-I (mouse) | IGF-I (human) |
|---------------------------------|----------|-----------------|------------------|-----------------|
| Glucose regulation | | | | |
| Plasma insulin | Reduced | Reduced | Greatly reduced | Elevated |
| Plasma glucose | Reduced | Reduced | Modestly reduced | Reduced |
| Somatotrophic axis | | | | |
| Plasma GH | Reduced | Absent | Elevated | Elevated |
| Plasma IGF-I | Reduced | Greatly reduced | Greatly reduced | Greatly reduced |
| Body size | Reduced | Reduced | Reduced | Reduced |
| Thyroid function and metabolism | | | | |
| Plasma thyroid hormones | Reduced | Greatly reduced | Reduced | N/A |
| Body core temperature | Reduced | Reduced | Slightly reduced | N/A |
| Reproduction | | | | |
| Sexual maturation | Delayed | Delayed | Delayed | Delayed |
| Fertility | Reduced | Suppressed | Reduced | Reduced/normal |
| Glucocorticoids and adiposity | | | | |
| Plasma corticosterone | Elevated | Normal | Normal | N/A |
| Percent body fat | Reduced | Elevated (old) | N/A | Elevated |

protection of CR against carcinogen-induced bladder cancer (62). Apoptosis in the tumor is decreased 10-fold in CR mice in which the levels of IGF-I are restored, indicating that the activation of antiapoptotic pathways contributes to tumor incidence (62).

CR extends further the life-span of Ames dwarf mice, suggesting that the mechanisms that regulate life-span extension in CR and dwarf mice are not identical (63). In flies, by contrast, dwarf mutations and CR slow aging through overlapping mechanisms (64). The apparent difference between these two models may be explained by phylogenetic differ-

ences in the role of normal levels of GH in cancer and cardiovascular diseases.

The dwarf phenotype of long-lived yeast, flies, and mice suggest that it will be difficult to extend human longevity without causing side effects (Fig. 1). In fact, GH deficiency in humans can lead to reduced life expectancy and is associated with increased fat mass, reduced muscle and bone mass, behavioral problems, increased prevalence of hypertension, insulin resistance, and premature atherosclerosis (68). Thus, the changes that accompany fat accumulation may counteract the putative beneficial effects of GH/IGF-I

deficiency in humans. The increased mortality is observed in GH deficient hypopituitary patients that, in most cases, also lack adrenocorticotrophic hormone (ACTH). By contrast, human mutations analogous to the *prop-1* mutations that extend longevity in rodents cause defects including dwarfism, wrinkled skin, and intellectual deficiency but do not appear to shorten life-span (69). Among the rare *prop-1* patients for whom life-span data are available, several surpassed the average life-span and one survived to age 91 (69). Unlike most patients with GH deficiencies, humans with *prop-1* mutations do not lack ACTH, raising the possibility that the increased mortality observed in hypopituitary patients is caused by ACTH and not GH deficiency.

The human Laron Syndrome (LS) is caused by a defect in the GH receptor and resembles GH deficiency clinically and biochemically (70). Laron Syndrome is characterized by high GH, but very low plasma IGF-I, very short stature, obesity, and impairments in physical and intellectual development (Table 1) (70). Later in life, LS causes hypercholesterolemia and glucose intolerance. In summary, GH and IGF-I deficiencies in humans are associated with major defects and diseases. However, the normal (and possibly longer) life-span of a few individuals with mutations analogous to those that extend longevity in mice suggest that it may be possible to extend human longevity by reducing plasma GH and IGF-I levels.

Although studies in rodent models point to GH and IGF-I as promoters of aging and age-related diseases, GH is prescribed extensively as an antiaging hormone (71). GH treatment can increase body mass and decrease adipose tissue in 61- to 81-year-old men with low plasma IGF-I concentration (72), and long-term GH replacement therapy causes some improvements in patients with GH deficiencies (73). However, the "antiaging" effects of GH therapy are typically observed after short-term treatment of patients with low plasma GH. By contrast, chronically high GH levels increase the incidence of diseases, including cancer and kidney diseases in rodents, and increase cardiovascular diseases and cancer in human acromegaly patients. GH administration also increases the development of diabetes and glucose intolerance in healthy, older women and men (71) and increases morbidity and mortality in patients that are clinically ill, even after short-term treatment (74). It is clear that a major and chronic increase in plasma GH/IGF-I levels increases morbidity and mortality.

Drug Targets

The data summarized here suggest that three categories of drugs may have the potential to prevent or postpone multiple age-related diseases:

es: drugs that (i) simulate dwarf mutations and therefore decrease GH production by pituitary cells, (ii) prevent IGF-I release from the liver, or (iii) decrease IGF-I signaling by acting on either extracellular or intracellular targets.

Dwarf mice eat normally and become obese in old age, yet they live 50% longer. Therefore, the pharmacological simulation of dwarf mutations should also increase obesity and extend longevity in mice. Although we do not know whether the longevity effect of dwarf mutations can be separated from the small body size, preliminary data suggest that it is possible. Dwarf mice treated transiently in early adult life with GH and thyroid hormone become much larger than untreated dwarf mice, but they live longer than littermate control mice (75). Studies in yeast and worms indicate that life-span can be extended without causing growth and reproductive defects. Yeast lacking Sch9 activity survive three times longer than wild-type but grow slowly, whereas yeast that have reduced Ras activity survive 100% longer but grow at normal rates (7, 13, 19). Worm *age-1* (*hx546*) and certain *daf-2* mutants can survive up to 150% longer than wild-type worms, but are fertile and have normal developmental rates and activity levels (17, 18, 76). Obesity and muscle weakness are other major side effects of GH deficiency that must be prevented before drugs that simulate dwarf mutations can be considered for human studies.

Studies in simple eukaryotes indicate that IGF-I-like factors activate a major pro-senescence pathway. Drugs that prevent IGF-I generation in response to GH would have the advantage of reducing plasma IGF-I levels without decreasing GH levels. Because GH can directly stimulate growth, activation, and differentiation in cells, e.g., phagocytes and lymphocytes (77), it is possible that, by reducing IGF-I but not GH levels, longevity may be extended while reducing side effects. Drugs that block IGF-I receptor activation may have a similar effect. However, mice with very low plasma IGF-I and elevated GH have a fourfold increase in insulin levels and muscle-specific insulin resistance, suggesting that reduction of only IGF-I is also associated with some adverse side effects (78).

Other drugs could target intracellular mediators of pro-senescence hormone and growth factors. Although a mammalian signal transduction pathway that regulates longevity has not been identified, the increased stress resistance and longevity of mice with *p66^{SHC-/-}* mutations suggests that a pro-senescence pathway is also present in mammalian cells (79). Studies in yeast point to Ras, PKA, and Sch9 as major intracellular pro-senescence proteins (Fig. 2). Akt-1/Akt2 and many other proteins that regulate longevity in worms are also shared with the mammalian IGF-I signaling pathway (Fig. 2) (8,

19). The well-characterized yeast and worm "longevity" pathways should provide templates for the identification of genes and drugs that regulate longevity and diseases in mammals.

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REVIEW

The Endocrine Regulation of Aging by Insulin-like Signals

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Reduced signaling of insulin-like peptides increases the life-span of nematodes, flies, and rodents. In the nematode and the fly, secondary hormones downstream of insulin-like signaling appear to regulate aging. In mammals, the order in which the hormones act is unresolved because insulin, insulin-like growth factor-1, growth hormone, and thyroid hormones are interdependent. In all species examined to date, endocrine manipulations can slow aging without concurrent costs in reproduction, but with inevitable increases in stress resistance. Despite the similarities among mammals and invertebrates in insulin-like peptides and their signal cascade, more research is needed to determine whether these signals control aging in the same way in all the species by the same mechanism.

Dozens of genes extend adult longevity. Remarkably, many of these genes are involved with hormonal signals, and both these genes and their endocrine systems are conserved among eukaryotes. Thus, insulin-like peptides, insulin-like growth factor (IGF), lipophilic signaling molecules, and sterols are all candidate effectors of aging in organisms as diverse as the nematode *Caenorhabditis elegans*, the fly *Drosophila melanogaster*, and the mouse *Mus musculus*. Suppression of these hormones or their receptors can increase life-span and delay age-

dependent functional decline. This regulation is likely to be adaptive because, at least among invertebrates, these hormones regulate the organism's capacity to survive during states of reduced metabolism coupled with high stress resistance and arrested development. Mutations that increase life-span through hormones are thought to initiate elements of this survival program independent of the appropriate environmental cues. Because mechanisms for survival often oppose the progress of aging, they can illuminate the cellular and molecular causes of senescence. The insulin/IGF system and its associated downstream hormones are likely to prove particularly instructive.

Genetics of *C. elegans* Dauer and Aging

The genetic dissection of aging rapidly advanced when strains of *C. elegans* with mutations in the dauer formation (Daf) pathway were found to have unusually long

lives (1). Dauer diapause is a nonfeeding, stress-resistant larval state evolved for endurance and dispersal under adverse conditions. Animals with weak alleles of Daf-constitutive mutants in the genes *age-1* and *daf-2* (2) can bypass dauer and become prodigiously long-lived adults in a manner dependent on the gene *daf-16* (1, 3, 4) (Fig. 1A). The products of these genes and others revealed insulin/IGF-like signal transduction as a central regulator of dauer and aging (5). The *C. elegans* genome contains 37 insulin-like ligands that are mainly expressed in neurons, but are also found in intestine, muscle, epidermis, and gonad (5, 6). Ligand bound to DAF-2 insulin-like receptor signals through a kinase cascade to phosphorylate forkhead transcription factor DAF-16, which is thus sequestered in the cytoplasm. In this state, adults directly reach reproductive maturity and age rapidly. Without activation of the pathway, DAF-16 promotes transcription and induces diapause and exceptional longevity (Fig. 1A).

DAF-16 also is a key regulator of heat and oxidative stress resistance, fat storage, developmental arrest, fertility, and metabolism (5, 7, 8). DAF-16 becomes localized to the nucleus in response to various stresses; this response is attenuated by insulin/IGF signaling (9). Elevated stress resistance combined with down-regulated central metabolism and reproduction may be coordinated physiological

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