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Biology of Healthy Aging and Longevity

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ABSTRACT

As human life expectancy is prolonged, age-related diseases are thriving. Aging is a complex multifactorial process of molecular and cellular decline that affects tissue function over time, rendering organisms frail and susceptible to disease and death. Over the last decades, a growing body of scientific literature across different biological models, ranging from yeast, worms, flies, and mice to primates, humans and other long-lived animals, has contributed greatly towards identifying conserved biological mechanisms that ward off structural and functional deterioration within living systems. Collectively, these data offer powerful insights into healthy aging and longevity. For example, molecular integrity of the genome, telomere length, epigenetic landscape stability, and protein homeostasis are all features linked to "youthful" states. These molecular hallmarks underlie cellular functions associated with aging like mitochondrial fitness, nutrient sensing, efficient intercellular communication, stem cell renewal, and regenerative capacity in tissues. At present, calorie restriction remains the most robust strategy for extending health and lifespan in most biological models tested. Thus, pathways that mediate the beneficial effects of calorie restriction by integrating metabolic signals to aging processes have received major attention, such as insulin/insulin growth factor-1, sirtuins, mammalian target of rapamycin, and 5' adenosine monophosphate-activated protein kinase. Consequently, small-molecule targets of these pathways have emerged in the impetuous search for calorie restriction mimetics, of which resveratrol, metformin, and rapamycin are the most extensively studied. A comprehensive understanding of the molecular and cellular mechanisms that underlie age-related deterioration and repair, and how these pathways interconnect, remains a major challenge for uncovering interventions to slow human aging while extending molecular and physiological youthfulness, vitality, and health. This review summarizes key molecular mechanisms underlying the biology of healthy aging and longevity. (REV INVES CLIN. 2016;68:7-16)

Key words: Aging. Sirtuin. Calorie restriction. Acetylome. Genome. Epigenetics.

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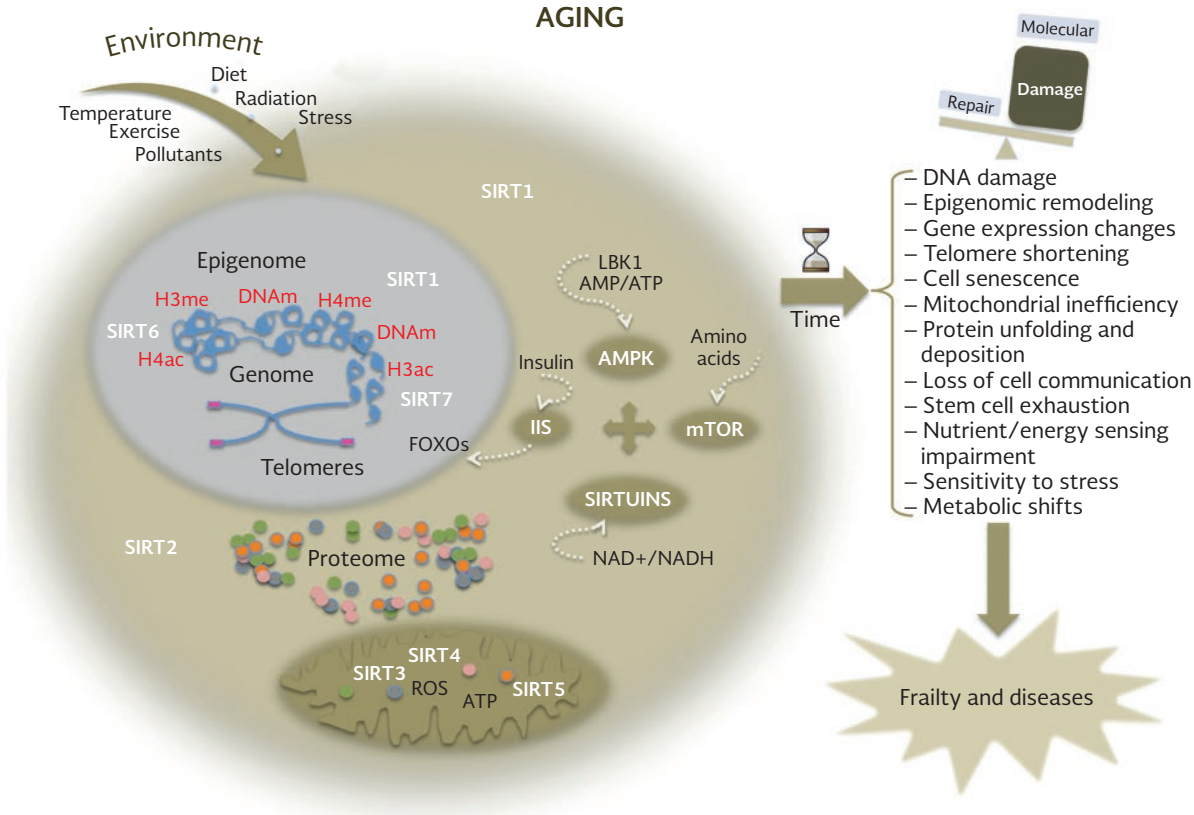
INTRODUCTION

Aging is a process of gradual physiological deterioration that all living beings experience with time. It is a heterogeneous and heterochronic process. As a heterogeneous process, aging may occur at different rates across diverse organisms, and even organisms of the same species can age at variable rates. Furthermore, the asynchrony by which various cells and tissues age within a single organism highlights the heterochronic nature of aging. At the biological level, aging is characterized by the accumulation of molecular and cellular damage, which leads to structural and functional aberrancies in cells and tissues, such as loss of mitochondrial homeostasis, impaired intercellular communication, senescence (cell arrest that hampers growth and division), and decreased regenerative capacity[1]. The dynamic interaction between a living being and its environment defines the rate and fate of aging, as is shown in figure 1. The ability of organisms to overcome stress and respond to external environmental challenges/insults is blunted within aged individuals when compared to younger counterparts[2,3]. Healthy aging, however, refers to the warding off of molecular and cellular decline for the longest length of the lifespan. Not surprisingly, healthy aging

Figure 1. Molecular mechanisms of aging. Homeostasis of the genome, telomeres, epigenome and proteome, all contribute to molecular integrity and healthy aging. Biological pathways that have the dual ability of sensing nutrients and/or energy levels, while also regulating cellular processes like epigenomic remodeling, gene expression, protein activity and organelle integrity –*i.e.*, mTOR, IIS, AMPK, sirtuins (SIRT1 in nucleus and cytoplasm; SIRT2 in the cytoplasm; SIRT3, SIRT4 and SIRT5 in the mitochondria; and SIRT6 and SIRT7 in the nucleus)– each play a key role in aging. Moreover, dynamic interaction between a living being and its environment also impacts the rate and fate of aging. Loss of molecular homeostasis leads to the cellular hallmarks of aging, ultimately contributing to frailty and diseases. Epigenetic marks such as methylation (me) or acetylation (ac) of histones H3 and H4 and DNA methylation (DNAm) are shown in red in the nucleus. The mitochondrion is the major source of energy production (ATP) and reactive oxygen species (ROS) generation. Mitochondrial fitness is thus an essential feature of healthy aging.
SIRT: sirtuin; FOXO: forkhead box protein; ATP: adenosine triphosphate; AMPK: AMP-activated protein kinase; IIS: insulin/insulin-like growth factor-1 signaling; mTOR: mammalian target of rapamycin; NAD+: nicotinamide adenine dinucleotide (oxidized); NADH: nicotinamide adenine dinucleotide (reduced form); ROS: reactive oxygen species.



has been associated with increased longevity. This claim is substantiated by the fact that genetic, dietary, and/or pharmacological interventions that promote cellular homoeostasis, stress resistance, and protection against age-related diseases also tend to extend lifespan and *vice versa*[4-6]. Overwhelming scientific evidence supports the claim that there is no single cause of aging. Indeed, notable advancements in the biology of aging, especially during the last few decades, have contributed to the identification of multiple mechanisms that modulate the aging process[1,7-9]. Despite this progress, uncovering interventions that can achieve healthy aging in humans is challenging. The conserved molecular and cellular mechanisms that underlie aging, especially how these pathways interplay and how complex lifestyles and environments to which humans are exposed modify them, are not completely understood.

Biological models such as yeast, worms, flies, and mice have contributed greatly towards understanding the biological mechanisms of aging as they offer many advantages over humans: e.g., they (i) have much shorter lifespans; (ii) are easier to manipulate genetically; and (iii) allow for better experimental control and study design, given the reduced environmental variability while studied. Diverse approaches can be achieved with the use of animal models such as the appealing contributions to the aging field of heterochronic parabiosis experiments in mice, which implies the fusion of an old and young mouse through their capillaries to share blood circulation. Heterochronic parabiosis has shown that systemic administration of young blood plasma into aged mice improves diverse aging features like cognitive impairments, neurogenesis, skeletal muscle regeneration, and cardiac hypertrophy. It also has allowed identifying circulating aging/pro-aging factors such as the growth differentiation factor 11 (GDF11) that acts in muscle and the cytokine CCL11 in the brain[10-12]. Experiments with animal models, especially rodents, have recently challenged the older paradigm of aging as an immutable process. Today, a growing body of evidence suggests that external/environmental manipulations related to diet, exercise, and changes in blood composition (due to the introduction of circulating factors from young animals into older ones) may rejuvenate diverse aspects of the physiology, including those associated with the aged central nervous system as reviewed by Buchard, et al., like the restoration of regenerative capacity and improvement of neuronal synaptic and cognitive function, besides the potential to extend health and lifespan[13].

In humans, most aging studies are performed using peripheral blood samples, biopsies, postmortem tissues, and various types of cells that can be propagated *in vitro* using artificial culturing conditions. In all of these cases, it is difficult to control for individual-level lifestyle/environmental factors between people, which adds substantial experimental variability. Furthermore, additional aging models are being reported in the literature, which hope to elucidate more about the multifactorial process of aging across species. Some of these emerging models include: the shortest-lived vertebrate killifish (~ four months), the longest-lived rodent mole nude rat (30 years), the bowhead whale (presumed to be the longest-lived mammal at 200 years), and bivalve mollusks (survive up to 500 years)[2,14,15]. Across all of these species, diverse experimental approaches have been used to study the biological signals of aging, ranging from the discovery of aging-associated biomarkers, genes/polymorphisms, regulatory proteins, hormones, compounds/metabolites, and various diets (from starvation and reduced calorie intake to intermittent fasting), which may modify lifespan and the aging process, as well as delay the onset of age-associated diseases and/or confer resistance to environmental challenges[8,16-18].

THE LIFELONG EFFECTS OF CALORIE RESTRICTION

Calorie restriction (CR) has endured for more than 75 years as the single most robust method to increase longevity and delay aging and disease across diverse organisms. Clive McCay at Cornell University performed the first CR experiments in 1935, using rats fed with 30% less food than the regular chow given to control-fed (*ad libitum*) rats. The CR diet increased both mean and maximum lifespan by more than 30%[19]. Since these initial experiments, all species tested by feeding them with 20-40% less food, from yeast to rodents, show lifespan extensions of up to 50%[20]. Experiments in rodents and monkeys have shown that CR decreases basal metabolic rate and energy expenditure, while physical activity remains unaltered. In these same animals, CR reversed key physiological biomarkers of aging like endometriosis,

osteoporosis, sarcopenia, high blood pressure, body fat accumulation, and glucoregulation imbalance[21,22]. Molecularly, data show that CR may reverse the nine typical cellular features of aging: (i) telomere erosion; (ii) epigenetic alterations; (iii) stem cells depletion; (iv) cellular senescence; (v) mitochondrial dysfunction; (vi) genomic instability; (vii) proteostasis imbalance; (viii) impaired nutrient sensing; and (ix) abnormal intercellular communication[23].

The beneficial effects of CR have also been documented in experiments using genetic and/or chemical models of disease linked to various cancers, neurodegeneration, cardiac disease, and inflammation[24-26]. Whether CR is sufficient to retard aging and extend longevity in humans is still unknown. Recent studies on a regimen of intermittent CR, induced by subjecting mice and humans to only a few days of fasting per week, have shown rejuvenation in the endocrine, immune, and nervous systems of mice and improvement in biomarkers of diseases (diabetes, cardiovascular disease, and cancer) and regeneration in humans, all without major adverse effects[27]. The "geroprotective" action of CR has led to a growing interest in searching for CR mimetics: i.e., small-molecule therapeutics or other chemicals/interventions that can recapitulate the rejuvenation effects attributed to CR. Examples of some of these compounds include resveratrol, rapamycin, and metformin, which will be discussed further below[28].

GENOME INTEGRITY, TELOMERES AND HEALTHY AGING

The genome contains the whole set of DNA, encoding the biological information (i.e., the genes) that determines the ability and extent to which organisms can develop within and respond, cope, and adapt to their environmental conditions. The genome not only includes genes but also non-coding sequences important for gene regulation. The major portion of the human genome –highly structured and ordered in the nucleus– is comprised of ~3 billion base pairs of linear DNA (called the "nuclear genome"); about less than 2% of these billion base pairs encode approximately 25,000 human genes. Lastly, a very small amount of circular DNA containing only 37 genes is confined to the mitochondria (called the "mitochondrial genome").

In the nucleus, the DNA is tightly looped around histone proteins organized into fundamental units named nucleosomes. Nucleosomes then package into the chromatin that is further ordered into discrete chromosomes. The ends (or "tips") of the chromosomes are protected by telomere "caps," which are repetitive sequences of DNA that conform to a highly specific motif (TTAGGG), spanning 8,000-15,000 base pairs in tandem[29]. Aging is classically associated with shortening of telomeres and high levels of DNA damage, including mutations, DNA breaks, and chromosomal rearrangements. Although the DNA experiences anywhere from 10,000 to 1,000,0000 molecular lesions per day, cells are equipped with a repair machinery that detects DNA lesions and repairs them[30]. With age, genome damage surpasses DNA repair capacity, causing genome instability. In line with this, studies have shown that DNA damage accumulates in old human tissues[31]. Strikingly, experiments in mice have demonstrated that accumulation of mutations in the mitochondrial genome also lead to premature aging[32]. As for the telomeres, 20-200 base pairs are lost per cell division. Once the telomeres reach a critically short length, the cells stop replicating, become senescent and die. Conversely, fetal tissues, stem cells, adult germ cells, and tumor cells –all with the capacity to propagate indefinitely– have some specialized machinery that allows them to maintain telomere length[33]. Several studies have shown that short telomeres in human leukocytes or peripheral blood mononuclear cells correlate with aging, unhealthy lifestyle habits, and diverse diseases like atherosclerosis, inflammation, and neurodegeneration[33]. It is widely documented that failures in telomere maintenance and/or DNA repair are associated with premature aging phenotypes, including cellular senescence, inflammation, cardiac disease, cancer, and neurodegeneration[30,34]. Thus, preservation of genomic integrity during the lifespan is essential for protecting against senescence, diseases, and for promoting healthy longevity. Importantly, CR favorably impacts the DNA repair and telomere machinery to enable these beneficial ends[35].

The genomics revolution continues to foster technological advancements that allow the sequencing of thousands of genomes from diverse organisms and facilitates their study simultaneously in an unprecedented manner. Genome-level analyses and comparisons, within the same or across different species,

continue to inform all branches of science, especially aging research[36]. To this end, comparative genomic studies of mammals that live longer than humans and that are more resilient to environmental challenges, senescence, and/or the development of age-related diseases may help to better elucidate the genes and molecular mechanisms that preserve health. For instance, the genome of the bowhead whale, which lives twice as long as the average person and has a body comprised of a thousand times more cells than us, shows striking modifications in the content of genes involved in DNA repair, cancer, cell cycle pathways, and aging[14]. Similarly, studies in the mole rat, a rodent resistant to senescence and cancer and that maintains elevated fecundity rates until death, show that this animal has powerful genomic traits such as anti-tumorigenic gene arrangements, which correlate with its resilience to cancer as it ages[37].

In the human genome, two canonical syndromes of accelerated aging, Werner syndrome (WS) and Hutchinson-Gilford progeria syndrome (HGPS), are caused by mutations in genes involved in DNA repair and mechanisms vital to preserving the nuclear envelop that protect the genome. Accordingly, cells derived from WS and HGPS patients exhibit higher levels of DNA damage, genomic instability, and telomere shortening[38,39].

EPIGENETIC INFLUENCE ON LONGEVITY VIA THE ENVIRONMENT

Although it is well accepted that the genome plays a key role in aging, and that experimental genetic manipulations in various species can increase or decrease their lifespans[40], studies in genetically identical human twins have shown that they do not necessarily develop similar diseases/phenotypes or age synchronously[41]. Data from twins suggest that the genome does not solely account for physiological traits or disease risk, but that additional layers of biological information may also shape cellular homeostasis, health, and aging. A new field has thus emerged: "epigenetics," which literally means "above the genes." The "epigenome" then is the entire collection of mechanisms that contribute to the modulation of gene expression, which has direct impact upon disease/phenotypes without changing the underlying sequence of the DNA.

Epigenomic regulation may occur at different levels: (i) at the DNA level itself (DNA methylation, abbreviated DNAm, which is the addition of a methyl group to cytosines); (ii) at the histone level (modifications of histones by methylation, acetylation, or phosphorylation); and (iii) at the nucleosome level (ATP-dependent chromatin remodelers regulate nucleosome positioning). Collectively, these epigenetic mechanisms regulate the genome's topology via chromatin structure to affect gene expression. For instance, tight and compact chromatin, packed densely by DNAm and specific histone modifications, may assemble into "closed" chromatin that can limit the accessibility of the machinery needed for gene expression. While a looser or "open" form of the DNA (usually facilitated by un-methylated DNA and a different set of histone modifications) is associated with active gene transcription[42]. The ability of various environmental signals (e.g., ambient toxins/pollutants, temperature, diet, etc.) to alter the genome via epigenetic alterations is well established in the literature[42,43]. As such, environmental signals can modulate chromatin remodeling and gene expression by adding/removing epigenetic marks on histones or the DNA itself[42-44].

Epigenetic alterations and gene expression changes are known to occur during human aging and disease. Studies in mice and in humans have shown that tissues experience gene expression changes, possibly due to epigenetic mechanisms across time in the young versus the old. For example, analysis of brains from 26- to 106-year-old humans shows that gene expression changes involved in neuronal function, mitochondrial fitness, DNA repair, antioxidant activity, and stress response occur after age 40[45]. In addition, Peters, et al. (2015) recently identified 1,497 genes that were differentially expressed with age in the whole blood of 14,983 individuals. These gene-expression profiles were used to determine the "transcriptomic age" of individuals. Interestingly, differences between transcriptomic age and chronological age were associated with key physiological hallmarks of aging such as cholesterol levels, body mass index, blood pressure, and fasting glucose[46]. Yet, it remains unclear how environmental factors orchestrate epigenetic and gene transcription changes in the brain, blood and/or in other tissues to affect health and the aging process itself. Disease susceptibility as a consequence of aging, therefore, is likely due to the combination of environmental "programming" via epigenetic

marks and predetermined factors, namely genetic mutations/polymorphisms, operating together to shape individual human health trajectories[44,47].

Human studies are inherently difficult to design and perform since they have to account for variations in lifestyle/environmental factors across people and time (e.g., changes in the type of diet, smoking, exercise, alcohol consumption, sleep, etc.), which are all self-reported measures subject to recall bias. Therefore, it is critically important to continue gaining biological insights from model organisms, where conserved genetic pathways relating to aging can be more easily disentangled from environmental/epigenetic factors, given better control of experimental variability in the laboratory setting. Despite these issues, human epigenetic research using robust, well-characterized cohorts has produced seminal contributions to the aging field. For example, accumulation of DNAm at specific loci across tissues can serve as a novel type of "biological clock" to inform key questions pertaining to development, cancer, and aging research[48]. Indeed, a recent application of this work shows that DNAm-calculated age from human blood samples could accurately and sufficiently predict all-cause mortality later in life[49]. Nevertheless, recent studies by Peters, et al., suggest that a combination of gene expression, epigenetic and telomeric data should be considered to refine age prediction[46].

Moreover, other epigenetic markers, such as methylation and/or acetylation in specific lysine residues of certain histone tails, have been shown to change with age[43]. Therefore, newer technological platforms and biocomputational methods are constantly being developed to better detect and map epigenetic changes (DNAm, histone modifications, transcriptomic-level profiles) to help elucidate more about the role of epigenetics and the environment within human aging and disease risk[42,43,46].

ENHANCED PROTEIN STABILITY ASSOCIATES WITH EXTREME LONGEVITY

Proteins are the factors that directly perform or enable cellular function and taken together they comprise the "proteome". Proteins build up diverse intracellular structures, establish metabolic networks through a diverse set of enzymatic activities, and integrate all physiological pathways to act in concert. Protein stability or proteostasis refers to the cellular capacity to protect protein structure and function against ambient stressors like changes in temperature, pH, oxidative stress, radiation, and aging. Vulnerability in proteostasis correlates with changes in aging and longevity rates among species[50]. Studies show that long-lived species are highly resistant to protein unfolding and to several environmental stressors, thereby maintaining endogenous enzymatic activities compared to short-lived organisms with less effective/robust proteostasis[15]. Cells contain an elaborate proteostasis network that involves: protein synthesis, chaperones, autophagy, the unfolded-protein response, and the ubiquitin-proteasome pathway. These network components collectively are aimed at maintaining protein turnover, counteracting protein misfolding, clearing-up unfolded proteins, and recycling of long-lived products[51,52]. Studies show that with age, this network can be compromised, leading to protein accumulation and the aggregation of anomalous unfolded and/or damaged proteins. Indeed, age is the major risk factor for cytotoxic deposition of protein aggregates. For instance, tau and beta-amyloid protein deposition are hallmarks in Alzheimer's disease and alpha-synuclein in Parkinson's disease[53]. In addition, differences in protein abundance and features that alter protein function, like protein cellular localization and gain/loss of protein marks or posttranslational modifications, can all occur with aging and at different rates in tissues. For example, protein alterations show that the brain ages more rapidly than the liver[54], thus allowing us to gaining deeper insights into the molecular basis of heterochrony during aging. Also, a set of proteins that are posttranslationally modified by reversible acetylation (known as the "acetylome") change with aging, among which is found the tau protein associated with Alzheimer's disease. Interestingly, CR as well as other types of diets including high fat diet, which alter aging, also impact the acetylome[55,56].

Various pathological states due to aging-related nutrient deregulation are also linked to protein damage. Aging markers at the organismal level, like hyperglycemia or hyperinsulinemia, enhance the generation of damaged proteins via glycosylation or oxidation, respectively. Molecules that are non-enzymatically modified by carbohydrates known as advanced glycosylation end products (AGEs) are linked to accelerated

aging, inflammation, and chronic diseases. Accordingly, untreated diabetic patients that maintain elevated glucose levels experience several physiological features consistent with accelerated aging, including osteoporosis, obesity, cataracts, altered wound healing, and vascular and microvascular deterioration[57].

MOLECULAR PATHWAYS THAT MODIFY THE RATE OF AGING

Some molecular pathways have the dual ability of sensing nutrients and/or energy levels while also regulating cellular processes like epigenome remodeling, gene expression, protein activity, and organelle integrity. Not surprisingly, such pathways have been found to act as key regulators of aging and diseases. For instance, the mechanistic target of rapamycin (mTOR) and insulin/insulin-like growth factor-1 (IGF-1) signaling (IIS) pathway are sensitive to nutrients, while AMP-activated protein kinase (AMPK) and the sirtuin enzymes sense energy levels[58-62] (Fig. 1). All of these pathways, to varying degrees, have been implicated in mediating the beneficial effects of CR in aging and a variety of age-related disorders. Accordingly, small molecules that target these pathways have been identified as CR mimetics, including resveratrol, rapamycin, and metformin[26,63-65].

Insulin/insulin-like growth factor-1 signaling pathway

The first pathway to alter the rate of aging, IIS, was identified in the worm model *C. elegans*[66]. The IIS pathway couples growth/survival signals with glucose nutrient status. In worms, flies, and mice, a reduction in IIS signaling increases longevity. In mice, a decrease in IGF-1 also delays the aging processes, including the onset of cancer and immune decline, as well as helping to maintain youthful cognitive ability[64]. The gene expression regulatory factors known as forkhead box proteins or FOXOs are key components of the IIS pathway. Nutrient conditions define FOXOs' cellular localization, which may be either in the cytoplasm or in the nucleus. Although the role of the IIS pathway in humans is poorly understood, genetic variants and/or combinations of small-nucleotide polymorphisms (SNP) in the human components of the IIS pathway correlate with low IGF-1 plasma levels in centenarians. Strikingly, in some human populations, various FOXO SNPs are associated with extreme longevity, thereby suggesting that this pathway may play a role in human lifespan extension[62].

Mammalian target of rapamycin and rapamycin

Rapamycin is a type of antibiotic produced by the bacteria *Streptomyces hygroscopicus* that has long been used as an immunosuppressant and in cancer therapy. Studies in yeast cells allowed the field to identify the TOR genes as important mediators of the antiproliferative effects of rapamycin. Later on, the mTOR gene in mammals was identified as the physical target of rapamycin. The mTOR acts as a serine/threonine protein kinase that responds to insulin, amino acids, and hormones to regulate a large range of cellular functions, including protein and lipid synthesis, autophagy, inflammation, mitochondrial function, and glucose metabolism[67]. In yeast cells, it was also first discovered that mTOR plays an important role in aging. Subsequently, a collection of data from different species, including worms, flies, and mice, have demonstrated that either genetic or pharmacological inhibition of mTOR (or components of the pathway) increases lifespan[61,68]. In zebra fish, mice, and humans, depletion of the mTOR pathway improves age-related disorders like cognitive decline, cancer, Alzheimer's disease, and kidney and heart diseases[69]. It has also been described, paradoxically, that mTOR inhibition may cause adverse effects *in vivo*, such as glucose intolerance, insulin resistance, and dyslipidemia[61]. In addition, genetic evidence shows that functional integrity of the mTOR pathway is necessary for conferring the effects of CR-mediated lifespan extension[70]. Thus, mTOR stands out as a key albeit complex modulator of longevity and health.

AMP-activated protein kinase

AMP-activated protein kinase is a kinase that senses changes in energy status. Both low levels of adenosine triphosphate (ATP) and phosphorylation by the liver kinase B1 (LKB1) activate AMPK to regulate a large number of physiological processes via modulation of molecular cascades that involve protein phosphorylation. The AMPK activation leads to a decrease in ATP utilization and an increase in energy production. Studies in rodents have shown that CR activates the AMPK pathway in heart, liver, and skeletal muscle[71]. Yet, data have

also shown that chronic CR fails to activate this pathway[26]. Overexpression of AMPK increases the lifespan of worms, flies, and mice prone to dying of cancer. Increased levels of LKB1 also promote longevity in flies. Interestingly, FOXO factors that are key regulators of the IIS pathway (as mentioned above), may also participate in mediating the longevity effects of AMPK[72]. Studies in worms demonstrate that metformin (a drug that disrupts mitochondrial function) requires AMPK to cause a 50% increase in worm lifespan. Pro-longevity effects of metformin have also been reported in both normal and cancer-prone mice. In humans, metformin has been widely used for the treatment of type II diabetes and also shows anti-tumorigenic effects. Although it is well substantiated that metformin activates AMPK, it is still unclear whether all the beneficial health effects of this drug depend entirely on the AMPK pathway[60].

Sirtuins

The sirtuin pathway was originally identified in yeast as a regulator of lifespan[73]. In humans, there are seven members of these proteins expressed ubiquitously in tissues, yet each of them is localized to distinct or shared cellular compartments. Three sirtuins regulate mitochondrial functions (SIRT3, SIRT4 and SIRT5), while SIRT1, the most comprehensively studied of the seven, modulates gene expression and the function of proteins involved in diverse cellular pathways. Both SIRT6 and SIRT7 are mainly confined to the nucleus to regulate gene expression, and SIRT2 functions in the cytoplasm. A unique aspect of sirtuins is that they require nicotinamide adenine dinucleotide (NAD^+) for their enzymatic activity, which is a coenzyme essential for metabolic homeostasis. By removing acetylation marks from the lysine residues of histones or non-histone proteins, sirtuins regulate the epigenome and the acetylome, respectively. Also, sirtuins modify the proteome by removing diverse types of acyl groups, including succinyl, malonyl, or fatty acids, or by adding an ADP-ribose moiety onto targets[55,65]. Thus, sirtuins have the remarkable ability to directly detect changing energy levels and orchestrate an enzymatic response to maintain cell homeostasis[59]. A large body of work has demonstrated that sirtuins play an important role in mediating aging and age-related diseases like cancer, inflammation, cardiac function, and cognition through the regulation of diverse molecular/cellular process, including genomic stability, senescence, DNA repair, mitochondrial function, metabolic homeostasis, and stem cell exhaustion. Using genetically engineered mouse models that express altered levels of SIRT1 in the brain, we have demonstrated that the integrity of this pathway is essential for normal learning and memory[74]. In addition, high levels of SIRT1 in the villi of the intestine protect against colon cancer[74,75]. In other experiments, ubiquitous overexpression of SIRT2 and SIRT6 has been reported to promote longevity in a mouse prone to cardiac disease and in normal mice, respectively[76,77]. Decreased levels of sirtuins, however, may also cause beneficial effects. For instance, genetic or pharmacological inhibition of SIRT2 protects against neurodegeneration in mouse models[78]. In humans, a variety of SNPs associated with sirtuin genes have been identified to correlate with healthy aging and longevity. For example, SNPs linked to the *SIRT1* and *SIRT3* genes have been found in long-lived populations of Chinese and Italian people, respectively[79,80]. Metabolic markers of aging, such as atherosclerosis, obesity, type II diabetes, and neurodegeneration (Alzheimer's and Parkinson's disease), also correlate with sirtuin polymorphisms[81,82]. Accordingly, small molecules that have been identified as activators of the sirtuin pathway, such as the natural polyphenol resveratrol[83], recapitulate CR-like effects in obese mice like improvements in insulin sensitivity, motor function, and endurance. At the molecular level, this polyphenol reduces IGF-I levels, increases AMPK enzymatic activity, and boosts mitochondrial number. However, resveratrol has so far failed to extend lifespan in lean mice[84].

CONCLUSIONS

Despite the impressive advancements made towards understanding more about the molecular basis of aging, there is still no definitive intervention for ensuring healthy aging in humans. To uncover new therapeutic avenues, we need to gain deeper knowledge about how different internal and external factors regulate the cellular hallmarks of aging, and how their regulation changes across time and individuals. All molecular pathways exhibit complex communication known as "crosstalk." The genome, epigenome, organelles, proteome, and pathways such as those involving sirtuins, mTOR, AMPK, and IIS-all integrate and process signals that must act coordinately to promote homeostasis

in cells and tissues (Fig. 1). It is unclear, however, how these complex molecular networks are affected by diverse environmental challenges and how they become impaired with aging. Lastly, in an effort to find beneficial interventions to delay aging-linked deterioration, the search for small molecules that can mimic CR –and the dissection of their pharmacological modes of action *in vivo*— is a growing area of research that merits more attention. Collectively, through all of these scientific efforts, we may someday achieve the longstanding human dream of living a long and healthy life.

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