Review

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Feature Review

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.