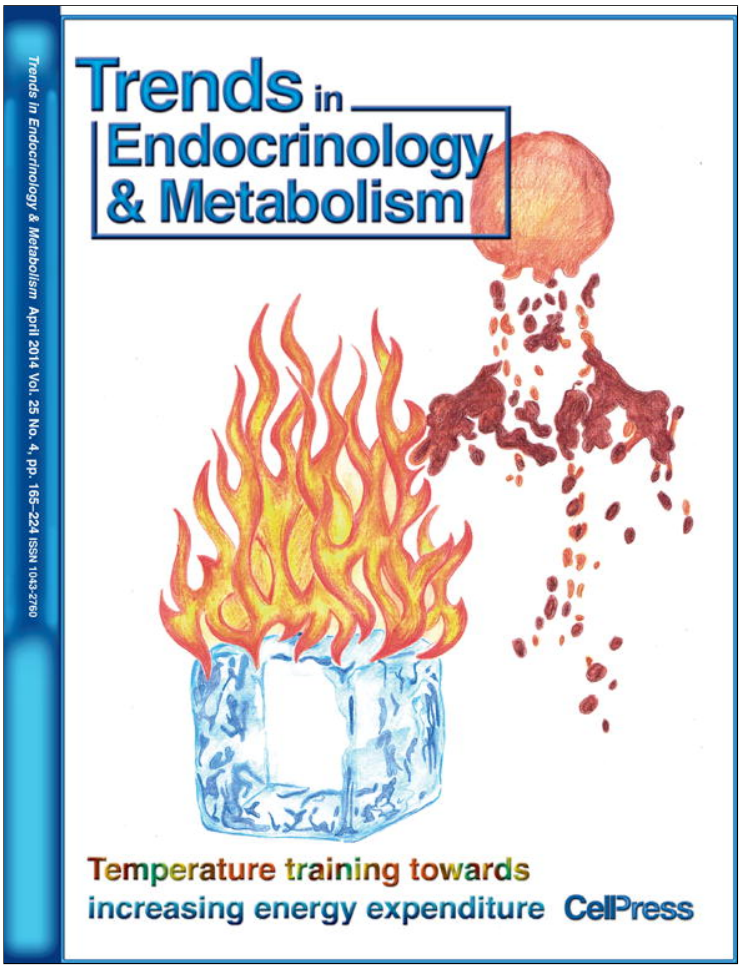

Review

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Cellular and molecular longevity pathways: the old and the new

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Human lifespan has been increasing steadily during modern times, mainly due to medical advancements that combat infant mortality and various life-threatening diseases. However, this gratifying longevity rise is accompanied by growing incidences of devastating age-related pathologies. Understanding the cellular and molecular mechanisms that underlie aging and regulate longevity is of utmost relevance towards offsetting the impact of age-associated disorders and increasing the quality of life for the elderly. Several evolutionarily conserved pathways that modulate lifespan have been identified in organisms ranging from yeast to primates. Here we survey recent findings highlighting the interplay of various genetic, epigenetic, and cell-specific factors, and also symbiotic relationships, as longevity determinants. We further discuss outstanding matters within the framework of emerging, integrative views of aging.

Pathways that control longevity across species: known mechanisms and new findings

Aging is a complex process defined as progressive functional deterioration associated with frailty, disease, and death. Over the past decades numerous genes and conditions have been revealed to influence aging across taxa. Among the most comprehensively studied pathways are the insulin/insulin-like growth factor 1 (IGF-1) signaling (IIS) pathway and dietary restriction (DR, see Glossary). In this review we focus on recently identified mechanisms influencing longevity, aiming to provide an overview of the relationships between different pathways that modulate lifespan, as well as the evolving concepts and new challenges pertinent to aging research.

Signaling out of the gonad

In the nematode *Caenorhabditis elegans*, removal of germline precursor cells either surgically or genetically (in *glp-1* mutants) significantly extends lifespan [1,2]. This initial observation has now been verified in different species [3]. Lifespan extension depends on the presence of the somatic gonad; removal of somatic gonadoblasts abrogates the longevity phenotype. Consistent with these findings, increased proliferation of germline precursor cells is detrimental and is inhibited by longevity-promoting mutations [4].

Recent work in *Drosophila melanogaster* indicates that ablation of the germline by forced differentiation of germline stem cells (GSCs) results in lifespan extension in

Glossary

Caloric restriction: a dietary regimen based on low calorie intake.
Cellular senescence: the phenomenon whereby normal dividing cells cease to divide after reaching a specific number of cell divisions (also known as replicative senescence). The term also describes the irreversible growth arrest that occurs when cells encounter stress. With the possible exception of embryonic stem cells [162], most division-competent cells, including some tumors cells, can undergo senescence when appropriately stimulated [163,164].
Dietary restriction (DR): refers to undernutrition without malnutrition. Does not imply reduced intake of a specific food group.
DNA methylation: an epigenetic signal that cells use to lock gene expression in the 'off' mode. Occurs at cytosine bases of eukaryotic DNA, which are converted to 5-methylcytosine by DNA methyltransferase enzymes. Some organisms, such as the yeast *Saccharomyces cerevisiae* and the nematode worm *Caenorhabditis elegans*, are thought to have no methylated DNA. In mammals, methylation is found sparsely but globally, distributed in defined CpG sequences throughout the entire genome, notably in CpG islands – stretches ~1 kb in length with high CpG content.
Epigenetic regulation: involves chromatin and DNA modifications that are heritable through cell division but that do not affect the DNA sequence itself. Given that aging also affects post-mitotic tissues and entails senescence, the term is used here more loosely to also include non-proliferative cells.
Hormesis: a phenomenon whereby favorable outcomes occur in response to low-dose toxins, drugs, or other stressors.
Immunosenescence: age-related decline of the immune response.
Inflammaging: chronic low-level inflammatory status associated with the elderly.
Microbiota: the microorganismal population colonizing the body of metazoans. Distinct microbiota are defined according to the origin of colonization (i.e., gut microbiota, skin microbiota, and oral microbiota refer to microorganisms populating the intestine, skin, and mouth, respectively).
Proteostasis: general protein homeostasis. It is controlled by biological processes that mediate protein synthesis, proper protein folding and trafficking, protein degradation, and clearing.
Stem cell niche: the microenvironment within a tissue where adult stem cells of that particular tissue reside. The niche interacts with the stem cell population via cell contact and/or secreted factors that play key roles in regulating stem cell function [165].
Telomeres: ribonucleoprotein complexes located at the ends of chromosomes and that are essential for chromosome protection and genome stability. They consist of tandem repeats of a G-rich DNA sequence (in vertebrates TTAGGG) which is bound by a six-protein complex known as shelterin. Telomeres also perform additional functions. In particular, they mediate the transcriptional silencing of genes located proximally to the telomeric region (a phenomenon termed subtelomeric silencing), and they ensure the proper segregation of chromosomes during mitosis (reviewed in [54]).

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males and females [5]. GSC ablation influences the metabolic homeostasis of the organism, resulting in hypoglycemia, while at the same time causing changes in the activity of the insulin pathway that are reminiscent of insulin resistance [5]. Further analysis indicates that signaling from the gonad controls longevity via multiple families of transcription factors, including in particular the vitamin D receptor ortholog *daf-12*, the FOXO ortholog *daf-16* [2], the HNF4α-like nuclear hormone receptor *nhr-80* [6], and the FOXA ortholog *pha-4* [7]. These transcription factors are involved in diverse processes, suggesting that germline removal globally impacts upon the physiology of the nematode to promote longevity [3]. One of the suggested mechanisms involves induction of autophagy by fatty acids [7], which in turn regulates energy homeostasis and protein quality control.

Additional findings indicate that a component of the pro-longevity mechanism induced by germline removal is the DAF-12 steroid receptor [8–10], which is involved in the transition between larval stages L2 to L3 and upregulates members of the *let-7* miRNA family. These miRNAs target the early larval nuclear factor *lin-14* as well as the *akt-1* kinase gene, resulting in the activation of DAF-16/FOXO, a key transcription factor that promotes lifespan extension under conditions of low IIS activation [11]. Thus, germline removal extends lifespan by stimulating DAF-12 signaling and the expression of *let-7* miRNAs, which diminish expression of the serine/threonine-protein kinase AKT-1 and LIN-14, thereby derepressing DAF-16/FOXO [12]. Collectively, these findings demonstrate that lifespan extension via the gonad is multifactorial and involves the regulation of several processes including glucose and lipid homeostasis.

DR: signaling through the IIS and TOR pathways

DR, where caloric intake is reduced without reaching the point of malnutrition, has been shown to extend the lifespan of multiple species across the evolutionary spectrum, including non-human primates [13–15]. DR is thought to trigger an evolutionarily ancient adaptive response to changes in the environment, allowing the shift of energy resources from anabolism and reproduction to somatic maintenance [16]. DR is often referred to as caloric restriction (CR) because of indications [17] that reduction of calories, not specific macronutrients (fat, carbohydrate, or protein) in the diet, is important. However, work in both *Drosophila* and rodents suggested that essential amino acids, and particularly tryptophan, play a key role in extending lifespan during reduced food intake [18,19].

Several nutrient-responsive signaling pathways have been implicated in mediating the pro-longevity effects of DR, most prominently the IIS and the target of rapamycin (TOR) pathways [20,21]. In mammals, growth hormone (GH) produced by the pituitary gland induces the production of IGF-1 in a variety of cell types, but primarily in hepatocytes. Similar signaling events are elicited by insulin. Genetic manipulations that result in a reduction in either of the components of this axis (including GH, IGF-1 receptor, insulin receptor, or downstream intracellular effectors such as AKT, mTOR, and FOXO) have been linked to longevity, both in model organisms and in humans [14,22–24].

The target of the IIS pathway, relevant to longevity is the transcription factor FOXO that is encoded in *C. elegans* by the *daf-16* gene and in *Drosophila* by the *foxo* gene [25,26]. In *C. elegans*, *daf-16* deficiency completely abrogates the lifespan extension observed in mutants for *daf-2*, the worm insulin/IGF receptor ortholog, or *age-1*, the worm phosphatidylinositol 3-kinase ortholog [25]. In the mouse there are four FOXO genes and, although their roles in regulating cell metabolic responses, particularly to insulin, have been studied in a variety of tissues, including the liver and the brain [27], their contribution to longevity at the organismal level still remains elusive. Genetic variations in the *FOXO3* gene have been associated with longevity in several different human populations, for example, among German centenarians [28–30]. However, it has yet to be shown whether the effects of reduced IIS on lifespan are directly dependent on FOXO activity in mouse models or other mammals.

Similarly to the IIS pathway, the role of the TOR pathway on aging is remarkably conserved. There is strong evidence that this pathway mediates the effects of DR on lifespan (reviewed in [31]). In *S. cerevisiae*, DR due to limitation of glucose has been shown to robustly extend lifespan. Replicative lifespan, measured by the number of replication events from a single mother yeast cell, is increased when TOR activity is abolished. Furthermore, lowering glucose levels does not further extend lifespan of a *tor1* deletion mutant [32]. The TOR kinase modulates a wide range of targets and biological processes. A component of the nutrient-responsive mTOR signaling pathway and TOR target is ribosomal protein S6 kinase (S6K), reduced activity of which extends lifespan in both worms and flies [33–36]. In flies, reduced S6K activity is required for rapamycin, a TOR inhibitor, to extend lifespan [37], whereas in mice, deletion of S6K1 extends lifespan and produces a broad-spectrum improvement in aging parameters [38], such as the induction of gene expression patterns similar to those seen in CR, whereas treatment with rapamycin extends lifespan ([39]. In *C. elegans*, longevity resulting from loss of RSKS-1 (S6K) depends on several factors, including PHA-4 (FOXA) [40], heat-shock factor protein 1 (HSF-1) [41], and AAK-2 (AMP kinase) [38]. Because S6K controls protein translation, and given that inhibition of protein translation increases lifespan [33,35,36], one possibility is that TOR and S6K influence lifespan via controlling protein synthesis. Indeed, the rate of protein synthesis is reduced in long-lived worms with reduced RSKS-1 activity [33,35,42]. Nevertheless, how reducing protein synthesis increases lifespan remains unclear. Reduced TOR activity also activates autophagy, which is required for DR and reduced IIS to increase lifespan in *C. elegans* [43,44] and for rapamycin to extend lifespan in *Drosophila* [37]. However, the exact mechanisms by which increased autophagy ameliorates aging have yet to be elucidated (reviewed in [45]). A recent study demonstrated a causal connection between induction of autophagy and lifespan extension, following frataxin downregulation, a mitochondrial protein with putative roles in iron homeostasis [46]. Further work is necessary to clarify whether specific forms of autophagy, and particularly mitophagy, are implicated in longevity pathways.

Genetic and genomic determinants of longevity

Genome integrity and stability

Accumulating evidence indicates that aging is accompanied by an increase in genome damage and instability [47]. In support of this notion, numerous progeroid diseases, rare genetic disorders that mimic physiological aging, such as the Werner and Bloom syndromes among others, have been associated with accumulated genomic damage [48]. Damage occurs during the course of a lifetime due to extrinsic or intrinsic causes: the former include environmental insults of physical, chemical or biological nature, whereas the latter include mutations, breakage, duplication, or relocation of pieces of chromosomes, inability to detect and correct errors that arise from DNA replication, as well as progressive loss of telomeric length. Several studies have demonstrated that experimental induction of DNA damage results in accelerated aging and compromised longevity. Conversely, a recent study demonstrated that increased expression of the serine/threonine-protein kinase BUB1 beta (BubR1), a regulator that acts as a mitotic checkpoint and safeguards the faithful segregation of chromosomes, improves healthspan and extends longevity in mammals [49], whereas, by contrast, BubR1 mutant mice have a markedly shortened lifespan, accumulate senescent cells, and exhibit a variety of age-related phenotypes [50].

In a similar vein, a recent study showed that deficiency of components of the nucleotide excision repair system (NER) in *C. elegans* further increases the lifespan of long-lived mutants (such as *daf-2* animals) [51], possibly through an adaptive activation of stress signaling, but it had no effect in a wild type background, in line with previous reports [52,53]. Moreover, NER deficiency leads to a striking transgenerational decline in replicative capacity and viability of proliferating cells.

Cellular senescence entails complex changes in cellular morphology, structure, and function. Among other features, a gradual decrease in telomeric length at the ends of chromosomes (known as telomere attrition) is a key contributor to the irreversible growth arrest of senescent cells. This occurs because DNA replicating polymerases are unable to operate at chromosomal ends [54]. These regions can only be replicated by the enzyme telomerase, which in somatic cells is not normally expressed or expressed at very low levels. Thus, unlike in stem cells that express telomerase, chromosomes of somatic cells are trimmed off their protective telomeric sequences during each cell cycle, contributing to telomeric exhaustion and senescence. The link between telomeric attrition and organismal aging has become clearer in the past few years with the analysis of mice engineered to carry either longer or shorter telomeric sequences. Confirming the hypothesis, mice with longer telomeres exhibit increased lifespans whereas, conversely, mice with shorter telomeres have shorter lifespans [55–58]. Moreover, recent work indicates that reactivation of telomerase in telomerase-deficient mice at an adult stage is sufficient to prevent the decreased lifespan of these mutants [59]. In addition, systemic viral transduction of telomerase in wild type mice resulted in extension of their lifespan without increasing the incidence of tumorigenesis [60].

A recent study provides additional mechanistic clues as to how telomerase deficiency can induce aging phenotypes. Analysis of mice deficient in the enzyme telomerase reverse transcriptase (TERT), exhibiting telomere dysfunction, revealed a marked compromise in mitochondrial biogenesis and function in diverse tissues, suggesting that a fundamental problem in energy maintenance might contribute to the premature aging phenotypes in these mice [61]. These marked mitochondrial changes seem to be caused by the combined suppression of the transcriptional co-activators peroxisome proliferator-activated receptor γ (PPAR-γ) coactivator 1α (PGC1α), PGC1β, and their downstream targets, and provide for the first time a link between telomeric attrition and the metabolic state of the cell. Indeed, telomere dysfunction activates p53, which in turn binds to the promoters of the genes encoding PGC1α and PGC1β and suppresses their expression; accordingly, telomere-dysfunctional mice lacking p53 have normal expression of PCGs, normal mitochondrial DNA content, and improved biomarkers of aging [61].

Although cellular senescence is unequivocally a key component of aging [62], its contribution to the process of aging remains controversial. Senescence involves the irreversible proliferative arrest of damaged or dysfunctional cells, contributing to restrict tumorigenesis, but also leading to the accumulation of senescent cells that impair the proper function of that tissue [62]. There is evidence that senescent cells accumulate during the course of aging in primates [63]; however, it has been difficult to establish whether cellular senescence contributes to organismal aging. In a recent study, life-long removal of senescent cells from the BubR1 mutant progeroid mouse, using an approach engineered to eliminate senescent cells positive for the cyclin-dependent kinase inhibitor p16Ink4a by administration of a drug, is sufficient to delay the onset of age-related pathologies; however, its effect on longevity has not been assessed [64].

In humans, premature aging syndromes also often occur due to mutations in nuclear proteins involved in maintaining genome integrity. For example, truncation of lamin A, a major component of the nuclear lamina and nuclear skeleton, or lack of Zmpste24, a metalloproteinase responsible for the maturation of prolamin A, cause Hutchinson–Gilford progerial syndrome (HGPS), a severe form of early-onset premature aging [65,66]. Expression of an aberrant isoform of prolamin A, named progerin, is also associated with aging in humans [67], and was recently shown to impair the differentiation of mesenchymal stem cells by inducing oxidative stress [68]. Consequently, defects in nuclear lamina that have been repeatedly associated with human aging or cellular senescence, such as changes in lamin-A, or decreased levels of lamin-B [69], are considered as biomarkers of aging [70,71]. Taken together, these findings demonstrate that loss of genetic and genomic stability is associated with aging and progeroid diseases. Moreover, mutations in key elements of the machinery that safeguards genetic and genomic stability result in accelerated aging and compromised lifespan.

miRNAs

At the post-transcriptional level, gene expression is regulated by micro RNAs (miRNAs) – small, non-coding RNAs that function either by blocking translation or mediating

the degradation of mRNA targets (reviewed in [72]). The primary miRNA transcript, transcribed by RNA polymerase II, is processed by the endonucleases Drosha and Dicer to generate a short RNA duplex. One strand of the duplex is loaded into the RNA-induced silencing complex (RISC) to bind to the target mRNA, whereas the other strand is degraded. *C. elegans* has been instrumental not only in facilitating the discovery of miRNAs [73] but also in unraveling their role in longevity. For example, loss of function of the miRNA *lin-4* significantly shortens lifespan, whereas *lin-4* overexpression extends the survival of nematodes. As demonstrated by epistasis analysis, lifespan modulation requires the *lin-4* target gene *lin-14* [74]. Although the mechanism downstream the *lin-4/lin-14* axis remains elusive, it is known to involve some components of the IIS pathway. Indeed, *lin-14* loss-of-function does not alter survival of long-lived insulin/IGF-1-deficient *daf-2* mutants, but it does require the downstream target DAF-16/FOXO to lengthen *C. elegans* lifespan.

Additional miRNAs that can affect the longevity of *C. elegans* through DAF-16/FOXO mediated gene transcription have recently been identified [75]. One such example is *miRNA-71*. Loss of *miRNA-71* function significantly decreases resistance to heat shock and oxidative stress, and abolishes the lifespan extension of animals that lack a germline. Thus, together with secreted signals from the reproductive system, *miRNA-71* likely targets a variety of factors across different tissues – culminating in the nuclear redistribution of DAF-16/FOXO in the intestine to extend lifespan [76]. Moreover, *miRNA-71* belongs to one of the three miRNAs whose patterns in early adulthood were recently found to predict lifespan differences significantly. Although the levels of all three miRNAs increase with aging, *miRNA-71* and *miRNA-246* levels correlate with lifespan, whereas *miRNA-239* levels show reverse correlation [75,77]. Confirming earlier findings, *miRNA-71* and *miRNA-239* act upstream of the IIS pathway. Thus, fluctuations in the insulin pathway during early life, due to variation in these miRNAs or due to other causes, may determine individual lifespan.

The levels of *miRNA-34* were also shown to increase with aging. In one study, *miRNA-34* was found not to affect *C. elegans* lifespan [75], whereas another study indicates that its inhibition enhances lifespan by regulating autophagy [78]. In flies, loss of *miR-34* triggers a decline in survival [79]. Therefore, there is still some controversy on the role of *miRNA-34* in regulating longevity. Another recent study demonstrates that a single miRNA, named *miRNA-80*, is responsible for shutting off DR pathways in *C. elegans* when food is available [80]. Deletion of *miRNA-80* leads to activation of the DR pathways, causing an extension of both the healthspan and lifespan of the nematode. Therefore, *miRNA-80* acts as a core regulator of the nutrient response system, orchestrating diverse and intersecting metabolic pathways. It is not known, however, whether the levels of this miRNA fluctuate among individuals or whether they are temporally regulated.

Several miRNA expression profiles have been generated to determine changes during aging in mammalian cells and tissues. However, because lifespan assays are not as trivial with mammals, most studies have focused on the effect of miRNAs in specific tissues or specific diseases. For example, a recent study in mouse models of senescence suggests that *miRNA-29*, which is increased with aging, targets type IV collagen genes [81]. Type IV collagen is important for the maintenance of the structure of the extracellular matrix. Increased *miRNA-29* levels in aged murine tissues decrease type IV collagen expression and weaken basement membranes. Interestingly, in mice, *let-7* targets various components of the IIS and mTOR pathways through the RNA-binding protein Lin28 [82], with important consequences for glucose metabolism. However, the effect of *let-7* miRNA in longevity has not yet been determined. Thus, although it is becoming increasingly appreciated that distinct miRNAs can either promote or decrease longevity, more work is necessary to unravel their targets and the pathways they regulate.

Stem cells and their niche during aging

Adult or tissue stem cells represent small and quiescent populations that are present in multiple tissues, and thus can specifically regenerate the cells of the tissue they belong to. In this way, they largely determine the ability of tissues to regenerate not only during the normal wear and tear but also in response to injury. Stem cell self-renewal, maintenance, and differentiation are ultimately regulated by the integration of local and systemic signals with intrinsic factors that determine stem cell behavior. Not surprisingly, aberrant stem cell function not only is a hallmark of aging but it is also thought to contribute to tissue senescence and failed function [83] (Figure 1).

Adult stem cells in *Drosophila* include both the GSCs in the ovary and the testis, and the intestinal stem cells (ISCs) in the midgut. They all reside in well-defined niches and have active roles in maintaining local tissue homeostasis, similarly to their mammalian counterparts [84]. Furthermore, it appears that additional stem-cell-like compartments exist in *Drosophila* and are beginning to be identified [85,86]. By contrast, *C. elegans* consists only of post-mitotic cells, with the exception of germline precursor cells, the removal of which, as already discussed, earlier confers lifespan extension. In mammals, stem cells are found in multiple tissues, including the hematopoietic system, the nervous system, the skin, and muscle.

A straightforward notion posits that aging is accompanied by an overall decline in the number of stem cells – and that tissues fail simply because they run out of stem cells. However, this is not universally true. For most tissues there is more than enough stem cell potential, as best demonstrated by serial transplantation of hematopoietic stem cells (HSCs), which can sustain mice across multiple lifespans [87]. In mice, the number of muscle stem cells seems to be relatively constant with age, but may either increase or decrease depending on the specific muscle examined [88]. In the *Drosophila* midgut, aging is characterized by an increase in the percentage of proliferative stem cells [89]. However, there are also clear examples of tissues in which stem cells do decline with age. In mammals, this is the case for neural stem cells, melanocyte stem cells, and spermatogonial stem cells [90–93], which are typically exhausted because they fail to self-renew. In neural stem cells, self-renewal is regulated by the FoxO

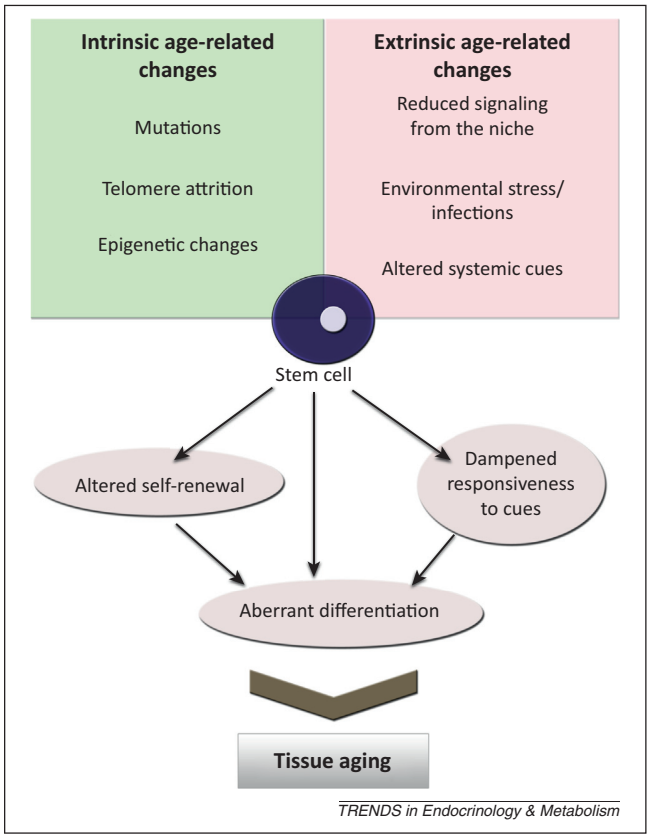

Figure 1. Overview of stem cell aging. Several factors can influence the aging of adult stem cells. These include intrinsic factors, such as the accumulation of mutations, telomeric attrition, and epigenetic changes, as well as extrinsic factors such as reduced signaling from the niche, exposure to stress or infections, as well as altered availability of systemic cues. Integrating these age-related changes, stem cells may exhibit a decreased potential for self-renewal, leading to stem cell exhaustion. Alternatively, they may fail to undergo differentiation or exhibit a biased differentiation potential, leading to the accumulation of stem cells or the accumulation of one somatic cell type. Another possibility is that the responsiveness of stem cells to specific cues in their environment is dampened, resulting in failure to function that is not accompanied by changes in stem cell number. Compromised stem cell function associated with aging leads to decreased tissue homeostasis and maintenance.

transcription factors [90,91], which are key effectors of the IIS pathway. In melanocyte stem cells, it appears that self-renewal is impaired as a result of precocious differentiation and sensitivity to DNA damage [94]. In *Drosophila*, there is an age-related decline in the number of GSCs, which results in reduced gametogenesis in aging males and females [95,96]. Both in mammals and in *Drosophila*, although the loss of stem cells clearly has functional implications for their associated tissue, there is no evidence that it results in a reduction in lifespan.

Accumulating evidence suggests that with aging stem cells lose their ability to respond to cues from their environment, or alternatively that the cues themselves are dampened. As already mentioned, stem cells reside in well-defined niches and depend on this environment for their proper function. For muscle stem cells (also known as satellite cells), it has been shown that with aging they fail to respond well to extrinsic signals from their niche or activate the necessary pathways, including Notch and Wnt signaling pathways. As a result they exhibit an age-related decline in proliferation and differentiation potential [97,98]. A recent study demonstrated that the aged muscle stem cell niche expresses fibroblast growth factor 2 (FGF2), compromising the quiescence and self-renewing capacity of a subset of satellite cells [99]. Sprouty1 (Spry1), an inhibitor of FGF signaling, was found to be expressed in dormant aged satellite cells. Increased FGF signaling in aged satellite cells, by removing Spry1, causes loss of quiescence, satellite cell depletion, and diminished regenerative capacity. Conversely, reducing niche-derived FGF activity through inhibition of fibroblast growth factor receptor 1 (FGFR1) signaling or overexpression of Spry1 in satellite cells prevents the age-related phenotypes. These findings indicate that an age-dependent change in the stem cell niche can directly influence stem cell characteristics and function [99]. Similarly, age-related depletion in the *Drosophila* testis is also largely induced by the aging of the niche. In young individuals, IGF-II mRNA-binding protein (Imp) counteracts endogenous small interfering RNAs to stabilize the cell adhesion molecule DE (*Drosophila* epithelial)-cadherin and a key self-renewal signal unpaired (upd) RNA. However, similarly to upd, Imp expression decreases in the niche cells of older males, and this is due to the targeting of Imp by the heterochronic miRNA *let-7*. In the absence of Imp, upd mRNA becomes unprotected and susceptible to degradation, leading to reduced self-renewal and stem cell depletion [100].

Additional mechanisms of age-related stem cell dysfunction have also been postulated. Recent work examined how both infectious and indigenous bacteria modulate stem cell activity in the *Drosophila* midgut. These studies revealed that some bacterial infections induce stem cell proliferation via the JAK-STAT (Janus kinase/signal transducer and activator of transcription) and JNK (c-Jun N-terminal kinase) signaling pathways. Similar effects, but of a smaller magnitude, could also be induced by indigenous gut microbiota. Indeed, altered control of gut microbiota in aged flies correlated with increased epithelium renewal [101]. The intrinsic and extrinsic changes that could contribute to aged stem cell phenotypes, including genetic and epigenetic changes that lead to altered gene expression profiles, are reviewed in detail elsewhere [102]. Future research in model organisms could test whether delaying age-related changes in stem cells or their niches is sufficient to delay organismal aging, presumably in a non-cell autonomous manner, and identify the systemic cues involved.

Immunity, inflammation, and microbiota in aging: the good, the bad, and the ugly

During aging, innate and adaptive immune responses gradually deteriorate, leading to immunosenescence. Upon invasion of pathogenic organisms, innate immunity is triggered as the first line of defense, through pattern recognition receptors (PRRs), including Toll-like receptors (TLRs) and cytoplasmic receptors, which recognize pathogen-associated molecular patterns (PAMPs). Innate immune responses are followed and complemented by the activation of the adaptive immune system, particularly when innate immunity is overwhelmed [103]. These defensive mechanisms decline progressively in the elderly, increasing susceptibility to infections and causing low-grade inflammation (Figure 2).

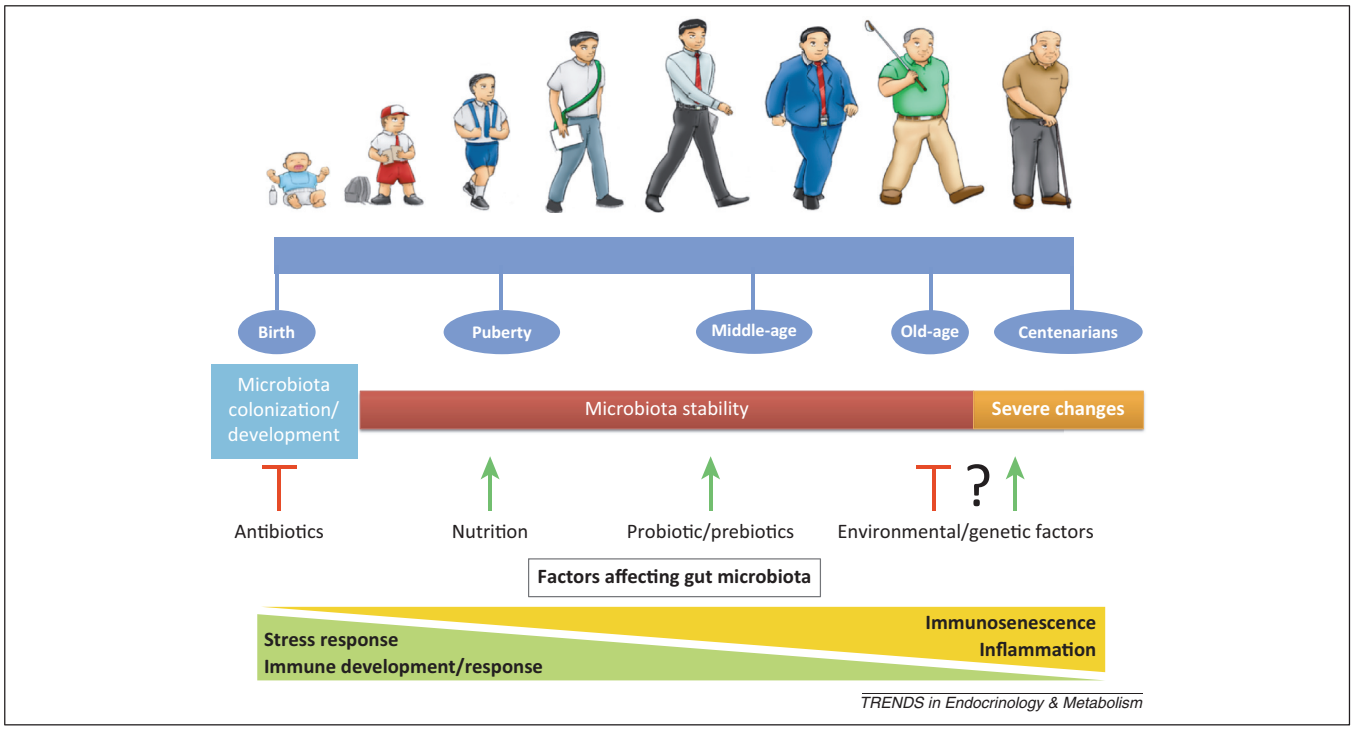

Figure 2. Human life cycle, microbiota, and stress responses. Recent studies shed light in the composition of the gut microbiota throughout life and elucidate their role in aging. It is now known that microbiota colonization is initiated already from birth, and that the development of gut microbiota has reached the highest level by the age of 2 years. After maturation, microbiota remain mostly stable during human life, and significant changes have only been observed in centenarians. Factors implicated in microbiota maturation and development are indicated. Stress and immune responses deteriorate during aging, causing low-grade inflammation and increased susceptibility to infections, which collectively lead to severe maladies.

This age-associated, chronic low-grade proinflammatory phenotype, referred to as inflammaging, is caused by an imbalance between inflammatory and anti-inflammatory mechanisms [104]. One such mechanism involves senescent cells which may initiate and induce chronic inflammation through a senescence-associated secretory phenotype (SASP) whereby senescent cells secrete a plethora of cytokines, chemokines, and proteases. SASP mechanisms as well as therapeutic interventions designed against cellular senescence and SASP are reviewed elsewhere [105]. The onset of immunosenescence transpires as early as at the age of 29 years in men and at the age of 43 years in women. It remains unclear how genetic, environmental and life style factors are associated with this difference [106]. This evolutionary conserved and multifaceted phenomenon becomes apparent in advanced age, where vaccine efficacy is immensely reduced [107]. Thus, healthy aging may be promoted through more efficient pharmacological and vaccination strategies, based on deeper understanding of the mechanisms and factors involved in immunosenescence.

Innate immunity pathways are evolutionary conserved from nematodes to mammals. In *C. elegans*, mechanisms regulating immunity include the longevity-regulating p38/MAPK, transforming growth factor β (TGF-β) and insulin-like receptor pathways [108]. Activating innate immunity with small molecules such as RPW-24, or the alkaloid compound harmane (2-methyl-β-carboline), leads to an increase in the lifespan of *C. elegans* under conditions of infection with pathogenic bacteria, via mechanisms that modulate the p38/MAPK pathway and upregulate immune effector genes [109,110]. Transcription factors such as SKN-1, DAF-16/FOXO, and DAF-19, that are involved in stress responses and sensory neuron cilia formation, have also been shown to play a role in immunosenescence and in pathogen resistance [111–113]. Similarly to human immunosenescence, *C. elegans* immune responses decline during aging, exacerbating mortality due to infection associated with a decline of the p38/MAPK pathway [114]. Recent findings in mammals have shown that hypothalamic immunity mediated by IκB kinase-β (IKK-β), nuclear factor κB (NF-κB), and related microglia–neuron immune crosstalk determines whole-body aging, highlighting the systemic impact of immunity on aging [115].

The molecular underpinnings of age-associated deterioration of the adaptive immune system remain elusive. An important emerging determinant that potentially influences the development and proper function of immune cells is commensal and symbiotic microbiota (Figure 3). Studies performed in germ-free animals demonstrate that, in the absence of gut microbiota, the immune system is compromised, exhibiting a smaller immune cell population and reduced expression of immune response molecules such as TLRs and class II major histocompatibility complex (MHC II) molecules [116–118]. Microbiota colonization is already established during birth, through exposure to the skin of the mother in case of caesarean section or through exposure to a more complex microbial population derived from the birth canal during natural birth, determining microbiota biodiversity [119]. After the first year, during which microbiota composition fluctuates, the microbiota matures to resemble that of an adult (Figure 2) [120]. The human body is dominated mainly by bacteria (Bacteroidetes,

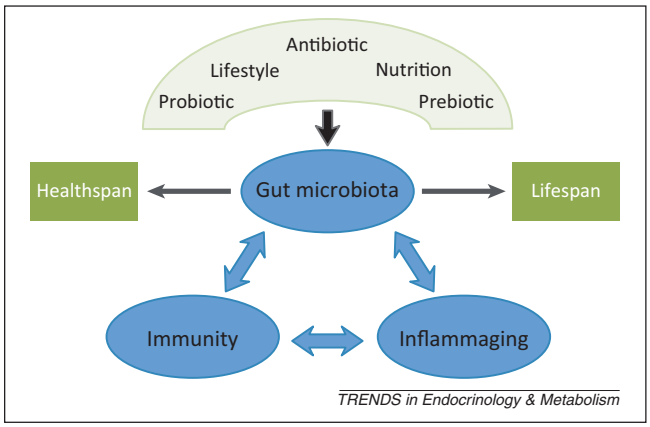

Figure 3. Gut microbiota, immune responses, and inflammation: implications in health and lifespan. Emerging evidence has revealed extensive crosstalk between microbiota, the immune system, and inflammation pathways that influences aging in humans. This interplay is mediated by various genetic and environmental factors in addition to lifestyle.

Firmicutes, and to a lesser degree Actinobacteria, Proteobacteria, Fusobacteria, and Verrucomicrobia), and a limited fraction of archaea, viruses, and eukaryota, forming a complex ecosystem that is becoming appreciated as an important modulator of host health and disease, by influencing host metabolism, immune responses, and physiology [119–121]. Indeed, microbial diversity and homeostasis has been linked to development, aging and diverse pathological conditions, including metabolic syndrome, cancer, cardiovascular diseases, stress, and obesity [122]. Compelling evidence derived from studies performed in *C. elegans* have shown clearly that bacterial metabolic activity is required for normal growth and metabolism, with alterations directly impacting upon animal development and aging [123–126]. Similarly, microbiota may exert direct or indirect effects upon host physiology by influencing drug bioavailability and efficacy, or by metabolizing drugs to beneficial or harmful metabolites. Several pharmacological substances have been found to exert adverse or secondary effect by altering the properties of microbiota [127–129]. For instance, metformin (a widely used antidiabetic drug) was found to increase lifespan in rodents and worms, and this effect was attributed to altered microbial metabolism [127,130]. Thus, gut microbiota diversity and activity has attracted much attention recently as a candidate determinant of longevity and healthspan. Several large-scale projects have been initiated to characterize customary microbiota and their irregularities that are linked to disease [131,132].

During aging, profound microbiota modifications occur, indicating that sustaining microbiota function and biodiversity during aging is important for healthy aging (Figure 2). Increasing efforts are focusing on how to preserve colonization of normal metabolically functional microbiota. To this end, pro/prebiotic consumption has been suggested towards maintaining human microbiota phylogenetic composition and suppressing frailty. Indeed, diet has been demonstrated to have a broad impact on microbiota by promoting their biodiversity [133–135]. Particularly during early childhood, extensive changes take place depending on diet composition [136]. Breast-fed infants exhibit a more favorable microbiota than formula-fed children, and changes also ensue with weaning. Evidence from model organisms support this notion and further outline the interaction between gut microbiota, nutrition, and healthy aging [137–139]. As discussed earlier, caloric restriction expands lifespan, but the underlying molecular mechanisms are still under debate. A recent study discusses how caloric restriction gut positively affects microbiota in C57BL/6J mice to exert a health benefit to the host [137]. Furthermore, it was demonstrated in non-human primates that the metabolic actions of gut microbiota were altered under conditions of caloric restriction, modulating the immune response of the host – and potentially influencing age-associated diseases and aging of the primates [138]. Thus, there is growing appreciation of the importance of symbiosis, and that interactions between host and microbiota may explain several phenomena that have so far eluded understanding.

Proteostasis-related stress response pathways: Dr Jekyll or Mr Hyde?

One of the major hallmarks of aging is the loss of proteostasis, allowing damaged proteins and organelles to accumulate, leading to diverse pathologies. The sophisticated machineries that restore protein homeostasis and normal cellular and organismal function become compromised during aging, leading to adverse outcomes including cell death. Some of these housekeeping mechanisms include the stress response pathways, the autophagy–lysosomal machinery. and the ubiquitin–proteasome system (UPS) degradation pathways.

Aging is accompanied by a progressive decline in autophagy [45,140]. It is now clear that normal autophagy is required for healthy old age and longevity. Accumulating evidence from animal models implicates autophagy in senescence-regulating cellular pathways [45]. These findings are fuelling novel strategies and approaches to prevent or treat human diseases and impede aging through induction of autophagy [141–144]. For example, peptides derived from the autophagy protein beclin 1, developed for direct and specific induction of autophagy, have been shown to reduce infection-related mortality [143]. Proteasomal degradation pathways also decline during aging, and similar interventions could be designed to treat pathological conditions [145]. Indeed, studies in yeast and *C. elegans* have shown that augmenting the UPS improves protein homeostasis and extends lifespan [146,147], suggesting that failure of proteostasis contributes to aging.

Alternative proteostasis and quality control mechanisms also exist in different cellular compartments. Components of the heat-shock response (HSR) neutralize aberrant protein folding, mainly in the cytoplasm, whereas distinct unfolded protein responses (UPRs) serve similar functions in the endoplasmic reticulum (ER) and mitochondria. The IIS pathway regulates lifespan in part by controlling stress responses via the transcription factor PQM-1 (paraquat/methylviologen- responsive) [148]. Protein homeostasis in the cytoplasm is regulated predominantly by the heat-shock factor family of transcription factors (HSFs) which coordinate the action of heat-shock proteins (HSPs) [149]. Mitochondrion-specific UPR mechanisms

and the stress sensors are currently under investigation (reviewed in [150,151]). In the ER, proteostasis is regulated by intricate pathways, mediated mainly by the three ER stress sensors: the protein kinase R (PKR)-like ER kinase (PERK), activating transcription factor 6 (ATF6), and the inositol-requiring protein 1 (IRE1) [149].

Emerging evidence implicates miRNAs in the regulation of stress pathways, probably by acting as rheostats of the UPR machinery, coupling different arms of the UPR and regulating ER stress-induced apoptosis [152–155]. ER stress has been implicated in the hormetic regulation of longevity and aging, whereby that mild and acute exposure to stressors may be beneficial for the long-term survival of organisms (the concept of hormesis). The main function of UPR is to restore ER proteostasis through the attenuation of *de novo* protein synthesis and augmentation of protein folding by inducing the transcription of chaperones. However, under extreme stress conditions, induction of cell death clears irreversibly damaged cells, limiting further systemic damage [156]. Recent findings in *C. elegans* indicate that cell non-autonomous control of proteostasis enhances adaptation to stress and promotes longevity [157–159]. Thus, mechanisms controlling proteostasis emerge as attractive targets for interventions against morbidities caused by aberrant proteostasis. To this end, several small molecules that augment the maintenance of proteostasis are being investigated for their effects on pathologies and aging in multiple model organisms [160,161].

Concluding remarks and future perspectives

Driven by changing demographics in the recent decades, the field of aging has built up solid resources to investigate the mechanisms that control longevity. These include experimental models from different phyla, including in particular *S. cerevisiae, C. elegans, D. melanogaster*, rodents, and primates. Based on these models, several signaling pathways have been identified that control aging in a well-conserved fashion across the evolutionary spectrum, such as the insulin and TOR signaling pathways, serving broad nutrient-sensing functions. Although indications are accumulating that these pathways may operate to influence similar traits in humans, more work is necessary to consolidate this hypothesis and develop molecules that can safely improve human healthspan and quality of life in the elderly. In aid of such efforts, ongoing whole-genome sequencing of centenarians may reveal novel genes that control human longevity or that can be alternatively used for the development of useful biomarkers of aging.

At the same time, it becomes increasingly appreciated that the process of aging can be modulated by several factors representing genetic, epigenetic, and environmental or tissue-specific components of an intricate control network. For example, age-associated changes in stem cell pools have recently been analyzed; however, whether these changes are actually responsible for tissue failure during aging is only beginning to be addressed (Figure 1). Moreover, although the importance of specific tissue-stem cells in organismal longevity is clear in some invertebrate models (for example in *Drosophila* [89]), the relationship between stem cell function and organismal longevity has yet to be demonstrated rigorously in mammals. Novel roles are also emerging for chromatin modifiers in the regulation of cellular and organismal senescence. With respect to the aforementioned putative role of stem cells in aging, it will be interesting to determine whether modulation of specific

Box 1. Epigenetic regulation of longevity

Chromatin is broadly divided into two types: transcriptionally inactive heterochromatin and transcriptionally active euchromatin. Whether chromatin forms euchromatin or heterochromatin depends on modifications of the histones and DNA. For example, acetylation of the N-terminal tails of histones promotes the formation of euchromatin, whereas DNA methylation promotes heterochromatin formation.

Histone acetylation is controlled by histone acetyltransferases (HATs), enzymes that add acetyl groups to histones, and histone deacetylases (HDACs) which remove acetyl groups [166]. In *S. cerevisiae*, inactivation of the histone deacetylase, Sir2, shortens replicative lifespan [167–169]. Conversely, activation of Sir2 extends lifespan. Orthologs of Sir2 have anti-aging functions in many other species, including nematodes and flies [167,169,170]. In mammals, decreased expression of the closest mammalian ortholog of Sir2, Sirt1, correlates with apparent premature aging of mice and increased activity of p53 family members [171]. Sirt1 protein levels decrease with age in mitotic tissues [172]. Thus, the ability of Sir2-like proteins to regulate aging appears to be conserved through evolution. However, Sirt1 has many non-chromatin substrates [169], and whether any effect on mammalian aging is epigenetically determined remains to be established. Moreover, new findings that overexpression of Sir2 does not extend lifespan in *Caenorhabditis elegans* and *Drosophila* have challenged this view [173]. A recent study demonstrated that male, but not female, transgenic mice overexpressing Sirt6 have a significantly longer lifespan than wild type mice. Gene expression analysis revealed significant changes in male Sirt6 transgenic mice, such as lower serum levels of IGF1, higher levels of IGF-binding protein 1, and altered phosphorylation levels of major components of IGF1 signaling, a key pathway in the regulation of lifespan [174].

It is long known that, during aging, mammalian cells undergo a global decrease in DNA methylation, whereas some promoters become aberrantly hypermethylated [175–177]. These include promoters of several tumor-suppressor genes [178], suggesting that age-related methylation changes increase cancer susceptibility. To determine whether histone methylation regulates lifespan, Brunet and colleagues performed a targeted RNA interference (RNAi) screen in *C. elegans* and identified the ASH-2 COMPASS complex which trimethylates histone H3 at lysine 4 (H3K4). Deficiency in members of the ASH-2 complex, including the H3K4 methyltransferase SET-2, extended the lifespan of the nematode. However, the H3K4 demethylase RBR-2 is also required for normal lifespan, consistent with the idea that excess H3K4 trimethylation – a mark associated with active chromatin – is detrimental for longevity [179]. Thus, histone methylation plays an important role in organismal aging. Additional evidence has also revealed that there is an interaction between the germline and the soma for the regulation of lifespan by chromatin regulators [180].

A fundamental question is whether epigenetic changes are transgenerational. Although some evidence of transgenerational epigenetic inheritance for simple traits exists, very little is known about the transgenerational inheritance of acquired complex traits. Surprisingly, recent work in *C. elegans* demonstrated that mutations in specific regulators of trimethylated lysine 4 on histone H3 (H3K4me3) in parents lead to lifespan extension in descendants for up to three generations, even after the initial mutation is no longer present [181]. These results can potentially revolutionize our understanding of the inheritance of integrative phenotypes, including aging and longevity.

Box 2. Outstanding questions

The gonad and nutrient-responsive pathways
- How does GSC ablation change the metabolic homeostasis of somatic cells?
- Are the relevant mechanisms evolutionarily conserved?
- By which mechanisms do increased autophagy and reduced protein synthesis promote longevity?

Genome integrity and stability
- Does removal of senescent cells promote longevity in diverse species?
- Through which signaling mechanisms does telomerase deficiency influence the metabolic state of cells?

miRNAs
- Which miRNAs influence longevity in mammals?
- Do centenarians exhibit distinct miRNA expression profiles?

Stem cells
- Are age-associated changes in stem cell pools responsible for tissue failure?
- Can modulation of specific chromatin regulators restore the properties of stem cells that become compromised with aging, thereby ameliorating age-dependent tissue dysfunction?

Immunity, inflammation, and microbiota in aging
- Do retrograde interactions (from the host towards microbiota) occur and what are their consequences?
- How crucial is host status (endocrine status, metabolic status, stress, etc.)? Do physiological (puberty, pregnancy, menopause, aging) or pathophysiological conditions have an impact on microbiota composition/activity?
- Are sanitation, easy access to antibiotics, and nutritional changes affecting health, disease, and aging via microbiota, and to what extent?
- To what extent does the microbiota affect the metabolism of pharmaceutical agents, altering their efficacy and toxicity?
- Do endangered microbiota species exist than need to be preserved?
- Because gut microbiota composition is mostly stable for most of the life of the host, is their activity responsible for the effects to the host and is there an age threshold above which their functionality is changed?
- What is the impact of diet, use of pro/prebiotics, and environmental factors on microbiota and immune development?
- To what extent can personalized medicine be achieved by interventions to preserve the phylogenetic composition of human microbiota?
- How do sex differences and modern lifestyle, including high calorie consumption and reduced exercise, influence immunosenescence?

Stress response pathways
- How are distinct stress pathways coordinated in response to different types and levels of stress in different tissues?
- How do stress response pathways and hormesis influence age-associated diseases?
- What is the role of miRNAs in the regulation of canonical and non-canonical stress pathways?

chromatin regulators can restore the proliferative and multipotential properties of stem cells that become compromised with aging, thereby ameliorating age-dependent tissue dysfunction. More generally, many questions regarding epigenetics and its role in aging remain open (Box 1). For example, it will be important to investigate whether 'epimutations' – errors in the elaborate apparatus of epigenetic silencing – accumulate stochastically with aging in different tissue types and what the consequences are of this accumulation. Moreover, identifying genes that are responsible for enhanced disease susceptibility when epigenetically deregulated is an attainable goal. Finally, understanding the interactions of the environment with the epigenome and their role in aging will facilitate the development of novel therapeutic approaches.

Over the past years our understanding of microbiota composition and function has advanced considerably, notwithstanding the novel challenges and many outstanding questions (Box 2). Their involvement in immunosenescence, immune mechanisms, and metabolism establishes the microbiota as an appealing target for therapeutic intervention of various diseases and aging. Thus, microbiota manipulation via simple interventions such as change of diet and lifestyle has the potential to augment healthy aging and ameliorate challenging and socioeconomic burdens associated with disease. An additional determinant of aging and age-associated disorders is the capacity to preserve protein homeostasis. Organisms are endowed with diverse mechanisms of adaptation against stress, and these mechanisms impinge on the course of aging. Manipulation of stress responses and the UPR is currently being evaluated as an intervention strategy to remedy various age-related pathologies such as diabetes, cancer, and cardiovascular and neurodegenerative diseases. Therefore, it becomes imperative to shed more light on the mechanisms underlying the interplay between different stress responses and their systemic effects in coordinating aging across different tissues in the context of the whole organism.

Undoubtedly, considerable progress in unraveling the multifactorial regulation of aging and longevity has been accomplished in recent years. Nevertheless, several questions have emerged from new findings, the most pertinent of which are summarized in Box 2. Answering these questions will be essential towards furthering our overall understanding of how aging is regulated at the organismal level. This in turn will facilitate the design of novel intervention strategies to ameliorate some of the aging-associated phenotypes and thereby improve human healthspan in the future.

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