Aging in the Nematode *Caenorhabditis elegans*

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Introduction

Aging is a complex, multifaceted process that takes place over time and is experienced by the vast majority of living organisms. Aging can be defined as the progressive accumulation of damage to molecules, cells and tissues, ultimately leading to physical and functional deterioration of the entire organism. As such, aging is associated with increased susceptibility to disease onset, including neurodegeneration, muscle atrophy, cancer and type II diabetes (Tosato et al., 2007; Lopez-Otin et al., 2013). Over the past decades, much effort has been invested in understanding the regulatory mechanisms and the key factors involved in the aging process. Evidence up to now obtained from studies performed in model organisms such as yeast, worms, flies and mice indicates that aging is affected not only by genetic alterations but also by epigenetic mechanisms and environmental factors. Research in the aging field focuses on the elucidation of the basic cellular and molecular mechanisms underlying the progressive decline in cellular function that accompanies aging. The ultimate goal of such studies is to develop efficient intervention strategies to slow the aging process, thereby prolonging healthspan (maintaining a healthy state) (Madeo et al., 2015). The most robust output of aging studies is the measurement of lifespan. Lifespan is the maximum time period an organism is alive. Several theories have been developed over time, trying to explain the fundamental biological phenomenon of aging. For instance, the "somatic mutation," "cellular waste accumulation," "rate of living," "free radical," and the "wear and tear" are among the most important theories of aging that have evolved within the last four decades of research (Vina et al., 2007). The fact that none of these theories can fully explain aging supports the notion that a multilevel and holistic view of the complex processes of aging and senescence is needed (Jin, 2010).

Over the past 20 years, much progress has been made in the field of aging. Research using model organisms revealed that aging is subject to regulation, like many other biological processes (Kenyon, 2010). This was first shown in the nematode *C. elegans* where genetic manipulations or changes in the way of living could reverse age-related phenotypes even when some of these were performed in already old animals (Smith et al., 2008). *C. elegans* has been widely used in aging studies during the last 100 years (Tissenbaum, 2015). The nematode offers unique advantages, highly relevant to the scientific questions to be tackled, which are not available in any other model system and this is proved by the fact that key findings in the field are derived from studies carried out in the worm. Specifically: It is short lived. *C. elegans* lives approximately 2 weeks, in contrast to other models which live several months or years. Additionally, it has a short life cycle and reproduces very easily in large numbers, creating genetic clones of itself due to self-fertilization. This is really important as it enables researchers to perform multitudinous experiments that improve data accuracy, thus increasing the impact of their studies (Gershon and Gershon, 2002). Also, elimination of the genetic variation among single individuals in a population allows robust interpretation of the results obtained by targeted manipulations. Another advantage is its transparent body at all stages, trait that enables optical monitoring and *in vivo* analysis of fundamental biological processes inside single cells and tissues during aging (Corsi et al., 2015). Also, *C. elegans* has approximately the same number of genes when

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compared to humans, most of which are evolutionarily conserved (Aboobaker and Blaxter, 2000). So, investigators can study and understand molecular mechanisms that are conserved in higher eukaryotes, including humans, using a very simple eukaryote instead. Importantly, its nervous system is fully charted, with the position and the connectivity of its 302 neurons precisely described. This unique trait among multicellular eukaryotic organisms allows a comprehensive study of age-related neurodegeneration events (Chew et al., 2013). Moreover, transgenic animals or viable mutants can be generated quite easily and efficiently, allowing the genetic dissection of signaling pathways and molecular mechanisms mediating aging (Wilkinson et al., 2012). It is worth noting that *C. elegans* is becoming an attractive platform for developing drug discovery and drug target identification methodologies. In this article, we will discuss the main advances in our understanding of the genetic and nongenetic modulators of the aging process. We will refer to the effector mechanisms and key factors that are activated downstream of signaling pathways focusing on studies pioneered in *C. elegans*. We will also discuss the contributions of epigenetics and environmental factors to aging phenotypes in the worm.

Genetic Regulation of Lifespan

Insulin/IGF-1 Signaling

One of the first lifespan-influencing pathways identified in *C. elegans* is the endocrine insulin/IGF-1 pathway. The first gene mutation in this pathway shown to significantly increase lifespan was in *age-1*, which encodes the nematode orthologue of the mammalian phosphoinositide-3 kinase (PI3K) *p110* subunit (Friedman and Johnson, 1988). A few years later, the *C. elegans* orthologue of the mammalian insulin and IGF-1-like receptors was identified. This is encoded by the *daf-2* gene (Kenyon et al., 1993; Dorman et al., 1995). Interestingly, it was shown that mutations in the insulin receptor gene that impair its function confer significant lifespan extension in a cell nonautonomous manner in various species, including worms, flies and mice (Apfeld and Kenyon, 1998; Selman et al., 2008; Partridge et al., 2011). The insulin/IGF-1 receptor is recognized by several insulin-like peptides (ILPs) which either act as agonists or antagonists of the receptor. Despite the fact that more than 40 different insulin-like peptides have been identified up to now, none of them is similar to human insulin. When an agonist peptide binds to and activates the receptor DAF-2, then AGE-1 is phosphorylated and activated. Subsequently, AGE-1 phosphorylates and activates the Akt/protein kinase B family of serine/threonine protein kinases, AKT-1 and AKT-2 in *C. elegans* and the serum- and glucocorticoid-inducible kinase 1(SGK-1). AKT-1, AKT-2 and SGK-1 phosphorylate the DAF-16/FOXO (controlled, germline tumour affecting-1) transcription factor (Lin et al., 2001). Phosphorylation of DAF-16 leads to its sequestration in the cytoplasm, with consequent inhibition of its transcription factor activity. Mutations in *daf-2*, which lead to lifespan extension, dauer larvae formation, and increased stress resistance, require the nuclear translocation of DAF-16 and activation of its downstream target genes (Honda and Honda, 1999; Barsyte et al., 2001; Scott et al., 2002; Lithgow et al., 1995). Microarray analysis of DAF-16 target genes and potential mediators of the *daf-2* mutant lifespan extension revealed an increase in the expression of genes involved in environmental stress responses and of antimicrobial genes mediating immune responses. Surprisingly, downregulation of each of these genes does not reverse the long lifespan of *daf-2* mutants. This finding supports the idea that not a single target/pathway, but instead, a global response mediates the *daf-2* longevity phenotype (Murphy et al., 2003).

Tissue-specific expression analysis revealed that *daf-16* expression in muscles is not needed for lifespan extension, while its neuronal expression only moderately enhances the longevity of *daf-16(−);daf-2(−)* mutants. On the other hand, intestinal expression of *daf-16* increases substantially the lifespan of these animals. Interestingly, however, the activity of DAF-16 in additional tissues, besides the intestine, is required to fully restore the lifespan of *daf-16(−);daf-2(−)* animals (Libina et al., 2003). Complementary studies proved that DAF-16 functions cell non-autonomously to promote longevity. Despite the fact that its activation in a single tissue (mainly the intestine) produces insulin-like molecules or hormones that can signal to the rest of the tissues, DAF-16 activity in these "downstream" tissues is also required for activation of, at least part of, its targets genes in these locations. Surprisingly, the signaling for lifespan extension differed from that for dauer formation. In the latter case, neuronal *daf-16* expression was needed (Libina et al., 2003). Concomitantly, it was found that neuronal *daf-2* expression harbors the signaling for longevity (Wolkow et al., 2000). Moreover, DAF-2 loss postdevelopmentally is sufficient to confer lifespan extension and stress resistance as shown by parallel experiments in which *daf-2* RNAi treatment was initiated at the young adult stage and at hatching. Indeed, DAF-2 depletion in young adults results in the same lifespan extension as DAF-2 deficiency from the time of hatching. The same effect was observed when adult animals were treated with paraquat. Enhanced resistance to oxidative stress is a trait of *daf-2* mutants and it was shown that animals subjected to *daf-2* RNAi from adulthood were as resistant to oxidative stress as the ones treated from larval stages (Dillin et al., 2002a).

Analysis of the pathways that influence lifespan extension downstream of the insulin/IGF-1 pathway showed that genes related to vesicle sorting, endocytotic trafficking to the lysosomes, autophagy, heat shock response (*hsf-1* upregulation) and proteostasis, energy metabolism homeostasis (*aak-2*), fat and lipid homeostasis, and transcription- and translation-related genes were among the most important regulators. Reducing their activity caused progeria-related phenotypes in *daf-2* mutant animals (Samuelson et al., 2007; Hsu et al., 2003; Li et al., 2013). The transcription factor SKN-1 (SKiNhead-1) is also required for the *daf-2* lifespan extension. SKN-1 and HSF-1 (Heat shock transcription factor-1) act synergistically to DAF-16 and activate distinct target genes (Tullet et al., 2008; Seo et al., 2013). More recently, SMK-1, a regulatory molecule that determines DAF-16 transcriptional specificity, and PQM-1, a zinc finger transcription factor, have assigned a role in longevity mediated by reduced DAF-2 activity. Interestingly, the nuclear localization of PQM-1 and DAF-16 appears to be mutually exclusive (Wolff et al., 2006; Tepper et al., 2013) (**Fig. 1**).

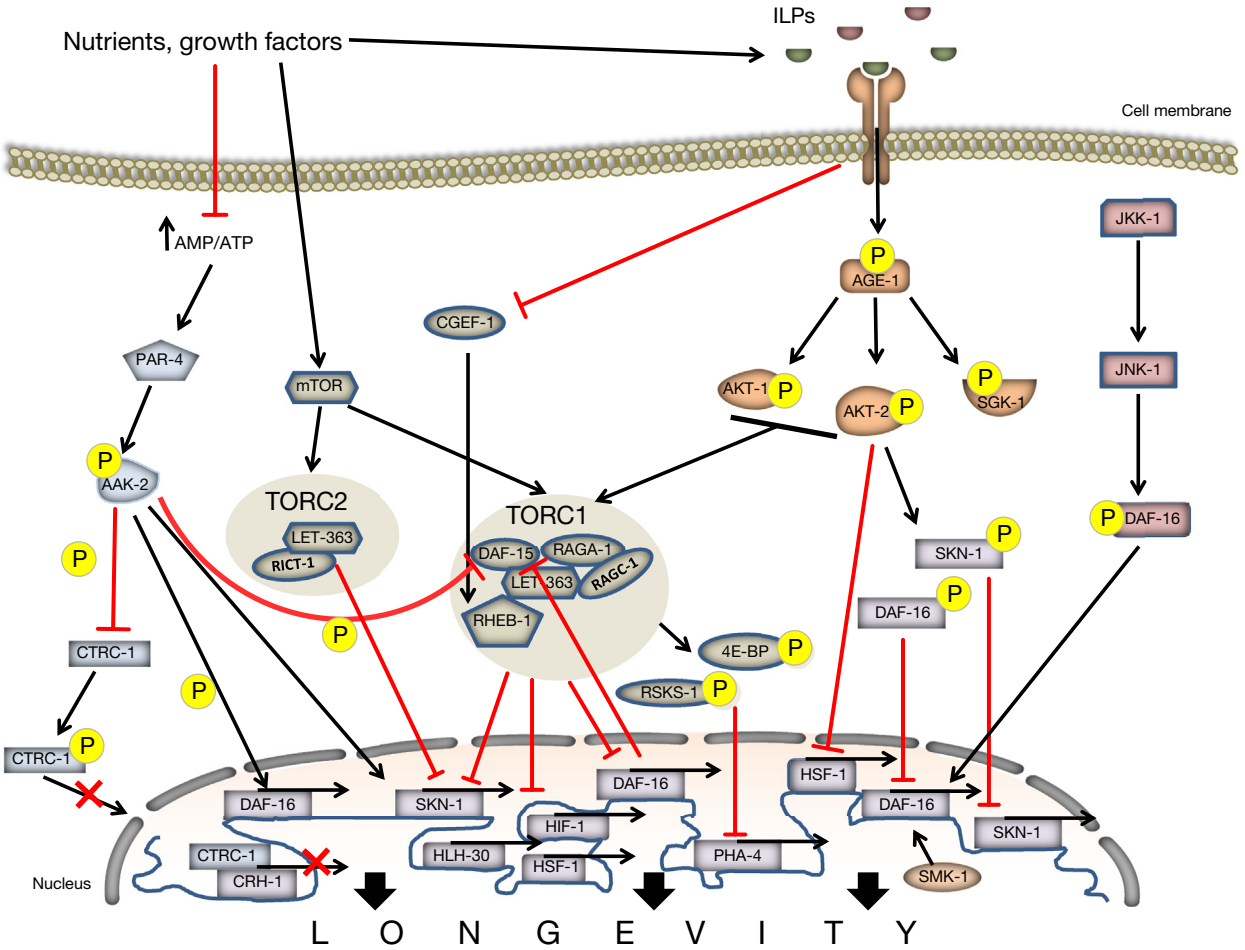

Fig. 1 Genetic regulation of lifespan. The insulin/IGF- (IGF-1), the target of rapamycin (TOR), and the AMPK signaling pathways act both in parallel and synergistically to affect lifespan. Phosphorylation events and pathway crosstalk fine-tune transcription factor activation in the nucleus and determine longevity. More details and description of additional signal transduction cascades that influence lifespan in *C. elegans* can be found in the text.

AMPK

AMPK (AMP-activated kinase) is a multimeric complex that consists of one catalytic (α subunit) and two regulatory (β and γ) subunits (Apfeld et al., 2004). Two homologues of the catalytic α subunit of AMPK were identified in *C. elegans*, AAK-1 and AAK-2 (Apfeld et al., 2004; Lee et al., 2008; Curtis et al., 2006). Despite their high sequence similarity, only AAK-2 plays a role in lifespan regulation (Apfeld et al., 2004). It acts as an energy sensor inside cells and is induced when energy levels drop. AAK-2 activation is mainly mediated by PAR-4 in *C. elegans*, the mammalian LKB1 (liver-kinase-B1) orthologue which phosphorylates AAK-2 at Thr^{243} (Lee et al., 2008; Narbonne and Roy, 2006). Activated AMPK phosphorylates multiple substrates to stimulate catabolic processes, ultimately restoring homeostasis (Moreno-Arriola et al., 2016). Indeed, transcriptomic analysis of *aak-2* mutant animals revealed that ribosomal and histone genes, translation-related and sperm genes, as well as oxidative phosphorylation-related genes were significantly downregulated as compared to their control counterparts (Shin et al., 2011). This is partially mediated through allosteric mechanisms, when the AMP/ATP ratio is significantly increased (Hardie, 2011). An elevation in the AMP/ATP ratio is monitored, for example when animals are exposed to stress, starvation, mitochondrial dysfunction, exercise, or upon dysfunction of the insulin/DAF-2 receptor (Apfeld et al., 2004; Lee et al., 2008; Curtis et al., 2006; Shin et al., 2011; Fukuyama et al., 2012; Chang et al., 2017). Regulation of the energy metabolism status of the cell through AMPK/AAK-2 is an important lifespan regulatory mechanism. This is supported by findings showing that *aak-2* mutation or downregulation either from hatching or in adulthood causes premature aging phenotypes and significantly decreases lifespan in *C. elegans* (Apfeld et al., 2004; Curtis et al., 2006). In contrast, lifespan is increased when *aak-2* is overexpressed or mutated to be constitutively active (Mair et al., 2011; Greer et al., 2007).

Furthermore, AAK-2 has been shown to mediate part of the *daf-2* longevity acting in parallel with DAF-16 (Apfeld et al., 2004). AAK-2 phosphorylates DAF-16 and enhances its transcription activity. Also, loss of AAK-2 function decreases the longevity of *daf-2* mutant animals (Greer et al., 2007). Reciprocally, DAF-16 is also needed for *aak-2*-induced lifespan extension as it upregulates two of the AMPK core subunits, AAKG-4 and AAKB-1, even though only AAKG-4 is linked with lifespan. As a result, AAK-2 and DAF-16

form a positive feedback loop which enhances the effect of DAF-16-dependent lifespan extension (Tullet et al., 2014). Apart from DAF-16, SKN-1 is also activated by AAK-2 (Onken and Driscoll, 2010). Interestingly, it was shown that the AAK-2 agonist, metformin induces SKN-1 nuclear translocation and enhances its transcriptional activity (Onken and Driscoll, 2010).

Moreover, AAK-2 activity triggers autophagy. This is achieved directly through phosphorylation of UNC-51 by AAK-2 and indirectly through activation of DAF-16 (Possik et al., 2014; Egan et al., 2011). Autophagy activation has also been linked to lifespan extension in various genetic backgrounds, such as *daf-2* and *rsks-1* mutants. Importantly, autophagy induction and enhanced stress resistance reportedly mediate *aak-2* lifespan extension. The longevity phenotype induced by *aak-2* overexpression or when AAK-2 is constitutively active has also been linked with CTRC-1 (cAMP response element binding protein-regulated transcriptional coactivator-1) inhibition. More specifically, it was shown that AAK-2 kinase directly phosphorylates CRTC-1, triggering its nuclear exclusion and inactivation. Neuronal inhibition of CTRC-1 is a prerequisite for AAK-2 dependent lifespan extension, as mutation of CRTC-1 at the AAK-2 recognition phosphorylation sites renders it unresponsive to AAK-2 phosphorylation and constitutively nuclear, blocking the lifespan extension phenotype triggered by *aak-2* overexpression (Mair et al., 2011; Burkewitz et al., 2015). More specifically, it was shown that neuronal CRTC-1 acts cell nonautonomously through the octopamine signaling to regulate lifespan by modulating metabolism and mostly mitochondrial metabolism in distal tissues. Also, NHR-49 activation in neurons is sufficient for AMPK-mediated longevity as it possesses antagonistic to CRTC-1 functions (Burkewitz et al., 2015). Complementary studies showed that NHR-49 and MDT-15 mediate the metabolic changes related to fat metabolism, fatty acid oxidation, and lipid synthesis downstream of AAK-2 activation (Moreno-Arriola et al., 2016). Nuclear exclusion of CTRC-1 leads to impaired activity of the CRH-1 transcription factor, which is the cyclic AMP response element binding (CREB) transcription factor family CREB homologue in *C. elegans* (Mair et al., 2011). This mechanism functions downstream of AAK-2, when the kinase is activated, to prolong lifespan (**Fig. 1**).

TOR Signaling

Increased availability of nutrients, growth factors, and amino acids has been linked to shortened lifespan in various model organisms. By contrast, lifespan is significantly increased upon nutrient or growth factor deprivation (Kapahi et al., 2017). The regulation of lifespan under these conditions has been consistently linked to TOR (target of rapamycin) signaling.

TOR, which is a highly conserved serine/threonine kinase, is inhibited when nutrients and growth factors are limited as well as when cellular energy levels drop (Sengupta et al., 2010). Increased amounts of nutrients and growth factors lead to activation of Akt, which phosphorylates its targets and activates TOR (Laplante and Sabatini, 2012; Sancak et al., 2007; Manning et al., 2002; Inoki et al., 2002). Moreover, under conditions where cellular energy levels decrease, AMPK is activated and phosphorylates either Tsc2 (which does not have a *C. elegans* homologue) or Raptor and subsequently represses TORC1 activity (Inoki et al., 2003; Gwinn et al., 2008). In *C. elegans*, the *let-363* gene encodes the mammalian homologue of TOR (Long et al., 2002). As known from mammals, and is also the case in *C. elegans*, TOR forms two distinct complexes with discrete functions, TORC1 and TORC2. TORC1 formation requires TOR/LET-363 association with its co-activator DAF-15 (the nematode homologue of Raptor), the Rag GTPases RAGA-1 and RAGC-1, and RHEB-1 (the nematode homologue of mammalian Rheb) (Hara et al., 2002; Lapierre and Hansen, 2012). Another, recently identified factor that regulates TORC1 activity in *C. elegans* is CGEF-1, the homologue of Dbl in mammals. Recent evidence indicates that CGEF-1 interacts with RHEB-1 and activates TORC1 (Li et al., 2018). TORC1 activation inhibits DAF-16 and SKN-1 activity, while DAF-16 can reciprocally inhibit DAF-15, forming a negative feedback loop (Robida-Stubbs et al., 2012; Jia et al., 2004). Placing additional complexity to this system, SKN-1 increases the expression of TORC1 genes (Robida-Stubbs et al., 2012; Zhao and Wang, 2016). Moreover, TORC2 formation requires the TOR/LET-363 interaction with its coactivator RICT-1, the homologue of the mammalian Rictor (Lapierre and Hansen, 2012). TORC2 formation and activation inhibits SKN-1 transcriptional activity (Robida-Stubbs et al., 2012; Ruf et al., 2013). On the other hand, it is not fully understood yet whether only one out of two complexes or both TORC1 and TORC2 contribute to PHA-4/FOXOA inhibition. Nevertheless, evidence up to now suggests that LET-363 suppresses PHA-4 through RSKS-1, the homologue of the mammalian S6 kinase (S6K) (Sheaffer et al., 2008).

Interestingly, it has been shown that TOR hyperactivity is linked to shortened lifespan while downregulation or inhibition of the TOR pathway leads to lifespan extension (Lamming and Sabatini, 2011; Vellai et al., 2003). Pharmacologic inhibition of TOR activity by rapamycin also leads to lifespan extension in several model organisms. Again in this case, TORC1 is more vulnerable to rapamycin while TORC2 is quite unaffected (Ballou and Lin, 2008). Furthermore, genetic inhibition of *rsks-1*, *raga-1* and *daf-15*/Raptor, among other components downstream of the TORC1/TORC2 complexes, also increases lifespan (Robida-Stubbs et al., 2012; Jia et al., 2004; Pan et al., 2007). This beneficial effect on lifespan is mediated by the initiation of a transcription program that includes activation of HLH-30/TFEB, SKN-1, DAF-16 and HIF-1α target genes (Robida-Stubbs et al., 2012; Lapierre et al., 2013; Land, 2007). The effect on lifespan is also governed by transcription-independent mechanisms which involve the phosphorylation of the translation regulators, RSKS-1/S6K and the translation initiation factor 4E binding protein 1 (4E-BP1) as well as ribosome biogenesis regulators (Showkat et al., 2014). Additionally, HSF-1 transcriptional activity is also needed for lifespan extension by *let-363* and *rsks-1* suppression, as HSF-1 depletion even from adulthood suppresses the extended lifespan of *rsks-1* mutants (Seo et al., 2013). Deeper analysis showed that the HSF-1 target gene *hsp-16* is partially needed for *rsks-1* longevity (Seo et al., 2013). The driving mechanisms of lifespan extension resulting from TOR downregulation rely on vital processes: translation inhibition, lipid metabolism, and autophagy induction (by PHA-4) (Long et al., 2002; Johnson et al., 2013). Both translation inhibition and autophagy induction have been directly associated with lifespan extension in various model organisms (Pan et al., 2007; Syntichaki et al., 2007; Nakamura and Yoshimori, 2018; Hansen et al., 2007). Inhibition of *let-363* has also been linked to elevated

lipolysis and LIPL-4 levels (Seah et al., 2016). Additionally, *let-363* deletion leads to fat accumulation as shown for monounsaturated fatty acids (MUFAs), a phenotype that has also been linked to long lifespan, while both *daf-15* and *rict-1* mutants contain increased number of lipid droplets (Shi et al., 2013; Han et al., 2017). These are adaptive responses to TORC1 inhibition which are activated to regulate organism metabolism by switching from anabolic, energy consuming processes to more catabolic ones (**Fig. 1**).

Mitochondria-Mediated Lifespan Control

Mitochondria are semiautonomous organelles containing their own genome, which encodes only about 1%–2% of their protein content. The rest of their components are encoded by the nuclear genome and after being translated in the cytoplasm, they are imported and targeted to the various suborganellar compartments. Mitochondria serve important functions that influence many aspects of cellular and organismal physiology, ultimately impinging on organismal healthspan and lifespan. Specifically, they primarily regulate energy production in the form of ATP through oxidative phosphorylation of the electron transport chain (ETC). They have essential roles in the maintenance of heme and iron/Sulphur cluster homeostasis, the regulation of lipid metabolism, calcium and copper homeostasis, amino acid production, apoptosis, and cell cycle (Guda et al., 2007; Lill et al., 2012; Alaynick, 2008; Duchen, 2000; Horn and Barrientos, 2008; Wang and Youle, 2009; Antico Arciuch et al., 2012). The reason why mitochondria have attracted so much interest is because their dysfunction is linked to several pathological conditions such as diabetes, premature aging, neurodegeneration, cancer, and cardiomyopathy as shown in several model organisms (Ventura and Rea, 2007; Rivera-Torres et al., 2013; Zong et al., 2016; El-Hattab and Scaglia, 2016; Newsholme et al., 2012).

To date, mild perturbation of mitochondrial function by either pharmacological treatments or genetic inhibition of electron transport chain components (ETC) has been linked to lifespan extension and increased stress resistance (Rea et al., 2007; Ristow and Schmeisser, 2014). This phenomenon is called mitohormesis. Interestingly, mutation or downregulation of ETC components (Mit mutants) associated with increased oxidative damage to mitochondrial proteins severely affects lifespan in *C. elegans* and other organisms. For example, mutation or genetic inhibition of either *mev-1* or *gas-1* shortens the worm lifespan (Baruah et al., 2014; Kayser et al., 2004). By contrast, downregulation of *atp-3*, *clk-1*, *cco-1*, *nuo-1*, *nuo-6*, *isp-1*, *frh-1* significantly increases lifespan independently of the *daf-2*/*daf-16* axis (Ventura and Rea, 2007; Rea et al., 2007; Cristina et al., 2009; Chin et al., 2014; Lee et al., 2010; Curran and Ruvkun, 2007). Surprisingly, some genetic inhibitions may not have the same effect with mutation in the same gene (Yang and Hekimi, 2010a). Nevertheless, the effect of downregulation of mitochondrial components on lifespan is evident only when the intervention occurs during development, unlike to what happens in the long-lived insulin mutants described previously (Dillin et al., 2002b). Lifespan extension, in response to a mitochondrial perturbation, is most probably mediated through altered production of oxidative phosphorylation metabolites such as ATP and ROS (Van Raamsdonk et al., 2010). For example, mutations that decrease respiration and ATP levels, thus metabolism, increase lifespan, as suggested in the "rate of living theory of aging" first introduced by R. Pearl in 1928. In other cases, ETC mutations cause elevated ROS levels and through mitohormesis enhance longevity (Lee et al., 2010). Initially, it was believed that ROS levels are a determinant of lifespan. According to this theory, increased ROS production due to oxidative phosphorylation causes mtDNA damage that contributes to cellular damage which over a lifetime triggers aging (Cui et al., 2012). This is the so-called free radical or oxidative damage theory of aging (Harman, 1956). Later, it was shown that, in contrast to what was initially believed, O_2^- levels are totally uncoupled from aging and mild ROS production is even beneficial as both mutation in the *sod-2* gene, which encodes a superoxide dismutase isoform, and mild paraquat treatment increase lifespan in *C. elegans* according to the "mitohormesis theory" (Lee et al., 2010; Van Raamsdonk et al., 2010; Doonan et al., 2008). This occurs because ROS act as signaling molecules inside cells and can either activate and stabilize HIF-1 or activate the intrinsic apoptotic pathway, among other responses (Lee et al., 2010; Yee et al., 2014).

Mitochondrial mutations trigger the activation of several pathways or transcription factors to counteract stress and confer the lifespan extension phenotype observed. A number of pathways are activated in response to mitochondrial perturbation, such as the selective mitochondrial autophagy (mitophagy), mitochondrial unfolded protein response (mtUPR), activation of the intrinsic apoptosis pathway, elevation of antioxidant genes and the detoxification response, mitochondrial biogenesis, fatty acid oxidation, metabolic reprogramming, and activation of the *aak-2* pathway (Burkewitz et al., 2015; Ventura and Rea, 2007; Yee et al., 2014; Jovaisaite et al., 2014; Sun et al., 2016; Weir et al., 2017; Butler et al., 2010, 2013; Palikaras et al., 2015; Yang and Hekimi, 2010b). Several transcription and transcription-related factors are also activated such as ATFS-1, HIF-1, FSTR-1/2, CHE-23, SKN-1, the TAF-4/TFIID complex, and *cep-1*, among others (Baruah et al., 2014; Cristina et al., 2009; Lee et al., 2010; Khan et al., 2013; Walter et al., 2011; Durieux et al., 2011). Nevertheless, discrete transcriptional programs and downstream mechanisms are activated in a mutation-specific manner as shown, for example in *clk-1*, *isp-1*, and *cyc-1* mutants (Cristina et al., 2009). Additionally, it was shown that in *isp-1* and *mev-1* mutants, *cep-1* oppositely affects longevity by activating different subsets of genes (Baruah et al., 2014). mtUPR is a stress response pathway that is induced when misfolded/unfolded proteins accumulate in the mitochondrial matrix. It consists of a transcription program that relies on ATFS-1 and DVE-1 transcription factors and the transcription coactivator UBL-5 to produce mainly chaperons which are transported to the organelle to alleviate stress (Haynes et al., 2013). mtUPR is also activated by ROS-induced mtDNA lesions and when components of the translation machinery of mitochondria are perturbed (Ventura and Rea, 2007; Moehle et al., 2018). To note, the lifespan extension of long-lived mitochondrial translation mutants such as *mrps-5* depends on the activation of mtUPR (Jovaisaite et al., 2014; Houtkooper et al., 2013). Furthermore, it has been shown that mtUPR induction even in a single tissue can signal and initiate the response to additional tissues through yet not well-understood mechanisms (Durieux et al., 2011). Nevertheless, artificial activation of mtUPR cannot extend lifespan

or be used as a longevity marker by itself (Bennett et al., 2014). Another age-related phenotype is the accumulation of mtDNA mutations during aging but their impact on the aging process is still debated (Hirose et al., 2016). Interestingly, besides the organelle's function, mitochondrial morphology, density and subcellular organization and localization are features that have been correlated with aging and age-related pathologies (Weir et al., 2017; Morsci et al., 2016; Yasuda et al., 2006; Chaudhari and Kipreos, 2017). On the other hand, despite being associated with the aging process, alterations in these characteristics are not sufficient to trigger cellular senescence by themselves (Regmi et al., 2014). Last, a recent study showed that dysfunctional mitochondria accumulate during aging in *C. elegans*. In young or long-lived animals, a healthy mitochondrial pool is retained through the balanced actions of mitochondrial biogenesis and mitophagy. In old animals, however, such quality control mechanisms decline. Moreover, mitophagy is partially required for the extended lifespan of specific long-lived *C. elegans* mutants (Palikaras et al., 2015).

The Jnk-1 Pathway

JNK-1 is a serine/threonine protein kinase, member of the broader family of the mitogen-activated protein kinases (MAPK). In *C. elegans*, *jnk-1* that encodes the homologue of the mammalian c-Jun N-terminal kinase (JNK) is expressed only in neurons. An additional component of the JNK pathway, which is also present in *C. elegans*, is JKK-1, a MAPKK family kinase which functions upstream of JNK-1 and activates it (Sakaguchi et al., 2004; Oh et al., 2005; Kawasaki et al., 1999). Besides its role in regulating stress responses, development, and inflammation, a role for JNK-1 in lifespan regulation has recently been established (Sakaguchi et al., 2004; Oh et al., 2005; Gerke et al., 2014). Indeed, overexpression of *jnk-1* increases lifespan, in contrast to its genetic inhibition. The longevity effect of *jnk-1* is also dependent on the presence and functionality of the upstream activating kinase JKK-1. JNK-1 regulates lifespan by directly interacting with and phosphorylating DAF-16 on its N-terminus (Oh et al., 2005; Gerke et al., 2014; Zhao et al., 2017). This phosphorylation event on DAF-16 seems to trigger its nuclear localization and transcriptional activation of target genes in a *daf-2*-independent manner. This is evident by the finding that *jnk-1* overexpression has an additive effect on *daf-2* longevity despite the fact that *jnk-1* is needed for *daf-2* lifespan extension (Oh et al., 2005).

Apart from DAF-16, JNK-1 also triggers the expression of *jun-1* and *fos-1* transcription factors which also regulate fasting-induced lifespan extension in *C. elegans* (Gerke et al., 2014; Uno et al., 2013). Last, the MAPK kinases p38/PMK-1 and ERK/MPK-1 also affect longevity in *C. elegans* (Okuyama et al., 2010; Park et al., 2018) (**Fig. 1**).

The Reproductive System

C. elegans gonad is a complex organ consisting of two distinct compartments: the somatic gonad and the stem cell niche, or the germline. Consistent with findings in several model organisms, where reduced reproduction positively affects lifespan, germline ablation, or genetic mutation in *gld-1* (encoding a protein containing a K homology RNA binding domain) increases the worm lifespan more than 50% (Yamawaki et al., 2008). Intriguingly, this positive effect on lifespan is lost when the somatic gonad is concomitantly ablated, uncoupling sterility *per se* from lifespan extension (Yamawaki et al., 2008). Subsequent studies indicated that germline loss triggers steroid signaling initiated by the somatic gonad which is targeted to distal tissues (Antebi, 2013). Core mediators of this steroid signaling are the nuclear hormone receptor DAF-12 and its ligands, the dafachronic acids (DAs) (Hsin and Kenyon, 1999; Dumas et al., 2010). DA production is mainly regulated by enzymes such as DAF-9 (cytochrome P450), DAF-36 (Rieske oxygenase), and DSH-16 (3-hydroxysteroid dehydrogenase). Mutation in these enzymes abrogates the lifespan extension of germline-less animals (Jia et al., 2002; Wollam et al., 2011, 2012). While DA supplementation alone is not enough to extend the lifespan of wild-type worms, it does restore lifespan extension of animals lacking both the somatic gonad and the germline (Yamawaki et al., 2010; Gerisch et al., 2001). Downstream of the DA/DAF-12 axis, DAF-16 is activated specifically in the intestine in a *daf-2* independent manner, while it is not clear yet whether DAF-12 mediates its effect through a single/specific tissue or not (Uno and Nishida, 2016). This was confirmed when lifespan extension of germline-less animals was abrogated by *daf-16* mutation. DAF-12 activation by DA triggers an increase in the levels of *mir-241* and *mir-84*, which negatively regulate AKT-1 and LIN-14 expression and finally trigger DAF-16 activity (McCormick et al., 2012). DAF-16 intestinal nuclearization and longevity of animals lacking the germline is regulated also by the Ankyrin repeat-containing protein KRI-1, which is also expressed in the intestine. KRI-1 is orthologous to the human KRIT1/CCM1 and its effects are *daf-2* independent (Berman and Kenyon, 2006). Additionally, DAF-16 nuclearization and lifespan extension of germline-less animals is enhanced by *mir-71* (Boulias and Horvitz, 2012). In addition, HSF-1, SMK-1/SMEK-1, DAF-18/PTEN, and the transcription elongation factor TCER-1/TCERG1 are also shown to be important for promoting longevity in germline less animals (Wolff et al., 2006; Yamawaki et al., 2008; Chen et al., 2013; Wang et al., 2008; Ghazi et al., 2009).

Furthermore, other factors needed for the enhanced longevity of germline deficient animals have been identified. For example, the nuclear receptors NHR-80 and NHR-49 which are fat metabolism regulators and the transcription factor PHA-4 (Goudeau et al., 2011; Ratnappan et al., 2014). NHR-80-dependent *fat-6* elevation is important for lifespan extension and is also dependent on DAF-16 (Goudeau et al., 2011; Ackerman and Gems, 2012). As far as PHA-4 is concerned, it mediates its effect by inducing autophagy in the intestine of germline-less animals. Lipolysis is also implicated because the triglyceride lipase LIPL-4 is induced in a DAF-16-dependent manner upon germline loss (Lapierre et al., 2011). This induction is necessary for the lifespan extension observed. Moreover, the lipase LIPS-17 and the fatty acid reductase FARD-1 also play a role, while it was shown that germline-less animals have increased fat content (Uno and Nishida, 2016; McCormick et al., 2012; Hansen et al., 2013).

Nongenetic Regulation of Aging

Epigenetic Alterations and Aging

As previously mentioned, the aging process is influenced by both genetic and nongenetic factors such as various environmental stimuli, including caloric restriction and stress. Importantly, emerging evidence supports a role for epigenetics in lifespan regulation in diverse species, including *C. elegans*. The term epigenetics strictly refers to long lasting and potentially heritable changes in phenotype or gene expression that are not due to changes in the underlying DNA sequences (Goldberg et al., 2007). In this regard, much excitement was generated by reports describing chromatin alterations that occur during aging. Additionally, several intriguing studies suggest that interfering with chromatin modifying enzymes influences longevity in diverse organisms ranging from yeast to mammals (Benayoun et al., 2015). These findings are not surprising given that chromatin modifications can eventually cause transcriptional changes that affect genomic stability, with consequences for healthspan and lifespan. Below, we summarize the most important mechanisms that link epigenetic modifications to lifespan regulation in *C. elegans*, starting with the role of chromatin modifiers (**Table 1**).

Chromatin modifications

Posttranslational modifications of histone proteins are accomplished by enzymes that add or remove modifications in vulnerable histone residues, causing alterations in chromatin features during aging.

DNA methylation in C. elegans. The nematode lacks DNA methylation on the fifth position of cytosines in CpG dinucleotide sites (5-mC), a modification that is frequent in mammals. This led to the conclusion that DNA methylation is absent in worms. However, a recent study revealed the presence of adenine N6-methylation (6mA) in the nematode DNA. Notably, 6mA levels increase transgenerationally in *spr-5* mutant animals that are deficient for the histone H3 lysine 4 dimethyl (H3K4me2) demethylase SPR-5 (Katz et al., 2009), which is a homologue of the mammalian LSD1/KDM1A (Shi et al., 2004). Further analysis identified a DNA demethylase, NMDA-1, and a potential DNA methyltransferase, DAMT-1, which regulate the levels of 6mA and the functional interplay between DNA 6mA and histone H3K4 methylation (Greer et al., 2015).

Histone methylation and modulation of aging. This form of modification in core histone proteins is associated with either active or repressed genome regions and is considered as a dynamic mark in health and disease (Benayoun et al., 2015). Histone methylation is regulated by histone methyltransferases and histone demethylases, some of which have already been implicated in the regulation of lifespan in model organisms (Benayoun et al., 2015). Indeed, accumulating findings support the notion that widespread alterations in heterochromatin organization occur during aging and may be causatively linked to the aging process. In this regard, and consistent with previous work suggesting that loss of repressive chromatin is detrimental to longevity, knockdown of the H3K27me3 demethylase UTX-1 extends lifespan in *C. elegans* and increases the levels of H3K27me3, implying their beneficial impact on longevity. Furthermore, UTX-1 genetically interacts with the Insulin/IGF-1 signaling pathway to regulate longevity independently of the germline (Jin et al., 2011; Maures et al., 2011). In contrast, overexpression of the H3K27me3 demethylase KDM6B/JMJD-3 prolongs lifespan, suggesting that accumulation of H3K27me3 at specific genes such as stress response genes negatively affects longevity. The fact that the removal of germline stem cells preserves *jmjd-3.1* expression, prevents the accumulation of H3K27me3 at stress gene loci and maintains the heat stress response (HSR) suggests that differential investment strategies between the soma and the germline compete with each other to promote organismal stress resistance at the onset of reproduction (Labbadia and Morimoto, 2015).

Table 1 Epigenetic regulators influencing lifespan in *C. elegans*.

Modulator	*Function*	*Genetic manipulation*	*Lifespan effect*
UTX-1	Chromatin modifier, H3K27me3 demethylase	Downregulation	Extension
JMJD-3	Chromatin modifier, H3K27me3 demethylase	Overexpression	Extension
MES-2	Chromatin modifier, Polycomb group protein	Downregulation	Extension
ASH-2	Chromatin modifier, methyltransferase	Downregulation	Extension
SET-2	Chromatin modifier, methyltransferase	Downregulation	Extension
WDR-5	Chromatin modifier, methyltransferase	Downregulation	Extension
RBR-2	Chromatin modifier, H3K27me3 demethylase	Downregulation	Shortening, extension (condition-dependent)
RBR-2	Chromatin modifier, H3K27me3 demethylase	Overexpression	Extension
MET-1	Chromatin modifier, H3K36me3 methyltransferase	Downregulation/mutation	Shortening
SIR-2.1	Chromatin modifier, histone deacetylase	Overexpression	Extension
SIR-2.4	Chromatin modifier, histone deacetylase	Overexpression	Extension
OGT-1	*O*-linked *N*-acetylglucosamine (*O*-GlcNAc) transferase	Downregulation	Shortening
OGA-1	*O*-linked *N*-acetylglucosamine (*O*-GlcNAc)-selective *N*-acetyl-beta-D-glucosaminidase (*O*-GlcNAcase)	Mutation	No effect in wild-type animals, extension in *daf-2* mutants
ATPH-2	Chromatin remodeler	Downregulation	Extension

However, downregulation of MES-2, which is a Polycomb group protein with an essential role for germline development and patterning, increases the lifespan of sterile worms. Interestingly, flies heterozygous for mutations in the Enhancer of zeste (E(z)) (the *Drosophila melanogaster* homologue of *mes-2*) are long-lived, suggesting that the role of MES-2 in longevity is evolutionarily conserved (Ni et al., 2012). Altogether, these data indicate that different H3K27me3 modifiers regulate longevity in a complex manner.

Moreover, downregulation of proteins that form the COMPASS (complex protein associated with SET1) chromatin complex, which catalyzes trimethylation of lysine 4 on histone 3 (H3K4me), increases lifespan in *C. elegans*. Specifically, it has been shown that knockdown or mutation in the *ash-2*, *set-2*, and *wdr-5* genes prolongs lifespan. On the other hand, knockdown of *rbr-2*, which encodes the demethylase H3K4me3, shortens lifespan, whereas the overexpression of RBR-2 in the germline results in lifespan extension, most likely by ameliorating the effects of stress imposed by reproduction (Greer et al., 2010). However, it was also shown that RNAi-mediated knockdown or mutation of *rbr-2* promotes longevity under certain experimental conditions (Greer et al., 2010; Alvares et al., 2014). Further analysis showed that RBR-2 together with the SPR-5 H3K4me demethylase contributes to the effect of Insulin/IGF-1 signaling on longevity. Specifically, RBR-2 and SPR-5 increased adult lifespan of stress-resistant *daf-2* mutants and enhances germ cell immortality at high temperature (Alvares et al., 2014). A recent study revealed a novel link between H3K4me3 modifiers and fat metabolism in regulation of lifespan in *C. elegans*. Indeed, depletion of H3K4me3 methyltransferase, which prolongs lifespan, leads to accumulation of MUFAs. The underlying mechanism relies, at least partially, on the downregulation of germline targets including *rsks-1* and on the upregulation of delta-9 fatty acid desaturases in the intestine (Han et al., 2017).

Another histone H3K36me3 methyltransferase, MET-1, was also shown to have a role in regulation of lifespan in *C. elegans*. Indeed, RNAi-mediated knockdown or mutation in the *met-1* gene shortens lifespan (Greer et al., 2010). This finding combined with the fact that H3K36me3 levels decrease with age suggests that equilibrium in H3K36me3 levels may have a crucial impact on the regulation of lifespan. Collectively, these data highlight the importance of specific histone methyltransferases or demethylases for aging research.

Further supporting the impact of histone methylation on the aging process, a recent study revealed that the histone lysine demethylases JMJD-1.2/PHF8 and JMJD-3.1/JMJD3 act as important regulators of lifespan in response to mild mitochondrial dysfunction both in *C. elegans* and mice. Indeed, these demethylases are required for electron transport chain-mediated longevity and the induction of (mtUPR) (Merkwirth et al., 2016).

Histone acetylation and impact on longevity. The extent of histone acetylation is regulated by histone acetyltransferases and deacetylases, several of which have been associated with aging modulation in diverse species, including *C. elegans*. For example, sirtuins, which are NAD^+-dependent protein deacetylases, were reported to extend lifespan in yeast, worms, flies, and mice, though their role as longevity regulators is somehow controversial (Kenyon, 2010; Burnett et al., 2011). Nevertheless, the mechanism through which sirtuins function to influence lifespan remains poorly characterized. At least in yeast, SIR2 (silent information regulator 2) was originally assigned a role as a chromatin silencing component that represses gene transcription at selected loci (Michan and Sinclair, 2007). Later, it was shown that genetic or pharmacological activation of SIR-2.1 and sirtuin 1 (SIRT1), which are the homologues of SIR2 in the nematode and mammals, respectively, enhances survival, at least partially, through autophagy (Morselli et al., 2010). In addition, SIR-2.4 (the nematode homologue of mammalian SRT6 and SRT7) is reportedly required for stress resistance and lifespan extension. SIR-2.4 regulates the nuclear localization of the DAF-16/FOXO transcription factor by antagonizing the acetyltransferase CBP-1, thereby attenuating DAF-16 acetylation and eventually promoting its activation under stress conditions (Chiang et al., 2012). Notably, sirtuins are located in distinct cellular compartments, that is, in the nucleus where they function to deacetylate histones, altering gene expression epigenetically, in the cytoplasm or in mitochondria where they regulate the activity of metabolic enzymes (Chang and Guarente, 2014). Together, these findings indicate that sirtuins exert their longevity effects not only through changes in the chromatin state but also by affecting the acetylation status of nonhistone substrates.

Other histone modifications and their role in modulation of aging. Besides histone methylation and acetylation, the addition of *O*-*N*-acetyl-glycosamine (*O*-GlcNAc) to H2A, H2B, and H4 and to promoters of genes linked to insulin-like signaling, metabolism, aging, stress, and pathogen-response pathways may epigenetically modulate gene expression in *C. elegans*, as resulted from whole-genome chromatin immunoprecipitation (ChIP)-on-chip tiling arrays, and transcriptional profiling (Love et al., 2010). In line with these findings, mutation in OGT-1, which is the enzyme that adds *O*-GlcNAc, shortens the lifespan of both wild-type and *daf-2* mutant animals. By contrast, mutation in OGA-1, the enzyme that removes *O*-GlcNAc, promotes longevity only in *daf-2* mutants (Love et al., 2010; Rahman et al., 2010).

To summarize, the mechanisms by which histone modifications regulate gene expression remain elusive. They may promote or inhibit the recruitment of transcriptional machinery or they may act to silence transcription through the formation of heterochromatin (i.e., H3K9me3, H4K20me2) and to regulate genome stability (i.e., H3K56ac, H3K14ac) (Lauberth et al., 2013). Whatever the mechanism is, it is important that these modifications change during aging (Benayoun et al., 2015).

Nucleosome remodeling and aging modulation. Nucleosome remodelers can change nucleosome positioning, chromatin state, and overall nuclear organization. Therefore, it is not surprising that their activity can influence aging. For example, the chromatin remodeler SWI/SNF was linked to DAF-16-mediated stress resistance and longevity in the nematode (Riedel et al., 2013). Moreover, inactivation of ATPH-2, a key component of the putative nematode homologue of yeast imitation switch complex (ISWI), which is an ATP-dependent chromatin remodeling complex, extends the worm lifespan mimicking the beneficial effects of calorie restriction in activating stress response genes during aging (Dang et al., 2014).

Environmental stimuli, epigenome remodeling, and more

Emerging findings suggest that various environmental stimuli such as food intake, nutrient, and energy sensing and physical exercise, among others, affect aging. Below, we will briefly discuss how dietary restriction and crucial energy sensors and metabolism regulators that influence longevity are linked with age-related epigenetic changes.

Dietary restriction. Reduced food intake without malnutrition is the only intervention that extends lifespan in all species tested so far. The beneficial effects of dietary restriction on healthspan and lifespan are mediated by multiple mechanisms. For example, the insulin/IGF-1 and TOR pathways have a predominant role, among the nutrient sensing pathways involved in the response to dietary restriction. On the other hand, sirtuins and AMPK have crucial roles in mediating the adaptation to decreased ratios of NADH/NAD and ATP/ADP, respectively, under dietary restricted conditions. As such, they are important players in dietary restriction-induced longevity. Another key player of the response to dietary restriction is chromatin. Indeed, dietary restriction induces changes in the expression of genes implicated in metabolism, stress responses, DNA damage repair and regulation of chromatin structure, among others. In brief, these gene expression changes not only contribute to the metabolic adaptation of the cell but also help to protect genome integrity and chromatin structure (Vaquero and Reinberg, 2009).

Adding another layer of complexity, dietary restriction extends *C. elegans* lifespan through SIR-2.1-dependent induction of autophagy (Morselli et al., 2010). Moreover, the PHA-4/FOXA transcription factor was shown to regulate adult lifespan extension by dietary restriction in *C. elegans*, and autophagy is induced specifically by PHA-4 activation under limited food conditions (Hansen et al., 2008). Interestingly, a recent study showed that autophagy specifically in the intestine is crucial for lifespan extension in dietary-restricted *eat-2* mutants. Furthermore, intestinal-specific inhibition of autophagy reduces the intestinal integrity of *eat-2* mutants and decreases their motility during aging (Gelino et al., 2016).

The nutrient and energy sensor AMPK is also involved in lifespan extension in response to dietary restriction. In this case, AMPK exerts its beneficial effects on lifespan in part by phosphorylating DAF-16/FoxO and activating DAF-16/FoxO-dependent transcription, suggesting that the AMPK-FoxO axis is involved in the antiaging effects of dietary restriction (Greer et al., 2007).

Stress response pathways in lifespan control

It is becoming apparent that lifespan is closely related to stress resistance (Morley and Morimoto, 2004). Evidence shows that long-lived mutants in *C. elegans* and other model organisms are more resistant to environmental stress, while the efficiency of most stress response pathways deteriorates with aging. This increases the probability of homeostatic collapse and disease onset, eventually leading to senescence and death. On the other hand, when the stress response pathways remain functional as the animal ages, age-prone dysfunction is alleviated and the organism bypasses the risk of disease-onset or death. According to this theory, long lifespan is itself a marker of increased stress resistance which also predicts that stress-response mechanisms are activated inside cells (Johnson et al., 2001). An excellent example is the insulin/IGF-1 mutants which are both long-lived and extremely resistant against several stressors as compared to their control counterparts (Lithgow and Walker, 2002). Effector mechanisms and factors activated in response to stress such as heat shock, oxidative stress, hypoxia, and/or DNA damage have also important roles in the regulation of the aging process (Haigis and Yankner, 2010; Kourtis and Tavernarakis, 2011). Also, hormetic responses, initiated upon mild exposure to the aforementioned stressors, increase lifespan in *C. elegans*, while very severe stress has the opposite effects (Cypser and Johnson, 2002; Epel and Lithgow, 2014; Gems and Partridge, 2008; Kumsta et al., 2017). For example, animals subjected to mild heat stress become long-lived. In parallel, long-lived mutants exhibit increased thermotolerance, effect that tightly couples heat stress resistance to lifespan (Lithgow et al., 1995). Upon heat stress, HSF-1 (the nematode homologue of mammalian HSF1-4) is activated (Morton and Lamitina, 2013). Upon its activation, HSF-1 trimerizes and forms distinct nuclear foci (Morton and Lamitina, 2013). Its activation triggers transcription of target genes, most of which encode chaperones which function in proteostasis restoration (Pirkkala et al., 2001). This finding combined with the fact that *hsf-1* expression and activity increase lifespan in *C. elegans* provides evidence that the HSF-1 pathway regulates lifespan by diminishing protein aggregation during aging (Morley and Morimoto, 2004). Indeed, wild-type animals accumulate toxic protein aggregates in contrast to the long-lived *daf-2* mutants in which activity and expression of HSF-1 is highly induced. On the other hand, *hsf-1* downregulation decreases the lifespan of wild-type animals and long-lived insulin mutants (Hsu et al., 2003; Morley and Morimoto, 2004). Recently, autophagy was identified as the effector mechanism downstream of HSF-1. In fact, either hormetic heat stress or *hsf-1* overexpression can induce autophagy, which in turn mediates the beneficial effects on proteostasis, lifespan, and stress resistance in *C. elegans* (Kumsta et al., 2017). HSF-1 activation in response to heat stress is posttranslationally regulated by the deacetylase SIR-2.1/SIRT1 (Brunquell et al., 2016). Inhibition of *sir-2.1* abrogates the expression of some of the HSF-1 target genes while its overexpression or allosteric induction by resveratrol enhances the heat stress response and increases lifespan (Viswanathan et al., 2005; Wood et al., 2004) (**Fig. 2**).

Another example is the oxidative stress response. It was first shown in *C. elegans* that long-lived mutants of the insulin/IGF-1 pathway retain a robust oxidative stress response at old ages as compared to their control counterparts (Honda and Honda, 1999). This was supported by the finding that aged *age-1* mutants display higher levels of catalase and SOD as well as enhanced activity of the enzymes compared to synchronous wild-type animals (Vanfleteren, 1993). Later on, it was revealed that the oxidative stress response is mediated by the activation of DAF-16 and SKN-1 as well as HIF-1 transcription factors (Park et al., 2009; Senchuk et al., 2018; Zhang et al., 2009). Interestingly, activation of SKN-1 target genes in response to oxidative stress is also required for normal lifespan (Park et al., 2009). Moreover, *skn-1* and *daf-16* are induced in several long-lived mutants while their downregulation severely shortens the lifespan both in these mutants and wild-type animals (Senchuk et al., 2018; Ewald et al., 2015). Also, as already mentioned, mild ROS elevation induces mitohormesis and triggers lifespan extension in *C. elegans*. This was also confirmed by paraquat treatment, which increases lifespan when administered at low doses (Yang and Hekimi, 2010a). Importantly though,

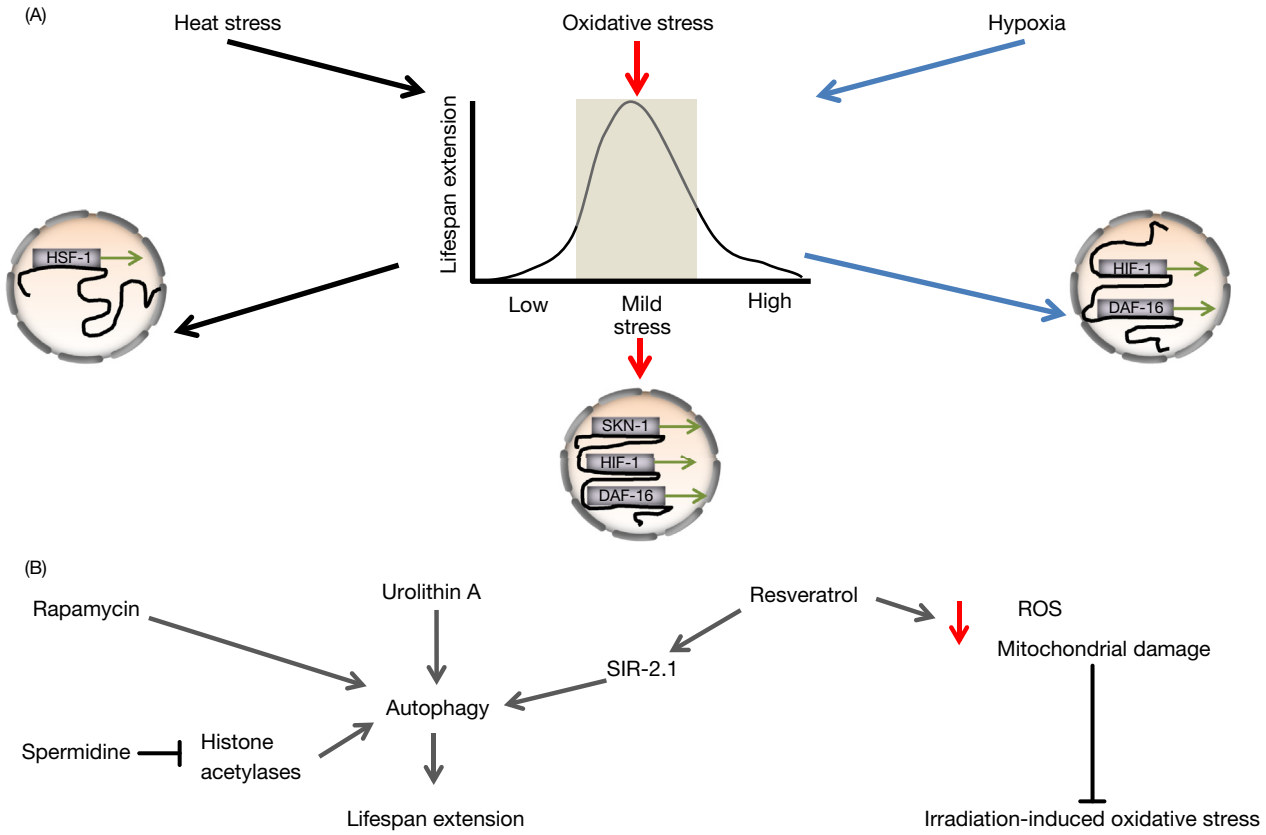

Fig. 2 Environmental and pharmacological factors that modulate lifespan. (A) Animals exposed to mild heat, oxidative, and hypoxic stress exhibit lifespan extension, which is mediated by the activation of distinct transcriptional programs (*black arrows* are representative of heat stress-related responses, *red arrows* of oxidative stress, and *blue arrows* of hypoxic stress); (B) treatment with rapamycin, resveratrol, urolithin A, and spermidine confer lifespan extension by inducing autophagy-related mechanisms.

oxidative stress is not linearly associated with lifespan extension. For example, *mev-1* mutants which are very oxidative stress challenged are short lived (Yang et al., 2007). Interestingly, treatment with antioxidants can alleviate their short lifespan, while it does not affect wild-type animals (Yang et al., 2007; Phulara et al., 2015). Moreover, antioxidant treatment could even decrease the lifespan of long-lived mutants (Yang and Hekimi, 2010a). These results indicate that only a specific window in ROS production triggers lifespan extension while overproduction of ROS can have the opposite effects on longevity (Fig. 2).

Last but not least, the hypoxia stress-related pathway has been recently implicated in the regulation of longevity. Hypoxia is a condition where cells and entire organisms are challenged with low oxygen levels. In response to hypoxia, the HIF-1 transcription factor is activated (Shen et al., 2005). Response to hypoxic stress is very well-conserved and involves the activation of HIF-1-target genes in *C. elegans* and other model organisms. Active HIF-1 exists in a heterodimeric complex which nuclearizes and through its target genes rewires metabolism toward anaerobic glycolysis (Jiang et al., 2001). Interestingly, it was recently shown that oxygen deprivation increases lifespan in *C. elegans* in a HIF-1- and DAF-16-dependent manner (Leiser et al., 2013). Exposure of animals to hypoxia during the late stages of larval development or even at the first day of adulthood is sufficient to positively influence lifespan by activating several downstream mechanisms (Leiser et al., 2013; Daskalaki et al., 2018). Supporting evidence indicates that genetic HIF-1 stabilization by VHL mutation can extend lifespan even under normal oxygen conditions (Leiser et al., 2013; Mehta et al., 2009). Also, *hif-1* overexpression is proportionally correlated with lifespan extension and increased heat and oxidative stress resistance. Further studies indicated that HIF-1 acts in parallel to DAF-16 and SKN-1 to increase lifespan (Zhang et al., 2009) (Fig. 2).

Pharmacological Interventions Modulating Aging

Pharmacological agents used to extend lifespan in model organisms act mainly as inducers of general autophagy. Also, agents that promote healthy aging by activating the selective mitochondrial autophagy known as mitophagy have been reported. Interestingly, many of the natural/pharmacological inducers of autophagy act by mimicking the effects of calorie restriction.

Although beyond the scope of this article, pharmacological modulators of autophagy, such as rapamycin, resveratrol, and spermidine, have been shown to extend the worm lifespan. Their beneficial effects on longevity are mediated by diverse mechanisms that often converge on autophagy (Fig. 2).

More specifically, rapamycin, which is the best characterized inducer of autophagy, extends lifespan independently of SIR-2.1 (Morselli et al., 2010). By contrast, resveratrol, a well-studied plant polyphenol found in grape berry skin, red wine, knotweed, peanuts, and other plants reportedly promotes longevity in *C. elegans* through SIR-2.1-mediated activation of autophagy (Morselli et al., 2010). Moreover, resveratrol protects worms against irradiation-induced oxidative stress, by lowering ROS levels and preventing mitochondrial damage (Ye et al., 2010).

Spermidine is a naturally occurring ubiquitous polyamine produced by putrescine and is the precursor of spermine. Several studies showed that the cellular content of polyamines decreases with age and this is often causatively associated with disease onset (Minois et al., 2011). Spermidine supplementation extends lifespan in several organisms, including yeast, worms and flies (Eisenberg et al., 2009). This beneficial effect on longevity is observed in both wild-type and *sir-2.1* mutant animals (although *sir-2.1* lesions attenuate autophagy induction by spermidine), suggesting that, in contrast to resveratrol, lifespan extension by spermidine is independent of *sir-2.1* (Eisenberg et al., 2009). In yeast, spermidine functions as an inhibitor of histone acetylases. Modulation of the acetylation state of histones affects the transcription of several genes, some of which are involved in the autophagic degradation machinery (Eisenberg et al., 2009). Together these findings indicate that spermidine and resveratrol enhance longevity by activating autophagy through distinct pathways that converge on acetylproteome (Morselli et al., 2010, 2011).

Ellagitannins, which belong to bioactive polyphenols, are found in fruits and nuts and have antioxidant, anti-inflammatory, and tumor suppressive properties (Losso et al., 2004). Pomegranate juice is the most prominent source of ellagitannins in nature (Johanningsmeier and Harris, 2011). Upon their consumption, ellagitannins are metabolized from gut microbiota into urolithins, which are subsequently absorbed and distributed to the entire human body through blood stream (Espin et al., 2013). Most of their effects have been tested in artificial conditions *in vitro* (Kang et al., 2016; Piwowarski et al., 2015; Seeram et al., 2007; Lansky et al., 2005). However, it was recently shown that urolithins can influence whole homeostasis during aging. Indeed, supplementation of urolithin A, which is the most prevalent ellagitannins-derived metabolite in humans, promotes mitophagy in nematodes and mammals (Ryu et al., 2016).

Concluding Remarks

Research using model organisms such as the lowly nematode *C. elegans* has culminated in the identification of several signaling pathways that control aging in a well-conserved fashion. In addition to genetic factors, a growing body of evidence indicates that epigenetic mechanisms and environmental factors can modulate healthspan and lifespan. These advances support the idea that similar mechanisms, albeit with variations, may operate in humans to regulate aging. However, more work is necessary to consolidate this notion. We anticipate that continued studies in *C. elegans*, where aging modulators can be easily manipulated, will further advance our understanding of aging and will facilitate the development of novel approaches aiming to maintain human health and quality of life in the elderly.

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