Murine Models of Life Span Extension

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Mice are excellent experimental models for genetic research and are being used to investigate the genetic component of organismal aging. Several mutant mice are known to possess defects in the growth hormone/insulin-like growth factor 1 (GH/IGF-1) neurohormonal pathway and exhibit dwarfism together with extended life span. Their phenotypes resemble those of mice subjected to caloric restriction. Targeted mutations that affect components of this pathway, including the GH receptor, p66Shc, and the IGF-1 receptor (IGF-1R), also extend life span; mutations that affect IGF-1R or downstream components of the pathway decouple longevity effects from dwarfism. These effects on life span may result from an increased capacity to resist oxidative damage.

A Question of Aging

Until recently, mammalian aging was thought to be too genetically complex to permit effective molecular analysis; however, it has now been established that longevity has a measurable genetic component (see "Aging Research Grows Up" at http://sageke.sciencemag.org/cgi/content/full/2001/1/oa1). Life span has been shown to be 15 to 25% heritable in humans (see Miller Viewpoint at http://sageke.sciencemag.org/cgi/content/full/sageke;2001/9/vp6) and as much as 48 to 79% heritable in mice (*1*, *2*), indicating that both genetic and environmental factors affect the aging process and may be open to manipulation.

Genetic studies in budding yeast, *Caenorhabditis elegans*, and *Drosophila* have identified many genes that influence life span (see Warner Subfield History at http://sageke.sciencemag.org/cgi/content/full/2003/6/re1 and SAGE KE Genes/Interventions database at http://sageke.sciencemag.org/cgi/genesdb), which has led in turn to insight into the relevant biochemical and physiological pathways. One theme to emerge has been that factors affecting basal metabolism, such as components of the growth hormone/insulin-like growth factor 1 (GH/IGF-1) pathway, are involved in the regulation of life span [for example *C. elegans* DAF-2 (http://sageke.sciencemag.org/cgi/genedata/sagekeGdbGene;38), which is related to the mammalian insulin receptor and IGF-1 receptor (IGF-1R); see Johnson Subfield History at http://sageke.sciencemag.org/cgi/content/full/sageke;2002/34/re4]. Selective breeding for longevity in *Drosophila* has revealed roles for proteins associated with oxidative stress resistance, including superoxide dismutases [SOD1 (http://sageke.sciencemag.org/cgi/genedata/sagekeGdbGene;141) (also known as Cu/Zn SOD) and SOD2 (http://sageke.sciencemag.org/cgi/genedata/sagekeGdbGene;144)], catalase (http://sageke.sciencemag.org/cgi/genedata/sagekeGdbGene;15), and certain heat shock factors (*3*–*9*), including hsp68 (http://sageke.sciencemag.org/cgi/genedata/sagekeGdbGene;475).

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However, research carried out in more complex organisms is likely to be more relevant for understanding aging-related processes that occur in humans. In this Review, we discuss results obtained from the study of genetically altered mice that exhibit changes in life span.

Mice as a Mammalian Model of Aging

Mice possess a number of favorable attributes for the experimenter: Their small size facilitates care and maintenance, and they have short generation times (about 10 weeks) and large litters (5 to 12 pups). Most laboratory strains have been bred for docility, which allows for high colony density, an important consideration for large-scale research (*10*) (although some researchers in the field of aging argue that inbred mice strains are inferior to wild mice as an experimental system; see "Give Me Liberty or Give Me an Early Death" at http://sageke.sciencemag.org/cgi/content/full/2002/26/nf8).

A wide variety of established inbred mouse strains exist, many of which have been extensively characterized for background phenotypes and common pathologies (see, for example, Jackson Laboratories Strain Information at http://jaxmice.jax.org/jaxmicedb/html/inbred.shtml). Furthermore, in April 2002 the mouse became the first mammalian model organism to have its genome completely sequenced (see the public announcement at http://www.ncbi.nlm.nih.gov/genome/guide/mouse/Mouse_Monthly_Newsletter_Apr02.pdf). Because of their predominance as a system for experimental genetic manipulation as well as their fertility, easy manipulability, and relatively short life span (2 to 3 years), mice are considered by some researchers to be the model organism of choice for researching the mechanisms that underlie mammalian aging.

Caloric Restriction in Mice

Caloric restriction (CR) (limiting food intake without causing nutritional deficiencies) was, until recently, the only agent or environmental factor known to result in a consistent, robust, and positive cross-species effect on life span [(*11*–*14*) and see Masoro Subfield History at http://sageke.sciencemag.org/cgi/content/full/sageke;2003/8/re2]. In the early 20th century, CR was found to decrease spontaneous tumor formation in mice (http://sageke.sciencemag.org/cgi/genedata/sagekeGdbIntrvn;9) and to result in an extended period of reduced fertility, acyclicity, reduced growth, and longer life span (*15*–*20*), although more recent research suggests that CR may confer certain negative health effects as well (see "Dietary Drawbacks" at http://sageke.sciencemag.org/cgi/content/full/2003/8/ns4).

CR from the time of weaning results in extended mean and maximal life spans, most likely as a result of lowered oxidative stress levels, at least in part (*11*–*13*, *18*, *21*, *22*). CR initiated in middle age also works to a lesser extent [(14) and see "Never Too Old" at http://sageke.sciencemag.org/cgi/content/full/2004/13/nf35]. Even in old mice, CR has potential health benefits, although life span may not be affected significantly (*23*–*29*). Leaner animals result from CR; correspondingly, it has

been found that rats (http://sageke.sciencemag.org/cgi/genedata/sagekeGdbIntrvn;11) undergoing CR display a higher per-unit lean body mass metabolism than do rats fed ad libitum (AL), despite a lowered caloric intake (*30*). CR-induced life span extension does not, therefore, appear to result from a reduced metabolic rate.

CR initiated in mice in young adulthood results in (i) lower plasma glucose and IGF-1 concentrations; (ii) protection against autoimmune disorders; (iii) elevated levels of apoptosis in tumors; (iv) reduced angiogenesis; (v) postponed or attenuated onset of cancer, immunosenescence, and inflammation; and (vi) delayed onset of other age-related diseases, all without irreversible side effects (*31–37*). A recent large-scale investigation of CR effects also revealed inhibition of cardiac, renal, and central nervous system pathologies, as well as decreased bone degeneration, atrophy of secretory organs, amyloid induction, and hyperplasia (*38*). In general, mice undergoing CR maintain robust physiological and immunological responses longer into life than do their AL-fed counterparts (*39*, *40*).

Reduced oxidative damage is one popular explanation for the effects of CR; however, because this effect does not result from CR-related changes in metabolic activity, other factors may cooperate with alterations in resistance to damaging reactive oxygen species (ROS) to extend life span (*41–43*). Reduced insulin levels lead to reduced glycemia-related glycation of proteins and nucleic acids; lowering insulin levels in *C. elegans* nervous tissue leads to an increase in life span (*11*, *44*, *45*). Mammals also exhibit a down-regulation of the GH/IGF-1 axis after CR, which might be related to the extended life span phenotype (*46–48*).

Another idea is that CR works by triggering an innate beneficial response to low-level stressors. This effect, in which a low caloric intake engenders a state of preparedness for other stresses, is known as hormesis. CR does enhance the induction of stress proteins in response to cellular damage (including ROS-induced damage). Furthermore, mice undergoing CR respond better to surgery and toxic chemical challenges than do AL-fed mice (*49*).

The effects of CR on human life span are still not known definitively, although long-term studies are in progress. There is evidence that humans with lower than normal body temperatures and reduced insulin levels might live longer than average (*50*). Researchers are also working on understanding the effects of CR in primates (*51*) (see also "Monkey in the Middle" at http://sageke.sciencemag.org/cgi/content/abstract/sageke;2002/31/nw108).

Mutant Mice That Live Longer Than the Wild Type

Researchers interested in aging have carried out a considerable amount of work using mice with the aim of understanding the genetic basis of life span determination and, in particular, of identifying alleles of specific genes that allow for long life span. Several long-lived mutants have been identified and characterized, including the Ames, Snell, Little, and Laron mice (see Bartke Viewpoint at http://sageke.sciencemag.org/cgi/content/full/sageke;2002/16/vp4).

Ames and Snell mice are long-lived dwarfs, exhibiting a phenotype similar to that seen in mice that undergo CR throughout their life spans. These mice lack GH-producing cells in the pituitary gland; in Ames mice because of a recessive point mutation in the *Prop1* (http://sageke.sciencemag.org/cgi/genedata/sagekeGdbGene;116) gene (which encodes a transcription factor involved in embryonic development of the anterior pituitary gland), and in Snell mice because of a mutation in the *Pit1* (http://sageke.sciencemag.org/cgi/genedata/sagekeGdbGene;113) gene, which also results in altered anterior pituitary development (*52–54*).

Ames mice

The first mammalian mutant found to have an increased average and maximal life span was the Ames mouse (http://sageke.sciencemag.org/cgi/content/full/sageke;2001/1/tg11). Depending on gender and genetic background, extensions of about 50% in average life span and 40% in maximal life span are observed. The Ames mouse is of considerable significance: Preliminary evidence suggests that humans with mutated *Prop1* may show increased longevity (*54*) (and see "Power to the People" at http://sageke.sciencemag.org/cgi/content/full/2003/50/ns8).

Ames mice display phenotypes of dwarfism (they are about one-third normal size), reduced growth rate, and deficiencies in GH, prolactin, thyroid-stimulating hormone (TSH), and IGF-1. Males exhibit variable fertility, but females are infertile as a result of a lack of prolactin (treatment with prolactin restores fertility). Both genders display delayed reproductive maturity. A number of aging-related phenotypes are also seen: renal pathology; changes in collagen; and declines in immune function, locomotor activity, learning, and memory. Reduced or delayed tumor development is also observed (*55–57*).

CR treatment of Ames mice results in a further life span extension, indicating separate (though probably not fully independent) underlying mechanisms. A mechanistic difference is also possible because CR decelerates aging (the slope of the mortality curve decreases), whereas the mutation in *Prop1* delays it [the mortality curve shifts to the right but maintains its shape (*43*)]. The general effect of CR on murine life span may therefore not be entirely a result of altered pituitary function, although this idea has been contested (*58*). The life span extension in Ames mice might also be caused by their lowered core body temperature (a reduction of 1.5°C as compared to the wild type) or increased expression of antioxidants [modest increases in the abundance of SOD1 and catalase are seen (*43*, *56*, *57*)].

Reduced prolactin abundance might also contribute to life span extension in these mice, because impaired bone mineralization and fat deposition are observed. These changes are consistent with the phenotype of the prolactin receptor knockout mouse (59). The sensitive glucose response of the Ames mouse might also be related to effects of reduced prolactin concentration on body fat (59). With respect to reduced TSH levels, artificially induced hypothyroidism in rats does extend life span. Longevity studies will need to be performed on genetically altered mice with specific defects in prolactin or TSH signaling in order to determine whether the altered prolactin and TSH phenotypes of the Ames dwarf influence life span.

Snell mice

Most of the phenotypes exhibited by Ames mice are shared by Snell dwarfs (http://sageke.sciencemag.org/cgi/content/full/sageke;2001/3/tg13). Snell mice have a mutation in the *Pit1* gene, which also results in deficient GH, prolactin, and TSH production. Their life spans are similarly extended (53). Other signs of slowed aging are also observed, including slower immune, joint, and connective tissue senescence (52). Skin-derived fibroblast cell cultures from Snell mice show increased re-

sistance to many forms of stress, including ultraviolet (UV) light, heat, paraquat (an ROS-producing herbicide), H_2O_2, and the toxic metal cadmium. This result supports the notion of a link between greater resistance to cellular stress and slowed aging, and also suggests that the altered hormonal profiles in dwarf mice cause long-lasting physiological changes that promote longevity in various cell types (*60*).

Lowered GH concentrations might be a major cause of the increased longevity observed in both Ames and Snell mice. GH overexpression shortens life span and is accompanied by symptoms of early aging, including shortened reproductive life span, increased astrogliosis and plasma corticosterone concentrations, and early-onset aging-related effects on cognitive function (*61*). Circulating insulin concentrations also decrease in Snell mice, resulting in reduced insulin/IGF-1 signaling. These signals are normally transmitted through phosphoinositide 3-kinase (PI3K) and Grb2 via insulin receptor substrate 2 (IRS-2), a tyrosine-phosphorylated protein functionally similar to but slightly larger than and immunologically distinct from IRS-1 (the primary mediator of insulin signaling). Consequences of reduced signaling include a smaller pool of IRS-2 and therefore less IRS-2–associated PI3K activity. The insulin receptor β-IRS-1-PI3K signaling pathway in Snell mouse livers is also attenuated. Interestingly, aged Snell mouse livers preferentially form PI3K complexes that have a p85α-p110α composition rather than a p85α-p110β composition; the preference for p110α complexes of PI3K has been proposed to be a potentially important characteristic of the longevity-signaling cascade (*62*, *63*).

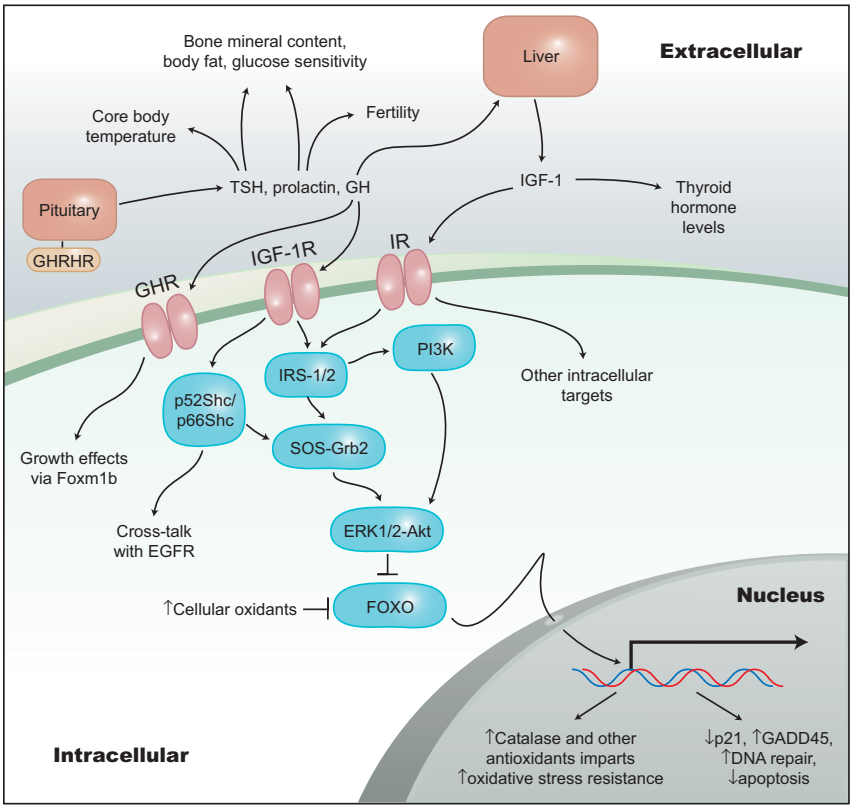

Fig. 1. Physiology and biochemistry of the GH/IGF-1 axis. The GH/IGF-1 neuroendocrine signaling pathway has been implicated in life span extension. Infertility is observed, but at a reduced level when genetic alterations affect either GHR or components that act after it in the pathway. Mutations that affect IGF-1R or downstream components effectively decouple life span extension from growth effects. Increased longevity is believed to be primarily caused by raised antioxidative capacity and decreased apoptosis. EGFR, epidermal growth factor receptor.

Little mice

The aptly named "Little" mice (http://sageke.sciencemag.org/cgi/content/full/sageke;2001/4/tg14) have a recessive mutation in *ghrhr* (http://sageke.sciencemag.org/cgi/genedata/sageke GdbGene;61), the gene encoding the GH-releasing hormone receptor. This mutation results in a profound but incomplete GH deficiency, making the observed phenotypes distinct from those resulting from the combined effects on prolactin and TSH concentrations in the Ames and Snell mice. Little mice also experience stunted growth, but grow to about one-half normal size rather than one-third, as seen in the Ames and Snell mice. Little mice also display a more modest 23 to 25% extension of life span, and only when maintained on a low-fat diet (*52*). The GH insufficiency causes lowered plasma IGF-1 concentrations (as in the Ames and Snell dwarfs), although this reduction is more predominant in the peripheral circulation than in many organs (*64*, *65*).

Laron mice

Whereas the mutant mice described above are GH-deficient, Laron mice (http://sageke.sciencemag.org/cgi/content/full/sageke;2002/8/tg1) lack a growth hormone receptor [GHR (http://sageke.sciencemag.org/cgi/genedata/sageke GdbGene;246)] because of a targeted knockout of the gene ($GHR^{-/-}$) and are thus GH-resistant. An important caveat to any observed phenotypes in these mice is that the gene targeted in Laron mice also encodes the GH binding protein (a product resulting from cleavage of the extracellular domain of GHR). These mice were developed to model the complete GH deficiency seen in human Laron dwarfism syndrome (http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=262500). Growth is stunted in Laron mice (adults are half the size of wild-type adults), and concentrations of circulating IGF-1 are very low to undetectable. The lack of negative feedback of IGF-1 on GH secretion results in higher concentrations of circulating GH and elevated prolactin production. Plasma insulin and glucose concentrations are lower than usual in the Laron mouse, but insulin responsiveness is augmented, and reduced insulin release causes lowered glucose tolerance. Because of a lack of IGF-1 action, thyroid hormone levels are low and a lower core body temperature results (*64*, *66*).

Despite delayed reproductive maturity, Laron mice are generally fertile, albeit at reduced levels, and are deficient in endocrine control of the gonads (*64*, *66*). They demonstrate delayed age-related cognitive decline, and a 37 to 55% increase in

both average and maximal life spans (*56*, *64*, *67*, *68*). Human sufferers of Laron syndrome are slightly immunodeficient; however, their growth is stunted, and preliminary evidence indicates increased life span in some patients (*54*, *69*).

The similar phenotypes of the Ames, Snell, Little, and Laron mice indicate that the GH/IGF-1 axis is a major contributor to longevity effects. But what occurs downstream of these signaling pathways to bring about the observed phenotypes? In invertebrates, DAF-2 is the primary effector molecule of the insulin-like signaling pathway, and because of its prior link to life span determination, many genes and gene products known to be affected by DAF-2 activity can serve as clues to mechanisms that might be operating in long-lived mice.

p66shc mutant mice

In 1999, the p66Shc protein (http://sageke.sciencemag.org/cgi/genedata/sagekeGdbGene;105) became the first downstream effector of the GH/IGF-1 pathway to be linked to life span extension in mice. p66Shc is derived from one of three alternate transcripts of the *shc* gene, and all the encoded proteins bind SOS (a guanine nucleotide exchange factor) upon tyrosine phosphorylation as part of a signaling pathway triggered by activated Ras (http://sageke.sciencemag.org/cgi/content/abstract/2002/32/nw114) (*70*). It is, therefore, a component of a central biochemical pathway linking cell surface receptors to alterations in gene expression. p46Shc and p52Shc, the proteins derived from the other two alternate *shc* transcripts, have been implicated in cytoplasmic propagation of growth, whereas p66Shc appears to be involved in apoptogenic signaling, which might indicate a basis for a distinction between the growth and life span extension phenotypes seen in the above mutants (*71*).

p66Shc is serine-phosphorylated upon binding SOS or upon induction by oxidative stress, in association with or independent of tyrosine phosphorylation, and this mechanism is lost in the *p66shc*$^{-/-}$ mouse (http://sageke.sciencemag.org/cgi/content/full/sageke;2003/8/tg4). Furthermore, the *p66shc* promoter is differentially histone-deacetylated and methylated as compared to the other *shc* splicing products, which specifically alters transcription of the *p66shc* alternate transcript (*71*–*73*).

The *p66shc*$^{-/-}$ mouse has a 30% longer life span than control mice, a very significant increase in life span in the complex and highly redundant mammalian system. This is equivalent to the life span extension observed in mice being treated with a 40% reduction in caloric intake. Cells from *p66shc*$^{-/-}$ mice were found to be resistant to UV- and H_2O_2-induced (via paraquat treatment) cell death but not resistant to gamma irradiation, indicating that an improved stress response results but is likely limited to oxidative damage (13, 73).

The actual molecular cascade leading to oxidative stress resistance in cell cultures from *p66shc*$^{-/-}$ mice is unclear. Although expression of the cell-cycle inhibitor p21 (waf/cip-1) is down-regulated in these mice (p21 is involved in cellular stress response via upregulation by p53), cells derived from *p21*$^{-/-}$ mice are known to have a normal oxidative stress response. However, *p66shc*$^{-/-}$ mice overexpress catalase, and derived cell cultures display reduced apoptosis in response to oxidative stress. The increase in longevity observed in the *p66shc*$^{-/-}$ mice could therefore be caused by an elevated damage response and/or reduced damage-induced apoptosis (*70*, *73*) (and see "One for All" at http://sageke.sciencemag.org/cgi/content/full/2002/49/nf15).

Perhaps the most important aspect of the *p66shc*$^{-/-}$ mouse is that it displays normal size, development, and fertility, in contrast with the negative physiological characteristics seen in the GH-deficient dwarf mice. This mouse therefore provides a model of life span extension and oxidative stress response without complicating physiological phenotypes. It indicates that oxidative damage resistance likely has a direct and separable influence on longevity and that other phenotypes observed in the dwarf mice result from distinct pathways. Negative side effects might still exist in *p66shc*$^{-/-}$ mice, but different conditions from those already examined would be required to evoke them (70).

Provision of a high-fat diet had practically no effect on *p66shc*$^{-/-}$ mutant mice, whereas this diet resulted in an increased aortic cumulative early lesion area, apoptotic vascular cells, systemic and tissue oxidative stress, and other signs of early atherogenesis in wild-type mice. The *p66shc*$^{-/-}$ mouse might therefore be an excellent model for vascular disease therapy as well as longevity (*70*, *73*, *74*). Crossing of *p66shc*$^{-/-}$ mutants with more robust outbred mice would determine whether the life span extension phenotype occurs in other, more natural genetic backgrounds.

IGF-1R knockout mice

The second genetically altered mouse to be produced in order to explore the significance of the GH/IGF-1 signaling pathway was the *IGF-1R* knockout (http://sageke.sciencemag.org/cgi/content/full/sageke;2003/8/tg1). This strain was created via a *Cre*-loxP system, which can be used to inactivate specific mouse genes in a tissue- or time-specific fashion. IGF-1R (http://sageke.sciencemag.org/cgi/genedata/sagekeGdbGene;287) is the mammalian equivalent of DAF-2. In higher organisms, the insulin and IGF-1 pathways are separate; the insulin receptor regulates energy metabolism (*75*), whereas IGF-1R mediates growth (*65*).

Mice that are homozygous for a knockout of the gene encoding IGF-1R are inviable, but female heterozygotes exhibit a phenotype of 30% life span extension. This effect is highly influenced by gender: Male life span extension is observed (16%) but is not statistically significant (*76*). As with other mutations that interfere with functions of the GH/IGF-1 axis, life span extension is associated with greater resistance to oxidative stress in derived cell cultures and occurs to a similar extent. Females display greater resistance to paraquat than males, and both genders are more resistant than wild-type controls (*76*).

Cultured *IGF-1R*$^{+/-}$ embryonic fibroblasts display a decrease in the phosphorylation of IGF-1R substrates such as IRS-1, p66Shc, and p52Shc. Furthermore, binding of major downstream targets of p66Shc—Grb2, ERK1/2 (http://sageke.sciencemag.org/cgi/content/abstract/2002/1/nw5), and Akt (http://sageke.sciencemag.org/cgi/content/full/2004/8/pe8)—to p66Shc is reduced by 50% in these cells. Any of these changes might contribute to the observed enhancement in oxidative stress response (*76*).

As with *p66shc*$^{-/-}$ mice, life span extension in *IGF-1R*$^{+/-}$ animals is uncoupled from growth reduction (the size and weight of both genders are decreased by only 6 to 8%). This uncoupling therefore occurs at the level of IGF-1R. Also, as seen in the *p66shc*$^{-/-}$ mice, there are no effects on metabolism, fertility, or development, and age-related diseases are not observed (*76*, *77*). No significant effect on voluntary food or water intake is observed in *IGF-1R*$^{+/-}$ mice, and metabolic rates are similar to controls, indicating unchanged efficiency of nutrient use. Furthermore, the knockout mutation has no effect on the onset or decline of repro-

System affected	Mouse strain	Mutation	Regular function	Life span extension	Other phenotypes	References
Pituitary	Ames	*prop1*$^{-/-}$	Transcription factor in anterior pituitary embryonic development	40-50%	Dwarfism (1/3 normal size); stunted growth; lowered core body temperature; GH,TSH, prolactin, IGF-1, insulin deficiencies; lowered IRS-2-associated PI3K activity; increased expression of Cu/Zn SOD and catalase; variable fertility in males but females infertile (treatable with prolactin); delayed reproductive maturity; retarded age-related increases in bone mineral density and percent body fat; hypersensitive glucose response; reduced or delayed tumour development, renal pathology, changes in collagen, and declines in immune function, locomotor activity, learning and memory; CR further extends life span	*43*, *52-59*
	Snell	*pit1*$^{-/-}$	Pituitary-specific transcription factor	40-50%	Dwarfism (1/3 normal size); stunted growth; GH,TSH, prolactin, IGF-1, insulin deficiencies; lowered IRS-2-associated PI3K activity; preferentially form IRS-2-p85α-p110α complexes over IRS-2-p85α-p110β; most symptoms of decreased aging seen in Ames mice; also slower immune, joint, and connective tissue senescence; increased resistance to UV, heat, ROS, toxic metal-induced stress	*52*, *53*, *60*, *61*, *63*
GH/IGF-1 axis	GH tg	*GH* overexpression	Growth and development	Decreased	Shortened reproductive life span; increased astrogliosis and plasma corticosterone levels; early onset of aging-related effects on cognitive function	*61*
	Little	*ghrhr*$^{-/-}$	Release of GH	23-25% when on low-fat diet	Dwarfism (1/2 normal size); incomplete GH deficiency; IGF-1 insufficiencies especially in the peripheral circulation; normal TSH and prolactin levels	*52*, *64*, *65*
	Laron	*GHR*$^{-/-}$, *GHBP*$^{-/-}$	Growth hormone receptor	37-55%	Dwarfism (1/2 normal size); GH-resistant; very low to undetectable IGF-1 levels; greater circulating GH levels and elevated prolactin production; lowered plasma insulin and glucose levels; augmented insulin responsiveness and lowered glucose tolerance; low thyroid hormone levels; lowered core body temperature; fertile but delayed reproductive maturity; deficient reproductive function and endocrine control of the gonads; delayed age-related cognitive decline	*54*, *56*, *64*, *66-69*
	IGF-1R	*IGF-1R*$^{+/-}$	IGF-1 receptor	26% (overall) 33% (females) 16% (males; change is not significant)	Virtually no growth effect (6-8% size reduction); no effect on metabolism, fertility, development; regular onset and decline of reproductive maturity; absence of age-related diseases; life span extension not significant in males; augmented resistance to oxidative stress in both genders but especially in females; unaltered metabolism and caloric intake; 50% reduced phosphorylation of IRS-1, p66Shc, p52Shc; 50% reduced p66Shc binding of Grb2, ERK1/2, Akt; homozygous KO lethal	*65*, *76-78*
	p66shc	*p66shc*$^{-/-}$	IGF-1R effector	30% (approx. equivalent to 40% reduction in caloric intake)	Normal size, development, and fertility; incapable of being serine-phosphorylated upon SOS binding due to cellular oxidative stress; differential promoter histone acetylation and methylation; p21 down-regulated; resistant to UV and ROS but not gamma irradiation; catalase overexpression; reduced apoptosis; increased vascular fitness	*70-74*, *78*

System affected	Mouse strain	Mutation	Regular function	Life span extension	Other phenotypes	References
Oxidative metabolism	$\Delta IR_{adipose}$	*IR*$^{-/-}$ (adipose tissue only)	Insulin receptor	18%	No effect on caloric intake or metabolism; reduced fat mass, less age-related obesity, fewer obesity-related metabolic abnormalities; indicates that some effects of CR may be separable from those of leanness	*75, 83-88*
	Peroxiredoxin	*Prdx1*$^{-/-}$	Antioxidant enzyme	Significantly reduced	Develop severe hemolytic anemia and several types of malignant cancers at ~9 months	*96*
	Thioredoxin (TRX-Tg)	*TRX* overexpression	Electron donor for peroxiredoxin	22-35%	Increased resistance to oxidative stress	*94, 95*
	Bcl-2 tg DC	*Bcl-2* overexpression in dendritic cells	Anti-apoptotic and antioxidative effects	Increased DC longevity	Elevated $CD4^+$ T cell and humoral immune responses	*97, 98*
Other	Klotho	*Klotho*$^{-/-}$	Membrane protein of unknown function; homologous to β-glucosidases and a mammalian hydrolase	Greatly decreased (<100 days)	Many aging-associated phenotypes: decreased activity, infertility, osteoporosis, arteriosclerosis, skin atrophy, emphysema; greatly reduced subcutaneous fat; atrophied thymus; slightly atrophic GH-, FSH- and LH-secreting cells in pituitary; lowered pancreatic insulin; lowered glycogen/lipid storage	*91-93*
	p53 mut	Increased expression/ activity	Tumor suppressor	Decreased 17-19%	Decreased cancer incidence; premature aging phenotypes: osteoporosis, generalized organ atrophy, diminished stress tolerance	*133-138*
	XPD TTD	R^{722}→W mut	5'→3' DNA helicase; part of NER pathway	Decreased >50%	Premature aging phenotypes: osteoporosis, osteosclerosis, kyphosis, cachexia, early graying, infertility, early cessation of development, cachectic dwarfism	*139*

Fig. 2. A summary of mice whose life span has been altered by genetic means. See the text for further discussion. tg, transgenic; KO, knockout; DC, dendritic cell; NER, nucleotide excision repair.

ductive maturity, unlike the delays seen in *GHR*$^{-/-}$ mice (*64*). Many theories of aging posit that fitness in youth is achieved at the cost of life span as a result of, for example, increased metabolic activity. However, the lack of any overt phenotypes associated with life span extension in the *IGF-1R* and *p66shc* mutant mice appears to represent "cost-free longevity" (*78*).

FOXO transcription factors

In *C. elegans*, the primary downstream effector of DAF-2 is the transcription factor DAF-16 (http://sageke.sciencemag.org/cgi/genedata/sagekeGdbGene;35), which influences longevity, proliferation, differentiation, and stress response (see Larsen Perspective at http://sageke.sciencemag.org/cgi/content/full/2003/17/pe9). The corresponding proteins in mammals are the FOXO (http://sageke.sciencemag.org/cgi/content/full/2003/28/nw99) family of forkhead transcription factors (Fox family, subgroup O), including AFX, FKHRL1, and FKHR (otherwise known as Akt-phosphorylated forkhead transcription factors). They appear to act by regulating expression of the gene encoding GADD45 (a p53-regulated controller of apoptosis, DNA repair, and cell-cycle progression) in response to oxidative stress, thereby contributing to the G_2/M cell-cycle checkpoint (*79*).

Forkhead proteins are regulated indirectly via a p66Shc-dependent pathway: Serine-phosphorylated p66Shc raises cellular oxidant levels, and FOXO proteins are negatively regulated by H_2O_2. In *p66shc*$^{-/-}$ cells, decreased redox-dependent inactivation results in greater FKHRL1 activity. This up-regulation results in augmented H_2O_2 scavenging and oxidative stress resistance (*80*). Furthermore, the methylation status of the *p66shc* promoter has been found to affect the expression of forkhead proteins (*72*).

A forkhead protein from a different subgroup, Foxm1b, restores proliferative capacity when overexpressed, alone or upon GH induction. However, GH treatment of *Foxm1b*$^{-/-}$ mice has no effect, indicating that Foxm1b is an essential downstream mediator of GH-induced proliferation (*81*).

Life span studies on *FOXO* or *Foxm1b* knockout mice have not yet been published; however, allelic variations between individuals that affect longevity might exist. Analysis of human populations for two different polymorphic variants of the genes encoding IGF-1 pathway components IGF-1R, PI3K, IRS-1, and FOXO1A revealed an *IGF-1R* allelic variant that is associated with lowered plasma IGF-1 concentrations and is more prevalent in longer lived people. An allelic combination of *IGF-1R* and *PI3K* gene variants had a similar effect (*82*). Longevity therefore appears to be influenced significantly by a complex set of overlapping gene functions within the GH/IGF-1 axis, both in humans and mice. This neurohormonal axis and some of the relevant intracellular signaling components are shown in Fig. 1.

Insulin receptor mutations

Systemic hormonal and neuroendocrine control over longevity has been most extensively examined in the GH/IGF-1 pathway; however, other relevant signaling pathways do exist. Mutations affecting normal functioning of the insulin receptor result in shortened life span in humans and mice (*83*–*87*). Therefore, the life span extension effects seen in hypoinsulinemic *C. elegans* mutants are likely caused by effects mediated by the counterparts of the GHR and IGF-1R signaling cascades.

On the other hand, *Cre*-loxP-mediated expression of a mutated insulin receptor (http://sageke.sciencemag.org/cgi/genedata/sagekeGdbGene;308) in adipose tissue results in an 18% extension of mouse life span. No effect is seen on caloric intake, but reduced fat mass, lower incidence of age-related obesity, and correspondingly few obesity-related metabolic abnormalities are observed. This result suggests that at least some of the effects of CR might be separable from those of leanness. The metabolic rates of the mice were not altered significantly, indicating that there is an unknown longevity-inducing factor at work resulting directly from lowered fat content in the body and not from diet (*88*).

Other mediators of murine longevity

Other factors might also play a role in the hormonal/neuroendocrine control of longevity. For example, mitochondrial membrane lipid saturation and therefore electromotive potential (which affects the rate of ROS accumulation) might be influenced by TSH via lipid desaturases (*89*). Additionally, a young ovary transplanted into an old ovariectomized mouse greatly affects ensuing life expectancy, indicating that the ovary also contributes to hormonal life span regulation (*90*).

The phenotypes of the mice discussed above are summarized in Fig. 2. Various other models of life span extension not directly related to hormone or neuroendocrine activity exist and are mentioned in Fig. 2, but a detailed discussion of these is beyond the scope of this Review. Other genetically altered mice in which longevity is affected include *Klotho* mice (http://sageke.sciencemag.org/cgi/content/full/sageke;2001/1/tg4): Inactivation of the *klotho* (http://sageke.sciencemag.org/cgi/genedata/sagekeGdbGene;81) gene is associated with premature death in mice, and single-nucleotide polymorphisms in the human homolog *KLOTHO* (http://sageke.sciencemag.org/cgi/genedata/sagekeGdbGene;222) have been identified and might be related to aging phenotypes and life span extension (*91*, *93*). Furthermore, antioxidants such as thioredoxin (*94*, *95*), peroxiredoxin (http://sageke.sciencemag.org/cgi/genedata/sagekeGdbGene;450) (*96*), and Bcl-2, which suppresses ROS generation and prevents apoptosis (*97*, *98*), have been linked to life span or cellular survival in mice. Elements of the Ras signaling pathway also display effects on both antioxidative capacity and apoptosis, and elevated Ras expression is associated with cellular senescence in a number of model systems (*99*–*109*).

Telomere length is another important factor that determines cellular life span (see "More Than a Sum of Our Cells" at http://sageke.sciencemag.org/cgi/content/full/2001/1/oa4). If cell senescence indeed contributes to organismal aging, then these effects must be localized to actively replicating cell types, such as cells of the immune system, as opposed to predominantly nonreplicative somatic cell types. Immunosenescence has been described as a characteristic of organismal aging that is altered by CR. Greater susceptibility to diseases in the elderly is likely a result of both a population shift from naïve to memory T cells (thus decreasing responses to novel antigens), and a lowered response in the remaining naïve T cells, which results in decreased T cell proliferation and cytokine reactivity (*110*–*113*) (and see "Immunity Challenge" at http://sageke.sciencemag.org/cgi/content/full/2003/23/oa1).

Consistent with this idea, the activity of telomerase (the enzyme that repairs telomere DNA sequences) is required for extended maintenance of T cell proliferative capacity (*114*), and humans with shorter telomeres experience an eight-fold higher incidence of death caused by infectious diseases (*115*) (and see

"When Tips Disappear, the End Is Near" at http://sageke.sciencemag.org/cgi/content/full/2003/5/nw24). Although mouse models in which the various components of immunosenescence have been genetically altered have not been examined for longevity effects, the mouse telomerase RNA knockout (http://sageke.sciencemag.org/cgi/content/full/2003/8/tg2) displays a decline in immune function after a number of generations. This is a very interesting emerging area of research that may reveal novel causal relations.

There is growing evidence in lower organisms to suggest that chromatin modification and structure (http://sageke.sciencemag.org/cgi/content/full/2003/14/re4) influence longevity via the proteins encoded by genes such as *SIR2* (http://sageke.sciencemag.org/cgi/genedata/sagekeGdbGene;137), *HDA1* (http://sageke.sciencemag.org/cgi/genedata/sagekeGdbGene;67), and *RPD3* (http://sageke.sciencemag.org/cgi/genedata/sagekeGdbGene;286) (*116*–*119*). It has been reported that the histone deacetylase Sir2 is required for CR effects in yeast (120) (see Kaeberlein Perspective at http://sageke.sciencemag.org/cgi/content/full/2001/1/pe1 but also "Calorie Restriction Un-SIR-tainty" at http://sageke.sciencemag.org/cgi/content/full/2004/19/nf48, which suggests an alternative hypothesis), and recent reports indicate that the related mammalian protein SIRT1 mediates some of the effects of CR in mice (*121*, *122*). Heat shock response pathways (especially involving hsp70/mot2, a putative heat shock protein and negative regulator of p53) might also affect life span (*123*, *124*). However, the task of generating and analyzing appropriate mouse models has thus far limited investigation of these effects in mammals.

Modern developments in genomics and proteomics are increasingly facilitating large-scale screening in mammalian model systems. Transcriptional profiling via DNA microarrays or serial analysis of gene expression allows for monitoring of physiological changes and their underlying genetic correlates over time (see Bengtsson Viewpoint at http://sageke.sciencemag.org/cgi/content/full/sageke;2001/12/vp8). Gene expression profiling of CR, Snell, and Laron mice has revealed a wide range of chromosomal loci with activities differing from those of control mice (*125*–*128*). Quantitative trait locus (QTL) analysis allows for detection of genetic loci associated with a given trait and of variations between alleles at these loci (*129*) (and see Service Perspective at http://sageke.sciencemag.org/cgi/content/full/2004/12/pe13 and Mackay Perspective at http://sageke.sciencemag.org/cgi/content/full/2004/17/pe17). QTL analysis of various inbred mouse strains has revealed several genetic regions associated with long-term survival and long life (*130*, *131*). A large-scale analysis of several genetically heterogeneous crossbred strains has also revealed that the strongest predictor of life span in mice is weight at 2 to 4 months of age (*132*). This finding, in association with the identification of specific alleles associated with longevity, should provide us with physiological and genetic early life predictors of longevity, which will facilitate long-term large-scale studies of life span extension.

Conclusion

Genes associated with longevity have been extensively studied in lower organisms such as *C. elegans*, *Saccharomyces cerevisiae*, and *Drosophila*. Many of the logistic and technological limitations associated with equivalent research in mammalian models have been overcome, thus allowing for better insight into the human aging process. Large-scale analysis is being facilitated by newer approaches involving gene expression arrays and QTL analysis, as well as determination of early predictors of longevity.

CR is the only environmental factor that has been found to significantly and consistently extend life span in almost all examined species. Naturally long-lived mouse mutants have been found: Ames, Snell, and Little mice all display extended life span and significant reduction in aging-associated phenotypes, but at the cost of impaired metabolic function, including adverse effects on growth, development, and fertility. These effects resemble but do not completely overlap with the effects of CR.

The importance of hormonal and neuroendocrine influences on longevity is well established, especially with regard to the GH/IGF-1 axis. Mutations in this signaling pathway at or downstream of IGF-1R separate life span extension phenotypes from associated negative effects, resulting in "cost-free longevity" (*78*). Analysis of mice in which components of the IGF-1 pathway such as IGF-1R and p66Shc have been genetically inactivated, together with work on other downstream effectors such as FOXO transcription factors, indicate that much of the observed extension of life span might be a result of increased antioxidative capacity. Inactivation of antioxidation enzymes such as thioredoxin and peroxiredoxin also affects life span. However, cultured human cells appear to be inherently more stress-resistant than mouse cells (see Hornsby Perspective at http://sageke.sciencemag.org/cgi/content/full/2003/30/pe21), which may call for caution in applying research carried out in mice to human biology. Other signaling molecules and pathways have also been implicated in the control of aging, including the insulin receptor, ovarian neuroendocrine control, Ras, the heat shock response, and factors affecting immunosenescence.

Research into aging in mammalian systems (beyond the investigation of aging-related diseases) is still in its infancy. Life span analyses must be performed on many more mouse strains in which components of the GH/IGF-1 signaling system have been inactivated. Similar studies will need to be carried out on other hormonal, neuroendocrine, and genetic pathways, particularly involving global regulators of gene expression such as histone deacetylases that may contribute to setting an "aging" pattern of gene expression. Results from these and other models of aging will contribute greatly to our understanding of organismal aging and will be fundamental to the development of future strategies of intervention in the ubiquitous group of processes we call aging.

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