Signaling Networks Determining Life Span

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Abstract

The health of an organism is orchestrated by a multitude of molecular and biochemical networks responsible for ensuring homeostasis within cells and tissues. However, upon aging, a progressive failure in the maintenance of this homeostatic balance occurs in response to a variety of endogenous and environmental stresses, allowing the accumulation of damage, the physiological decline of individual tissues, and susceptibility to diseases. What are the molecular and cellular signaling events that control the aging process and how can this knowledge help design therapeutic strategies to combat age-associated diseases? Here we provide a comprehensive overview of the evolutionarily conserved biological processes that alter the rate of aging and discuss their link to disease prevention and the extension of healthy life span.

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INTRODUCTION

Aging has long been considered a default state occurring after an animal fulfills the requirements of natural selection, which is mostly to ensure the continuity of its species through reproduction (1). Efforts in geroscience research have proven that aging is more than just a passive process and can be regulated in well-defined settings and isolated genetic backgrounds using model organisms (2). The complexity of this process arises from the observation that time does not affect all the cells within the body homogeneously, but selectively or stochastically incapacitates a subset of biological processes that differ among individuals, thus causing them to age differently. The large variability in the pathologies of aging accounts for the heterogeneity of the causes of death within the aged human population (3). Indeed, diseases of aging include a wide range of cardiovascular diseases, neurodegenerative diseases, infectious diseases, and cancers.

A fundamental challenge in the aging field is to isolate the source of the heterogeneity underlying this process, and pinpoint whether it originates from an equal variety of age-associated factors. Despite the complex nature of the senescence process, simple genetic and environmental alterations can result in an increase in healthy life span, or health span, in laboratory model organisms. More than two decades of multidisciplinary research using a variety of genetic, genomic, biochemical, and behavioral approaches has provided a plethora of molecular and cellular factors

that may directly cause aging. The findings survey signaling elements involved in DNA damage, stem cell maintenance, proteostasis, energy and oxidative metabolism, and environmental regulation. In this review, we investigate the role of each of these signaling networks in their contribution to aging and integrate research from multiple model organisms, including the budding yeast *Saccharomyces cerevisiae*, the nematode *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster*, and the laboratory mouse. We discuss the conservation of these environmental and genetic players in mammalian models and highlight the role of these components in their ability to promote healthy life span and detail the potential cross talk between these many pathways in the regulation of aging.

NUTRIENT-SENSING PATHWAYS

Insulin and IGF-1 Signaling

Robust genetic alterations impacting aging were discovered in *C. elegans* as mutations of *age-1*; phosphatidylinositol 3-kinase (PI3K), a downstream effector of insulin/insulin-like signaling; and *daf-2*, the insulin/insulin-like receptor (4, 5). The identification of these long-lived mutant animals uncovered insulin/insulin-like signaling (IIS) as the metabolic pathway first discovered to modify the aging process (**Figure 1**). In *C. elegans*, increased life span from reduced IIS requires phosphorylation and nuclear translocation of the forkhead box protein O (FOXO) transcription factor DAF-16, which regulates protective genes involved in stress response, antimicrobial activity, and small heat-shock protein (HSP) chaperones (6).

Analogously, deletion of components of the IIS in flies and mice increases longevity. In mice, decreased IIS is observed in mutants of the somatotropic axis, essentially consisting of growth hormone (GH) and insulin-like growth factor (IGF-1). IGF-1 is predominantly produced by the liver in response to circulating GH levels and elicits insulin-like signaling through IGF-1 receptor (IGF-1R) activation in many tissues. Reduction of circulating IGF-1 has been positively correlated with increased longevity in inbred mouse strains (7) but is also linked to reduced body size or even dwarfism. Spontaneous mutations that result in GH deficiency produced dwarf animals with exceptional longevity. The dwarf Ames ($Prop1^{df}$), Snell (Pit^{dw}), GH-releasing hormone (GHRH) defective "little," and GH receptor/binding protein (GHR/BP) knockout mice display decreased IGF-1 and fasted insulin levels and live between 30 to 50% longer than wild-type animals (8, 9).

Although decreased GH secretion substantially extends life span, a debate exists on the actual health span of these animals. Ames and Snell dwarf mice exhibit deficiencies in anterior pituitary function, resulting in additional hormonal changes that negatively affect fertility, metabolic fitness, adiposity, glucose tolerance, and insulin secretion despite considerable life extension (10, 11). In humans, hypothyroidism is also characterized by diminution of the acute insulin response, resulting in impaired glucose tolerance (12), but the causal effects of this condition on human longevity are unclear. Long-lived Ames dwarf mice and GHR/BP knockout mice display a lower incidence and delayed onset of certain cancers and increased insulin sensitivity, and GHR/BP knockout animals also show improved cognitive function at old age (13).

Other long-lived mice of the IIS pathway that do not display defective pituitary function include IGF-1R heterozygous knockout ($Igf\text{-}1R^{+/-}$) (14), insulin receptor substrate 1 knockout ($Irs1^{-/-}$) (15), whole-body and brain-specific IRS2 knockout (16), fat-specific insulin receptor knockout (FIRKO) (17), hypomorphic PI3K ($p110\alpha^{D933A/WT}$) (18), $Pten^{tg}$ (19), and AKT heterozygous knockout ($Akt^{+/-}$) (20) mice. In conflicting reports, the IRS2 knockout animals were found to be short lived (15) and $Igf\text{-}1R^{+/-}$ mice had a life span similar to wild-type animals, which could be due to differences in husbandry and genetic backgrounds. Potential

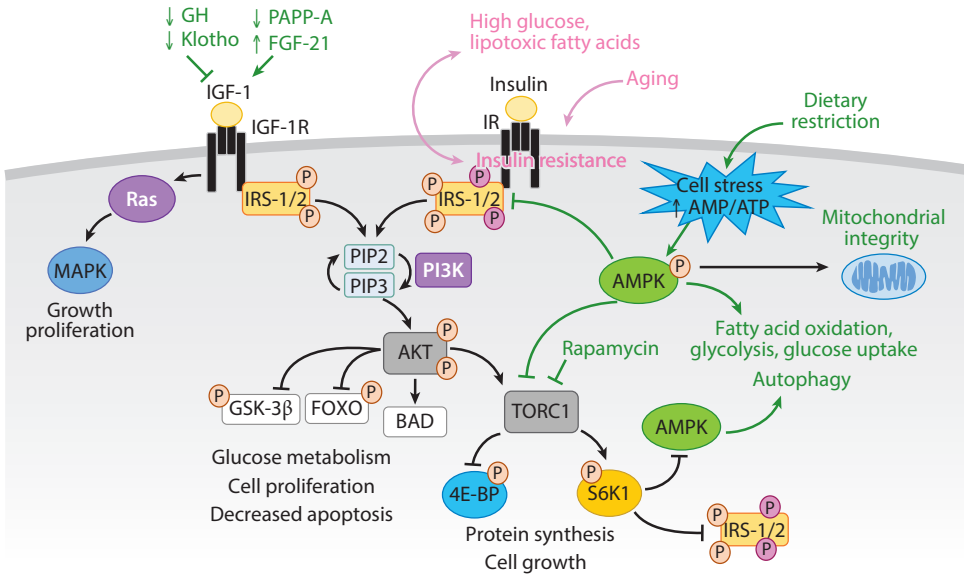

Figure 1

Nutrient-uptake pathways and aging. Stimulation of insulin receptor (IR) and insulin-like growth factor receptor (IGF-1R) results in phosphorylation of insulin receptor substrates (IRS) with phosphorylation of phosphatidylinositol 3-kinase (PI3K) and activation of the p110 catalytic subunit, resulting in the formation of phosphatidylinositol 3,4 phosphate (PIP2) and phosphatidylinositol 3,4,5 phosphate (PIP3). PIP3 then activates protein kinase B (AKT). AKT inhibits apoptosis by inactivating BCL-2 antagonist of cell death (BAD), induces glucose metabolism through glycogen synthase kinase 3β (GSK-3β), suppresses a wide range of cellular responses via the forkhead box protein O (FOXO) transcription factor and stimulates protein synthesis by activating the mammalian target of rapamycin complex 1 (TORC1). TORC1 activates the ribosomal S6 kinase 1 (S6K1) and eukaryotic initiation factor 4E binding protein (4E-BP), leading to protein synthesis and cell growth. S6K1 also negatively regulates IRS proteins to shut down nutrient import upon overload. Signaling through the IGF-1R also activates the Ras/MAPK (rat sarcoma/mitogen-activated protein kinase) signaling pathway, which results in cell proliferation. In the absence of cellular nutrients and in low energetic conditions such as dietary restriction, AMP-activated kinase (AMPK) levels increase and inhibit protein synthesis through TORC1, shutting down anabolic processes and maintaining cellular energy by promoting mitochondrial respiration. Aging is associated with impaired insulin signaling through serine phosphorylation of IRS proteins, leading to impaired glucose transport and systemic damage from fat metabolism (*pink arrows*). Positive modulators of longevity comprise reduced bioavailability of IGF-1, activation of AMPK, blockade of TORC1, and growth through increased autophagy and preserved mitochondrial integrity (*green arrows*). Abbreviations: AMP, adenosine monophosphate; ATP, adenosine triphosphate; FGF-21, fibroblast growth factor-21; GH, growth hormone; PAPP-A, pregnancy-associated plasma protein-A.

causes for these contradictory findings are discussed elsewhere (21). Globally, the impact on longevity of mutations affecting IGF-1 levels and downstream IGF-1 signaling events is smaller than disrupting GH biosynthesis or actions. Consistent with improved life span, most of these models display various degrees of enhanced health span, including improved metabolic function and decreased adiposity as observed in the FIRKO, $p110\alpha^{D933A/WT}$, $Pten^{tg}$, and $Akt^{+/-}$ mice. Disruption of *Irs1* and *Irs2* leads to insulin or IGF-1 resistance without inducing hyperglycemia or diabetes in mice. Of note, these animals develop compensatory pancreatic β-cell expansion and enhanced insulin secretion to cope with the resistance and thus present various aspects of

delayed aging, such as improved immune and bone function for the IRS2 knockout and improved activity and substrate utilization in the brain-specific IRS2 null animals (16).

An attractive avenue to manipulate IGF-1 without interfering with downstream insulin signaling is to directly reduce IGF-1 bioavailability in circulation. Pregnancy-associated plasma protein-A (PAPP-A) induces the proteolysis of inhibitory IGF binding protein-4 (IGFBP-4), an IGF-1 binding partner that sequesters IGF-1 and thus enhances IGF-1 action. Strikingly, PAPP-A knockout mice live 20 to 40% longer than wild-type animals and maintain a healthy immune system (22), without impacting cancer incidence. A second inhibitor of IGF-1 signaling is fibroblast growth factor-21 (FGF-21), a liver hormone that is secreted during fasting and modulates hepatic fatty acid oxidation and ketogenesis, thus increasing insulin sensitivity while blocking somatic growth. Remarkably, FGF-21 overexpression increased mouse longevity by 36% in combined genders and improved metabolic health compared with controls (23). PAPP-A and FGF-21 overexpression both negatively impact bone mass and body growth, a probable consequence of inhibiting IGF-1 during development. A third approach to reduce IGF-1 bioavailability is the overexpression of the Klotho protein (or α-Klotho), a transmembrane protein that may undergo cleavage to release a bioactive hormonal signal that inhibits IGF-1. Although Klotho-overexpressing mice live extremely long (increased longevity of 20–31% in males and 19% in females), they present some of the adverse effects of insulin-signaling inhibition, such as insulin resistance and hyperinsulinemia (24). However, whether Klotho's only role is to inhibit insulin signaling remains unclear, as it has been proposed to interact with Wnt signaling and multiple membrane-bound receptors.

These mouse models of constitutively decreased IIS may point toward a beneficial role of decreasing GH or IGF-1 levels to extend life span. However, the therapeutic potential of this pathway to treat age-associated diseases remains questionable, as GH and IGF-1 naturally decline with age, inducing a somatopause that may account for age-associated decrease in muscle mass, increased adiposity, and reduced sexual steroid levels (25). Thus, further evaluation of the timing requirements of reducing GH and IGF-1 is necessary.

Target of Rapamycin Signaling

The target of rapamycin (TOR) is a major cellular nutrient-sensing pathway that regulates cell growth and integrates insulin signaling and cell stress signals (26). TOR is a highly conserved serine/threonine kinase that is part of two structurally and functionally distinct complexes, TORC1 and TORC2, and is inhibited by the immunosuppressive drug rapamycin. In metazoans, rapamycin inhibits TORC1, which controls growth-related processes such as ribosome biogenesis, protein synthesis, transcription, nutrient uptake, and autophagy in response to nutrients, growth factors, and cellular energy status (**Figure 1**). TORC2 is not directly inhibited by rapamycin, although long-term rapamycin treatment can inhibit this complex indirectly in certain cell types (27).

The role of TOR in regulating the rate of aging is well documented. Genetic or pharmacologic inhibition of TOR through rapamycin treatment extends life span in yeast, worms, flies, and mice (28–32). Prolonged rapamycin treatment delays cancer incidence in aged mice and extends life span (29). The method by which rapamycin regulates the rate of aging is complex. Short-term treatment results in immunodeficiency and favors glucose intolerance and insulin resistance (27), whereas prolonged treatment results in improved metabolic profiles, increased oxygen consumption and ketogenesis, and markedly enhanced insulin sensitivity (33).

Insight into rapamycin's mode of action on longevity came from findings in *D. melanogaster*. These findings showed rapamycin's effect on aging was independent of decreased IIS or dietary restriction (DR); instead, it acted on the TORC1 branch of TOR signaling to modulate autophagy and protein synthesis (28). TORC1 regulates translation and growth through phosphorylation of

two key downstream effectors, the ribosomal protein S6 kinase 1 (S6K1) and the eukaryotic translation initiation factor 4E-binding protein (4E-BP). The contribution of these substrates has been investigated in various model organisms. The effects of S6K1 on life span have been observed across taxa (30, 31, 34, 35). Robust genetic inhibition of S6K1 leads to increased longevity coupled with improved glucose tolerance and insulin sensitivity at mid-age in mice (35). S6K1 plays a prominent role in translation initiation (36) and also regulates cellular energy levels through the activity of the AMP-activated kinase (AMPK). Interestingly, S6K1 can phosphorylate IRS1 and IRS2 to dampen insulin signaling upon nutrient overload, therefore establishing a link between mTOR and IIS in the control of longevity (31, 35). Surprisingly, protein synthesis is not affected in S6K1 null muscle cells, but deletion of S6K1 elicits gene expression patterns similar to pharmacological activation of AMPK in liver, skeletal muscle, and white adipose tissue; this finding suggests that the S6K1 effects on life span may be due to increased AMPK activity, rather than forestalling translation (35).

Another downstream substrate of TORC1 is the eukaryotic translation initiation factor 4E-binding protein (4E-BP), which represses cap-dependent mRNA translation initiation by sequestering the eukaryotic translation initiation factor 4E (eIF4E). The fly equivalent of 4E-BP (d4E-BP) is upregulated upon DR and mediates DR-dependent changes in mitochondrial activity and life span extension (31). Specific activation of d4E-BP in muscle suppresses age-related tissue degeneration and enhances life span in the fly (37). Similarly, enhanced 4E-BP activity in mouse skeletal muscle protects mice against age- and diet-induced insulin resistance and metabolic decline. Specifically, these animals present increased translation of PPARγ coactivator-1α (PGC-1α) and increased mitochondrial respiration (38). Paradoxically, whole-body disruption of 4E-BP has been reported to improve metabolic health in the same genetic background (39), a difference that might be explained by the tissue specificity of 4E-BP activity in mammals. Indeed, adipose-activated 4E-BP animals display insulin resistance, as opposed to skeletal muscle–activated 4E-BP animals (38), thus supporting the theories that 4E-BP affects metabolism differently in various tissues and that the role of 4E-BP in metabolic health upon TOR inhibition might be primarily achieved in muscle cells through improved translation of the mitochondrial machinery.

Independent repression of S6K1 and activation of 4E-BP in skeletal muscle both lead to enhanced macroautophagy (autophagy), promoting skeletal muscle atrophy (38, 40). Autophagy is a process of cellular degradation in which cellular constituents are engulfed in membrane-bound autophagosomes then targeted to lysosomes for proteolytic breakdown. Enhanced autophagy through overexpression of *Atg5*, a critical autophagy-related gene involved in autophagosome formation, increases median life span by 17% coupled to improved metabolic health in mice (41). The tissue-specific knockout of ATG (autophagy-related) genes reveals a premature onset of age-related symptoms in model organisms and highlights the role of this pathway in quality control of organelles and proteins in nondividing cells (42).

AMP-Activated Protein Kinase

A crucial nutrient sensor is AMPK, which becomes activated when cellular energy levels are low and inhibits anabolic processes such as protein synthesis through mTOR blockade (43). AMPK activation is therefore beneficial for longevity in worms (44, 45) and is positively correlated with improved metabolic health in mice (46), even though direct evidence for AMPK's role in mammalian aging is lacking at the moment. How AMPK may impact longevity is complex, as this kinase is a central player in many signaling pathways (**Figure 1**). As described in the previous section, AMPK modulates the output of TORC1 inhibition. Mutation of *C. elegans* AMPK (*aak-2*) shortens the increased life span of S6K1 mutants (*rsks-1*) (35), suggesting that *aak-2* might be

required for the longevity of *rsks-1* mutants, even though more evidence is necessary to prove this point. Additionally, AMPK may also act upstream of TOR, as evidence suggests that it can phosphorylate Raptor to directly regulate TORC1 and tuberous sclerosis 2 (TSC2), an inhibitor of TORC1 (43, 47). Therefore, both TOR and AMPK may act as cellular checkpoints of energy levels to regulate metabolism and growth and be part of a complex feedback network that remains poorly understood.

Mechanistically, under conditions of nutrient scarcity, activation of AMPK rapidly turns on glycolysis and fatty acid oxidation to restore ATP levels and implements a long-term metabolic switch by increasing mitochondrial content and the use of mitochondrial substrates as an energy source (48). This switch is achieved by phosphorylation of PGC-1α (49), a master regulator of mitochondrial biogenesis and energy metabolism (50), under conditions of low energy. Increasing PGC-1α content in mouse skeletal muscle preserves oxidative phosphorylation (OxPhos), preventing muscle wasting and improving metabolic fitness by inhibiting insulin resistance and fat accumulation with age (51). The safeguarding of mitochondrial fitness is gaining more attention in aging research as many studies indicate that perturbations of mitochondrial biogenesis, homeostasis, and dynamics are central to the maintenance of healthy cellular and whole-body aging (see section on Mitochondrial Health).

Finally, another emerging player in longevity functioning downstream of AMPK is the cAMP-responsive element binding protein (CREB) signaling pathway and its associated cAMP-regulated transcriptional coactivators (CRTCs) (52). CRTCs are direct AMPK targets and are recruited to modulate the longevity output of AMPK activation in *C. elegans* (45). However, the link between AMPK and CRTCs in mammalian aging remains to be tested. Mammalian systems have evolved three members of the CRTC family, and their respective roles in longevity are mostly unknown (52). We discovered that CRTC1, the most predominant CRTC in neuronal tissues, regulates CREB signaling and transcriptional activation of calcitonin gene-related peptide (CGRP), a neuropeptide secreted from nociceptive sensory neurons that inhibits insulin secretion, and negatively impacts mouse life span and metabolic flexibility (53). As these results greatly suggest that the role of CRTC in life span is conserved, more insight is required to understand how these proteins may function in regulating metabolic health and life span as well as their connection with AMPK.

DIETARY RESTRICTION

To date, DR is the most robust intervention to increase life span in model organisms including rodents and primates and to delay the emergence of age-related diseases (54). Yet the molecular mediators underlying the extended longevity of DR animals remain elusive, partially because of the use of different DR regimens, ranging from moderate to severe DR, that affect aging differently in model organisms (54). Although moderate DR (8% reduction) weakly influenced mouse life span, a more severe DR regimen (30% reduction) increased median life span by up to 50% and drastically delayed the onset of chronic diseases (55).

At the molecular level, the mechanisms of DR appear embedded in the response to reduce energy availability, resulting in the emergence of an altered metabolic state that promotes health and longevity. The beneficial effects of DR may represent an adaptive evolutionary response designed to maximize the chances of reproduction in periods of low food abundance, awaiting future exposure to nutrients. In line with this theory, reprogramming of mitochondrial energy metabolism and inhibition of anabolic processes appear as strong candidates to mediate the beneficial effects of DR. This theory is consistent with reduced TOR signaling (31), decreased IIS (54), as well as activation of AMPK (44) that occur while protein synthesis is maintained to preserve somatic tissue homeostasis (56). Common features of these DR-dependent mechanisms

include energy-saving processes, such as the maintenance of mitochondrial function to ensure the integrity of existing cellular components; increasing oxidant scavenging, such as catalase activity (57); and the induction of autophagy to recycle macromolecules and organelles (58). In *C. elegans*, the longevity response to DR is specifically controlled by the *pha-4* transcription factor [ortholog of the mammalian forkhead box protein A (FOXA)], which regulates the induction of various autophagy genes required for life span extension (58, 59).

One common feature of many DR regimens across species is the restriction of the sulfur amino acids methionine and cysteine, which confer overlapping functional benefits in various organisms (60, 61). In a mouse model of DR-mediated stress resistance, restriction of sulfur amino acids was found to elicit production of hydrogen sulfide through increased activity of the transsulfuration pathway (TSP) and to protect the animals against hepatic ischemia reperfusion injury (62). The TSP controls the conversion of methionine into cysteine, and activation of this pathway is required for DR-mediated life span extension in flies (63). As multiple DR regimens elicit different molecular responses (54), it is unclear whether other components dependent on the TSP are required for DR-induced life span extension.

MITOCHONDRIAL HEALTH

Mitochondrial Integrity and Biogenesis

Mitochondria have represented a cornerstone in aging research for the past 50 years. Within the eukaryotic cell, these essential organelles form an intricate network that is dynamically remodeled in response to both internal and external cues (**Figure 2**). Their most prominent role is the generation of energy in the form of ATP through OxPhos, exemplifying them as cellular powerhouses. Mitochondria control a plethora of pathways involved in cell signaling and metabolic homeostasis. Concomitant with the progressive loss of cellular integrity experienced during the aging process, mitochondrial biogenesis and function undergo a major impairment resulting in extensive changes in mitochondrial morphology and architecture (64). Mitochondrial homeostasis is controlled by the balance between mitochondrial biogenesis and disposal through mitophagy, the lysosomal degradation of dysfunctional mitochondria. Reduced biogenesis and impaired removal of damaged mitochondria have therefore been extensively linked to the aging process (65). Age-related changes in mitochondrial integrity in various organisms have often been associated with a decrease in mitochondrial number, reflecting not only defects in mitochondrial biogenesis but also higher rates of autophagic removal (66). On the contrary, stimulation of mitochondrial biogenesis through exercise and endogenous signaling molecules improve health span (67). Key molecular players in these processes have been identified. Physical activity and low caloric diets improved mitochondrial biogenesis and function through activation of sirtuin 1 (SIRT1) and its downstream target, the transcriptional coactivator PGC-1α (68, 69). Both PGC-1α and SIRT1 directly modulated life span by regulating mitochondrial metabolism (70, 71). Overexpression of PGC-1α in mouse skeletal muscle improved OxPhos, thereby protecting animals from metabolic decline during aging (51). Taken together, the beneficial effects of DR and exercise are likely mediated by increased mitochondrial activities, and autophagy induction may also contribute to these effects (65).

Natural aging comes with a reduced efficiency of mitochondrial energy metabolism that can be attributed to several mechanisms. A decline in mitochondrial biogenesis was found to be a consequence of telomere attrition in telomerase-deficient mice that resulted in the p53-dependent repression of PGC-1 isoforms (72). Remarkably, reactivation of telomerase in wild-type mice reverted this effect, suggesting that telomere dysfunction directly impacts aging

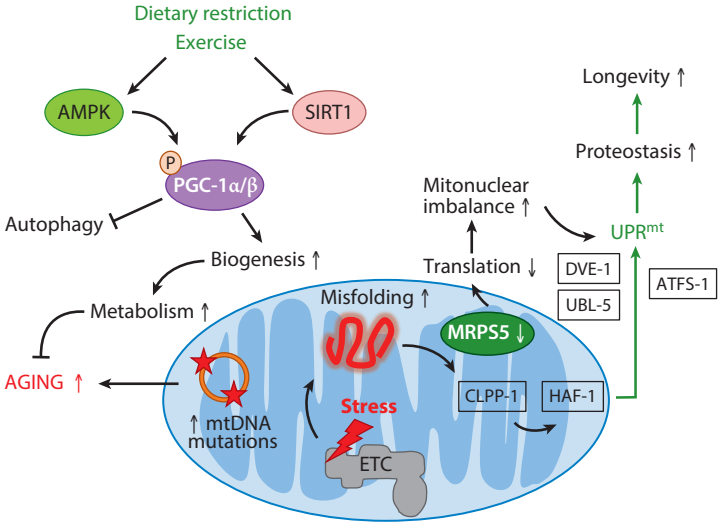

Figure 2

Mitochondrial health during aging. Mitochondrial biogenesis in response to environmental cues such as dietary restriction or exercise is regulated through activation of sirtuin 1 (SIRT1) and its target PPARγ coactivator-1α (PGC-1α). In addition, AMP-activated kinase (AMPK) can stimulate PGC-1α activity through direct phosphorylation and thereby repress autophagy. In turn, increased mitochondrial biogenesis leads to stimulation of mitochondrial metabolism, which suppresses aging. The aging process is associated with the accumulation of mutations in mitochondrial DNA (mtDNA). Perturbation of mitochondrial activities can result in mitochondrial proteotoxic stress through protein misfolding, which triggers activation of the mitochondrial unfolded protein response (UPR^{mt}), a conserved signaling pathway that promotes mitochondrial health. In addition, impaired mitochondrial translation through reduction in MRPS5 results in mitonuclear protein imbalance that activates the UPR^{mt}. The UPR^{mt} in *Caenorhabditis elegans* requires the mitochondrial matrix-localized protease CLPP-1, the basic leucine zipper protein (bZIP) transcription factor ATFS-1, the homeobox transcription factor DVE-1, and the ubiquitin-like protein UBL-5. These factors stimulate gene expression of mitochondrial quality control factors that promote mitochondrial proteostasis and positively regulate longevity. Abbreviations: ATFS-1, activating transcription factor associated with stress; DVE-1, defective proVEntriculus; ETC, electron transport chain; HAF-1, half transporter-1 (P-glycoprotein-related); MRPS5, mitochondrial ribosomal protein S5.

through mitochondrial activities (detailed in the section on Telomere and Cellular Senescence, below). A direct impairment of mitochondrial bioenergetics during aging is also due to other mechanisms involving defective mitochondrial membrane dynamics, increased mutational load in mitochondrial DNA (mtDNA), deficits in the assembly of the respiratory chain, mitochondrial phospholipid metabolism, and oxidative stress (64).

Mitochondrial DNA

Mitochondrial function requires the coordinated expression of both nuclear and mitochondrial genomes. The mitochondrial genome harbors genetic information for 13 polypeptides and 22 tRNAs required for mitochondrial gene expression and synthesis from mitochondrial ribosomes. A large body of evidence supports a direct role for the accumulation of both mtDNA mutations and deletions as key drivers of aging in various model organisms. The first indication that mtDNA damage might cause aging and age-associated disorders came from the observation that mtDNA mutations underlie multisystem disorders that resemble human aging (73). In fact, mutations in

mtDNA accumulate with human aging and increase at a higher rate than mutations in nuclear DNA across various tissues (74). To verify whether mtDNA mutations directly cause mitochondrial dysfunction and promote aging in mammals, mouse models with increased mtDNA mutation rates have been engineered. Homozygous knockin mice expressing a proofreading-deficient version of the mitochondrial DNA polymerase γ (PolG), also known as mtDNA mutator mice, display a 2,500-fold higher mutation frequency than wild-type animals. These mice exhibit extensive mitochondrial dysfunction, develop several early onset age-associated pathologies, and have a median life span reduced to only 48–60 weeks (75, 76). Remarkably, defective mitochondrial respiration in these mice is not accompanied by enhanced reactive oxygen species (ROS) production or oxidative damages. However, the causality of mtDNA mutations in regular aging was challenged by recent reports demonstrating that the amount of mtDNA mutations in homozygous mutator mice is several orders of magnitude higher than normal old mice. Specifically, heterozygous PolG mice, which carry 30-fold more mitochondrial mutations than normally aged, very old mice have a normal life span and normal health (76). In addition, mtDNA deletor mice, which carry a mutation in the mtDNA helicase Twinkle, harbor an organism-wide increase in mtDNA deletions. Surprisingly, these mice have a normal life span and show no signs of premature aging despite exhibiting progressive respiratory chain dysfunction and late-onset mitochondrial myopathy (77). Currently, how various alterations in mtDNA contribute differentially to the aging process remains unresolved.

Another puzzling aspect in the connection of mitochondria to aging is the multiplicity of mitochondrial genomes and the varying degree of mutation load upon each genome (heteroplasmy). The low degree of transmission of heteroplasmic mtDNA variants led to the mitochondrial bottleneck hypothesis, which postulates that only a limited number of selected mtDNA molecules in the germline are maternally transmitted. This hypothesis further implies that de novo mtDNA mutations acquired early in life may be clonally propagated and cause mosaic respiratory defects in aging tissues (78). These findings indicate the existence of a biochemical threshold for critically high mtDNA mutations that ultimately results in mitochondria dysfunction and premature aging symptoms. Along this line, recent work has demonstrated that germline-transmitted mtDNA mutations can induce aging phenotypes in offspring and aggravate aging induced by preexisting somatic mitochondrial mutations (79). A recent experimental approach provides a molecular explanation by which mtDNA mutagenesis contributes to premature aging in mtDNA mutator mice. In this model, the accumulation of mtDNA point mutations might cause instability of respiratory chain complexes owing to the amino acid substitutions in newly synthesized mitochondrial-encoded electron transport chain (ETC) subunits (80). In support of this idea, loss-of-function mutations in the *Ndufs4* subunit of ETC complex I recapitulated premature aging phenotypes and age-associated encephalopathy, likely due to impaired assembly or stability of mitochondrial complex 1 (81). Of note, the administration of the mTOR inhibitor, rapamycin, delayed the onset of aging phenotypes in *Ndufs4*-deficient mice, suggesting that blocking mTOR signaling may provide a therapeutic strategy for the treatment of mitochondrial diseases (82).

In the early 2000s, it came as a surprise when several labs reported the discovery that the RNAi-mediated reduction of mitochondrial ETC subunits could substantially extend life span in several model organisms (83, 84). Strikingly, this ETC pathway of longevity has strict temporal requirements during larval development in worms, suggesting that mitochondrial dysfunction during a critical time window during embryogenesis sets the rate of aging for the entire life of the organism (83). This phenomenon further suggests the perpetuation of a longevity signal from the mitochondria that regulates life span throughout life. Current work is underway to investigate the biochemical nature of this signal, termed mitokine (85). The extended life span of worms with reduced ETC activity, however, comes with severe problems: animals are smaller, have impaired movement, and exhibit reduced fecundity. Follow-up work on this pathway further revealed the existence of key

tissues, mainly nerve cells, that determine the rate of aging downstream of mitochondrial dysfunction in both worms and flies (85, 86). Mechanistically, the depletion of individual subunits may lead to a stoichiometric imbalance in ETC complexes, which are built of proteins encoded by both nuclear and mitochondrial genomes, thereby imposing a proteostatic stress on the mitochondrial environment. These stress conditions were found to lead to the activation of the mitochondrial unfolded protein response (UPR^{mt}) that senses the accumulation of damaged or misfolded proteins in the organelle and responds with a signaling cascade that culminates in the transcriptional activation of mitochondrial chaperone genes in the nucleus that are subsequently imported into the mitochondria again to defeat the proteotoxic stress (87) (**Figure 2**). General defense mechanisms that protect mitochondria from various damages are discussed in the following section.

Mitochondrial Quality Control and Homeostasis

An extensive arsenal of surveillance strategies has evolved to ensure mitochondrial quality control in the defense against potentially harmful insults that are ultimately linked to aging and age-associated disease. These elaborate mechanisms protect the mitochondria from continuous damage and constantly monitor mitochondrial integrity (88). Molecular chaperones and ATP-dependent proteases survey the folding and assembly of newly imported mitochondrial proteins and selectively degrade misfolded or damaged proteins within the organelle. Highly conserved proteolytic machineries within mitochondria provide quality control of respiratory chain subunits and regulate their biogenesis. Deficiencies in this system itself are linked to aging and age-associated neurodegenerative disorders. The first hint that defects in mitochondrial proteolysis are linked to age-associated disorders came with the discovery that mutations in SPG7 (paraplegin), which encodes a subunit of the ATP-dependent *m*-AAA protease in the mitochondrial inner membrane, is associated with neurodegeneration in humans (89). Mutations in the *SPG7* gene were found in patients affected by an autosomal recessive form of hereditary spastic paraplegia. The prohibitins PHB1 and PHB2 form a high-molecular-weight supercomplex with the *m*-AAA protease in the mitochondrial inner membrane and regulate its proteolytic activity (90). Notably, loss of prohibitins in mice compromised mitochondrial architecture and led to premature aging symptoms accompanied by neurological defects and shortened life span (91, 92). Interestingly, yeast cells deficient in prohibitins exhibited a shortened replicative life span and elevated proteotoxicity (93). In response to DR, however, mitochondrial proteotoxic stress in prohibitin mutants was suppressed and resulted in a robust life span extension, an observation that has also been made in worms (93, 94). The above mentioned examples involve rather high levels of mitochondrial impairment, and molecular thresholds that allow surveillance and repair dependent on the quality of the stress level are believed to exist (95). Under mild stress conditions, such as the reduction of mitochondrial ETC subunits, activation of the UPR^{mt} provides a defense mechanism that aims to combat intraorganellar proteotoxicity through transcriptional activation of mitochondrial HSPs that can alleviate stress and thereby protect the organelle from harmful damage. The UPR^{mt} signaling pathway has been mainly characterized in *C. elegans*, and key players include the mitochondrial matrix-localized ATP-dependent protease CLPP-1, a homeobox-like transcription factor DVE-1, a small ubiquitin-like protein UBL-5, and the basic leucine zipper protein (bZIP) transcription factor ATFS-1 (95) (**Figure 2**). Individual components of the UPR^{mt} are required for increased longevity of worms, and reduced ETC function suggests that stress-responsive signaling is pivotal for longevity in response to mitochondrial dysfunction (85). Similarly, the discovery that impairment of the mitochondrial translation apparatus by genetic reduction of mitochondrial ribosomal protein S5 (MRPS5) caused UPR^{mt} activation and resulted in increased longevity in both worms and mice lead to the concept of mitochondria-nuclear imbalance that might drive aging in a conserved manner (96).

DNA DAMAGE AND OXIDATIVE STRESS

DNA Damage and Aging: Cause or Consequence?

More than 60 years ago, Denham Harman (97) proposed the free radical theory of aging, which suggested that the generation of oxidative stress within cells triggers molecular damage that irreversibly accumulates with time and ultimately causes aging. In this theory, both mitochondrial and nuclear DNA are among the molecules damaged by oxidative stress. Soon after the discovery that mitochondria were transforming oxygen into water, a process that, when deficient, resulted in the generation of a superoxide anion radical, Harman developed the mitochondrial theory of aging (98). This theory postulates that a lifelong accumulation of mtDNA mutations in multiple tissues eventually results in mitochondrial failure, which, in synergy with downstream processes such as apoptosis, results in the loss of cellularity and the progressive decline of tissue integrity during aging. In this section, we review the current understanding of DNA damage, at both the nuclear and the mitochondrial level in the process of aging, in relation to oxidative stress.

There are at least two dozen types of DNA damage induced by ROS, among which 8-oxo-2-deoxyguanosine (8oxodG), an oxidized form of guanosine, has been by far the most studied owing to its mutagenicity and because of the correlation between its accumulation and pathological processes such as cancer, degenerative diseases, and aging. Studies from the 1990s indicated increases in 8oxodG in nuclear and/or mitochondrial DNA in several organs with age in various organisms, including flies, gerbils, mice, and rats. Flies that presented physiological symptoms of aging exhibited increased levels of 8oxodG. In mice, 8oxodG accumulated with age in a tissue-specific manner. Strikingly, the rate of DNA mutations within brain and heart tissues in mice grew exponentially, reaching a 10-fold increase at 24–33 months of age. As mortality rates also increase exponentially with time, these observations support the idea that DNA damage can predict life span. Interestingly, DR mice and rats have a lower concentration of 8oxodG in all tissues compared with controls fed ad libitum, suggesting that an altered metabolic state may protect cells from DNA damage (99). Improved methods of 8oxodG detection have confirmed these discoveries linking DNA damage and aging in different species (99, 100).

Although unrepaired 8oxodG modification leads to a G to T transversion, this type of change appears to be relatively rare, comprising no more than 5% of all DNA lesions. Other types of DNA damage, including DNA double-strand breaks, which are among the most deleterious damage occurring to DNA, mtDNA deletions, and nuclear chromosome translocations, also increase with age (101). Cells possess mechanisms to repair damaged DNA molecules. DNA repair systems include base excision repair (BER) and nucleotide excision repair (NER) for single-strand lesions, whereas homologous recombination (HR) and nonhomologous end joining (NHEJ) repair double-strand breaks. BER protects DNA in both the nucleus and mitochondria and is the main defense against endogenously derived DNA lesions, including oxidative lesions. Some reports have shown that BER and NHEJ activity slightly decreases with age in the mouse brain (102). However, the redundancy of several enzymes involved in DNA repair might help genome integrity despite decreases in the activity of certain DNA repair enzymes. In humans, sequencing of genes involved in DNA damage repair has revealed that single nucleotide polymorphisms in the *Wrn* helicase gene were associated with short life span. Mice deficient for enzymes involved in double-strand break repair, including *Ku70*, *Ku80*, *Xpf1*, *Xpd*, *Xrcc5*, *Wrn*, or *Ercc2*, are short lived and display early symptoms of some pathologies normally seen during aging (102). Several of these enzymes, when mutated in humans, also lead to progeroid syndromes. However, drawing conclusions about the involvement of these enzymes in normal aging solely based on the short life span of these knockout mice and a few early aging pathologies observed in these mice might

be premature. Most defects caused by mutations of these genes are absent during normal aging. Therefore, although these models help better characterize the pathways involved in humans inheriting the same genetic diseases, they might be limited to understanding normal aging per se (103). In that sense, mouse models that specifically improved DNA repair are expected to provide answers about the role of DNA repair in normal aging. To date, such models have been difficult to engineer, owing to the fact that DNA repair is dependent on multiprotein complexes and that overexpression of a single DNA repair protein is not sufficient to promote activity of the entire process.

In summary, although the accumulation of damage to nuclear and mitochondrial DNA during the normal adult life span has been observed, the causality of this phenomenon in the aging process has yet to be clearly proven. To date, it appears to be rather a consequence of aging than a cause. The generation of novel animal models may pave the way for a better understanding of the complex interactions between DNA damage and life span regulation in the future.

Role of Oxidative Stress in Aging

What are the repercussions of modulating the natural defenses against oxidative stress on aging? As observed with DNA damage and aging, positive correlations were found between maximum life span of many mammalian models and superoxide dismutase (SOD), catalase activity, and free-radical scavenging enzyme levels. Mechanistic studies in *C. elegans* (104, 105) and mice (106, 107) have greatly contributed to the understanding of how ROS can modulate life span.

To protect cellular components from the deleterious effects of sustained oxidative stress, cells possess an arsenal of antioxidant small molecules and enzymes, including Cu/ZnSOD (SOD1) or MnSOD (SOD2), thioredoxin (TRX), peroxiredoxin (PRX), glutathione peroxidase (GPX), and catalase. Several of these enzymes are essential for mouse viability, including MnSOD, TRX1, TRX2, and GPX4. Surprisingly, the life span of heterozygotes of *Gpx1*$^{+/-}$ (in a *Sod2*$^{+/-}$ or wild-type background), *Trx2*$^{+/-}$, *Sod1*$^{+/-}$, and *Sod2*$^{+/-}$ appears normal, with no signs of premature aging, despite decreased resistance to oxidative stress and DNA damage associated with a higher incidence of cancer (107). Similarly, the deletion of each or combination of the five SODs in *C. elegans* does not shorten life span despite increasing sensitivity to oxidative stress (105). More surprising, *sod-2* mutant worms are long-lived and exhibit phenotypes reminiscent of other long-lived mitochondrial mutants; these phenotypes include altered mitochondrial function, low oxygen consumption, slow development, and low brood size (108).

Paradoxically, heterozygous mutation of *Gpx4*, the primary enzymatic defense against oxidative damage to cellular membranes, increased life span in mice (109). The heterozygous *Gpx4*$^{+/-}$ mutation conferred an increased sensitivity to oxidative stress, but no difference in oxidative DNA damage was found. It appears that the faster elimination of cells upon ROS elevation in *Gpx4*$^{+/-}$ mice prevents these cells from carrying dysfunctional damages and possibly from becoming tumorigenic. Accordingly, fatal lymphoma occurrence is delayed in these mice. Similarly, depleting the thioredoxin defense from the cytosol, via *Trx1* heterozygous knockout, decreases cancer incidence and ultimately increases life span (110). However, a conflicting report from the same group recently stated that the *Trx1*$^{+/-}$ mutation failed to increase life span. Preliminary results also indicated that the *Trx1* and *Trx2* double heterozygous deletion increases life span while still sensitizing these animals to oxidative stress (111). During the last decade, it has been demonstrated in *C. elegans* and mice that slight amounts of oxidative stress due to specific disruption of mitochondrial ETC or by direct treatment of animals with H_2O_2 can result in a hormetic effect, producing upregulation of stress-response pathways and ultimately increased life span (112). Another

organism, the naked mole rat, exhibited greater levels of oxidative damage than physiologically age-matched mice; nevertheless, these animals still lived an order of magnitude longer than mice (113).

Addressing the question of ROS involvement in aging from another angle, some research groups aimed at verifying whether attenuation of oxidative stress could indeed prolong life span. In *C. elegans*, some studies initially reported an increased life span upon SOD/catalase mimetic treatment, whereas more recent studies dismissed those results by showing SOD mimetics have no positive effect on life span (114). Transgenic *Drosophila* overexpressing SOD enzymes alone or in combination with catalase exhibited reduced amounts of 8oxodG and increased resistance to oxidative stress with age, ultimately causing life span extension (115). However, increasing the levels of several antioxidant enzymes in mice has proven unsuccessful to slow down the aging process as overexpression of SOD1, SOD2, catalase, or GPX4 does not increase life span despite increasing resistance against oxidative stress. The only exceptions were mice with increased catalase specifically targeted to the mitochondria (MCAT mice) or the peroxisome (PCAT mice) and *Trx2* transgenic mice (116). Levels of 8oxodG were reduced and the incidence of some age-related pathologies were also delayed. Furthermore, overexpression of the SOD1 enzyme in addition to the PCAT transgene resulted in a further increased life span compared with PCAT mice alone (116).

Taken together, these studies indicate that sustained oxidative stress may be deleterious for mice, resulting in pathologies ranging from neonatal lethality to increased cancer rates and decreased life span. However, less severe depletions in antioxidant defenses rarely decrease life span even if overall oxidative damages are increased. Eventually, depletion in these enzymes actually improves life span of mice as observed for *Gpx4* heterozygous and *Trx1* and *Trx2* double heterozygous knockout models. Moreover, models of increased antioxidant defenses have proven, in many cases, that decreased oxidative damage can be uncoupled from life span extension. In summary, these experimental observations indicate that the free radical theory of aging might not be entirely accurate. An alternative interpretation of ROS effects on life span is that the redox status of the cell could be more important in regulating life span than the actual oxidative damage occurring over the course of regular aging. The threshold of oxidative damage required to impact negatively on life span likely is, in the absence of exogenous damage such as irradiation or toxins, never reached. It is also becoming clear that some ROS, such as H_2O_2, can serve as signaling molecules in several important signaling pathways and that alteration of the redox balance might be the route by which antioxidant defense can increase life span in *C. elegans* and mice. Future studies are required to better understand the specific longevity pathways regulated by ROS and whether antioxidant enzymes can increase life span independently of an action on ROS, for example, by reducing the activity of specific proteins, including transcription factors.

TELOMERE AND CELLULAR SENESCENCE

Telomeres are tandem DNA repeats ($TTAGGG_n$) sequences located at the end of chromosomes. They associate with shelterin proteins to form a T-loop that prevents the end of chromosomes to be recognized as double-strand breaks and elicit a DNA damage response, thereby ensuring chromosomal stability (117). First recognizing their role in aging in vitro, Hayflick observed that human somatic cells could divide a limited number of times before reaching an arrested state termed senescence (118). Subsequently, senescence was found to be caused by critically short telomeres that were not fully replicated after each mitosis, resulting in a loss of a small amount of DNA during each division (119). However, when telomere length was maintained, through expression of telomerase, a specific DNA polymerase that replicates telomeres, cells could bypass the Hayflick limit and divide indefinitely (120). Interestingly, human cancer and germ cells retain a high expression of telomerase and thereby maintain telomere length during divisions. At the

cellular level, several important signaling pathways triggered by telomere shortening have been elucidated. The detailed mechanisms have been described in depth elsewhere (120). In brief, when telomeres become sufficiently short, p53 and DNA damage response pathways are activated. In highly proliferative tissues, it seems that the major consequence of p53 activation is a cellular checkpoint of growth arrest, senescence, and/or apoptosis in stem and progenitor cells. In this scenario, the failure of stem cells harboring short telomeres to replace differentiated or effector cells dying by apoptosis induced also by short telomeres or by other damages could impair, as a whole, organ or tissue homeostasis and contribute to organismal aging (121). Accordingly, p53 deficiency in those organs ameliorates this phenotype. In quiescent tissues, telomere dysfunction leads to a reduction of PGC-1α and PGC-1β levels, resulting in decreased mitochondrial function. Interestingly, p53 is also known to interact with longevity pathways such as IGF-1, insulin, mTOR, and AMPK signaling, further explaining how p53 activation upon telomere attrition can modulate aging.

Several correlative studies have linked in vivo telomere length to cellular senescence and organismal aging. First, most studies in humans have shown that telomere length progressively declines with age in several tissues, including proliferative compartments and more quiescent tissues. When placed in culture, cells with short telomeres from old donors display a limited proliferative capacity compared with cells from young donors. Interestingly, either telomere length, telomere shortening rate, or the percentage of short telomeres can be a good predictor of mortality and life span (122, 123). In another model, only one critically short telomere, rather than the average length of telomeres in the cell, is critical for cell viability and chromosome stability (124).

In contrast to humans, yeast telomere length remains constant during the life span of normal cells, suggesting that aging is not due to telomere attrition. However, telomere elongation mutants showed a rapid loss of cell viability, which suggests that telomere maintenance is at least required for normal yeast life span (125). Telomere length in mice is greater than in humans in part due to the telomerase expression that stays high in most somatic cells of the mouse. In some tissues such as brain or spleen, however, telomerase activity is undetectable. Telomerase is a ribonucleoprotein consisting of two components: the telomerase reverse transcriptase (TERT) and the RNA component (TERC). Mice deficient in TERC (*mTerc*$^{-/-}$) exhibit fast telomere attrition compared with wild-type mice but do not present with obvious abnormalities in the first generation owing to reserve telomere length. However, from the third generation on, mice present with increased genomic instability and early onset of age-associated pathologies. Organs with less proliferative rates do not display an accelerated aging phenotype, further supporting the prevalent role of telomere attrition in highly proliferative organs. From the fourth generation on, these phenotypes are aggravated and tumor incidence is increased by 4–6 times compared with wild-type mice, arising principally from highly proliferative organs; this finding suggests that chromosomal instability due to telomere shortening is likely to play a role in the initiation of tumorigenesis and represents one mechanisms through which cancer incidence increases with age (126). Notably, telomerase-deficient mice do not recapitulate the full spectrum of aging pathologies seen in wild-type mice, indicating that additional mechanisms play important roles in aging.

In humans, hereditary loss of function in telomerase component genes (*hTERT* or *hTR*) results in congenital dyskeratosis. Patients present accelerated rates of telomere shortening and suffer from premature adult stem cell dysfunction, premature age-related diseases, and decreased longevity (127). Mutations in telomerase component genes have also been implicated in idiopathic pulmonary fibrosis, liver disorders, and bone marrow failure syndromes. However, studies in centenarians have found specific variations in human telomerase genes are linked with longer telomere maintenance, healthy aging, and exceptional longevity (128).

Mice with critically short telomeres due to telomerase deficiency can be rescued by telomerase reactivation in adults. This reactivation reduces DNA damage signaling and the associated cellular

checkpoint responses, eliminates degenerative phenotypes across multiples organs, and reverses neurodegeneration (129). In wild-type mice, overexpression of *mTert* increased telomere length but promoted the rate of spontaneous cancers in old animals, reminiscent of the overexpression of TERT often found in human cancers (130). This finding is not surprising, considering the unlimited proliferative capacity thereby given to cells from these transgenic mice with overexpression of telomerase. Interestingly, when *mTert* transgenic mice are crossed with cancer-resistant mice, whose life span is by itself not different from wild-type mice, the double mutant mice displayed delayed aging and a 40% increase in median longevity (131). More recently, telomerase reactivation by gene therapy in old wild-type mice increased telomere length in several tissues, improved several parameters of aging, and extended life span without increasing cancer (132). Moreover, the TERT-based gene therapy in those mice reverted their metabolic profile to that of younger mice, further suggesting that telomerase reactivation can exert an antiaging role. As a pharmacological approach, a small molecule activator of telomerase, TA-65, has recently been discovered. When given to mice, it increased telomerase activity in several tissues, restored telomere length, and ultimately improved health span without increasing cancer incidence (133). However, treated mice did not outlive control animals.

In summary, these observations indicate that telomere attrition might play a major role in mammalian aging. Maintaining telomere length during aging or reversing telomere shortening results in beneficial effects on health span and life span. Nevertheless, it appears that telomerase activity should be tightly regulated because of its implication in cancer. Surprisingly, although more than 50 mouse models of extended life span have been published to date, few data exist regarding the telomere attrition in those models. Measuring telomere length with age in several organs of these mouse models is likely to help better understand the signaling pathways involved in telomere length regulation during aging.

EPIGENETIC MODIFICATIONS

The research of epigenetics is gaining increasing attention within the aging field and contributes significantly to our understanding of how environmental cues impinge on the regulation of the natural aging process (2, 134). Alterations in the epigenetic landscape include a large variety of modifications that affect both the DNA itself as well as the chromatin structures that provide higher order compaction of DNA within a cell (**Figure 3**). These changes include alterations in DNA methylation, posttranslational modifications of histones, as well as structural remodeling of nucleosomal architecture. The modification of histones is widespread and complex and involves not only acetylation and methylation but also ubiquitylation, phosphorylation, and citrullination. Several well-characterized histone modifications have been associated with aging; these modifications include increased histone H4K16 acetylation, H4K20 trimethylation, or H3K4 trimethylation, and decreased H3K9 methylation or H3K27 trimethylation (135). Multiple classes of enzymes, including DNA methyltransferases, histone acetyltransferases, deacetylases, methyltransferases, and demethylases, shape the epigenomic landscape and confer an extensive plasticity to the aging process. The key players and signaling networks that mediate these epigenetic patterns in response to external cues are just being unraveled.

Histone Modifications

The methylation of histones is one of the best-characterized modifications that directly affects life span. The first evidence came with the discoveries that genetic inactivation of either H3K4 or H3K27 histone methylation complexes can regulate longevity. Specifically, deficiencies in the H3K4 methyltransferases ASH-2, WDR-5, and SET-2 extend *C. elegans* life span in a

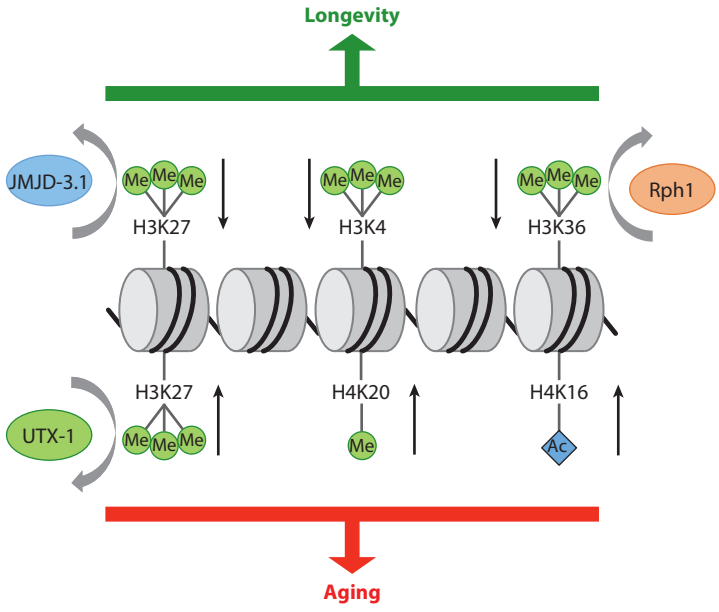

Figure 3

Epigenetic regulation of aging. Global levels of several histone modifications, including increased histone H4K16 acetylation and H4K20 trimethylation, have been associated with aging, whereas decreased H3K4 di- and trimethylation, H3K36 trimethylation, and H3K27 trimethylation can lead to longevity in model organisms. Notably, in some genetic models either a decrease or an increase in H3K27 trimethylation is linked to longevity. Nematodes lacking the H3K27 demethylase UTX-1 showed increased levels of H3K27 trimethylation and extended longevity, whereas overexpression of the H3K27 demethylase JMJD-3.1 led to a decrease in H3K27 trimethylation and life span extension. In *Saccharomyces cerevisiae*, reduction of the H3K36 histone demethylase Rph1 caused an increase in H3K36 trimethylation and an extension of replicative life span. Direction of arrows indicates increase or decrease in levels of histone mark. Abbreviations: JMJD-3.1, jumonji C-domain-containing histone demethylase-3.1; Rph1, damage-responsive repressor of PHR1; UTX-1, ubiquitously transcribed tetratricopeptide repeat, X chromosome.

germline-dependent manner (136), whereas the genetic reduction of H3K27 trimethylation complex members modulates longevity of *Drosophila* (137). Enzymes involved in reversing histone methylation also have direct roles in the regulation of longevity. Loss of the H3K27 demethylase UTX-1 in worms extends life span by regulating the insulin/IGF-1 signaling axis (138, 139).

It has become increasingly evident that organellar stress responses shape the aging process and are intricately linked to longevity assurance in various organisms. The epigenetic control of stress signaling is therefore an intriguing avenue under active investigation. In yeast, mitochondrial stress-induced longevity is regulated by H3K36 trimethylation and requires the H3K36 demethylase Rph1 (140). In contrast, the heat shock response (HSR) is repressed during early adulthood in nematodes through an increase in H3K27 trimethylation, which was associated with reduced levels of the H3K27 demethylase *jmjd-3.1* (141) (**Figure 3**). However, the precise role of this mark in aging, independent of longevity, awaits further characterization.

Sirtuins

The sirtuin family of NAD-dependent protein deacetylases has been proposed to be a family of potent aging regulators. Early studies in yeast, flies, and worms claimed that the single sirtuin gene

Sir2 mediated a robust increase in longevity (142). Several of these findings are controversial, and independent groups discovered that *Sir2* overexpression did not affect life span in *C. elegans* and *Drosophila*. Conversely, repetition from the Guarente lab did find increased life span in nematodes that overexpress *sir2*, albeit the increase was modest at best and only present in a few transgenic lines (143, 144). In mammals, several of the seven sirtuin protein family members (SIRT1-7) can modulate parameters of aging in mice (145). Transgenic overexpression of *Sirt1* does not increase murine life span but positively impacts overall health (146). Mice deficient in SIRT6 have a reduced life span (147), whereas overexpression of *Sirt6* has prolongevity effects through regulation of genomic stability, nuclear factor κB (NF-κB) signaling, and glucose homeostasis (148). SIRT3, which localizes to mitochondria, appears to be required for DR-mediated longevity through deacetylation of mitochondrial proteins (149). Recently, SIRT7 has been implicated in the control of hematopoietic stem cell aging through modulation of protein-folding stress in mitochondria (150). Therefore, several members of the sirtuin family have reported beneficial effects on certain aspects of longevity in mammals. In summary, evidence that the chromatin state directly impacts the aging process and is associated with age-associated disorders is emerging. A more detailed understanding of chromatin networks shaping the epigenetic landscape as well as their precise molecular mechanisms may pave the way for novel treatment strategies of age-related disorders and the improvement of healthy aging.

PROTEOSTASIS IMBALANCE

As cells and organisms age, accumulation of damaged and misfolded proteins increases due to a functional decline in the protein homeostasis (proteostasis) machinery upon aging, causing reduced cellular viability and the development of protein-misfolding diseases such as neurodegenerative diseases (151). Malfunction of the proteostasis network impairs cellular homeostasis through impaired coordination of the rate of protein synthesis, folding, trafficking, secretion, and degradation. Thus, postmitotic cells accumulate oxidative damage such as carbonylation, oxidized methionine, glycation, and aggregation of proteins with age. Interventions that slow aging have beneficial effects on proteostasis and alleviate accumulation of toxic aggregates, as observed upon reducing IIS levels in nematodes (152) and in a mouse model of Alzheimer disease (153–155).

Protein Synthesis

With age, differentiated cells exhibit aberrant changes in translation. Decreasing the rate of translation is sufficient to extend life span in many model organisms, as documented upon TOR inhibition in yeast, worms, and mice (30, 31, 34, 35) and reduction of ribosomal proteins levels or depletion of translation initiation factors in yeast and worms (34, 156). But is it the global reduction in protein synthesis that really causes life span extension? Emerging evidence reveals that reducing translation causes preferential translation of certain mRNAs, without necessarily impairing the amount of protein synthesized. In yeast, deletion of 60S ribosomal subunits increased life span through the preferential translation of the Gcn4 transcription factor, inducing a transcription profile necessary for full life span extension by DR (156). Similarly, flies subjected to DR exhibited preferential translation of mitochondrial genes in a 4E-BP–dependent manner (157). However, in several mouse studies of reduced TOR activity, overall translation was not affected (35, 38, 56), but the increased AMPK and PGC-1α activity observed in these mice may underlie a conserved improved translation of select genes that encode mitochondrial components (56).

Protein Folding

To acquire their functional conformation, proteins require the assistance of molecular chaperones. The presence of misfolded proteins is detected by chaperone networks to initiate a proteostasis response. Distinct cellular stress-response pathways guard the proteome of the cytosol, endoplasmic reticulum (ER), and mitochondria. Upon heat stress, which induces protein misfolding, the cytosolic response is initiated by the HSR, regulated primarily by heat shock factor 1 (HSF-1). In worms, HSF-1 is required for a reduced IIS longevity pathway: Its overexpression powerfully extends life span and enhances the folding of protein aggregates by transcriptionally regulating molecular chaperones and stabilizing the actin cytoskeleton (158, 159), whereas reduction of HSF-1 induces accelerated aging (160).

A specific set of chaperones is dedicated to guiding the folding of secretory proteins exiting the ER. A specialized stress response named the unfolded protein response, or UPR^{ER}, enables the ER to cope with an imbalance between secretory load and protein-folding capacity, or during stresses such as heat shock. The UPR^{ER} is mediated by three signaling pathways, IRE1, PERK, and ATF6, and has several outcomes, including reduced translation rates, mRNA degradation, transcriptional upregulation of many chaperones, and, after excessive or prolonged ER stress, apoptosis. As with the HSR, activity of the UPR^{ER} declines with age (161). Activation of the UPR^{ER} through overexpression of the spliced and active form of the transcription factor XBP-1 in nervous tissue is sufficient to confer extended life span and resistance to chemical stress in worms (161), highlighting the preponderant role of this stress response in proteostasis and aging.

A third cellular proteostasis pathway aims at protecting mitochondria from disturbances in the stoichiometry of mitochondrial proteins (see the section on Mitochondrial Health for details). Mitochondrial stress induced by knockdown of mitochondrial ETC complexes increases worm longevity (83) and, as with the UPR^{ER}, can be activated cell nonautonomously from neuronal to peripheral tissues (85). This pathway is part of the mitochondrial quality control machinery that detects misfolded proteins in the mitochondria, initiating a signaling cascade that leads to the transcription of protective genes (87).

Proteolytic Degradation

Damaged proteins are degraded by the two principal proteolytic systems: the ubiquitin–proteasome system and the autophagic–lysosomal system. Their efficiency declines with age, supporting the idea that protein clearance mechanisms are directly linked to aging and age-associated diseases (162). Additionally, the functional decline of cellular stress-response pathways, such as the HSR, contributes to increasing the proteolytic load and overwhelming the proteolytic machineries. The age-dependent dysfunction in proteasome activity is the result of decreased subunit expression, loss and replacement of certain subunits and disassembly, as well as obstruction by protein aggregates (162). Overexpression of the proteasome subunit *rpn-6*, a target of FOXO/DAF-16, enhances worm survival upon proteotoxic stress (163). Stem cells, which do not undergo replicative senescence, presented high proteasome activity compared with their differentiated counterparts and enhanced levels of 26S proteasome non-ATPase regulatory subunit 11 (PSMD11), the mammalian ortholog of *rpn-6* (164). Conversely, deficiency in the proteasome E3 ubiquitin ligase C terminus of HSC70-interacting protein (CHIP) caused a reduction in mouse longevity and health span (165).

Similarly, upon normal aging, the autophagic–lysosomal proteolytic system undergoes a progressive dysfunction. Many reasons could cause this decline: Aging impairs the clearance of autophagosomes, results in lower levels of critical ATG proteins, and elicits insensitivity to hormonal

cues in mammalian cells that regulate autophagy. Consequently, increasing autophagy extends life span and protects organisms from extrinsic environmental stress or intrinsic age-related deterioration, suggesting that protein clearance mechanisms are directly linked to aging and age-associated diseases (65).

CELL NONAUTONOMOUS CONTROL OF AGING

Evidence in Model Organisms

Even though aging affects individual tissues differently, overall organismal demise occurs in a coordinated fashion. Precisely, the rate of aging in individual tissues shows synchronization in the distinct transcriptional patterns for neural, vascular, and steroid-responsive tissues, as revealed by the Atlas of Gene Expression in Mouse Aging Project (AGEMAP) (166). The importance of systemic factors in the regulation of aging has been established in invertebrate models of proteostasis (167), as observed in cell nonautonomous regulation of the UPR^{mt}, the UPR^{ER}, and more recently the HSR, all of which require a prolongevity signal to be sent from neuronal to distal tissues in order to activate protective stress-signaling pathways and prolong life span (85, 161, 168). Similarly, in mammals the secretion of GH, IGF-1, and FGF-21 tightly controls metabolic cues to regulate longevity (see the section on Insulin and Insulin-Like Growth Factor Signaling for details). Cell nonautonomous control of aging is also powerfully illustrated in models of deficient chemosensory signaling in which alterations in canonical sensory perception can acutely influence normal aging. For example, ablation of sensory neurons in the worm extends life span and increases adipose storage and insulin-like signaling (169, 170), and mutations that impair chemosensory signal transduction increase longevity in both worms and flies (169, 171). Likewise, in mice, impairment of transient receptor potential cation channel subfamily V member 1 (TRPV1) sensory receptors expressed in afferent neurons from the dorsal root ganglia is sufficient to extend life span and improve metabolic health, acting remotely on pancreatic β-cells to improve β-cell mass and insulin secretion (53). Intriguingly, the nature of chemosensory signals that can affect mammalian longevity remain uncharacterized.

Systemic Inflammation

Which systemic factors could transmit an aging signal from tissue to tissue? Heterochronic parabiosis experiments (young–old mouse pairs) point toward the existence of rejuvenation factors of blood-borne origin that can be transmitted from young to old, counteracting age-induced chemokine increases or brain vascular deterioration and stem cell loss (172, 173). Accumulation of systemic low-grade inflammation, a phenomenon known as inflammaging, is a hallmark of aging, and increased levels of multiple inflammatory cytokines, including tumor necrosis factor α (TNFα), interleukin-6 (IL-6), IL-1β, cytokine antagonists, and acute phase proteins such as C-reactive protein (CRP), may underlie the activation of pathological senescence processes (174). The accumulation of these proinflammatory agents (or inflammaging agents) characterizes multiple age-induced pathologies, such as sarcopenia, neurodegeneration, arthritis, atherosclerosis, and insulin resistance (175). Lifelong administration of the anti-inflammatory agent aspirin (acetylsalicylic acid) results in an 8–12% median life span extension in male mice, highlighting the therapeutic potential of anti-inflammatory therapies (176). The origin of inflammaging is still poorly understood and may result in part from persistent viral and bacterial infections that are not cleared properly by a defective adaptive immune system (175). Senescent cells present in aging tissues constitute another significant source of inflammation, their appearance being due to a

decline in apoptotic capacity with age and/or a gradual decrease in the immune system's ability to recognize and degrade these cells. Despite the relatively small number of senescent cells in aged tissues, a unique senescence-associated secretory profile (SASP) including many proinflammatory cytokines might broadly impact organismal aging through endocrine or other cell nonautonomous regulation (177).

Additionally, both age-derived adipose tissue expansion and macrophage recruitment in inflamed tissues increase the levels of proinflammatory cytokines that contribute to chronic insulin resistance and metabolic inflexibility (178). The presence of low-grade chronic inflammation, common in obesity-associated diseases, has been termed metainflammation (179). Metabolic dangers such as circulating cytokines, lipotoxic fatty acids, ceramides, free cholesterol, and ROS have the capacity to activate multiple molecular pathways, such as the NLRP3 inflammasome, JNK/AP-1, and IκBα kinase-β (IKKβ)/NF-κB signaling, that coordinate the increase in inflammatory genes (179). The NLRP3 inflammasome, an innate immune sensor that triggers sterile inflammation in the absence of infection in both peripheral and central tissues, has recently been linked to functional decline with age. Ablation of the NLRP3 inflammasome protects mice from age-related increases in glucose intolerance, innate immune activation, alterations in central nervous system transcriptome, and astrogliosis (180). Another fundamental endpoint connecting metabolic inflammation with aging is the NF-κB–dependent activation of inflammatory gene transcription, which is activated downstream of Toll-like receptors and cytokine receptors, such as tumor necrosis factor receptor (181). Preventing the deterioration of hypothalamic immunity in aged mice was sufficient to extend life span (182). Active NF-κB and IKK-β in microglia drove hypothalamic TNF-α production via microglia–neuron paracrine communication and induced overall aging of peripheral tissues, including bone loss, muscle, and skin atrophy. This report also identifies the age-dependent reduction in the reproductive hormone gonadotropin-releasing hormone (GnRH) to be induced by IKK-β/NF-κB enhanced activity (182). Restoring GnRH levels with age had beneficial effects on skin thickness, muscle endurance, and brain neurogenesis, suggesting that hypothalamic immune health functions as a neuroendocrine regulator to adjust physiological aging in peripheral tissues. The nature of the aging signal induced in receiving cells remains to be determined. Of note, diet-induced obesity studies reveal a similar hypothalamic inflammation, generated from saturated fat-dependent microglial activation in the hypothalamus. This initial cell-autonomous inflammation is sufficient to generate an inflammatory milieu that disables insulin signaling in tissues, systemically exacerbates metainflammation, and predisposes the organism to disease. Thus, it is quite tempting to think that this vicious metainflammation cycle may be recapitulated upon aging. Nevertheless, the mediators of inflammaging, such as the SASP and age deficiency in the adaptive immune response, might differ in the nature of their signaling pathways.

CONCLUSIONS

In this review, we provide evidence that aging is an integrative homeostatic process resulting from the combination of exogenous and endogenous cumulative insults. Various forms of damage originate from individual cells or systemic demise, as observed in chronic inflammation (**Figure 4**). Although there are a number of hypotheses as to where aging begins and how it propagates, no single theory is sufficient to embrace all aspects of this complex process. The literature on aging provides countless evidence that targeted alterations of one particular signaling network, either from external or genetically encoded damage, impacts several or all other systems. A good example of this concept is illustrated by the pathophysiology of Parkinson disease, a neurodegenerative condition of motor neurons mostly caused by death of dopaminergic neurons in the substantia

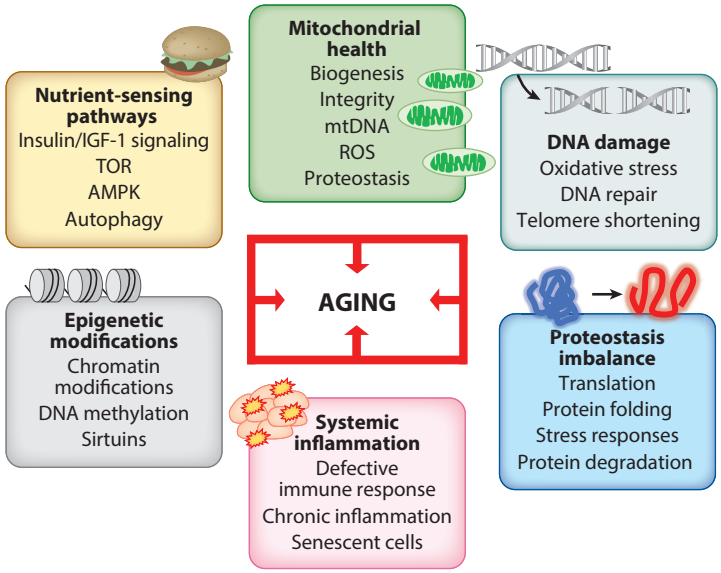

Figure 4

Signaling networks of aging. Model depicting an overview of the signaling pathways involved in the regulation of longevity in various model organisms. Abbreviations: AMPK, AMP-activated kinase; IGF-1, insulin-like growth factor; ROS, reactive oxygen species; TOR, target of rapamycin.

nigra. Environmental changes surrounding the affected neurons, as well as oxidative stress, mitochondrial dysfunction, and impairment in protein degradation within these neurons, all constitute linked hallmarks of the disease with the capacity to coinfluence each other (183). However, progressive understanding of the signaling networks of aging raises the possibility of manipulating human life span and the particular possibility of combating age-associated pathologies. Pioneering work on blood-borne systemic factors offers the fascinating perspective of rejuvenation strategies that may function therapeutically (172, 173). Although appreciating the unique features of any given age-related pathology is critical to designing therapies, strategies aiming at elucidating the common denominator, aging, will be fundamental to the advancement of medicine. If we can identify a faulty biological process at an early stage of its development, resolving small-scale defects and avoiding progressive disruption of whole-body homeostasis and the onset of fatal disease may be much easier.

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