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Extending Healthy Life Span—From Yeast to Humans

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When the food intake of organisms such as yeast and rodents is reduced (dietary restriction), they live longer than organisms fed a normal diet. A similar effect is seen when the activity of nutrient-sensing pathways is reduced by mutations or chemical inhibitors. In rodents, both dietary restriction and decreased nutrient-sensing pathway activity can lower the incidence of age-related loss of function and disease, including tumors and neurodegeneration. Dietary restriction also increases life span and protects against diabetes, cancer, and cardiovascular disease in rhesus monkeys, and in humans it causes changes that protect against these age-related pathologies. Tumors and diabetes are also uncommon in humans with mutations in the growth hormone receptor, and natural genetic variants in nutrient-sensing pathways are associated with increased human life span. Dietary restriction and reduced activity of nutrient-sensing pathways may thus slow aging by similar mechanisms, which have been conserved during evolution. We discuss these findings and their potential application to prevention of age-related disease and promotion of healthy aging in humans, and the challenge of possible negative side effects.

Aging is a complex process of accumulation of molecular, cellular, and organ damage, leading to loss of function and increased vulnerability to disease and death. Despite the complexity of aging, recent work has shown that dietary and genetic alterations can substantially increase healthy life span of laboratory model organisms (Fig. 1).

Many of the mutations that extend life span decrease activity of nutrient-signaling pathways, such as the Igf (insulin-like growth factor)/insulin and the TOR (target of rapamycin) pathways, suggesting that they may induce a physiological state similar to that resulting from periods of food shortage. Indeed, dietary restriction, a reduction in food intake without malnutrition, extends life span of diverse organisms, including yeast, flies, worms, fish, rodents, and rhesus monkeys. The level of restriction usually ranges from 10 to 50% below the level in mammals fed ad libitum, but longevity extension can be achieved by complete starvation in yeast and worms. The beneficial effects of dietary restriction in mammals are obtained by reducing glucose consumption, but also by reducing fat or protein intake. Dietary restriction also protects against age-related decline in function and disease in rodents and monkeys (*1*), and in humans, it reduces risk factors for diabetes, cardiovascular disease, and cancer (*2*). Hence, understanding how dietary restriction exerts these effects could reveal targets for drugs and therapies for a broad-spectrum prevention of aging-related loss of function and disease.

Here we consider the role of nutrient-sensing signaling pathways in mediating the beneficial effects of dietary restriction. We focus on processes that are evolutionarily conserved in multiple organisms and discuss the evidence for their potentially protective, and detrimental, effects in humans.

Signaling Pathways and Dietary Restriction

Involvement of pathways in dietary restriction is usually tested by determining if a genetic mutation alters the response. Some methods of dietary restriction allow exploration of a range of food intakes. In worms, flies, and mice, life span rises to a maximum as food intake is lowered, but then declines rapidly through starvation with further reduction of food intake (Fig. 2). The degree of dietary restriction that maximizes life span, and the amplitude of the response, can thus be determined and used to test for genetic effects (*3*).

It is unlikely that a single, linear pathway mediates the effects of dietary restriction in any organism. Matching of metabolism, growth, and fecundity to food intake is crucial for survival and reproduction in nature, and parallel and redundant pathways appear to be involved (Box 1). Different organisms grow and reproduce at different rates and experience different degrees of food shortage in nature. The nutrient content of food and the way it is sensed are also variable. Thus, responses of organisms to dietary restriction may differ in mechanisms, and extent. When developing new or improved model systems to

	Life-span increase		Beneficial health effects	
	Dietary restriction	Mutations/ drugs	Dietary restriction	Mutations/ drugs
Yeast	3-fold	10-fold (with starvation/ DR)	Extended reproductive period	Extended reproductive period, decreased DNA damage/mutations
Worms	2- to 3-fold	10-fold	Resistance to misexpressed toxic proteins	Extended motility Resistance to mis-expressed toxic proteins and germ-line cancer
Flies	2-fold	60–70%	None reported	Resistance to bacterial infection, extended ability to fly
Mice	30–50%	30–50% (~100% in combination with DR)	Protection against cancer, diabetes, atherosclerosis,cardio-myopathy, autoimmune, kidney, and respiratory diseases; reduced neurodegeneration	Reduced tumor incidence; protection against age-dependent cognitive decline, cardio-myopathy, fatty liver and renal lesions. Extended insulin sensitivity
Monkeys	Trend noted	Not tested	Prevention of obesity; protection against diabetes, cancer, and cardiovascular disease	Not tested
Humans	Not determined	Not determined (GHR-deficient subjects reach old age)	Prevention of obesity, diabetes, hypertension Reduced risk factors for cancer and cardiovascular disease	Possible reduction in cancer and diabetes

Fig. 1. Experiments on dietary restriction (DR) and genetic or chemical alteration of nutrient-sensing pathways have been performed on a range of model organisms. The results differ widely, and little is known about the long-term effects in humans.

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An enhanced version of Fig. 3 and a slide show on aging can be found at www.sciencemag.org/cgi/content/full/328/5976/321.

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study aging and healthy life span, it will be important to combine studies that mimic the various environments encountered by the organism in the wild with those that simplify the method and facilitate molecular studies.

Studies of Yeast

The single-celled budding yeast is an excellent organism for genetic screens for mechanisms that are candidates for the evolutionarily conserved effects of dietary restriction on life span in multicellular organisms. Both the number of daughter cells generated by a single mother cell (replicative life span) and survival of a population of nondividing cells (chronological life span) have been analyzed.

Nutrient-sensing pathways. Reduced activity of two major nutrient-sensing pathways can extend both types of yeast life span.

The first is centered on an amino acid–sensing pathway, including the target of rapamycin (TOR) and the serine-threonine kinase Sch9. Deletion of SCH9, which has sequence and functional similarity to the ribosomal protein S6 kinase (S6K), causes an increase of up to several-fold in both chronological (Fig. 3) and replicative life span (*4*, *9*), as does deletion or inhibition of *TOR1* (*4*–*6*), probably by inactivating the downstream Sch9 (*4*, *6*, *7*, *9*). Alterations to protein synthesis are strongly implicated in extension of replicative life span by reduced TOR/Sch9 and may play a key role in chronological life span as well (*8*). Extension of chronological life span by reduced activity of the TOR pathway depends on the transcription factor Gis1, which activates many protective systems including Mn-SOD (*6*, *9*). Understanding exactly how reduced activity of this pathway and the activation of the Gis1 transcription factor extend yeast life span will be crucial, because similar effects are seen in worms, flies, and mice.

The second pathway includes three proteins: Ras, adenylate cyclase (AC), and protein kinase A (PKA) (Fig. 3) (*9*, *10*). The activation of two transcription factors (Msn2 and Msn4) that control cellular protection systems is required to mediate the effect of reduced Ras-AC-PKA signaling on chronological life-span extension (*9*) and may also mediate the extension of replicative life span (*11*). Again, analysis of mechanisms in yeast will illuminate the role of this pathway in mice.

Extension of yeast life span by these pathways requires the antioxidant enzyme Mn-SOD (superoxide dismutase), which scavenges the superoxide free radical (*12*). Superoxide level increases during yeast aging and is reduced in long-lived mutants deficient in Ras-AC-PKA or Tor-Sch9 signaling (*9*). However, overexpression of both the antioxidant enzymes SOD1 and SOD2 or catalase in yeast results in a minor extension of mean survival, indicating that many other systems, including DNA-repair genes, are important in longevity modulation (*9*, *13*).

Nondividing yeast cells deplete glucose and produce ethanol and/or acetic acid, which contribute to chronological aging (*13*, *14*). Deletion of *TOR1*or *SCH9* promotes removal of ethanol and acetic acid and accumulation of glycerol in the medium (*6*, *13*). Because acetate and ethanol can be used as nutrients by yeast, their removal may therefore extend chronological life span by mechanisms similar to those of dietary restriction (*6*, *13*, *14*). However, the chronological life span of cells deficient in *TOR-SCH9* or *RAS-AC-PKA* is also extended in media where acetic acid and ethanol are absent (*6*, *13*), indicating that these pathways can increase life span independently of acetic acid (Fig. 3). These findings may point to mechanisms relevant to aging in multicellular eukaryotes, in which analogous effects of the pro-aging pathways on the utilization and storage of carbon sources are observed (Fig. 3).

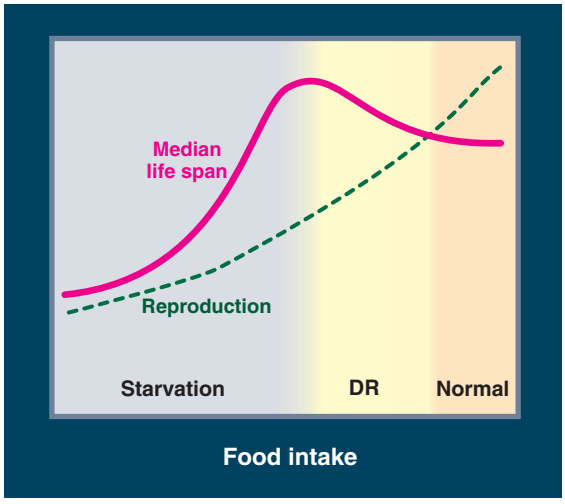

Fig. 2. The median life span and fecundity of higher eukaryotes are negatively affected by a very low food intake. However, life span but not fecundity is optimized by dietary restriction (DR). Unlike what is observed in most higher eukaryotes, starvation extends the yeast chronological life span.

Dietary restriction. In budding yeast, starvation implemented by switching the cells from a medium containing nutrients to one containing water doubles chronological life span (*12*, *15*). More moderate reductions in the concentration of glucose in the growth medium from the standard 2% to 0.5 or 0.05% also extend both replicative and chronological life span, apparently by the regulation of overlapping pathways and mechanisms described below (*4*, *10*, *12*, *16*).

Decreased signaling by the Ras-AC-PKA and Tor-Sch9 pathways is important in the response to glucose restriction (Fig. 3), with increased transcriptional activity of Msn2 and Msn4, and the consequent expression of Pnc1 [a nicotinamide deaminase that regulates the activity of the nicotinamide adenine dinucleotide (NAD)–dependent deacetylase Sir2] (*11*, *17*). Reduced protein synthesis and the breakdown of proteins and intracellular organelles are thought to also contribute to replicative longevity (*17*).

Inhibition of both the Ras-AC-PKA and Tor-Sch9 pathways and the activation of the protective transcription factors described above are also implicated in dietary restriction–dependent extension of chronological life span (Fig. 3) (*12*). It will be important to identify additional mediators of the effects of dietary restriction on life span (Fig. 3).

Studies of the Nematode Worm *Caenorhabditis elegans*

C. elegans was one of the first organisms that allowed the identification of mutations that extend life span. Unlike the unicellular yeast, it allows studies of different cell types and organs, such as the nervous or digestive systems. It is also more closely related to mammals.

Nutrient-sensing pathways. With the onset of multicellularity, nutrient-sensing systems evolved a systemic element that allowed communication at a distance between different parts of the body, while the exact nature of the response could also vary among the cells of different tissues. The worm *C. elegans* is a relatively simple multicellular organism in which to unravel these effects.

Reduced activity of the IIS (insulin/Igf-like signaling) pathway extends life span in *C. elegans* and other multicellular organisms (*18*). This life-span increase requires the Forkhead FoxO transcription factor *daf-16* (Fig. 3), which regulates genes involved in a wide range of defensive activities including cellular stress response, antimicrobial activity, and detoxification of xenobiotics and free radicals. As in yeast, overexpression of the antioxidant SOD-1 causes only a minor extension in life span (*19*). Extension of life span by reducing the activity of the IIS pathway also requires the heat shock factor *hsf-1*, which can itself extend life span and regulates expression of small heat shock proteins (*18*). Reduction of IIS only in specific tissues has revealed that the nervous system and the gut, which includes the white adipose tissue (fat) of the worm, are important. Regulation of insulin ligands by DAF-16 acts systemically to coordinate the rate of aging in the whole worm (*18*).

The TOR pathway interacts intimately with IIS, and inhibition of its activity, including reduction of TOR and S6 kinase, as in yeast, can increase life span in *C. elegans* (Fig. 3) (*18*, *20*, *21*). Autophagy, a process inhibited by TOR that involves digestion of cellular components, is required for the increase in survival (*21*), and altered activity of several other targets of the TOR pathway, such as translation (*22*) and the activity of the HIF-1 (hypoxia-inducible factor 1) transcription factor (*23*, *24*), can independently extend life span.

Dietary restriction. Multiple methods of dietary restriction are used in *C. elegans*, including mutations that reduce pharyngeal pumping, removal of the bacterial food source, dilution of live or dead bacteria in solid or liquid cultures, peptone dilution, and axenic culture (*25*), and these extend life span by both distinct and overlapping

mechanisms (*25*, *26*). Chemosensation may be important in the response to dietary restriction, because diffusible substances from bacteria that are sensed by the worm can reduce life span without any change in food intake (*27*). As in rodents, at least one method of dietary restriction, requiring heat shock factor–1, can protect against age-related proteotoxicity (*28*).

The key IIS effector DAF-16 is required for the response only to some methods of dietary restriction, and for these methods, the adenosine 5′-monophosphate (AMP)–activated protein kinase (AMPK) is also required (Fig. 3) (*26*). Testing of the role of TOR has yielded inconsistent results (*25*). However, targets of the TOR pathway, such as autophagy and HIF-1, clearly contribute to dietary restriction responses (*25*).

Two other transcription factors, Pha-4 and SKN-1, are required for the response to some methods of dietary restriction in *C. elegans* (Fig. 3) (*25*). Pha-4 is the single worm ortholog of the mammalian FOXA family of forkhead transcription factors, which play key roles in metabolic homeostasis. SKN-1 is the worm ortholog of the mammalian Nfe2l1 and Nfe2l2 transcription factors, which induce the phase II detoxification pathway, as does SKN-1 in *C. elegans*. Increased activity of SKN-1 can increase worm life span (*29*).

Although IIS and TOR play a clear role in aging in *C. elegans*, the types of age-related damage ameliorated and the molecular mechanisms by which they are reduced are unknown, as is the extent to which these or other pathways mediate the responses to different forms of dietary restriction.

Studies of the Fruit Fly *Drosophila*

The fly is important for establishing evolutionary conservation of mechanisms; for studying events in different tissues, which are more numerous and differentiated than in *C. elegans*; and for examining sex differences.

Nutrient-sensing pathways. Reduced IIS can extend life span in *Drosophila*, establishing its evolutionarily conserved role (Fig. 3) (*22*). It is not yet known whether, as in *C. elegans*, the fly FOXO is required for extension of life span (Fig. 3).

Changes in gene expression in long-lived IIS mutants of the worm, fly, and mouse have implicated phase 1 and 2 detoxification as evolutionarily conserved targets of IIS (*22*, *30*). Long-lived IIS mutants in all three organisms are resistant to xenobiotics, and up-regulation of transcription factors that regulate xenobiotic metabolism can extend life span in worms and flies (*22*, *29*, *30*).

The *Drosophila* genome contains seven genes encoding *Drosophila* insulin-like peptides (*dilps*), and genetic deletion of three of them made in neuroendocrine cells in the brain extends life span (*31*). Stress signaling and ablation of the germ line reduce expression of one or more *dilps* in the neuroendocrine cells and also extend fly life span (*32*). Identification of the tissues mediating the response to reduced IIS activity, by overexpression of the FOXO transcription factor, which is negatively regulated by IIS, has implicated tissues similar to those in *C. elegans*, namely, the fat body (equivalent of mammalian white adipose tissue and liver) and/or gut.

Down-regulation of TOR pathway activity genetically (*33*) or by rapamycin (*34*) (Fig. 3) extends life span in *Drosophila*, as it does in yeast and *C. elegans*. Extension of life span by rapamycin requires autophagy, reduced S6K activity, and eukaryotic initiation factor 4E binding protein (4E-BP) and is associated with reduced protein turnover (*34*), similar to what is seen in *C. elegans*.

Dietary restriction. Dietary restriction in *Drosophila* is commonly implemented by dilution of the food (*35*, *36*), for which the flies do not compensate by increased food intake (*37*). Per calorie, reduction of amino acid consumption extends life span substantially more than does reduction of sugar intake, with essential amino acids mediating most of the response (*38*). Volatiles from live microorganisms alone can shorten life span, similar to findings in worms, and olfactory mutants can be long-lived (*39*).

As in *C. elegans*, deletion of *Drosophila* FOXO (dFOXO) shortens life span, but the flies continue to respond to dietary restriction (*40*, *41*). However, reduced activity of both IIS and TOR can protect against shortening of life span by increased food intake (*33*, *34*) (Fig. 3). The response to dietary restriction may be mediated by insulin-like peptides, and transcript levels of *dilp5* respond to nutrition (*41*). Thus, reduced IIS plays a role in extension of life span by dietary restriction, but loss of FOXO can be compensated by other pathways such as the TOR pathway (Fig. 3). Reduced IIS and dietary restriction act acutely in *Drosophila* to increase survival (*25*, *40*), and it will be important to determine if this also occurs in mammals, because it would imply that drugs to improve health during aging should be taken over long periods of time. Life span is in general extended more in females than in males, for reasons that are not yet understood.

Studies of Rodents

Invertebrate model organisms have acted as engines of discovery for genes and mechanisms involved in extension of life span, but the mouse is the most practical mammal for establishing if homologous genes and processes can extend healthy life span, and hence lead to human clinical trials.

Nutrient-sensing pathways. As in yeast, worms, and flies, reduced activity of nutrient-sensing pathways can increase mouse life span. Mutations in growth hormone (GH) and IIS genes can substantially increase life span in mice (*42*) (Fig. 3), although precisely how is as yet poorly understood. GH-deficient mice show increased expression of antioxidant enzymes and increased stress resistance in muscle cells and fibroblasts, whereas GH administration decreases antioxidant defenses in the liver, kidney, muscle, and heart (*43*). As observed in yeast, disruption of type 5 adenylyl cyclase (AC5), which is predominantly expressed in the heart and brain, also increases stress resistance and longevity in mice, although GH has not been linked to AC (Fig. 3) (*44*). However, there is little experimental evidence for a conserved role of specific reduction in oxidative stress alone in life-span extension. As in yeast, worms, and flies, inhibition of the mTOR pathway, either with rapamycin (*45*) or deletion of ribosomal S6 protein kinase 1 (S6K1), increases mouse life span, the latter also reducing incidence of age-related pathologies, including bone, immune, and motor dysfunction and insulin resistance (*46*). Nutrient-sensing pathways may extend life span in mammals through transcription factors orthologous to those described in lower eukaryotes (Fig. 3), but data are at present lacking. Systems other than antioxidant enzymes downstream of these transcription factors including heat shock proteins, endoplasmic reticulum (ER) stress and autophagy proteins, apoptosis enzymes, xenobiotic metabolism, and others may have key functions as mediators of life-span extension (*47*).

Remarkably, the up to 50% life-span extension in mice that lack the GH receptor–binding protein (GHR-BP) is associated with lower morbidity and disease-related mortality, with ~47% of long-lived mice dying without obvious lethal pathological lesions and only about 10% of their wild-type

Box 1. Nutrient-sensing pathways and aging.

Nutrient-sensing pathways are fundamental to the aging process. Different nutrients can activate different pathways directly or indirectly. For example, in yeast, glucose activates the Ras-AC-PKA pathway, but in mice nutrients increase the level of IGF-1, which, in turn, activates pro-aging pathways in various mammalian cells (Fig. 3). Dietary restriction partially inactivates one or several nutrient-signaling pathways, thereby causing life-span extension in model organisms. In yeast and worms, this effect on longevity requires one or more transcription factors (proteins that regulate the expression of many genes involved, for example, in cellular protection, metabolic pathways, and processing of damaged proteins). In mice, dietary restriction or the inhibition of nutrient signaling can also reduce various age-related diseases, including cancer. These effects on diseases are believed to be a result of delaying the aging process in the various cells associated with the disease. The reason why these pathways are inactivated or partially inactivated by reduced nutrients is apparently simple: During periods of food scarcity, cells and organisms must be able to enter a standby mode in which cell division and reproduction are halted or minimized to allow energy to be available to maintenance systems. The conserved composition and function of anti-aging pathways in the different organisms indicate that most species have developed anti-aging systems to overcome periods of starvation.

siblings doing so, similar to findings in GH-deficient mice (*42*, *48*). GHR-BP knockout and GH-deficient mice display lower incidence and delayed occurrence of fatal neoplasms, increased insulin sensitivity, and a reduction in age-dependent cognitive impairment (*42*). The reduction in neoplastic diseases may be explained in part by lower somatic mutation frequency in the liver, kidney, and intestine of GH-deficient dwarf mice (*49*).

Some of the effects of dietary restriction can potentially be obtained pharmacologically and in-

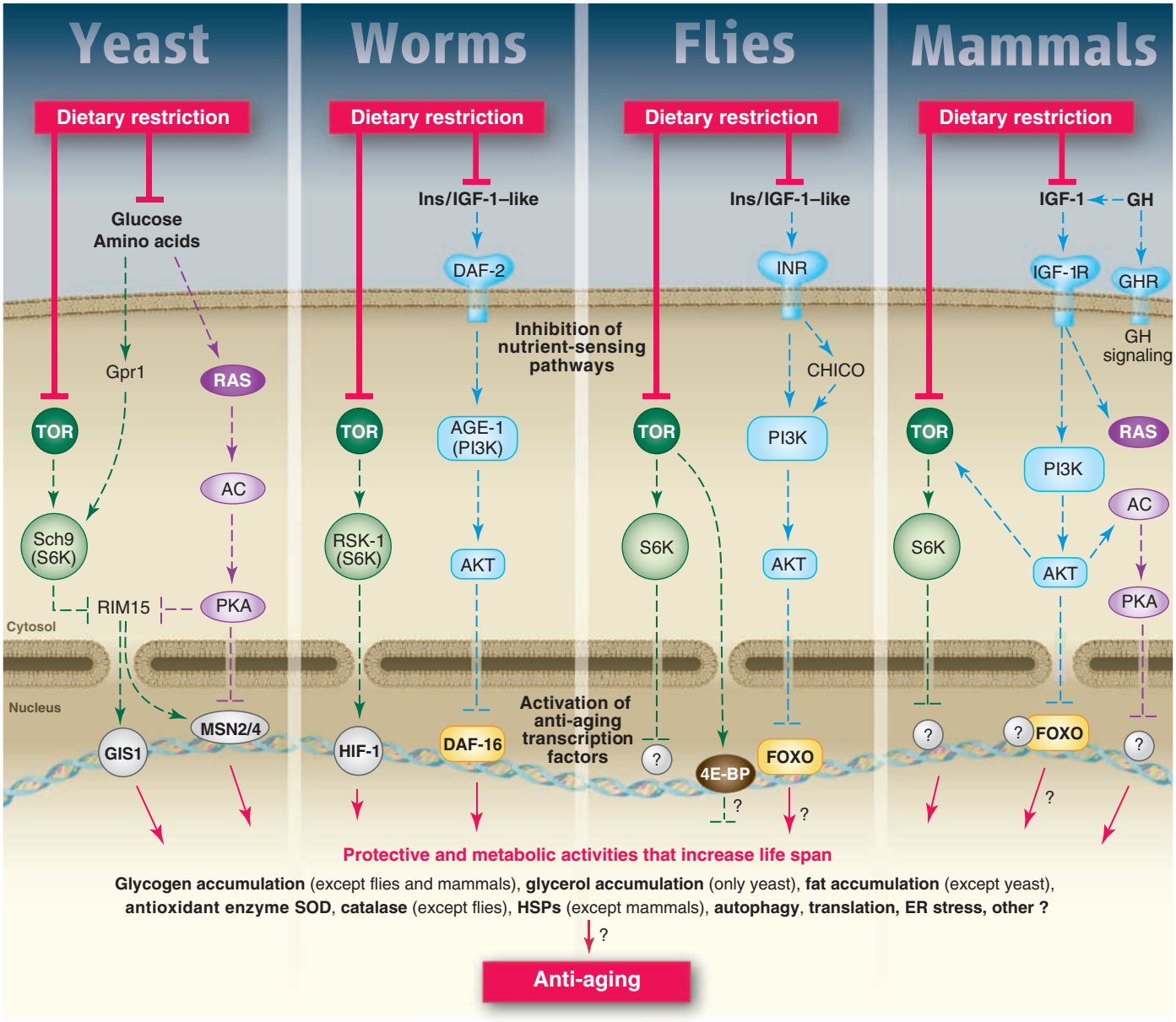

Fig. 3. A model for the conserved nutrient signaling pathways that regulate longevity in various organisms and mammals. Dietary restriction reduces the activity of various signal transduction pathways either directly (yeast) or indirectly through the reduced levels of growth factors such as IGF-1 (worms, flies, mammals). The role of TOR and S6K in promoting aging appears to be conserved in yeast, worms, flies, and mice. By contrast, the AC-PKA pathways and the TOR-S6K pathway promote aging in yeast and mammals, whereas an insulin/IGF-1–like receptor or the upstream growth hormone (mammals) accelerates aging in worms, flies, and mice. Similar transcription factors (GIS1, MSN2/4, DAF-16, FOXO) inactivated by either the AC/PKA, IGF-1/AKT, or TOR/S6K pathways affect cellular protection and/or aging in all the major model organisms. Notably, in the multicellular worms, flies, and mice, these genes may promote aging within the cells in which they are expressed but also in other cells through the regulation of circulating factors. The mechanisms proposed for the longevity extension caused by inhibition of these nutrient signaling pathways include a decrease in the free radical superoxide (mediated in part by SODs) and of its damage to macromolecules, protection of proteins by chaperones (Hsp70), decreased translation, the activation of autophagy, and the switch to hypoxia-associated gene expression patterns (in yeast and mice). In yeast, the effects of DR on life-span extension are also associated with reduced activities of the Tor-Sch9 and Ras-AC-PKA pathways and require the serine-threonine kinase Rim15 and transcription factors Gis1, Msn2, and Msn4. In worms, transcription factors regulated by the TOR-S6K and AGE-1-AKT pathways are implicated in the anti-aging effects of DR and, in flies, reduced activity of both Ins/IGF-1 and TOR can protect against shortening of life span by increased food intake, although in both worms and flies deletion of DAF-2/FOXO shortens life span, but the animal continues to respond to DR. In mice the longevity effects of DR appear to involve reduced activity of the GHR/IGF-1 pathways because DR does not extend further the life span of GHR-deficient mice (*69*). An enhanced version of Fig. 3 is at www.sciencemag.org/cgi/content/full/328/5976/321.

dependently of the inhibition of GH/IGF-1 (insulin-like growth factor 1). For example, treatment of mice on a high-fat diet with resveratrol, a natural compound found in grapes that mimics some of the effects of dietary restriction, results in reduced mortality or protection against morbidity, possibly by activating a set of genes affected by dietary restriction, but resveratrol did not increase survival of mice or flies fed a standard diet (*50*, *51*). Disruption of PKA, downstream of adenylyl cyclase, also causes life-span extension in mice, as it does in yeast (Fig. 3) (*52*), and causes a reduction in age-dependent tumors and insulin resistance (*9*, *52*). It will be important to determine whether superoxide and error-prone polymerases, implicated in the Tor-Sch9- and age-dependent DNA damage/mutations in yeast, are mediators of the GH–IGF-1–dependent genomic instability and cancer in mammals.

Dietary restriction. Dietary restriction increases rodent life span by up to 60%, in part by delaying the occurrence of many chronic diseases (*1*). As in GH–IGF-1 axis mutant mice, ~30% of animals on dietary restriction die without evidence of severe organ pathology, whereas only 6% of controls do so (*53*). Attenuated IIS may mediate some of the anti-aging effects of dietary restriction in mice (Fig. 3). In particular, the reduction of IGF-1 signaling could be responsible for the reduced incidence of spontaneous mutations and tumors in the kidney and small intestine of GH-deficient and dietary-restricted mice (*49*). Part of this protection appears to be mediated by a transcription factor (Nrf2) (*54*), the *C. elegans* and *Drosophila* orthologs of which increase life span when overexpressed.

Dietary restriction in mice greatly increases insulin sensitivity and attenuates β-amyloid deposition in a model for Alzheimer's disease (*55*), in agreement with the effect of reduced IIS signaling in protection against β-amyloid toxicity in mice (*56*) (Fig. 3). Severe dietary restriction or starvation may also be applicable to disease treatment in certain contexts. For example, fasting protects mice against high-dose chemotherapy, in part by reducing serum IGF-1 signaling, but does not protect cancer cells, in which the constitutive activity of pro-aging pathways (oncogenes) blocks the activation of stress resistance in response to nutrient deprivation (*57*).

However, dietary restriction can also impair function such as immunity and wound healing. For example, the healing of skin wounds is reduced in long-term dietary-restricted mice and is greatly accelerated by a short period of ad lib feeding before the wound is inflicted (*58*). Also, dietary-restricted mice are more susceptible to infections by bacteria, virus, and worms, even though dietary restriction can delay the age-dependent decline in certain immune functions (*59*).

Studies of Primates

Findings from model organisms have begun to be tested in monkeys and also in human volunteers, including subjects with mutations analogous to those that extend the healthy life span of laboratory animals.

Nutrient-sensing pathways. Some individuals have naturally occurring deficiencies in the GH–IGF-1 axis (Fig. 4A). In GH receptor–deficient individuals, diabetes or cancer is uncommon, yet these individuals do not appear to have an advantage in reaching very old ages (*60*) (Fig. 4A), possibly because of developmental defects and the increased mortality at younger ages (*61*). In subjects with GH receptor and IGF-1 deficiencies, cancer was absent whereas 9 to 24% of the relatives developed malignancies (*60*), although this report is inconclusive because the mean age of the IGF-1–deficient subjects was much lower than that of controls. Although GH and/or IGF-1 deficiency promotes obesity and hyperlipidemia, whereas treatment with GH has a beneficial effect on both traits, replenishment of GH increases intima media thickness and the number of atherosclerotic plaques. The dyslipidemia and obesity from GH–IGF-1 axis deficiency may therefore not exacerbate vascular aging (*62*).

An overrepresentation of heterozygotes for mutations in the IGF-1 receptor gene was found among Ashkenazi Jewish centenarians as compared to controls (*63*), whereas subjects with a genotype associated with reduced concentration of free IGF-1 in plasma were overrepresented among long-lived people (*64*), indicating that specific polymorphisms that down-regulate the GH and IGF-1 signaling may promote human longevity. Furthermore, genetic variants of FOXO transcription factors, orthologs of the key IIS effector *daf-16* in *C. elegans*, have repeatedly been shown to associate with human life span (*65*).

Further studies of GH/IIS mutants in humans are needed to establish the role of these pathways in cancer and also in diabetes, cardiovascular, and neurodegenerative diseases. If the multiple beneficial effects of reduced signaling observed in mice are confirmed in humans, drugs that block these pathways could be considered for prevention of specific diseases.

Dietary restriction. Recently, a 20-year 30% dietary restriction applied to adult rhesus monkeys was shown to reduce age-related deaths (*66*). The incidence of neoplasia and cardiovascular disease was 50% lower than in the controls (*66*), and 16 of the initial 38 controls but none of the dietary-restricted monkeys developed diabetes or prediabetes. Many metabolic, hormonal, and structural adaptations in dietary-restricted rodents, including a major reduction in body fat mass, higher insulin sensitivity, and reduced inflammation and oxidative damage, were also observed in dietary-restricted monkeys (*1*). In addition, immune senescence, sarcopenia, and brain atrophy of the gray matter were attenuated (*1*, *66*). Possible downsides of dietary restriction, such as reduced resistance to infection or attenuated wound healing, have not been investigated.

In humans, dietary restriction provides major and sustained beneficial effects against obesity, insulin resistance, inflammation, oxidative stress, and left ventricular diastolic dysfunction, in agreement with the metabolic and functional changes observed in dietary-restricted rodents (Fig. 4B) (*2*). Moreover, dietary restriction in humans induces some of the hormonal adaptations observed in dietary-restricted rodents (e.g., increased adiponectin and reduction in triiodothyronine, testosterone, and insulin) and reduced cholesterol, C-reactive protein, blood pressure, and intima-media thickness of the carotid arteries, all risk factors for cardiovascular disease (*2*, *67*).

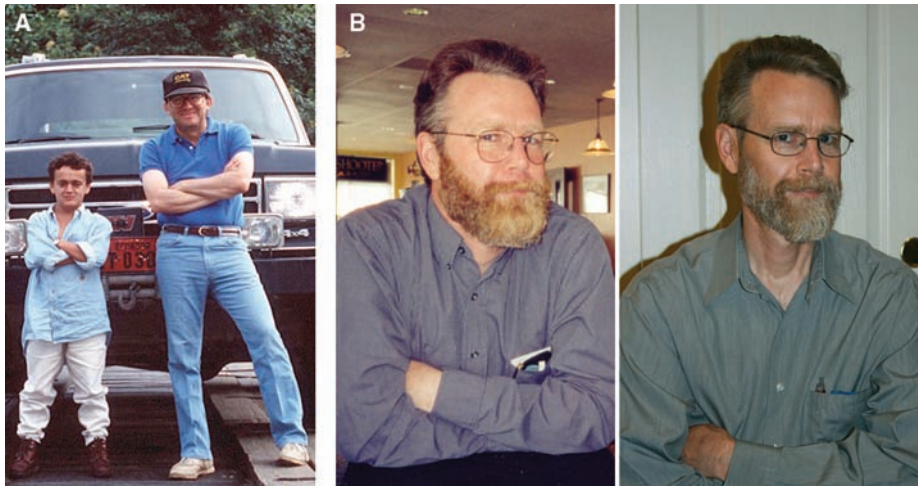

Fig. 4. (**A**) Individual with normal levels of GH receptor (right) and a GH receptor–deficient subject in the mountains of southern Ecuador (left). (**B**) Composite photograph of a dietary restriction practitioner before starting dietary restriction with adequate nutrition [left: weight 180 lb, or 81.6 kg; body mass index (BMI) 26.0 kg/m^2) and after 7 years of dietary restriction (right: weight 134 lb, or 60.8 kg; BMI 19.4 kg/m^2).

However, there are major differences in effects of dietary restriction between rodents and humans. Dietary restriction decreases serum IGF-1 concentration by ~30 to 40% in rodents but does not reduce IGF-1 levels in humans, unless protein intake is also reduced (*68*) (Fig. 4B), raising the possibility that protein restriction alone may provide some benefits, as in *Drosophila* (*38*).

Outlook

Extreme dietary restriction can lead to several detrimental health effects such as amenorrhea, infertility, sarcopenia, osteoporosis, and immune deficiencies. Thus, it will be important to examine these negative side-effects in dietary-restricted subjects that are not malnourished. Indeed, experimental studies are required to evaluate the optimal calorie intake and macro- and micronutrient composition needed for healthy aging in humans, on the basis of age, sex, genotype, and energy expenditure.

Although adjustment of dietary intake and composition may be realistic and beneficial, the severe dietary restriction that induces major health benefits is not a desirable option for most people. Drugs that target nutrient-sensing pathways to obtain the health benefits of dietary restriction are realistic, but the effects of chronic administration require study. For instance, rapamycin, the TOR inhibitor that extends mouse life span (*45*), is an immunosuppressant and may not produce an overall health benefit in humans living in an environment with pathogens. However, genetic deletion of the GH receptor or of the downstream S6 kinase in mice extends life span and induces a broad-spectrum improvement in health (*42*, *46*, *48*). More testing of potential disadvantages is required and many open questions remain (Box 2), but these seem promising drug targets and are hopefully the first of many.

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Box 2. Some questions for future research.

1. Can aging be delayed and diseases prevented by blocking cellular and molecular damage, or must gene expression be "reprogrammed" to achieve healthy life-span extension?

All four model organisms should help answer this question, but the powerful screening methods in yeast, worms, and flies are more likely to lead to unbiased answers.

2. Which collection of molecular events leads to cellular and organismal aging?

Yeast studies should allow a more in-depth molecular and genetic approach, whereas studies of worms and flies should lead to a better understanding of the relative role of different cell types and tissues in aging. Mouse research should help establish whether these molecular mechanisms are relevant to humans and age-associated diseases.

3. Can protein restriction mimic the effects of dietary restriction and limit cancer growth?

Protein restriction is much less difficult to maintain than dietary restriction and may be more powerful than dietary restriction in reducing the serum IGF-1 concentration in humans. Moreover, protein restriction, by lowering intracellular amino acid levels, reduces mTOR activity and stimulates autophagy, two key processes involved in aging and cancer. Randomized clinical trials of healthy volunteers and patients with cancer are needed to understand the long-term metabolic and clinical effects of protein restriction in humans.

4. Does exercise-induced leanness induce the same metabolic adaptations as dietary restriction and slow aging?

More studies in both rodents and humans are needed to elucidate the metabolic and molecular mechanisms responsible for the different effects of calorie restriction (low-energy flux) and exercise (high-energy flux) on aging and longevity.

5. Can these discoveries be translated into health benefits for humans?

We need to identify candidate drug targets and determine if targeting them with drugs can achieve health benefits in the absence of undesirable side-effects. It will also be essential to determine if long-term drug intervention is required for a full benefit, or if instead the full subsequent benefit can be achieved with drug treatment initiated at later ages.

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