

Genetics of Longevity in Model Organisms: Debates and Paradigm Shifts

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Annu. Rev. Physiol. 2013. 75:621–44

First published online as a Review in Advance on November 26, 2012

The *Annual Review of Physiology* is online at <http://physiol.annualreviews.org>

This article's doi:
10.1146/annurev-physiol-030212-183712

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Keywords

aging, dietary restriction, disposable soma, evolution, hyperfunction, insulin/IGF-1 signaling, oxidative damage, target of rapamycin (TOR)

Abstract

Discovering the biological basis of aging is one of the greatest remaining challenges for science. Work on the biology of aging has discovered a range of interventions and pathways that control aging rate. A picture is emerging of a signaling network that is sensitive to nutritional status and that controls growth, stress resistance, and aging. This network includes the insulin/IGF-1 and target of rapamycin (TOR) pathways and likely mediates the effects of dietary restriction on aging. Yet the biological processes upon which these pathways act to control life span remain unclear. A long-standing guiding assumption about aging is that it is caused by wear and tear, particularly damage at the molecular level. One view is that reactive oxygen species (ROS), including free radicals, generated as by-products of cellular metabolism, are a major contributor to this damage. Yet many recent tests of the oxidative damage theory have come up negative. Such tests have opened an exciting new phase in biogerontology in which fundamental assumptions about aging are being reexamined and revolutionary concepts are emerging. Among these concepts is the hyperfunction theory, which postulates that processes contributing to growth and reproduction run on in later life, leading to hypertrophic and hyperplastic pathologies. Here we reexamine central concepts about the nature of aging.

Hyperfunction:

purposeless continued activity of developmental programs in later life, leading most lethally to diseases of hypertrophy and hyperplasia

Dietary restriction

(DR): reduction of food availability without starvation, leading to extended late-life health and life span and, typically, reduced fecundity

THE NATURE AND MALLEABILITY OF THE AGING PROCESS

Loss of vitality as organisms traverse their intrinsically limited life spans is a truism of our everyday experience. Indeed, aging is so inexorable and inevitable that one might think that it is not amenable to explanation or experimental analysis. Machines manufactured by humans deteriorate and eventually break down, so why not living organisms too? Yet the aging process is highly malleable. Aging also remains one of the great mysteries of science. We do not understand exactly what goes wrong during aging, nor do we understand the mechanistic causes of such failure, and, although we have found ways of slowing aging in laboratory animals, we do not understand how these interventions work. Aging is very complicated, with many different changes occurring in parallel in different parts of the body and at different levels of biological organization: macromolecules, organelles, cells, tissues, and the systemic environment (1). A major challenge is to distinguish between bystander effects that probably do not contribute much to loss of function, such as graying hair in humans, and those that are important for vitality, including the pathways leading to aging-related disease.

Fundamental questions about aging thus remain unanswered, and the field is enlivened by vigorous debate even over how we should think about the process. Is it caused by accumulation of damage and, if so, by what kind of damage? Are the oxygen and other reactive molecules postulated as key to aging by the free radical theory really of paramount importance, or are other kinds of damage to macromolecules as or more important? For instance, do endogenously or exogenously generated toxins build up in cells during aging? Or is the key to aging damage to levels of organization other than that of macromolecules—for instance, the level of chromosomes, mitochondria, cell membranes, the extracellular matrix, or the systemic environment? Is aging caused by damage at all, or is it a result of a different kind of malfunction, such as overactivity of processes that were beneficial earlier in life? We address these questions in this review, focusing particularly on the roles of oxidative damage, toxins, and the runaway activity of processes that were useful in youth but have become harmful in old age (the so-called hyperfunction theory).

We have learned much about aging from laboratory model organisms: the budding yeast *Saccharomyces cerevisiae*, the nematode worm *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster*, and the mouse. These organisms all age and die, although on very different timescales: approximately 3 weeks in *C. elegans*, 3 months in *Drosophila*, and 3 years in the mouse. A key breakthrough has been the discovery that the life spans of these organisms can be extended, and their health during aging improved, by quite simple environmental and genetic interventions (2). This finding provides key evidence that the aging process has been targeted; shortening of life span, although potentially an informative acceleration of normal aging, may instead be a consequence of novel pathology. One of the main achievements of this work on extension of life span has been to demonstrate that there are commonalities of the aging process in these very different organisms. The same types of interventions can improve health and function during aging, protect against aging-related diseases, and extend life span. This evolutionary conservation is most firmly established for the effects of nutrition and of the molecular mechanisms involved in the sensing of nutrients (**Figure 1**) (2, 3).

In the 1930s, dietary restriction (DR) was discovered to extend life span of rats subjected to reduced food intake that fell short of malnutrition (4). The effects of DR on aging were reproduced in *S. cerevisiae*, *C. elegans*, and *Drosophila*, as well as in rhesus monkeys (5–7) and in a number of nonmodel organisms. DR is often referred to as calorie or caloric restriction because of suggestions, on the basis of work with rodents, that reduction in intake of calories, rather than of any specific macronutrient (fat, carbohydrate, or protein) in the diet, is important (8). However, work with

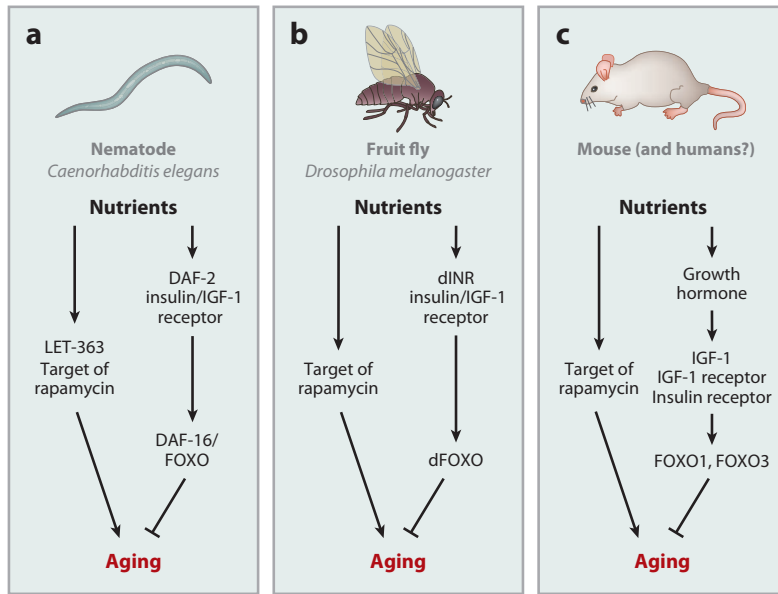


Figure 1

An ancient nutrient-sensitive signaling network plays an evolutionarily conserved role in the regulation of aging rate. Interventions in this pathway can slow aging in (a) nematodes, (b) insects, and (c) mice. Studies of the way that allele frequencies change with increasing age suggest that this network may also influence aging rate in humans. Abbreviations: FOXO, forkhead box O transcription factor; IGF-1, insulin-like growth factor 1.

both *Drosophila* and rodents has shown that specific amino acids play a key role in extending life span during reduced food intake (9–13).

Because the effects of DR have long been known, the health status of aging DR rodents has been very thoroughly investigated, as has that of rhesus monkeys. These animals show a remarkably broad spectrum improvement in function and delay or amelioration of aging-related pathology. For instance, their handling of glucose, insulin sensitivity, and inflammatory profiles are improved, and they are protected against brain atrophy, sarcopenia, cardiovascular disease, type 2 diabetes, neoplasia, and endometriosis (6, 14, 15).

Because of the substantial increases in life span and improvements in health during aging that can be conferred by DR in animals, there is considerable interest in its potential health benefits for humans. However, randomized controlled trials of food intake are difficult in humans because of the extraordinarily high levels of self-discipline that are required to comply with a regime of low food intake over an extended period. Nonetheless, shorter-term studies with volunteers show benefits that are consistent with many of the same health benefits as are seen in nonhuman primates (16–18). For instance, in humans on DR, multiple risk factors for atherosclerosis are reduced (19), and age-related increase in heart rate variability, a marker of decline of cardiac autonomic function, is delayed (20). Further trials of effects on DR in humans are ongoing (14, 21).

As a result of systematic mutagenesis, single-gene mutations that extend life span were first discovered in *C. elegans*, and the initial mutations were in genes that were part of the insulin/insulin-like growth factor 1 (IGF-1) signaling (IIS) pathway (2, 22–25). This pathway, which has both systemic and cellular components, matches nutrient-consuming processes such as growth, metabolism, and reproduction to the nutrient status of the animal. The IIS network

Insulin/insulin-like growth factor 1 (IGF-1) signaling (IIS): downregulation of the single invertebrate IIS pathway slows aging

Target of rapamycin (TOR): the TOR kinase is activated by nutrients and promotes protein synthesis and aging in animals ranging from nematodes to rodents

seems to have evolved with the onset of multicellularity (26) and is important in coordinating nutritional responses in different parts of the organism. Subsequently, mutations in orthologous genes in *Drosophila* and the mouse were also found to increase life span (27–30). Importantly, during aging the long-lived mutant mice showed improved glucose homeostasis, immune profile, and neuromuscular performance and were protected against osteoporosis, cataracts, and ulcerative dermatitis (31), a broad-spectrum improvement in health reminiscent of the effects of DR. In humans, natural genetic variants associated with genes encoding components of the insulin/IGF-1 pathway are associated with survival to advanced ages (32–39), sometimes in multiple independent studies (40), although also sometimes with low repeatability (41). This nutrient-sensing pathway thus has an evolutionarily conserved effect on aging, implying that simpler laboratory animals could be used to make discoveries about mechanisms of aging relevant to humans.

The IIS pathway forms a signaling network with an intracellular nutrient-sensing network that includes the target of rapamycin (TOR) kinase. The TOR network is also involved in sensing cellular nutritional conditions, particularly specific amino acids and energy status, and it has multiple interactions and feedback loops with the IIS network. In recent years, mutations in TOR pathway components have been shown to extend life span in *S. cerevisiae*, *C. elegans*, *Drosophila*, and mice (42–45). Mice with a null mutation in the S6 kinase (S6K)1, a downstream effector of the TOR kinase, are long lived and show a broad-spectrum improvement in health during aging similar to that seen in animals with reduced IIS (46). The insulin/IGF-1/TOR signaling network thus plays a conserved role in aging (**Figure 1**) (2, 47).

This signaling network has also been implicated in mediating at least some of the effects of DR on life span in yeast, *C. elegans*, and *Drosophila*. These studies have typically demonstrated that the response of life span to DR is abrogated or altered in animals mutant for insulin/IGF-1/TOR signaling. However, the real situation is more complex than any single experiment can reveal; different nutritional manipulations that go under the name of DR clearly work through different mechanisms, both because they produce different phenotypes in the animals and because they give different results in epistasis experiments with mutants (48). Epistasis data on life span can be difficult to interpret, particularly where additive and synergistic effects are seen (49). Furthermore, multiple parallel mechanisms could mediate the effects of DR. A full understanding of the mechanisms mediating extension of life span by DR in any organism is therefore probably some way off.

However, the discovery that single-gene mutations in this nutrient-sensing signaling network can ameliorate aging raises at least two important questions. (a) How deep is the evolutionary conservation? Is it confined to the cellular signaling domain, or are the effector mechanisms and the types of aging-related malfunction that are ameliorated also conserved? The answers will both reveal a great deal about the aging process and determine the extent to which the laboratory model organisms can act as useful models for human aging. (b) Can we use drugs to alter the activity of the signaling network so as to obtain health benefits during aging? Here, the answer is a clear yes, at least in animal models.

Rapamycin is a macrolide drug approved for human use as an immune suppressant, in cancer chemotherapy, and to prevent restenosis after cardiac surgery. As its name implies, rapamycin inhibits the activity of TOR kinase in a highly potent and specific manner. Rapamycin has remarkably broad effects, both against aging and against aging-related diseases (50). For instance, the drug can extend life span both in *Drosophila* and in mice (51–53). Indeed, mice given rapamycin starting when they were middle aged showed extended life span (54). In addition, in humans aspirin is protective not only against cardiovascular disease but also against cancer and targets at least two components of the insulin/IGF-1/TOR network (55–57), while metformin, which targets the AMP kinase upstream of TOR kinase (58) is protective against both type 2 diabetes and various cancers (59).

HOW DO NUTRIENT-SENSING PATHWAYS CONTROL AGING? IDENTIFYING PROXIMAL MECHANISMS

DR and lowered activity of nutrient-sensing signaling pathways must somehow alter downstream biochemical mechanisms, either reducing the activity of processes that impair function and health during aging or elevating activities that protect against aging. The identity of these processes is still unclear, but the search for them has often been guided by a central paradigm: Aging is caused by the accumulation of damage (60–65). From this point of view, the aging rate is determined by the relative intensity of accumulation of damage, on the one hand, and of somatic maintenance mechanisms, on the other. Much effort has thus been devoted to understanding what biochemical processes lie downstream of the effects of DR and nutrient signaling (66–77).

One approach to identifying such processes is to characterize the downstream elements of the signaling pathways concerned. In the case of IIS, this approach has led to identification of a number of gene-regulatory proteins, particularly transcription factors (TFs). Among these, much attention has focused on the forkhead box O (FOXO) TFs. However, many other TFs contribute to IIS effects on aging, e.g., in the worm the HSF-1 heat shock TF (71), the DAF-12 nuclear receptor (78, 79), the SKN-1 Nrf2 TF homolog (80), and the SMK-1 and HCF-1 activators of FOXO (81, 82; reviewed in References 2 and 83).

FOXO TFs play a central downstream role in the control of aging. In both worms and flies, the extension of life span caused by reduced IIS requires FOXO: DAF-16 in the worm and *dFOXO* in the fly (84–86). It is not yet known whether increased life span upon reduced IIS in mice requires any of the four mouse FOXOs. However, population genetic association studies in humans have found enrichment for genetic variants in both FOXO1a and, particularly, FOXO3a in individuals who survive to late ages (32–35), suggesting that the role of FOXOs in aging may extend to humans.

Identifying the processes regulated by FOXO that control aging rate is important but difficult. Changes in the transcriptional activity of FOXO during extension of life span from reduced IIS have been characterized in both *C. elegans* and *Drosophila* by measuring binding sites of TFs genome wide and changes in transcript representation (67, 76, 77, 87–90). Cross-referencing of mRNA and chromatin profiling data suggests that in both worms and flies FOXO has relatively few direct gene-regulatory targets and acts within a regulatory subnetwork, i.e., by regulating a second tier of other regulators (76, 77). Possible effector mechanisms of IIS/FOXO that cause longer life include upregulated antioxidant defense (91) [although this now seems unlikely (72, 92)], prevention of yolk accumulation (67, 93), increased expression of molecular chaperones (66, 71, 94, 95), and increased xenobiotic metabolism (76, 80, 89, 96).

Like effects of IIS, effects of the TOR pathway on aging show evolutionary conservation. Moreover, there is strong evidence that this pathway mediates the effects of DR on life span (reviewed in Reference 47). The TOR kinase modulates several potentially relevant targets and biological processes. One target is ribosomal S6K, reduced activity of which extends life span in both worms and flies (44, 97–99). In flies, reduced S6K activity is required for rapamycin to extend life span (51), while in mice, deletion of S6K1 extends life span and produces a broad-spectrum improvement in health (46). How reduced S6K activity contributes to amelioration of aging remains unclear. In the worm, longevity resulting from loss of *rsk-1* (S6K) depends on several factors, including *pha-4* (FOXA) (100), *hsf-1* (101), and *aak-2* (AMP kinase) (46). Because S6K controls protein translation and inhibition of protein translation increases life span (97–99, 102), one possibility is that TOR/S6K control aging via controlling protein biosynthesis rate. Rate of protein synthesis is reduced in long-lived worms with reduced *rsk-1* (74, 97, 99). But how reducing protein synthesis increases life span remains unclear. Reduced activity of TOR also activates autophagy, which is required for DR and reduced IIS to increase life span in *C. elegans*

(68, 73) and for rapamycin to extend life span in *Drosophila* (51). But again, exactly how increased autophagy ameliorates aging is unclear.

4E-binding protein (4E-BP), an inhibitor of cap-dependent translation initiation, is also a direct target of TOR and is inhibited by TOR activity. The *C. elegans* genome does not possess a predicted 4E-BP (98), but one is present in *Drosophila*. However, in the fly 4E-BP is not required for increased life span in response to rapamycin or for the response to one type of DR (103), although it and its effects on translation have been implicated in a second type of DR through modulation of mitochondrial activity (104). TOR may affect aging through effects on other processes such as stress resistance, endoplasmic reticulum stress signaling, and altered metabolism (47), but exactly how any of these make an organism age more slowly remains unclear.

DOES THE PROCESS OF MOLECULAR WEAR AND TEAR CAUSE AGING?

Several findings are consistent with the idea that reduced food availability or nutrient-dependent signaling extends life span by reducing the rate of accumulation of molecular damage. Such interventions typically delay the increase in accumulation of molecular damage with age (e.g., References 105–109), although not always (e.g., References 110–112). Such interventions also often increase resistance to a variety of stressors (28, 30, 63, 85, 113–115) and activate a range of functions predicted to promote somatic maintenance (66, 69, 71, 76, 89, 91, 94, 96, 116).

Although supportive of the damage/maintenance paradigm, such correlative evidence does not prove it. For example, if molecular damage accumulation were a sign, rather than a cause, of aging, then interventions that slow aging would still be expected to retard the increase in molecular damage during aging. Moreover, aging leads to death through elevation of a broad spectrum of pathologies, and molecular damage levels are typically increased by pathology (117). Thus, whatever the primary cause of aging, one might expect an intervention that slowed aging to lead to lowered levels of molecular damage, even though such damage is not a cause of aging. Furthermore, transcript and chromatin profiling studies imply that many hundreds of genes show altered expression under longevity-inducing conditions (67, 87–89, 118, 119), weakening the conclusion that altered expression of any single gene, e.g., that encoding superoxide dismutase (SOD), is evidence of its role in longevity assurance.

To verify the damage/maintenance paradigm would require evidence that induction of reduced or increased levels of molecular damage is sufficient to slow or accelerate aging rate, respectively. If, in contrast, treatments that increase levels of damage are found to not accelerate or even retard aging rate, the paradigm could be refuted. Over the past 20 years many such tests have focused on the role of antioxidant defense in longevity assurance, with mixed results. Initial reports tended to support the paradigm. For example, in *Drosophila* simultaneous overexpression of genes encoding SOD and catalase appeared to be sufficient to increase life span (120). In *C. elegans*, treatment with a synthetic compound with SOD activity was reported to increase life span (121). However, in each case the initial conclusions appear to have been the result of experimental artifact (122–124). It is possible that publication bias has affected this topic; i.e., researchers seeing increases in life span under conditions expected to increase antioxidant defense may be more likely to submit their work for publication, and journals more likely to publish it.

Is the Oxidative Damage Theory Wrong?

During the past decade, further rigorous tests of the role of molecular damage, particularly oxidative damage, in aging were performed. For example, several research groups independently

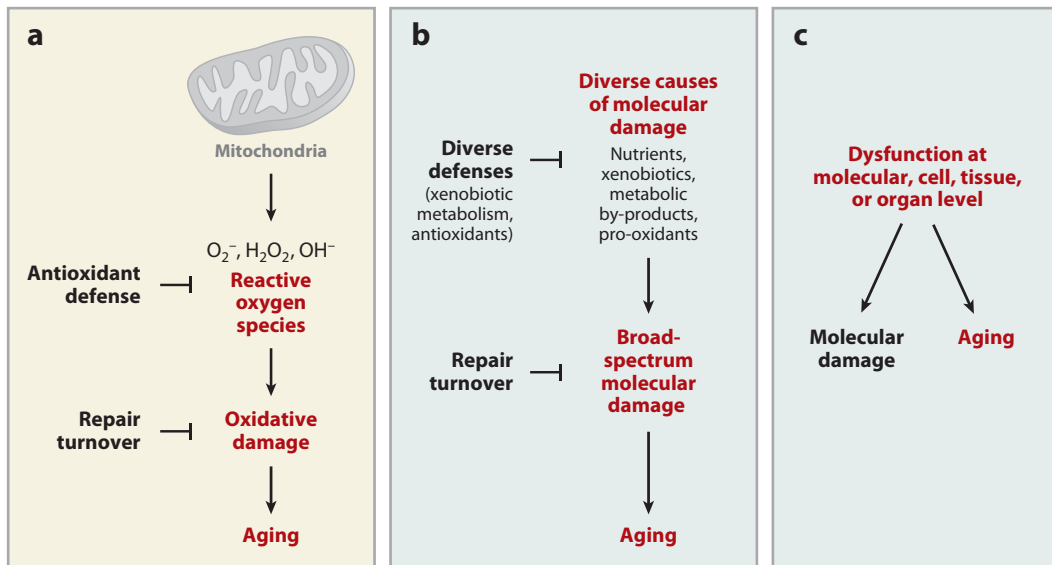


Figure 2

Alternative mechanistic theories of aging. (a) The oxidative damage theory of aging has been highly influential for several decades, but recent studies suggest that it may not be correct. (b) The molecular damage theory more broadly suggests that reactive oxygen species are not the major cause of the molecular damage underlying aging. (c) Aging is caused not by molecular damage but rather by a different type of dysfunction.

found that abrogation of *sod* gene function in *C. elegans* did not shorten life span (reviewed in Reference 125) and even, in one case, increased it, despite increasing levels of oxidative damage (126). Simultaneous deletion of all five *C. elegans* *sod* genes produced worms that were hypersensitive to oxidative stress but, strikingly, had normal life spans (127). Moreover, in a number of other cases treatments that increased levels of reactive oxygen species (ROS) and/or oxidative damage in *C. elegans* led to increases rather than to decreases in life span (127–131).

Similar studies have been performed in the mouse. When care is taken to maintain a constant genetic background and to perform life span studies under nonstressful animal housing conditions, manipulation of antioxidant gene expression often has little effect on life span (132). For example, tests were performed on the effects on life span of overexpression of cytosolic Cu/ZnSOD, catalase, Cu/ZnSOD and catalase combined, and Cu/ZnSOD and mitochondrial MnSOD combined; in no case did life span increase (133). Moreover, loss of one copy of the *sod-2* MnSOD caused increased levels of DNA damage but did not accelerate aging (134), suggesting that levels of mitochondrial ROS and DNA damage do not limit life span.

Although these many studies have certainly left the status of the oxidative damage theory of aging uncertain, their broader significance is a subject of much discussion (124, 125, 132, 135, 136). What are these findings really telling us? In the remainder of this review, we consider three possibilities (**Figure 2**). First, these studies are misleading, and oxidative damage is, in fact, a major determinant of aging. Second, damage from ROS such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2) is only a small part of a broader spectrum of aging-causing molecular damage. Third, and most radically, molecular damage is not a major primary cause of aging; i.e., the molecular damage/somatic maintenance paradigm is untrue (124).

Could these new studies be missing something important about the role of oxidative damage in aging? One possibility is that they have been focusing on the wrong kinds of ROS. One could

Oxidative damage theory: theory that reactive oxygen species cause stochastic molecular damage, whose accumulation is a major cause of aging

Disposable soma

theory: proposes that evolution diverts energy from somatic maintenance to early-life fitness traits (e.g., reproduction), thereby shortening life span

imagine that damage from mitochondrial O_2^- and H_2O_2 does not cause aging but that damage from other forms of ROS does. The latter forms might include the targets of detoxification by the many enzymes of phase 2 xenobiotic metabolism that are upregulated in a number of long-lived animal models (89, 96, 118, 119, 137).

As an aside, recent studies show that ROS clearly do play a role in modulating aging, but likely not as the result of induction of molecular damage. For example, in *C. elegans*, slight increases in ROS production increase life span (127–129). ROS also play important physiological roles, including in cellular signaling pathways. For example, in mammals H_2O_2 is generated to strengthen insulin signaling by inactivating redox-sensitive signal-quenching phosphatases (138). O_2^- is also generated as an immune defense mechanism (the oxidative burst), which may also occur in invertebrates such as *C. elegans* (139). Of course, treatments that greatly increase ROS levels can decrease life span (reviewed in References 135 and 140), but the relevance of this finding to the mechanisms of aging under nonstressed conditions is questionable.

Broad-Spectrum Molecular Damage: Xenobiotic Metabolism and Longevity Assurance?

A second perspective is that the fundamental mechanism of aging is not oxidative damage but a broader spectrum of causes of damage. According to this broader molecular-wear-and-tear theory, reducing levels of damage from selected forms of ROS such as O_2^- and H_2O_2 will have little effect because other forms of molecular damage will continue to accumulate unabated and will cause aging.

One argument against the idea that O_2^- and H_2O_2 are major drivers of aging is that the cell possesses enzymatic means to detoxify them. Moreover, antioxidant enzymes such as SOD and catalase do not require energy input for their operation. Thus, it is unclear why dealing with these chemical species should be a particular challenge to the cell. By contrast, the diversity of potentially damaging species in the environment (including food) is very great. This chemical diversity represents a major challenge to the cell. The evolutionary response to this challenge is the xenobiotic detoxification system, which is complex and costly. This system principally comprises a large battery of detoxification enzymes [drug-metabolizing enzymes (DMEs)] and cellular pumps that chemically alter and excrete toxic chemical moieties (141) and is part of a broader biotransformation system that also functions in chemical synthesis in the cell. This system includes cytochrome P450 (CYP) oxidases and carbonyl reductases, which render more reactive and soluble lipophilic moieties and form phase 1 of the detoxification system, and glutathione-S-transferases (GSTs) and UDP-glucosyltransferases (UGTs), which add hydrophilic side chains that reduce toxicity and increase solubility, thereby aiding excretion. DME gene expression is highly regulated, perhaps because these genes are numerous (for example, *D. melanogaster* has 83 CYP and 27 GST genes) (142), and their activity is costly in energy terms (141).

When whole-genome microarray studies began to be used to investigate long-lived animal models, the many genes showing alteration of expression included numerous DME genes (67, 87, 118, 119). In particular, in studies looking at functional classes overrepresented among genes upregulated in several long-lived models, gene categories linked to xenobiotic metabolism were salient (89, 96). This finding led to speculation about the role of xenobiotic metabolism in longevity assurance. In particular, it was proposed that (a) the chemical diversity of the causes of molecular damage presents an insurmountable challenge to the cell, making damage accumulation unavoidable, and that (b) because of the costly nature of xenobiotic metabolism, natural selection lowers its activity to free up resources for reproduction (64, 143), consistent with the disposable soma theory of aging (61). Meanwhile, further studies have underscored the association between longevity and

enhanced xenobiotic metabolism. For example, long-lived dwarf mice show increased resistance to the toxicity of several xenobiotics (115), and increased DME gene expression has now been seen in a number of long-lived mouse models, including crowded-litter mice (137).

A critical test of this theory is whether enhancement of xenobiotic metabolism is sufficient to extend life span. A number of observations support this possibility. Activation of transcriptional activators of DME gene expression can increase life span. For example, in *C. elegans* the SKN-1 Nrf2-type TF activates expression of phase 2 DME gene expression, and its overexpression or chemical activation can increase worm life span (80, 144). In *Drosophila* activation of Nrf2 (CnC) by mutational inhibition of the repressive cofactor Keap1 increases life span (145). The mechanisms downstream of Nrf2 that affect life span remain undemonstrated, although regulatory targets of Nrf2 in *C. elegans* have been identified (69, 80). These targets include *gst-10*, whose gene product detoxifies the electrophilic aldehyde 4-hydroxynon-2-enal (4-HNE). Overexpression of *gst-10* increases 4-HNE-conjugating activity in worm lysates, decreases levels of 4-HNE-protein adducts (i.e., damage), and increases life span (146). These findings suggest that GST-10 slows aging by reducing molecular damage levels. However, it is also possible that 4-HNE influences aging through its action as a second messenger (147).

Studies of the role of xenobiotic metabolism in aging seem to support a picture of broad-spectrum molecular damage causing aging, and this theory is consistent with the finding that manipulation of SOD and catalase activity has failed to support the oxidative damage theory. Therefore, molecular damage may play a key role in aging, and the systems that detoxify damaging molecules may be potential targets for amelioration of human aging. However, recent work has suggested that molecular damage may not be the whole story.

BEYOND THE DAMAGE/MAINTENANCE PARADIGM

Recent studies probing proximal mechanisms have increasingly followed leads that do not obviously involve the damage/maintenance paradigm. One such mechanism is alteration of fat metabolism, which increases life span in several contexts (reviewed by Reference 148). For example, in *C. elegans*, germline removal increases both life span and expression of the lipase LIPL-4, and the increase in life span is partially LIPL-4 dependent (149, 150). Moreover, increased LIPL-4 levels are sufficient to increase life span. The increase in lipase activity also requires active autophagy, suggesting a role for lipophagy in longevity assurance (151).

Yet this effect on life span is not simply a function of lowered fat levels. In fact, overall lipid levels are increased, not decreased, in worms lacking a germline. Likewise, some long-lived mice, such as the S6K1 mutant, are lean, whereas others, such as the Ames and growth hormone receptor knockout (GHRKO) dwarfs, are plump (46, 152, 153). Moreover, surgical removal of visceral fat improves longevity-associated parameters (e.g., insulin sensitivity) in wild-type mice but worsens them in GHRKO mice (154). Thus, it does not seem to be a case of less fat good, more fat bad. Instead, altered signaling appears to increase life span by promoting forms of lipid or states of adipose tissue that are more healthy (148). For example, growth hormone (GH) appears to increase production by visceral fat of the proinflammatory cytokine IL-6 but to decrease production of adiponectin, which is anti-inflammatory and increases insulin sensitivity (154).

Changes in secretory phenotype may also mediate the effect on organismal aging of late-life accumulation of senescent cells (155). Such cells result from activation of tumor suppressor pathways by a variety of stimuli, including telomere shortening. Senescent cells are hypertrophic and secrete a range of proteins—the senescence-associated secreted phenotype (155)—that include proteases and inflammatory cytokines and that can promote tumor growth (156). Thus, antitumor surveillance in early life enhances fitness by reducing cancer risk but contributes to aging (157).

Age changes such as these occur at the levels of cells, tissues, and the systemic environment and are therefore distinct from stochastic molecular damage as primary drivers of aging.

Another possibility is that slow epigenetic changes during the course of adulthood lead to dysregulation of gene expression and hence to aging (158, 159). For example, in *C. elegans* deficiency in the histone H3 lysine 4 trimethylation (H3K4me3) complex can increase life span (160). Moreover, reduced histone methylation caused by transient loss of H3K4me3 can be transmitted to progeny, resulting in increased life span in several subsequent generations of worms (161). In mammals, similar mechanisms may underlie the effects of diet during pregnancy on health of offspring in later life (162).

Aging as Hyperfunction

Blagosklonny (136, 163, 164) recently proposed the hyperfunction theory as an alternative to the damage/maintenance paradigm. According to this theory, processes that contribute to early-life fitness through growth and reproduction continue in later life at too high a level (hence the term hyperfunction, or twisted growth), leading to pathology and eventually to death. Many of these activities are promoted by the nutrient-sensing regulatory network that includes GH, IGF-1, and TOR. Moreover, many lethal pathologies of aging involve gain of biomass. Hypertrophy or hyperplasia underlies cardiovascular disease; type II diabetes; and, particularly, cancer and contributory or associated factors, such as inflammation, atherosclerosis, increased adiposity, and hyperglycemia (136, 163, 164). However, hyperfunction can also cause atrophy; e.g., osteoclast hyperactivity can lead to bone loss and osteoporosis.

Short-lived invertebrate models also show late-life pathologies consistent with hyperfunction, particularly the very short lived *C. elegans* (165). One example involves yolk synthesis. Worms exist largely as protandrous hermaphrodites that switch from sperm to egg production early in adulthood and then self-fertilize. To provision the developing oocytes, yolk (vitellogenin complexed with lipid) is synthesized in the intestine in large quantities and is then transported to the gonad via the body cavity (166). After several days of reproduction, the store of self-sperm becomes depleted, and reproduction ceases. However, bulk production of yolk continues, and consequently it accumulates in large pools within the body cavity (93, 167, 168). This does not happen in long-lived *daf-2* insulin/IGF-1 receptor mutants (93). Moreover, life span is extended by RNAi of several *vit* genes encoding vitellogenin (67), implying that yolk overproduction contributes to mortality. Pathologies of aging *Drosophila* are less well described, but old flies do show testicular and intestinal hyperplasia (169, 170).

The theory suggests that a variety of different forms of hyperfunction cause diverse pathologies whose sum effect is the cause of death in aging organisms. Until now, in model organism aging studies, late-life pathologies in worms, flies, and mice have been relatively little studied. A common view has long been that the underlying aging process (e.g., stochastic damage accumulation) is distinct from the pathologies whose risk is increased by aging and that only the former process is interesting. Arguably, this view is reinforced by the questionable clinical distinction drawn between aging and age-related disease (171). But the perspective discussed here suggests that aging is the sum of these pathologies. Another concern has been that pathologies of aging in nematodes and insects may not be informative about the biology of aging in higher animals. Yet although such pathologies (e.g., cuticular hypertrophy in *C. elegans*) may appear to be lineage specific (167), the processes (e.g., IIS hyperfunction) that lead to them may not be.

Upregulation of autophagy and reduced or redirected protein translation have been implicated in extension of life span by reduced activity of nutrient signaling. The importance of autophagy in longevity assurance has been interpreted as promoting turnover of damaged molecular constituents

of the cell (172). Explaining why reducing protein synthesis might increase life span in terms of the damage/maintenance paradigm is not straightforward, but several explanations have been proposed (173, 174). By contrast, the hyperfunction theory suggests that both increased autophagy and reduced protein synthesis retard aging by suppressing hypertrophy and hyperplasia.

Integrating Ultimate and Proximate Theories of Aging: Disposable Soma or Bloating Soma?

Although the proximate causes of aging remain murky, its ultimate causes are well defined. On the whole, salient features of animal biology (e.g., embryogenesis, possession of sensory systems and of a circulatory system) reflect evolutionary adaptations. However, the same is not true of aging. Darwin's contemporaries August Weismann and Alfred Russel Wallace proposed that aging has evolved to remove worn-out older individuals, thereby reducing competition for scarce resources and resulting in benefits for the species. However, such an evolutionary mechanism would require group selection of a sort that makes this an untenable scenario.

Aging is now understood to evolve as the result of the declining force of natural selection acting to maintain fitness and remove harmful effects in later life (175–177). Because of the impact of extrinsic hazards such as disease, predation, and accidents, most individuals will survive to be young, but few will survive to be old, and genetic variants that affect events later in life will hence be less subject to natural selection. Deleterious mutations that affect later life can thus accumulate to higher equilibrium frequencies, genes that benefit the young but at the cost of a higher rate of aging can enter the population by natural selection, and both processes lead to the evolution of aging.

Although support for the evolutionary theory is robust, the genes and processes underlying the evolution of aging, and the trade-offs between increases in early-life fitness and aging, remain unclear. A major theory that integrates evolutionary (ultimate cause) and mechanistic (proximate cause) theories is that of disposable soma (61, 178). As its proximate component, this theory incorporates the damage/maintenance paradigm. Pivotal to this theory is the assumption that somatic maintenance is costly in resources. Given that the life histories of organisms typically evolve under conditions of variable and, at times, limited resource availability, the division of resources between growth and reproduction on the one hand and somatic maintenance (longevity assurance) on the other hand must be carefully optimized. Thus, at a given level of extrinsic mortality, organisms will invest just sufficient resources in somatic maintenance to live long enough to reach the end of the reproductive period, resulting in a short-lived (or disposable) soma (61, 178).

The disposable soma theory provides a plausible account that integrates ultimate and proximate mechanisms of aging. This theory predicts how aging biology operates. However, although reproduction–life span trade-offs are often seen, e.g., in *Drosophila* (179), it remains unclear whether these trade-offs are attributable to plasticity in resource partition between reproduction and somatic maintenance (reviewed in detail in Reference 180). For example, manipulation of nutritional conditions in fruit flies has established that the effects of DR on life span and those on fertility can be uncoupled (12).

If we turn to alternatives to the damage/maintenance paradigm, the idea that aging results from runaway activity of early-life programs for growth and reproduction (i.e., hyperfunction) fits well with evolutionary ideas about aging (164, 181, 182). Studies of DR and the genetics of aging have revealed the close connection between nutrition and aging. An important contributor to fitness is the capacity to rapidly adjust to the inevitable fluctuations of the food supply. Such fluctuations have led to the evolution of marked phenotypic plasticity that allows an organism to focus on physiological states geared for growth and reproduction when there is ample food and to postpone reproduction (diapause) when food is scarce. A major mediator of this plasticity

Proximate theory of aging: theory of how aging occurs in terms of biological mechanisms

Ultimate theory of aging: theory of why aging exists (i.e., why it has evolved)

Antagonistic

pleiotropy: in the evolution of aging, a mutation increases fitness in early life but has detrimental effects later, increasing aging

is the GH/IGF-1/TOR pathway. Here one can see how antagonistic pleiotropy could lead to the evolution of aging. Mutations that affect GH/IGF-1/TOR and that increase rates of biosynthesis in early life may enhance fitness by accelerating growth and increasing reproductive output. However, such mutations will also increase hyperfunction and exacerbate pathologies caused by hypertrophy and hyperplasia, consequently accelerating aging (182). By this view, poor maintenance does not cause the demise of the soma; rather, excess biosynthesis does. That is, the soma is not disposable but bloated. Thus, the hyperfunction theory can effectively link Williams's (177) trade-off theory with empirical findings about the control of aging rate by GH, IGF-1, and TOR, thereby generating a new integrated account of the causes of aging (**Figure 3**).

Although the original evolutionary theory did not specify a mechanism for pleiotropy in aging in terms of biological processes, it did suggest a hypothetical example: "a mutation arising that has a favorable effect on the calcification of bone in the developmental period but which expresses itself in a subsequent somatic environment in the calcification of the connective tissue of arteries" (177). This scenario, although inaccurate in its specifics (arteriosclerosis is not caused by calcium deposition), to some extent anticipates the concept of hyperfunction.

An important phenomenon that an integrated theory of aging must explain is DR, whereby reduction of food levels leads to increased life span and, typically, to reduced fertility. These effects of DR have been explained in terms of the disposable soma theory: When food becomes limiting, resources are diverted from reproduction to somatic maintenance (183). But why should natural selection favor the limitation of resource investment into somatic maintenance under such conditions? Arguably, if more resources are available, then organisms should invest more, not less, in somatic maintenance, in which case DR ought to shorten life span (164). By contrast, the hyperfunction theory provides a more straightforward explanation: Low food levels are associated with reduced levels of nutrient-sensitive signaling (e.g., TOR). This reduced signaling lowers biosynthesis levels, reducing overall biomass and suppressing hypertrophy and hyperplasia. The end result is retardation of aging (**Figure 4**).

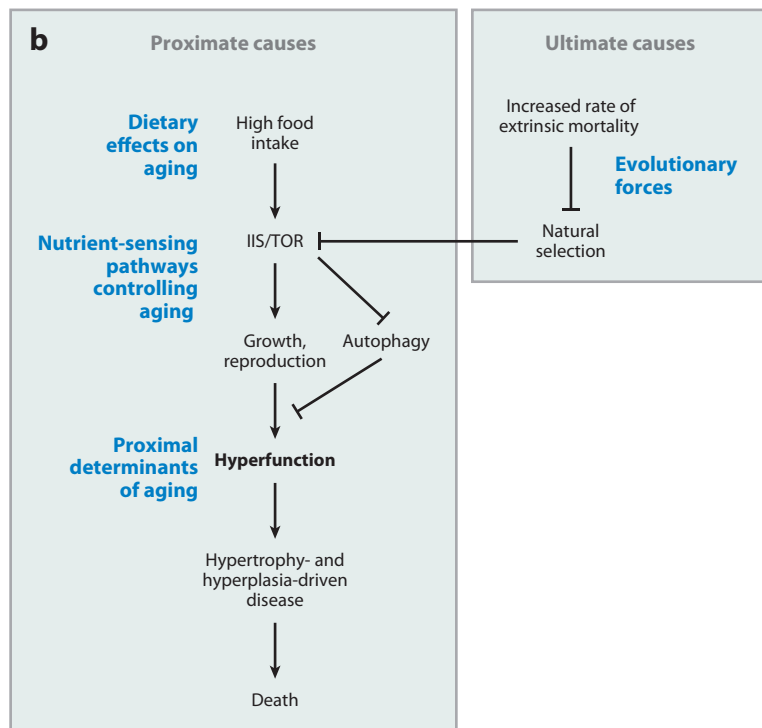
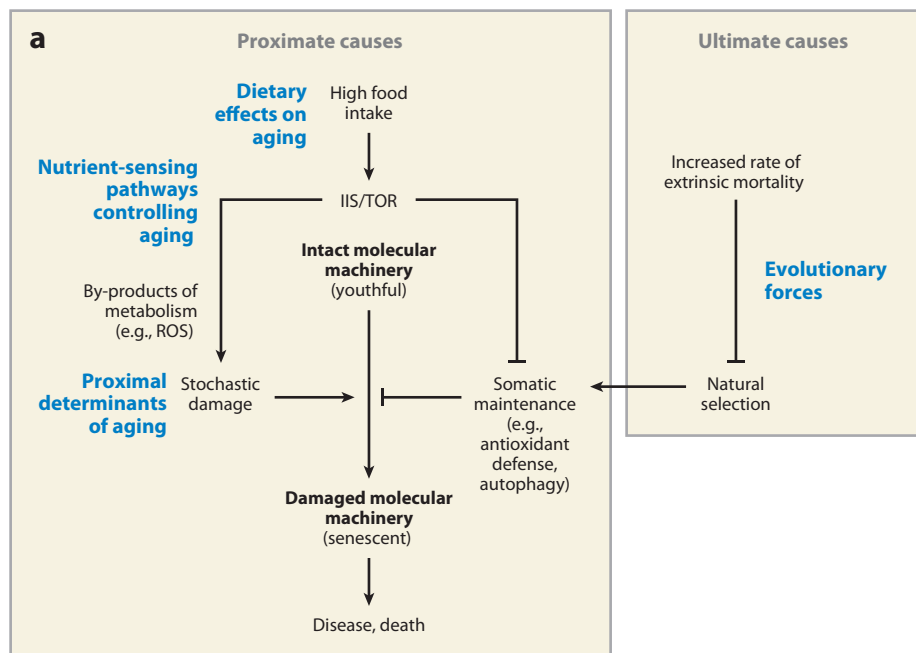
CONCLUSIONS

The past few years have seen a whirlwind of rapid development and conceptual turnover in the biology of aging. Such developments include many issues not dealt with in this review, such as the major advances achieved in developing DR mimetic drugs, with the discovery that DR slows aging in primates (5–7); the control of mammalian aging by S6K1 in the TOR pathway (which mediates DR effects) (46); and the slowing of aging in multiple animal models, including rodents, by the TOR inhibitor rapamycin (51–54, 184). The past few years have also seen controversies in the resveratrol-sirtuin-DR-aging paradigm (185–189). In this review, we explore the current debates surrounding the nature of the mechanisms of aging that are regulated by diet and nutrient-sensitive signaling pathways. We highlight the crisis in the oxidative damage theory

Figure 3

Central conceptual framework for the biology of aging, encompassing proximate and ultimate causes.

(a) Under the standard model, aging is a wear-and-tear process and is caused by the accumulation of molecular damage to biomolecules. Pathways and natural selection affecting aging rate alter the rate of accumulation of damage. (b) Under an alternative model, aging is caused by hyperfunction or overactivity in late life of biochemical processes (e.g., growth). Such hyperfunction leads to hypertrophy- and hyperplasia-driven pathologies. Pathways and natural selection affecting aging rate alter hyperfunction levels. Abbreviations: IIS, insulin/insulin-like growth factor 1 signaling; ROS, reactive oxygen species; TOR, target of rapamycin.



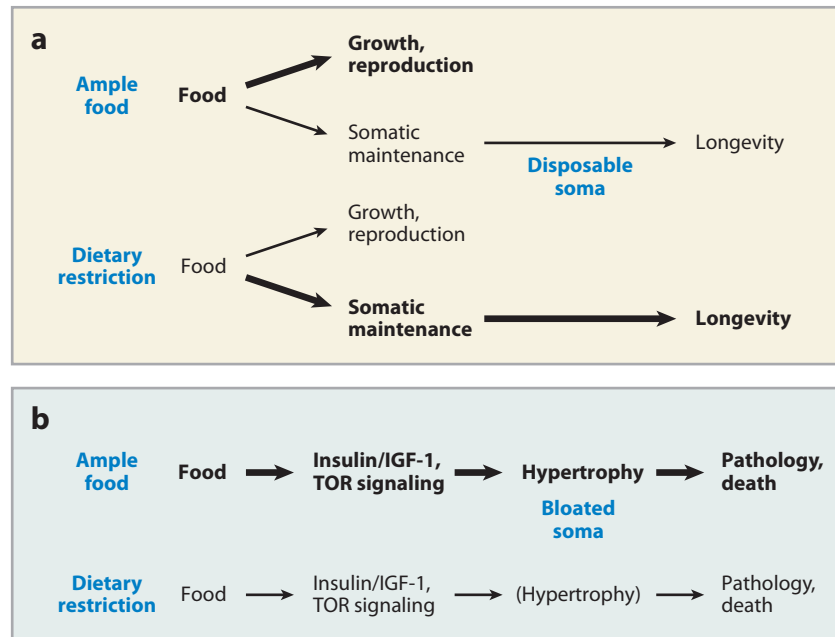


Figure 4

Evolutionary explanation for the mechanism of dietary restriction (DR) in terms of the standard model and alternative model outlined in **Figure 3a** and **Figure 3b**, respectively. (a) Disposable soma theory, derived from the damage/maintenance paradigm. (b) Bloated soma theory as part of the hyperfunction theory. Arrow thickness denotes level of activity (e.g., in resource investment or signal intensity). After Reference 164.

and explore its possible significance. One exciting outcome of these intellectual upheavals is the appearance of the new hyperfunction paradigm as an alternative to the damage/maintenance paradigm.

These new developments raise many new questions and motivate a reexamination of the older paradigms. For example, if treatments that retard aging act by suppressing hyperfunction, then why is longevity so often associated with enhanced stress resistance (28, 30, 63, 85, 113–115)? The strength of the association suggests that it is not purely coincidental. Yet this association may be neither coincidental nor causal. Phenotypic plasticity in response to varying food availability is also linked to survival in the face of other forms of hardship. By this view, shutting down growth and enhanced resistance to stress are linked as part of the broader response to environmental stress. For example, *C. elegans* dauer larvae are adapted to endure—to achieve longevity in a harsh world (190, 191). Thus, reduced IIS may retard aging by suppressing hyperfunction and may protect against acute causes of mortality by upregulated stress resistance mechanisms.

Among the most salient and lethal symptoms of mammalian senescence is cancer, a disease that originates with DNA damage. Thus, at least one form of molecular damage, that to DNA, must be a primary contributor to the aging process. Yet even here, the etiology of disease is a combination of molecular damage and hyperfunction (136). The problem with cancer is not that the cell function is weakened by damage but that cell function is too vigorous, and the process of carcinogenesis is one of the somatic evolution of an overly robust state. Moreover, the ontogenesis and progression of cancer, particularly its age increase, are driven in part by age-related changes in the tissue microenvironment, e.g., accumulation of senescent cells. These

changes are also the product of a combination of DNA damage and hyperfunction because DNA damage is a major cause of cellular senescence but involves formation of a hypertrophic cell, one that is giant in size and with a copious secretory phenotype (reviewed in Reference 155).

Another possible challenge to the hyperfunction theory is the influence on aging in worms and flies of Nrf2-type TFs (80, 144, 145, 192). This influence appears to be more consistent with molecular damage as a driver of aging. Yet even the Nrf2-aging link may be interpreted in terms of the hyperfunction model. Xenobiotic metabolism is only one facet of a broader biotransformation system in which DMEs participate and that also includes diverse biosynthetic functions (141). For example, *gst-4*, the mostly strongly upregulated SKN-1 regulatory target in the worm (69), is a predicted glutathione-dependent prostaglandin D synthase (193). SKN-1 also upregulates lysosomal genes, suggesting that it may promote autophagy (69), and it is also required for development of the worm pharynx (reviewed in Reference 194).

In *Drosophila*, Nrf2 also influences aging via its effects on cell proliferation. In the fruit fly intestine, aging is associated with hyperproliferation and misdifferentiation of intestinal stem cells (170). These cells are maintained in a resting, quiescent state by fruit fly Nrf2 (195). This quiescent state is achieved by shifting the redox balance toward a more reduced state. In mammals, FoxO can act in a similar way, reducing ROS levels and preventing hyperproliferation in hematopoietic stem cells (196). These findings raise the possibility that SKN-1 promotes worm longevity by preventing age-associated hypertrophy.

In conclusion, the hyperfunction theory provides a clear and simple explanation for many observations within biogerontology and a framework to unite key central concepts. Arguably, the theory picks up clues that in the past were missed. In the mid-1990s the hope was that the nature of aging might be revealed by cloning and sequencing genes that control aging, such as *age-1* and *daf-2* in *C. elegans* (23, 84). By 2001 the emerging picture was that IIS, with GH in mammals, plays an evolutionarily conserved role in controlling mammalian aging (24, 25, 27, 28, 152, 197, 198). Investigators noted that IIS affects both growth and stress resistance but quickly assumed that only the latter was relevant (199, 200), for several reasons. One of the first genes identified as controlling longevity in *Drosophila* was *chico*, which encodes an insulin receptor substrate homolog. Both *chico*^{-/-} homozygote flies and *chico*^{-/+} heterozygote flies were long lived, but only *chico*^{-/-} flies had reduced body size (28, 201). Thus, the effects of *chico* on body size and aging could be dissociated. However, despite their normal body size, adult *chico*^{-/+} flies may show reduced levels of biosynthesis that are sufficient to suppress IIS-associated hyperfunction. In fact, *chico*^{-/+} flies do show reduced egg production (28).

Another likely reason for disregarding this vital clue linking growth and aging is the power of a dominant paradigm (here that of damage/maintenance) to prevent us from seeing what is obvious: The pathways controlling aging, including *growth* hormone and insulin-like *growth* factor 1, also control *growth*, therefore suggesting that *growth* causes aging. It is remarkable how we can sometimes fail to see what is right under our noses, as in the case of the Paris police searching for the purloined letter of Edgar Allen Poe's story.

SUMMARY POINTS

1. The rate of aging is plastic and subject to alteration by manipulations of diet and nutrient-sensitive signaling.
2. The core mechanism of aging remains poorly defined but was long thought to reflect a balance between stochastic molecular damage and somatic maintenance.

3. Challenges to the oxidative damage theory have led to alternative ideas, e.g., that broad-spectrum molecular damage is a cause of aging, a view supported by the control of aging by regulators of xenobiotic metabolism (e.g., Nrf2-type transcription factors).
4. A new theory views aging as the result of hyperfunction, which results in hypertrophy- and hyperplasia-driven pathologies in late life.
5. Integration of the evolutionary and hyperfunction theories has led to the new bloated soma theory of aging.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

LITERATURE CITED

1. Kirkwood TBL, Martin GM, Partridge L. 1999. Evolution, senescence, and health in old age. In *Evolution of Health and Disease*, ed. SC Stearns, pp. 219–30. Oxford, UK: Oxford Univ. Press
2. Kenyon C. 2010. The genetics of ageing. *Nature* 464:504–12
3. Fontana L, Partridge L, Longo VD. 2010. Extending healthy life span—from yeast to humans. *Science* 328:321–26
4. McCay C, Crowell M, Maynard L. 1935. The effect of retarded growth upon the length of life and upon ultimate size. *J. Nutr.* 10:63–79
5. Bodkin NL, Alexander TM, Ortmeier HK, Johnson E, Hansen BC. 2003. Mortality and morbidity in laboratory-maintained rhesus monkeys and effects of long-term dietary restriction. *J. Gerontol. A* 58:212–19
6. Colman RJ, Anderson RM, Johnson SC, Kastman EK, Kosmatka KJ, et al. 2009. Caloric restriction delays disease onset and mortality in rhesus monkeys. *Science* 325:201–4
7. Mattison JA, Roth GS, Beasley TM, Tilmont EM, Handy AM, et al. 2012. Impact of caloric restriction on health and survival in rhesus monkeys from the NIA study. *Nature* 489:318–21
8. Masoro EJ. 2006. Caloric restriction and aging: controversial issues. *J. Gerontol. A* 61:14–19
9. Miller RA, Buehner G, Chang Y, Harper JM, Sigler R, Smith-Wheelock M. 2005. Methionine-deficient diet extends mouse lifespan, slows immune and lens aging, alters glucose, T4, IGF-I and insulin levels, and increases hepatocyte MIF levels and stress resistance. *Aging Cell* 4:119–25
10. De Marte ML, Enesco HE. 1986. Influence of low tryptophan diet on survival and organ growth in mice. *Mech. Ageing Dev.* 36:161–71
11. Zimmerman JA, Malloy V, Krajcik R, Orentreich N. 2003. Nutritional control of aging. *Exp. Gerontol.* 38:47–52
12. Grandison RC, Piper MD, Partridge L. 2009. Amino-acid imbalance explains extension of lifespan by dietary restriction in *Drosophila*. *Nature* 462:1061–64
13. Sun L, Sadighi Akha AA, Miller RA, Harper JM. 2009. Life-span extension in mice by preweaning food restriction and by methionine restriction in middle age. *J. Gerontol. A* 64:711–22
14. Anderson RM, Weindruch R. 2012. The caloric restriction paradigm: implications for healthy human aging. *Am. J. Hum. Biol.* 24:101–6
15. Kemnitz JW. 2011. Calorie restriction and aging in nonhuman primates. *ILAR J.* 52:66–77
16. Fontana L, Weiss EP, Villareal DT, Klein S, Holloszy JO. 2008. Long-term effects of calorie or protein restriction on serum IGF-1 and IGFBP-3 concentration in humans. *Aging Cell* 7:681–87
17. Meyer TE, Kovacs SJ, Ehsani AA, Klein S, Holloszy JO, Fontana L. 2006. Long-term caloric restriction ameliorates the decline in diastolic function in humans. *J. Am. Coll. Cardiol.* 47:398–402

18. Soare A, Cangemi R, Omodei D, Holloszy JO, Fontana L. 2011. Long-term calorie restriction, but not endurance exercise, lowers core body temperature in humans. *Aging* 3:374–79
19. Fontana L, Meyer T, Klein S, Holloszy J. 2004. Long-term calorie restriction is highly effective in reducing the risk for atherosclerosis in humans. *Proc. Natl. Acad. Sci. USA* 101:6659–63
20. Stein PK, Soare A, Meyer TE, Cangemi R, Holloszy JO, Fontana L. 2012. Caloric restriction may reverse age-related autonomic decline in humans. *Aging Cell* 11:644–50
21. Rochon J, Bales CW, Ravussin E, Redman LM, Holloszy JO, et al. 2011. Design and conduct of the CALERIE study: comprehensive assessment of the long-term effects of reducing intake of energy. *J. Gerontol. A* 66:97–108
22. Klass MR. 1983. A method for the isolation of longevity mutants in the nematode *Caenorhabditis elegans* and initial results. *Mech. Ageing Dev.* 22:279–86
23. Friedman DB, Johnson TE. 1988. A mutation in the *age-1* gene in *Caenorhabditis elegans* lengthens life and reduces hermaphrodite fertility. *Genetics* 118:75–86
24. Morris JZ, Tissenbaum HA, Ruvkun G. 1996. A phosphatidylinositol-3-OH kinase family member regulating longevity and diapause in *Caenorhabditis elegans*. *Nature* 382:536–38
25. Kimura KD, Tissenbaum HA, Liu Y, Ruvkun G. 1997. *daf-2*, an insulin receptor-like gene that regulates longevity and diapause in *Caenorhabditis elegans*. *Science* 277:942–46
26. Skorokhod A, Gamulin V, Gundacker D, Kavsan V, Muller IM, Muller WE. 1999. Origin of insulin receptor-like tyrosine kinases in marine sponges. *Biol. Bull.* 197:198–206
27. Tatar M, Kopelman A, Epstein D, Tu M-P, Yin C-M, Garofalo RS. 2001. Mutations in the *Drosophila* insulin receptor homologue retard senescence and impair neuroendocrine function. *Science* 292:107–10
28. Clancy D, Gems D, Harshman LG, Oldham S, Hafen E, et al. 2001. Extension of lifespan by loss of CHICO, a *Drosophila* insulin receptor substrate protein. *Science* 292:104–6
29. Bluher M, Kahn B, Kahn C. 2003. Extended longevity in mice lacking the insulin receptor in adipose tissue. *Science* 299:572–74
30. Holzenberger M, Dupont J, Ducos B, Leneuve P, Geloën A, et al. 2003. IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice. *Nature* 421:182–87
31. Selman C, Lingard S, Choudhury AI, Batterham RL, Claret M, et al. 2008. Evidence for lifespan extension and delayed age-related biomarkers in insulin receptor substrate 1 null mice. *FASEB J.* 22:807–18
32. Willcox BJ, Donlon TA, He Q, Chen R, Grove JS, et al. 2008. FOXO3A genotype is strongly associated with human longevity. *Proc. Natl. Acad. Sci. USA* 105:13987–92
33. Anselmi CV, Malovini A, Roncarati R, Novelli V, Villa F, et al. 2009. Association of the *FOXO3A* locus with extreme longevity in a southern Italian centenarian study. *Rejuvenation Res.* 12:95–104
34. Flachsbarth F, Caliebe A, Kleindorp R, Blanché H, von Eller-Eberstein H, et al. 2009. Association of *FOXO3A* variation with human longevity confirmed in German centenarians. *Proc. Natl. Acad. Sci. USA* 106:2700–5
35. Li Y, Wang WJ, Cao H, Lu J, Wu C, et al. 2009. Genetic association of *FOXO1A* and *FOXO3A* with longevity trait in Han Chinese populations. *Hum. Mol. Genet.* 18:4897–904
36. Lunetta KL, D’Agostino RB Sr, Karasik D, Benjamin EJ, Guo CY, et al. 2007. Genetic correlates of longevity and selected age-related phenotypes: a genome-wide association study in the Framingham Study. *BMC Med. Genet.* 8(Suppl. 1):S13
37. Kojima T, Kamei H, Aizu T, Arai Y, Takayama M, et al. 2004. Association analysis between longevity in the Japanese population and polymorphic variants of genes involved in insulin and insulin-like growth factor 1 signaling pathways. *Exp. Gerontol.* 39:1595–98
38. Suh Y, Atzmon G, Cho M, Hwang D, Liu B, et al. 2008. Functionally significant insulin-like growth factor I receptor mutations in centenarians. *Proc. Natl. Acad. Sci. USA* 105:3438–42
39. Tazearslan C, Huang J, Barzilai N, Suh Y. 2011. Impaired IGF1R signaling in cells expressing longevity-associated human IGF1R alleles. *Aging Cell* 10:551–54
40. Slagboom PE, Beekman M, Passtoors WM, Deelen J, Vaarhorst AA, et al. 2011. Genomics of human longevity. *Philos. Trans. R. Soc. Lond. Ser. B* 366:35–42
41. Soerensen M, Dato S, Tan Q, Thinggaard M, Kleindorp R, et al. 2012. Human longevity and variation in GH/IGF-1/insulin signaling, DNA damage signaling and repair and pro/antioxidant pathway genes: cross sectional and longitudinal studies. *Exp. Gerontol.* 47:379–87

42. Kaeberlein M, Powers RW 3rd, Steffen KK, Westman EA, Hu D, et al. 2005. Regulation of yeast replicative life span by TOR and Sch9 in response to nutrients. *Science* 310:1193–96
43. Powers RW 3rd, Kaeberlein M, Caldwell SD, Kennedy BK, Fields S. 2006. Extension of chronological life span in yeast by decreased TOR pathway signaling. *Genes Dev.* 20:174–84
44. Kapahi P, Zid B, Harper T, Koslover D, Sapin V, Benzer S. 2004. Regulation of lifespan in *Drosophila* by modulation of genes in the TOR signaling pathway. *Curr. Biol.* 14:885–90
45. Jia K, Chen D, Riddle D. 2004. The TOR pathway interacts with the insulin signaling pathway to regulate *C. elegans* larval development, metabolism and life span. *Development* 131:3897–906
46. Selman C, Tullet JM, Wieser D, Irvine E, Lingard SJ, et al. 2009. Ribosomal protein S6 kinase 1 signalling regulates mammalian lifespan. *Science* 326:140–44
47. Kapahi P, Chen D, Rogers AN, Katewa SD, Li PW, et al. 2010. With TOR, less is more: a key role for the conserved nutrient-sensing TOR pathway in aging. *Cell Metab.* 11:453–65
48. Mair W, Dillin A. 2008. Aging and survival: the genetics of life span extension by dietary restriction. *Annu. Rev. Biochem.* 77:727–54
49. Gems D, Pletcher S, Partridge L. 2002. Interpreting interactions between treatments that slow ageing. *Aging Cell* 1:1–9
50. Menzies F, Rubinsztein D. 2010. Broadening the therapeutic scope for rapamycin treatment. *Autophagy* 6:286–87
51. Bjedov I, Toivonen JM, Kerr F, Slack C, Jacobson J, et al. 2010. Mechanisms of lifespan-extension by rapamycin in *Drosophila melanogaster*. *Cell Metab.* 11:35–46
52. Miller R, Harrison D, Astle C, Baur J, Boyd A, et al. 2011. Rapamycin, but not resveratrol or simvastatin, extends life span of genetically heterogeneous mice. *J. Gerontol. A* 66:191–201
53. Wilkinson JE, Burmeister L, Brooks SV, Chan CC, Friedline S, et al. 2012. Rapamycin slows aging in mice. *Aging Cell* 11:675–82
54. Harrison DE, Strong R, Sharp ZD, Nelson JF, Astle CM, et al. 2009. Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. *Nature* 460:392–95
55. Algra AM, Rothwell PM. 2012. Effects of regular aspirin on long-term cancer incidence and metastasis: a systematic comparison of evidence from observational studies versus randomised trials. *Lancet Oncol.* 13:518–27
56. Rothwell PM, Price JF, Fowkes FG, Zanchetti A, Roncaglioni MC, et al. 2012. Short-term effects of daily aspirin on cancer incidence, mortality, and non-vascular death: analysis of the time course of risks and benefits in 51 randomised controlled trials. *Lancet* 379:1602–12
57. Din FV, Valanciute A, Houde VP, Zibrova D, Green KA, et al. 2012. Aspirin inhibits mTOR signaling, activates AMP-activated protein kinase, and induces autophagy in colorectal cancer cells. *Gastroenterology* 142:1504–15 e3
58. Ben Sahra I, Regazzetti C, Robert G, Laurent K, Le Marchand-Brustel Y, et al. 2011. Metformin, independent of AMPK, induces mTOR inhibition and cell-cycle arrest through REDD1. *Cancer Res.* 71:4366–72
59. Gallagher EJ, LeRoith D. 2011. Diabetes, cancer and metformin: connections of metabolism and cell proliferation. *Ann. NY Acad. Sci.* 1243:54–68
60. Harman D. 1956. Aging: a theory based on free radical and radiation chemistry. *J. Gerontol.* 11:298–300
61. Kirkwood TBL. 1977. Evolution of ageing. *Nature* 270:301–4
62. Sohal RS, Weindruch R. 1996. Oxidative stress, caloric restriction, and aging. *Science* 273:59–63
63. Lithgow G, Walker G. 2002. Stress resistance as a determinate of *C. elegans* lifespan. *Mech. Ageing Dev.* 123:765–71
64. Partridge L, Gems D. 2006. Beyond the evolutionary theory of ageing, from functional genomics to evo-gero. *Trends Ecol. Evol.* 21:334–40
65. Schumacher B, Garinis GA, Hoeijmakers JH. 2008. Age to survive: DNA damage and aging. *Trends Genet.* 24:77–85
66. Morley J, Brignull H, Weyers J, Morimoto R. 2002. The threshold for polyglutamine-expansion protein aggregation and cellular toxicity is dynamic and influenced by aging in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA* 99:10417–22

67. Murphy CT, McCarroll SA, Bargmann CI, Fraser A, Kamath RS, et al. 2003. Genes that act downstream of DAF-16 to influence the lifespan of *C. elegans*. *Nature* 424:277–84
68. Meléndez A, Tallóczy Z, Seaman M, Eskelinen E-L, Hall DH, Levine B. 2003. Autophagy genes are essential for dauer development and life-span extension in *C. elegans*. *Science* 301:1387–91
69. Oliveira RP, Porter Abate J, Dilks K, Landis J, Ashraf J, et al. 2009. Condition-adapted stress and longevity gene regulation by *Caenorhabditis elegans* SKN-1/Nrf. *Aging Cell* 8:524–41
70. Cohen E, Bieschke J, Perciavalle RM, Kelly JW, Dillin A. 2006. Opposing activities protect against age-onset proteotoxicity. *Science* 313:1604–10
71. Hsu A, Murphy C, Kenyon C. 2003. Regulation of aging and age-related disease by DAF-16 and heat-shock factor. *Science* 300:1142–45
72. Doonan R, McElwee JJ, Matthijssens F, Walker GA, Houthoofd K, et al. 2008. Against the oxidative damage theory: Superoxide dismutases protect against oxidative stress but have little or no effect on lifespan in *C. elegans*. *Genes Dev.* 22:3236–41
73. Hansen M, Chandra A, Mitic LL, Onken B, Driscoll M, Kenyon C. 2008. A role for autophagy in the extension of lifespan by dietary restriction in *C. elegans*. *PLoS Genet.* 4:e24
74. Ching TT, Paal AB, Mehta A, Zhong L, Hsu AL. 2010. *drr-2* encodes an eIF4H that acts downstream of TOR in diet-restriction-induced longevity of *C. elegans*. *Aging Cell* 9:545–57
75. Rogers A, Chen D, McColl G, Czerwieńiec G, Felkey K, et al. 2011. Life span extension via eIF4G inhibition is mediated by posttranscriptional remodeling of stress response gene expression in *C. elegans*. *Cell Metab.* 14:55–66
76. Alic N, Andrews TD, Giannakou ME, Papatheodorou I, Slack C, et al. 2011. Genome-wide dFOXO targets and topology of the transcriptomic response to stress and insulin signalling. *Mol. Syst. Biol.* 7:502
77. Schuster E, McElwee JJ, Tullet JMA, Doonan R, Matthijssens F, et al. 2010. DamID in *C. elegans* reveals longevity-associated targets of DAF-16/FoxO. *Mol. Syst. Biol.* 6:399
78. Larsen PL, Albert PS, Riddle DL. 1995. Genes that regulate both development and longevity in *Caenorhabditis elegans*. *Genetics* 139:1567–83
79. Gems D, Sutton AJ, Sundermeyer ML, Larson PL, Albert PS, et al. 1998. Two pleiotropic classes of *daf-2* mutation affect larval arrest, adult behavior, reproduction and longevity in *Caenorhabditis elegans*. *Genetics* 150:129–55
80. Tullet JM, Hertweck M, An JH, Baker J, Hwang JY, et al. 2008. Direct inhibition of the longevity-promoting factor SKN-1 by insulin-like signaling in *C. elegans*. *Cell* 132:1025–38
81. Wolff S, Ma H, Burch D, Maciel GA, Hunter T, Dillin A. 2006. SMK-1, an essential regulator of DAF-16-mediated longevity. *Cell* 124:1039–53
82. Li J, Ebata A, Dong Y, Rizki G, Iwata T, Lee SS. 2008. *Caenorhabditis elegans* HCF-1 functions in longevity maintenance as a DAF-16 regulator. *PLoS Biol.* 6:e233
83. Antebi A. 2007. Genetics of aging in *Caenorhabditis elegans*. *PLoS Genet.* 3:1565–71
84. Kenyon C, Chang J, Gensch E, Rudener A, Tabtiang R. 1993. A *C. elegans* mutant that lives twice as long as wild type. *Nature* 366:461–64
85. Slack C, Giannakou ME, Foley A, Goss M, Partridge L. 2011. dFOXO-independent effects of reduced insulin-like signaling in *Drosophila*. *Aging Cell* 10:735–48
86. Yamamoto R, Tatar M. 2011. Insulin receptor substrate chico acts with the transcription factor FOXO to extend *Drosophila* lifespan. *Aging Cell* 10:729–32
87. McElwee J, Bubb K, Thomas J. 2003. Transcriptional outputs of the *Caenorhabditis elegans* forkhead protein DAF-16. *Aging Cell* 2:111–21
88. Halaschek-Wiener J, Khattra JS, McKay S, Pouzyrev A, Stott JM, et al. 2005. Analysis of long-lived *C. elegans daf-2* mutants using serial analysis of gene expression. *Genome Res.* 15:603–15
89. McElwee JJ, Schuster E, Blanc E, Piper MD, Thomas JH, et al. 2007. Evolutionary conservation of regulated longevity assurance mechanisms. *Genome Biol.* 8:R132
90. Oh SW, Mukhopadhyay A, Dixit BL, Raha T, Green MR, Tissenbaum HA. 2006. Identification of direct DAF-16 targets controlling longevity, metabolism and diapause by chromatin immunoprecipitation. *Nat. Genet.* 38:251–57
91. Vanfleteren JR. 1993. Oxidative stress and ageing in *Caenorhabditis elegans*. *Biochem. J.* 292:605–8

92. Honda Y, Tanaka M, Honda S. 2008. Modulation of longevity and diapause by redox regulation mechanisms under the insulin-like signaling control in *Caenorhabditis elegans*. *Exp. Gerontol.* 43:520–29
93. Depina A, Iser W, Park S, Maudsley S, Wilson M, Wolkow C. 2011. Regulation of *Caenorhabditis elegans* vitellogenesis by DAF-2/IIS through separable transcriptional and posttranscriptional mechanisms. *BMC Physiol.* 11:11
94. Walker GA, Lithgow GJ. 2003. Lifespan extension in *C. elegans* by a molecular chaperone dependent upon insulin-like signals. *Aging Cell* 2:131–39
95. Chiang WC, Ching TT, Lee HC, Mousigian C, Hsu AL. 2012. HSF-1 regulators DDL-1/2 link insulin-like signaling to heat-shock responses and modulation of longevity. *Cell* 148:322–34
96. McElwee JJ, Schuster E, Blanc E, Thomas JH, Gems D. 2004. Shared transcriptional signature in *C. elegans* dauer larvae and long-lived *daf-2* mutants implicates detoxification system in longevity assurance. *J. Biol. Chem.* 279:44533–43
97. Pan KZ, Palter JE, Rogers AN, Olsen A, Chen D, et al. 2007. Inhibition of mRNA translation extends lifespan in *Caenorhabditis elegans*. *Aging Cell* 6:111–19
98. Syntichaki P, Troulinaki K, Tavernarakis N. 2007. eIF4E function in somatic cells modulates ageing in *Caenorhabditis elegans*. *Nature* 445:922–26
99. Hansen M, Taubert S, Crawford D, Libina L, Lee S-J, Kenyon C. 2007. Lifespan extension by conditions that inhibit translation in *Caenorhabditis elegans*. *Aging Cell* 6:95–110
100. Sheaffer KL, Updike DL, Mango SE. 2008. The target of rapamycin pathway antagonizes pha-4/FoxA to control development and aging. *Curr. Biol.* 18:1355–64
101. Chen D, Thomas EL, Kapahi P. 2009. HIF-1 modulates dietary restriction-mediated lifespan extension via IRE-1 in *Caenorhabditis elegans*. *PLoS Genet.* 5:e1000486
102. Henderson ST, Bonafe M, Johnson TE. 2006. *daf-16* protects the nematode *Caenorhabditis elegans* during food deprivation. *J. Gerontol. A* 61:444–60
103. Partridge L, Alic N, Bjedov I, Piper MD. 2011. Ageing in *Drosophila*: the role of the insulin/Igf and TOR signalling network. *Exp. Gerontol.* 46:376–81
104. Zid BM, Rogers AN, Katewa SD, Vargas MA, Kolipinski MC, et al. 2009. 4E-BP extends lifespan upon dietary restriction by enhancing mitochondrial activity in *Drosophila*. *Cell* 139:149–60
105. Adachi H, Fujiwara Y, Ishii N. 1998. Effects of oxygen on protein carbonyl and aging in *Caenorhabditis elegans* mutants with long (*age-1*) and short (*mev-1*) life spans. *J. Geront. A* 53:B24044
106. Chen J, Velalar CN, Ruan R. 2008. Identifying the changes in gene profiles regulating the amelioration of age-related oxidative damages in kidney tissue of rats by the intervention of adult-onset calorie restriction. *Rejuvenation Res.* 11:757–63
107. Heilbronn LK, de Jonge L, Frisard MI, DeLany JP, Larson-Meyer DE, et al. 2006. Effect of 6-month calorie restriction on biomarkers of longevity, metabolic adaptation, and oxidative stress in overweight individuals: a randomized controlled trial. *JAMA* 295:1539–48
108. Meydani M, Das S, Band M, Epstein S, Roberts S. 2011. The effect of caloric restriction and glycemic load on measures of oxidative stress and antioxidants in humans: results from the CALERIE Trial of Human Caloric Restriction. *J. Nutr. Health Aging* 15:456–60
109. Santin K, da Rocha RF, Cechetti F, Quincozes-Santos A, de Souza DF, et al. 2011. Moderate exercise training and chronic caloric restriction modulate redox status in rat hippocampus. *Brain Res.* 1421:1–10
110. Edman U, Garcia AM, Busuttill RA, Sorensen D, Lundell M, et al. 2009. Lifespan extension by dietary restriction is not linked to protection against somatic DNA damage in *Drosophila melanogaster*. *Aging Cell* 8:331–38
111. Page MM, Withers DJ, Selman C. 2012. Longevity of insulin receptor substrate1 null mice is not associated with increased basal antioxidant protection or reduced oxidative damage. *Age*. In press. doi: 10.1007/s11357-012-9395-9
112. Jacobson J, Lambert AJ, Portero-Otin M, Pamplona R, Magwere T, et al. 2010. Biomarkers of aging in *Drosophila*. *Aging Cell* 9:466–77
113. Murakami S, Salmon A, Miller R. 2003. Multiplex stress resistance in cells from long-lived dwarf mice. *FASEB J.* 17:1565–66

114. Broughton SJ, Piper MD, Ikeya T, Bass TM, Jacobson J, et al. 2005. Longer lifespan, altered metabolism, and stress resistance in *Drosophila* from ablation of cells making insulin-like ligands. *Proc. Natl. Acad. Sci. USA* 102:3105–10
115. Amador-Noguez D, Dean A, Huang W, Setchell K, Moore D, Darlington G. 2007. Alterations in xenobiotic metabolism in the long-lived Little mice. *Aging Cell* 6:453–70
116. Honda Y, Honda S. 1999. The *daf-2* gene network for longevity regulates oxidative stress resistance and Mn-superoxide dismutase gene expression in *Caenorhabditis elegans*. *FASEB J.* 13:1385–93
117. Halliwell B, Gutteridge JMC. 2007. *Free Radicals in Biology and Medicine*. Oxford, UK: Oxford Univ. Press
118. Tsuchiya T, Dhahbi J, Cui X, Mote PL, Bartke A, Spindler SR. 2004. Additive regulation of hepatic gene expression by dwarfism and caloric restriction. *Phys. Genomics* 17:307–15
119. Amador-Noguez D, Yagi K, Venable S, Darlington G. 2004. Gene expression profile of long-lived Ames dwarf mice and Little mice. *Aging Cell* 3:423–41
120. Orr WC, Sohal RS. 1994. Extension of life-span by overexpression of superoxide dismutase and catalase in *Drosophila melanogaster*. *Science* 263:1128–30
121. Melov S, Ravenscroft J, Malik S, Gill M, Walker D, et al. 2000. Extension of life-span with superoxide dismutase/catalase mimetics. *Science* 289:1567–69
122. Kaiser M, Gasser M, Ackermann R, Stearns SC. 1997. P-element inserts in transgenic flies: a cautionary tale. *Heredity* 78:1–11
123. Partridge L, Gems D. 2007. Benchmarks for ageing studies. *Nature* 450:165–67
124. Gems D, Doonan R. 2009. Antioxidant defense and aging in *C. elegans*: Is the oxidative damage theory of aging wrong? *Cell Cycle* 8:1681–87
125. Van Raamsdonk JM, Hekimi S. 2010. Reactive oxygen species and aging in *Caenorhabditis elegans*: causal or casual relationship? *Antioxid. Redox Signal.* 13:1911–53
126. Van Raamsdonk JM, Hekimi S. 2009. Deletion of the mitochondrial superoxide dismutase *sod-2* extends lifespan in *Caenorhabditis elegans*. *PLoS Genet.* 5:e1000361
127. Van Raamsdonk JM, Hekimi S. 2012. Superoxide dismutase is dispensable for normal animal lifespan. *Proc. Natl. Acad. Sci. USA* 109:5785–90
128. Schulz TJ, Zarse K, Voigt A, Urban N, Birringer M, Ristow M. 2007. Glucose restriction extends *Caenorhabditis elegans* life span by inducing mitochondrial respiration and increasing oxidative stress. *Cell Metab.* 6:280–93
129. Heidler T, Hartwig K, Daniel H, Wenzel U. 2010. *Caenorhabditis elegans* lifespan extension caused by treatment with an orally active ROS-generator is dependent on DAF-16 and SIR-2.1. *Biogerontology* 11:183–95
130. Yang W, Hekimi S. 2010. A mitochondrial superoxide signal triggers increased longevity in *Caenorhabditis elegans*. *PLoS Biol.* 8:e1000556
131. Cabreiro F, Ackerman D, Doonan R, Araiz C, Back P, et al. 2011. Increased lifespan from overexpression of superoxide dismutase in *C. elegans* is not caused by decreased oxidative damage. *Free Radic. Biol. Med.* 51:1575–82
132. Perez VI, Bokov A, Van Remmen H, Mele J, Ran Q, et al. 2009. Is the oxidative stress theory of aging dead? *Biochim. Biophys. Acta* 1790:1005–14
133. Perez VI, Van Remmen H, Bokov A, Epstein CJ, Vijg J, Richardson A. 2009. The overexpression of major antioxidant enzymes does not extend the lifespan of mice. *Aging Cell* 8:73–75
134. Van Remmen H, Ikeno Y, Hamilton M, Pahlavani M, Wolf N, et al. 2003. Life-long reduction in MnSOD activity results in increased DNA damage and higher incidence of cancer but does not accelerate aging. *Physiol. Genomics* 16:29–37
135. Muller FL, Lustgarten MS, Jang Y, Richardson A, Van Remmen H. 2007. Trends in oxidative aging theories. *Free Radic. Biol. Med.* 43:477–503
136. Blagosklonny MV. 2008. Aging: ROS or TOR. *Cell Cycle* 7:3344–54
137. Steinbaugh MJ, Sun LY, Bartke A, Miller RA. 2012. Activation of genes involved in xenobiotic metabolism is a shared signature of mouse models with extended lifespan. *Am. J. Physiol. Endocrinol. Metab.* 303:E488–95
138. Goldstein BJ, Mahadev K, Wu X. 2005. Redox paradox: Insulin action is facilitated by insulin-stimulated reactive oxygen species with multiple potential signaling targets. *Diabetes* 54:311–21

139. Chavez V, Mohri-Shiomi A, Maadani A, Vega LA, Garsin DA. 2007. Oxidative stress enzymes are required for DAF-16-mediated immunity due to generation of reactive oxygen species by *Caenorhabditis elegans*. *Genetics* 176:1567–77
140. Gems D, Doonan R. 2008. Oxidative stress and aging in the nematode *Caenorhabditis elegans*. In *Oxidative Stress in Aging*, ed. S Miwa, K Beckman, F Muller, pp. 81–110. Totowa, NJ: Humana
141. Gibson GG, Skett P. 2001. *Introduction to Drug Metabolism*. Bath, UK: Nelson Thornes
142. Lespinet O, Wolf Y, Koonin E, Aravind L. 2002. The role of lineage-specific gene family expansion in the evolution of eukaryotes. *Genome Res.* 12:1048–59
143. Gems D, McElwee JJ. 2005. Broad spectrum detoxification: the major longevity assurance process regulated by insulin/IGF-1 signaling? *Mech. Ageing Dev.* 126:381–87
144. Powolny AA, Singh SV, Melov S, Hubbard A, Fisher AL. 2011. The garlic constituent diallyl trisulfide increases the lifespan of *C. elegans* via *skn-1* activation. *Exp. Gerontol.* 46:441–52
145. Sykiotis GP, Bohmann D. 2008. Keap1/Nrf2 signaling regulates oxidative stress tolerance and lifespan in *Drosophila*. *Dev. Cell* 14:76–85
146. Ayyadevara S, Engle MR, Singh SP, Dandapat A, Lichti CF, et al. 2005. Lifespan and stress resistance of *Caenorhabditis elegans* are increased by expression of glutathione transferases capable of metabolizing the lipid peroxidation product 4-hydroxynonenal. *Aging Cell* 4:257–71
147. Gueraud F, Atalay M, Bresgen N, Cipak A, Eckl PM, et al. 2010. Chemistry and biochemistry of lipid peroxidation products. *Free Radic. Res.* 44:1098–124
148. Ackerman D, Gems D. 2012. The mystery of *C. elegans* aging: an emerging role for fat. *BioEssays* 34:466–71
149. Hsin H, Kenyon C. 1999. Signals from the reproductive system regulate the lifespan of *C. elegans*. *Nature* 399:362–66
150. Wang MC, O'Rourke EJ, Ruvkun G. 2008. Fat metabolism links germline stem cells and longevity in *C. elegans*. *Science* 322:957–60
151. Lapiere L, Gelino S, Meléndez A, Hansen M. 2011. Autophagy and lipid metabolism coordinately modulate life span in germline-less *C. elegans*. *Curr. Biol.* 21:1507–14
152. Brown-Borg HM, Borg KE, Meliska CJ, Bartke A. 1996. Dwarf mice and the ageing process. *Nature* 384:33
153. Berryman DE, List EO, Coschigano KT, Behar K, Kim JK, Kopchick JJ. 2004. Comparing adiposity profiles in three mouse models with altered GH signaling. *Growth Horm. IGF Res.* 14:309–18
154. Masternak M, Bartke A, Wang F, Spong A, Gesing A, et al. 2012. Metabolic effects of intra-abdominal fat in GHRKO mice. *Aging Cell* 11:73–81
155. Coppé J, Desprez P, Krtolica A, Campisi J. 2010. The senescence-associated secretory phenotype: the dark side of tumor suppression. *Annu. Rev. Pathol.* 5:99–118
156. Krtolica A, Parrinello S, Lockett S, Desprez P, Campisi J. 2001. Senescent fibroblasts promote epithelial cell growth and tumorigenesis: a link between cancer and aging. *Proc. Natl. Acad. Sci. USA* 98:12072–77
157. Campisi J. 2005. Senescent cells, tumor suppression, and organismal aging: good citizens, bad neighbors. *Cell* 120:513–22
158. Berdasco M, Esteller M. 2012. Hot topics in epigenetic mechanisms of aging: 2011. *Aging Cell* 11:181–86
159. Heyn H, Li N, Ferreira HJ, Moran S, Pisano DG, et al. 2012. Distinct DNA methylomes of newborns and centenarians. *Proc. Natl. Acad. Sci. USA* 109:10522–27
160. Greer E, Maures T, Hauswirth A, Green E, Leeman D, et al. 2010. Members of the H3K4 trimethylation complex regulate lifespan in a germline-dependent manner in *C. elegans*. *Nature* 466:383–87
161. Greer EL, Maures TJ, Ucar D, Hauswirth AG, Mancini E, et al. 2011. Transgenerational epigenetic inheritance of longevity in *Caenorhabditis elegans*. *Nature* 479:365–71
162. Ozanne S, Constância M. 2007. Mechanisms of disease: the developmental origins of disease and the role of the epigenotype. *Nat. Clin. Pract. Endocrinol. Metab.* 3:539–46
163. Blagosklonny MV. 2006. Aging and immortality: quasi-programmed senescence and its pharmacologic inhibition. *Cell Cycle* 5:2087–102
164. Blagosklonny MV. 2007. Paradoxes of aging. *Cell Cycle* 6:2997–3003
165. Gems D, de la Guardia Y. 2012. Alternative perspectives on aging in *Caenorhabditis elegans*: reactive oxygen species or hyperfunction? *Antioxid. Redox Signal.* In press. doi: 10.1089/ars.2012.4840

166. Kimble J, Sharrock WJ. 1983. Tissue-specific synthesis of yolk proteins in *Caenorhabditis elegans*. *Dev. Biol.* 96:189–96
167. Herndon L, Schmeissner P, Dudaronek J, Brown P, Listner K, et al. 2002. Stochastic and genetic factors influence tissue-specific decline in ageing *C. elegans*. *Nature* 419:808–14
168. McGee MD, Weber D, Day N, Vitelli C, Crippen D, et al. 2011. Loss of intestinal nuclei and intestinal integrity in aging *C. elegans*. *Aging Cell* 10:699–710
169. Salomon RN, Jackson FR. 2008. Tumors of testis and midgut in aging flies. *Fly* 2:265–68
170. Biteau B, Hochmuth C, Jasper H. 2008. JNK activity in somatic stem cells causes loss of tissue homeostasis in the aging *Drosophila* gut. *Cell Stem Cell* 3:442–55
171. Gems D. 2011. Tragedy and delight: the ethics of decelerated ageing. *Philos. Trans. R. Soc. Lond. Ser. B* 366:108–12
172. Koga H, Kaushik S, Cuervo AM. 2011. Protein homeostasis and aging: the importance of exquisite quality control. *Ageing Res. Rev.* 10:205–15
173. Hiplkiss AR. 2007. On why decreasing protein synthesis can increase lifespan. *Mech. Ageing Dev.* 128:412–14
174. Kaerberlein M, Kennedy BK. 2011. Hot topics in aging research: protein translation and TOR signaling, 2010. *Aging Cell* 10:185–90
175. Haldane JBS. 1941. *New Paths in Genetics*. London: Allen & Unwin
176. Medawar PB. 1952. *An Unsolved Problem of Biology*. London: HK Lewis
177. Williams GC. 1957. Pleiotropy, natural selection and the evolution of senescence. *Evolution* 11:398–411
178. Kirkwood TB. 2005. Understanding the odd science of aging. *Cell* 120:437–47
179. Partridge L, Gems D, Withers DJ. 2005. Sex and death: What is the connection? *Cell* 120:461–72
180. Flatt T. 2010. Survival costs of reproduction in *Drosophila*. *Exp. Gerontol.* 46:369–75
181. Blagosklonny MV. 2010. Why the disposable soma theory cannot explain why women live longer and why we age. *Aging* 2:884–87
182. Blagosklonny MV. 2010. Revisiting the antagonistic pleiotropy theory of aging: TOR-driven program and quasi-program. *Cell Cycle* 9:3151–56
183. Masoro E, Austad S. 1996. The evolution of the antiaging action of dietary restriction: a hypothesis. *J. Gerontol. A* 51:B387–91
184. Robida-Stubbs S, Glover-Cutter K, Lamming D, Mizunuma M, Narasimhan S, et al. 2012. TOR signaling and rapamycin influence longevity by regulating SKN-1/Nrf and DAF-16/FoxO. *Cell Metab.* 15:713–24
185. Pacholec M, Bleasdale JE, Chrnyk B, Cunningham D, Flynn D, et al. 2010. SRT1720, SRT2183, SRT1460, and resveratrol are not direct activators of SIRT1. *J. Biol. Chem.* 285:8340–51
186. Dai H, Kustigian L, Carney D, Case A, Considine T, et al. 2010. SIRT1 activation by small molecules: kinetic and biophysical evidence for direct interaction of enzyme and activator. *J. Biol. Chem.* 285:32695–703
187. Ledford H. 2010. Ageing: much ado about ageing. *Nature* 464:480–81
188. Burnett C, Valentini S, Cabreiro F, Goss M, Somogyvári M, et al. 2011. Absence of effects of Sir2 over-expression on lifespan in *C. elegans* and *Drosophila*. *Nature* 477:482–85
189. Kanfi Y, Naiman S, Amir G, Peshti V, Zinman G, et al. 2012. The sirtuin SIRT6 regulates lifespan in male mice. *Nature* 483:218–21
190. Klass MR, Hirsh DI. 1976. Nonaging developmental variant of *C. elegans*. *Nature* 260:523–25
191. Riddle DL. 1988. The dauer larva. In *The Nematode Caenorhabditis elegans*, ed. WB Wood, pp. 393–412. Plainview, NY: Cold Spring Harb. Lab.
192. An JH, Blackwell TK. 2003. SKN-1 links *C. elegans* mesendodermal specification to a conserved oxidative stress response. *Genes Dev.* 17:1882–93
193. Kubagawa H, Watts J, Corrigan C, Edmonds J, Sztul E, et al. 2006. Oocyte signals derived from polyunsaturated fatty acids control sperm recruitment in vivo. *Nat. Cell Biol.* 8:1143–48
194. Maduro MF. 2009. Structure and evolution of the *C. elegans* embryonic endomesoderm network. *Biochim. Biophys. Acta* 1789:250–60
195. Hochmuth C, Biteau B, Bohmann D, Jasper H. 2011. Redox regulation by Keap1 and Nrf2 controls intestinal stem cell proliferation in *Drosophila*. *Cell Stem Cell* 8:188–99

196. Tothova Z, Gilliland D. 2007. FoxO transcription factors and stem cell homeostasis: insights from the hematopoietic system. *Cell Stem Cell* 1:140–52
197. Coschigano KT, Clemmons D, Bellush LL, Kopchick JJ. 2000. Assessment of growth parameters and life span of GHR/BP gene-disrupted mice. *Endocrinology* 141:2608–13
198. Gems D, Partridge L. 2001. Insulin/IGF signalling and ageing: seeing the bigger picture. *Curr. Opin. Genet. Dev.* 11:287–92
199. Partridge L, Gems D. 2002. Mechanisms of ageing: public or private? *Nat. Rev. Genet.* 3:165–75
200. McCulloch D, Riddle DL, Gems D. 2002. Increased longevity is not associated with reduced body size in the nematode *Caenorhabditis elegans*. *Exp. Gerontol.* 38:129–36
201. Tu M-P, Epstein D, Tatar M. 2002. The demography of slow aging in male and female *Drosophila* mutant for the insulin-receptor substrate homolog *chico*. *Aging Cell* 1:75–80