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Review

Mitochondrial-to-nuclear communication in aging: an epigenetic perspective

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Age-associated changes in mitochondria are closely involved in aging. Apart from the established roles in bioenergetics and biosynthesis, mitochondria are signaling organelles that communicate their fitness to the nucleus, triggering transcriptional programs to adapt homeostasis stress that is essential for organismal health and aging. Emerging studies revealed that mitochondrial-to-nuclear (mito-nuclear) communication via altered levels of mitochondrial metabolites or stress signals causes various epigenetic changes, facilitating efforts to maintain homeostasis and affect aging. Here, we summarize recent studies on the mechanisms by which mito-nuclear communication modulates epigenomes and their effects on regulating the aging process. Insights into understanding how mitochondrial metabolites serve as prolongevity signals and how aging affects this communication will help us develop interventions to promote longevity and health.

Highlights

Mito-nuclear communication plays an integral role in cellular homeostasis and aging.

Mitochondrial metabolites are substrates or mediators of epigenetic modifications.

Mitochondrial-to-nuclear stress signals modulate lifespan via epigenetic regulations.

The emerging role of mito-nuclear communication in the epigenome and aging

Mitochondria are the central core of energy metabolism within the cell, producing **adenosine triphosphate (ATP)** (see Glossary) through the **tricarboxylic acid cycle (TCA cycle)** and **oxidative phosphorylation (OXPHOS)**. The mitochondrial proteome is mostly encoded by the nucleus, with only 13 subunits of the **electron transport chain (ETC)** encoded by the mitochondrial DNA (mtDNA). Thus, constant mito-nuclear communication is required to coordinate the expression, translation, and assembly of mitochondrial OXPHOS complexes encoded by the mitochondrial and nuclear genomes to ensure optimal mitochondrial function [1]. During aging, a decline in mitochondria function is associated with decreased OXPHOS activity, altered levels of mitochondrial TCA cycle enzymes, accumulation of mtDNA mutations, increased **reactive oxygen species (ROS)** production, and dysregulated mitochondrial proteostasis [2]. As a result, mitochondrial dysfunction may disrupt communication between mitochondria and the nucleus, resulting in changes in gene expression associated with aging.

Primarily, mitochondrial function is mediated by the nuclear-encoded genes through anterograde (nuclear-to-mitochondrial) signals, which promote mitochondrial biogenesis or increase mitochondrial activity to meet cellular needs. This regulation mainly depends on nuclear-encoded transcription factors, such as PCG1α, NRF1, and other coregulators, to induce the expression of mtDNA-encoded genes [3]. In contrast, retrograde signaling is transmitted from mitochondria to the nucleus in response to perturbations within mitochondrial, such as the proteostasis stress, energy deficits, and increased ROS production, which triggers transcription reprogramming for metabolic adaptions [4–6] (Figure 1). While there are several major regulators involved in mito-nuclear communication under stress conditions, only SIRT1, AMPK, and ATFS-1 have been studied in great detail [3]. Emerging evidence showed that mitochondrial metabolites and other stress signals can serve as signaling molecules to mediate epigenetic modifications, which in turn facilitate the expression of metabolic genes essential for cellular homeostasis

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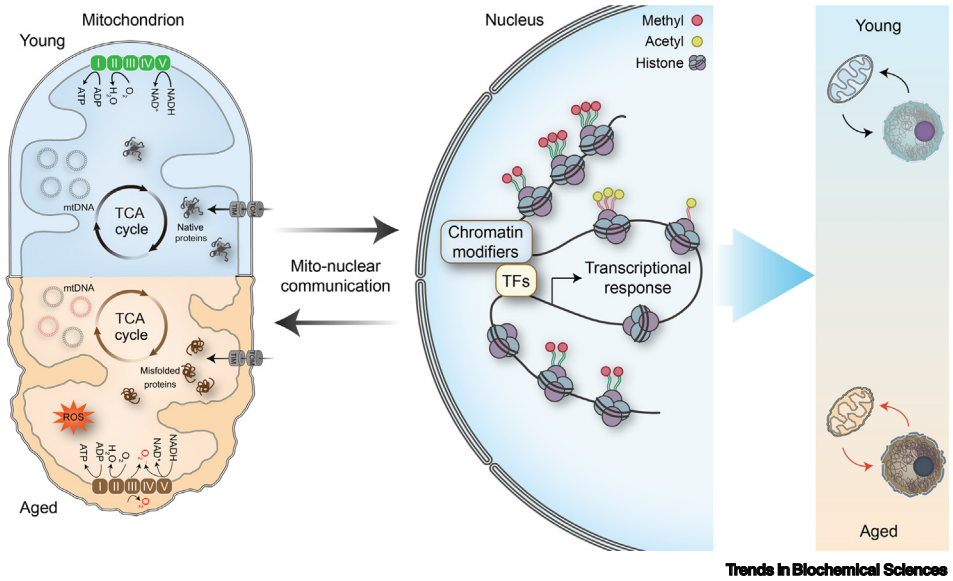

Figure 1. Mito-nuclear communication and aging. The anterograde (nuclear-to-mitochondrial) signals mediate the expression of mtDNA-encoded genes to promote mitochondrial function. The retrograde signals (mitochondrial-to-nuclear) allow mitochondria to communicate their fitness status to the nucleus in response to various stress conditions, such as proteostasis stress, energy deficits, and increased ROS production, to regulate the expression of nuclear-encoded genes for metabolic adaptions. In addition to transcriptional factors (TFs), mitochondrial metabolites and stress signals could serve as secondary messengers to communicate with multiple chromatin modifiers that affect gene expression for stress adaptations. Therefore, mitochondrial–nuclear signaling plays a crucial role in cellular homeostasis, and disruption of the interplay between mitochondria and the nucleus contributes to aging and age-related disease. Abbreviations: mtDNA, mitochondrial DNA; NAD^+, nicotinamide adenine dinucleotide; NADH, NAD^+ hydrogen; TCA, tricarboxylic acid.

and aging regulation [7–9]. Notably, aging is accompanied by extensive epigenetic alterations and metabolic changes, which are more complex than a simple constant decline [10,11]. Thus, communication between mitochondria and the nucleus provides cells with a dynamic regulatory network that allows them to respond to ever changing metabolic conditions or stresses associated with aging [5,9]. In this review, we discuss examples of how mitochondrial metabolites and stress signals modulate aging or longevity via epigenetic alterations and summarize the current state of knowledge about how mitochondrial metabolites could serve as potential prolongevity signals.

The interplay between mitochondrial metabolites and epigenetics modulates lifespan

In addition to providing the energy for cells, mitochondria also serve as one of the metabolic hubs responsible for the biosynthesis of macromolecules such as nucleotides, lipids, and proteins [7]. The intermediate metabolites derived from mitochondria are generally considered to be by-products of cellular metabolism. It is increasingly recognized that metabolic signals originating from the mitochondria can initiate epigenetic modifications in the nucleus through nonmetabolic mechanisms [12] (Figure 2). The interplay between mitochondrial metabolites and epigenomes allows for alterations in nuclear gene expression, which in turn regulates cellular homeostasis and modulates the aging process [9] (Table 1). In this section, we discuss examples of mitochondrial metabolites, including acetyl-coenzyme A (acetyl-CoA), alpha-ketoglutarate (α-KG), nicotinamide adenine dinucleotide (NAD^+), and methionine, as potential longevity regulators and how changes in their abundance influence the epigenomes and the aging process in different organisms.

Glossary

Adenosine triphosphate (ATP): a molecule that carries energy within cells. ATP consists of adenine, ribose, and three groups of phosphoric acid, which release energy during hydrolysis.
Electron transport chain (ETC): a series of protein complexes that transfer electrons from electron donors to electron acceptors via redox reactions and couple this electron transfer with the transfer of protons across a membrane.
Mitochondrial unfolded protein response (UPR^{mt}): a protective transcriptional response used to promote organelle-specific proteostasis during mitochondrial dysfunction.
Mitokine: a molecule (secreted protein, peptides, or others) produced and secreted from cells experiencing mitochondrial stress, eliciting an organismal mitochondrial stress response in distal tissues that have not been directly affected by the stress stimulus.
One-carbon metabolism: comprises a series of interlinking metabolic pathways that include the methionine and folate cycles that are central to cellular function, providing 1C units (methyl groups) for the synthesis of DNA, polyamines, amino acids, creatine, and phospholipids.
Oxidative phosphorylation (OXPHOS): a metabolic pathway in which cells use enzymes to release the chemical energy stored within the nutrients through oxidation in order to produce ATP.
Reactive oxygen species (ROS): by-products of cellular metabolism, primarily in the mitochondria; oxygen-containing radicals that are capable of independent existence with one or more unpaired electrons, which is essential for cell physiology and participates in many pathological processes.
Tricarboxylic acid cycle (TCA cycle): a cyclic series of enzymatic reactions occurring in the mitochondrial matrix, through which organisms produce energy.

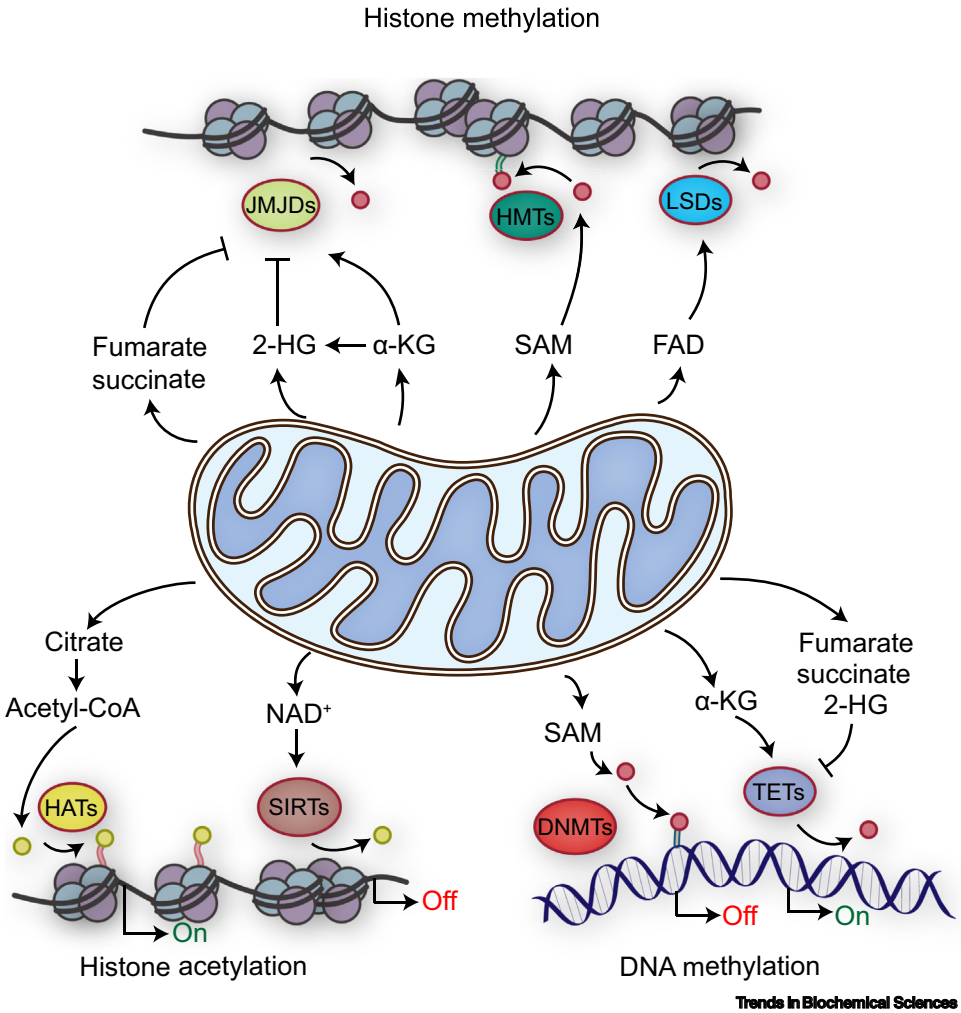

Figure 2. Mitochondrial metabolites for epigenetic modifications. Metabolites generated by the tricarboxylic acid (TCA) cycle, the electron transport chain (ETC), or one-carbon cycle within the mitochondria can act as substrates or cofactors to control epigenetic modification, especially histone acetylation and methylation and DNA methylation. Acetyl-CoA, which is produced from multiple sources within mitochondria, including pyruvate, amino acids, and fatty acids, is the source of acetyl groups used by the histone acetyltransferases (HATs) to effect histone and protein acetylation. Variations in acetyl-CoA are thus mitochondrial signals that can modulate broad gene expression programs. Alpha-ketoglutarate (α-KG) produced in the TCA cycle serves as an essential cofactor for the chromatin-modifying Jumonji C (JmjC) domain-containing lysine demethylases (JMJDs) and ten-eleven translocation (TETs) DNA demethylases, while fumarate, succinate and 2-hydroxyglutarate (2-HG) inhibit both JMJDs and TETs. Nicotinamide adenine dinucleotide (NAD^+) and flavin adenine dinucleotide (FAD), which link the TCA cycle to the ETC via oxidative phosphorylation (OXPHOS), also influence the epigenome. The level and redox state of FAD, an essential cofactor of Lys-specific demethylases (LSD), a class of histone demethylases, is regulated by mitochondria. NAD^+ is a cofactor for the protein deacetylases sirtuins (SIRTs) that regulate the level of histone acetylation. In addition, *S*-adenosyl-L-methionine (SAM), which is generated in the cytosol and sustained by the one-carbon metabolism in mitochondria, provides the source of methyl groups for histone and DNA methyltransferases (HMTs and DNMTs). 'Off' denotes transcriptional inhibition; 'on' denotes transcriptional activation.

Acetyl-CoA

The TCA cycle, also known as the citric acid cycle or the Krebs cycle, releases stored energy through the oxidation of acetyl-CoA derived from carbohydrates, fatty acids, and proteins. Acetyl-CoA enters the TCA cycle to generate citrate and oxaloacetate within mitochondria [12]. As the cycle runs, the intermediates produced from the TCA cycle can be transported into the

Table 1. Mitochondrial metabolites that are implicated in longevity in various organisms

Metabolites		Action	Pathways affected	Epigenetic modifications	Lifespan	Organism	Refs
TCA cycle	Acetyl-CoA	Deletion of the mitochondrial CoA-transferase ACH1	Hyperactivation of Acs2p/ACSS2	H2A/B, H3 acetylation	Decreased chronological lifespan (CLS))	*Saccharomyces cerevisiae*	[21]
		ETC impairment-induced decrease in acetyl-CoA levels	UPR^{mt} activation	H3Ac	Increased	*C. elegans*	[91]
		Inhibition of ACLY activity to reduce acetyl-CoA levels	Unknown	H3Ac, H4Ac	Increased	*Drosophila melanogaster*	[20]
		Brain-specific knockdown of ACSS2	Activation of autophagy	Unknown	Increased	*D. melanogaster*	[21]
		Attenuation of hippocampal ACSS2 expression	Reduction in neuronal plasticity	H3K9ac, H3K27ac, H4K12ac	Cognitive impairment	*Mus musculus*	[15]
	Citrate	ETC impairment-induced decrease in citrate levels	UPR^{mt} activation	H3Ac	Increased	*C. elegans*	[91]
		Lower temperature-induced decrease in citrate/isocitrate ratio	Unknown	H3Ac, H4Ac	Increased	*D. melanogaster*	[20]
	α-KG	ETC impairment of mit mutants have increased α-KG levels	Inhibition of α-ketoacid dehydrogenases	Unknown	Increased	*C. elegans*	[32]
		α-KG supplementation	ATP synthase and TOR inhibition	Unknown	Increased	*C. elegans*	[29]
		α-KG supplementation	Activation of AMPK and inhibition of mTOR	Unknown	Increased	*D. melanogaster*	[30]
		α-KG supplementation	Decreased levels of inflammatory cytokines	Unknown	Increased	*M. musculus*	[31]
	Succinate	Mutation of succinate dehydrogenase complex (SDH) to increase succinate levels	Inhibition of multiple α-KG-dependent dioxygenases	Genome-wide histone and DNA methylation	Contribute to tumorigenesis	*M. musculus*	[97]
	Fumarate	Fumarate supplementation	*daf-16*/FOXO and *sir-2.1*/Sirt1 requirement	Unknown	Increased	*C. elegans*	[98]
		Mutation of fumarate hydratase (FH) to increase fumarate levels	Inhibition of multiple α-KG-dependent dioxygenases	Genome-wide histone and DNA methylation	Contribute to tumorigenesis	*M. musculus*	[97]
OXPHOS	NAD^+	NR supplementation to increase NAD^+ levels	Sir2 activation	Unknown	Increased replicative lifespan (RLS)	*S. cerevisiae*	[39]
		NR supplementation to increase NAD^+ levels	SIR-2.1 and UPR^{mt} activation; increased mitochondria content and ATP levels; improved metabolism	Unknown	Increased	*C. elegans*	[54]
		Boost *de novo* NAD^+ synthesis	SIR-2.1 activation, UPR^{mt} activation	Unknown	Increased	*C. elegans*	[99]

Table 1. (continued)

Metabolites		Action	Pathways affected	Epigenetic modifications	Lifespan	Organism	Refs
		NR supplementation to increase NAD^+ levels	SIRT1, SIRT2 activation; UPR^{mt} activation	BubR1 deacetylation	Increased	*M. musculus*	[40,100]
		Boost *de novo* NAD^+ synthesis	SIRT1 activation, UPR^{mt} activation	Lowered acetylation levels of FOXO1	Protect the liver and kidneys	*M. musculus*	[99]
		Regenerating mitochondrial NAD^+ from bolstering MC1 activity	Prevent neural inflammation	Unknown	Increased	*M. musculus*	[52]
	ROS	Menadione and rapamycin supplementation to increase mtROS levels	Inactivation of a histone H3K36 demethylase Rph1p	H3K36me3	Increased CLS	*S. cerevisiae*	[82]
		Exposure to ROS naturally during early development	Inactivation of the SET1/MLL histone methyltransferases	H3K4me3	Increased	*C. elegans*	[83]
Methionine metabolism	Methionine	Methionine restriction	Retrograde response activation, increased stress tolerance, autophagy-dependent	Unknown	Increased CLS	*S. cerevisiae*	[64]
		Metformin supplementation to inhibit microbial folate and methionine metabolism	Decreased in methionine levels	Unknown	Increased	*C. elegans*	[101]
		Methionine restriction with reduced levels of amino acids	TOR signaling involvement	Unknown	Increased	*D. melanogaster*	[102]
		Methionine restriction	Decreased levels of glucose, T4, IGF-I, and insulin; increased levels of hepatocyte MIF; improved stress resistance	Unknown	Increased	*M. musculus*	[63]
	SAM	Deletion of methionine adenosyltransferase *sam1*	Unknown	Unknown	Increased RLS	*S. cerevisiae*	[103]
		Knock-down of methionine adenosyltransferase *sams-1*	Mimicking dietary restriction	Unknown	Increased	*C. elegans*	[65]
		Knock-down of methionine adenosyltransferase Sams	Increased in methionine levels	Unknown	Decreased	*D. melanogaster*	[104]
		Overexpression of glycine *N*-methyltransferase gnmt to decrease SAM levels	Reduced insulin/IGF-1 signaling	Unknown	Increased	*D. melanogaster*	[104]
	Spermidine	Spermidine supplementation	Inhibition of histone acetyltransferases activity, suppression of oxidative stress, activation of autophagy	H3Ac	Increased	*S. cerevisiae*, *C. elegans*, *D. melanogaster*	[69]

cytosol for biosynthetic purposes. For example, citrate can exit the mitochondria through the mitochondrial citrate transporter SLC25A1 (tricarboxylate antiporter solute carrier family 25, member 1) and be converted back to acetyl-CoA and oxaloacetate by ATP-citrate lyase (ACLY) both in the cytosol and in the nucleus [13]. Alternatively, ACSS2, the cytosolic acyl-CoA synthetase short-chain family member 2, utilizes acetate to generate acetyl-CoA under nutrient-limited conditions [14,15]. The cytosolic acetyl-CoA can then be used to synthesize fatty acids, steroids, and certain amino acids. In addition to its prominent role in metabolism and biosynthesis, the signaling role of acetyl-CoA is related to its ability to provide acetyl groups for protein acetylation, including histone acetylation, a process catalyzed by histone acetyltransferases (HATs) [16]. Acetylation neutralizes the positive charge on lysine residues, which leads to an open chromatin structure, facilitating access for transcription factors and affecting gene expression [17]. The abundance and distribution of acetyl-CoA in distinct subcellular compartments changes in response to various mitochondrial perturbations or pathological conditions during aging [9,16]. Thus, acetyl-CoA serves as a second messenger that relays signals from mitochondria to the nucleus to regulate metabolic adaptations that may contribute to the aging process.

Although acetyl-CoA is a central metabolite for both catabolic reactions and anabolic metabolism, very few studies have investigated how levels of acetyl-CoA change with age due to its unstable nature. Many mitochondrial-related functions are found to be impaired during aging and in age-related neurodegenerative diseases [18]. In line with this notion, a study reported that acetyl-CoA levels are decreased in the aging mouse brain and administration of two compounds, CMS121 or J147, restored the levels of acetyl-CoA in the brain; this increase was associated with the acetylation of histone H3 at a site specific for memory enhancement [19]. Despite the tendency of an overall decline in mitochondrial function associated with aging, studies in fruit flies surprisingly found that levels of acetyl-CoA and citrate increase in midlife [20]. Knock-down of ACLY reduces the level of acetyl-CoA, leading to metabolic changes and increased longevity in *Drosophila* [20]. It seems that the effects of acetyl-CoA on lifespan regulation are not consistent among different organisms; recent studies have indicated that compartmentalized acetyl-CoA and its effects on gene expression in specific tissues are key to lifespan regulation.

In yeast, blocking the mitochondrial route to acetyl-CoA resulted in the cytosolic accumulation of acetate, which hyperactivated acetyl-CoA synthesis (Acs2p) and triggered histone acetylation, resulting in a shortened lifespan [21]. Pouikli and colleagues observed an unexpected age-dependent change in the localization of the acetyl-lysine signal, shifting from nuclear to mitochondrial upon aging in mesenchymal stem cells (MSC) [22]. They found that acetyl-CoA was trapped inside mitochondria of aged MSCs due to a decrease in the level of citrate carrier, leading to a reduction in histone acetylation and age-dependent chromatin compaction. Restoration of cytosolic acetyl-CoA levels, either by exogenous expression of the citrate carrier or via acetate supplementation, remodels the chromatin landscape and rejuvenates the MSCs [22]. The results of this study highlight the effect of mitochondrial-to-nuclear communication on stem cell aging.

Given that the histone acetylation landscape changes with age in different tissue across various organisms [23], future studies focusing on the distribution and relative abundance of compartmentalized acetyl-CoA will shed new light on the connection between mitochondrial metabolites and histone marks that ultimately change gene expression, resulting in aging-related phenotypes.

α-KG

α-KG is an endogenous intermediary metabolite that is generated from isocitrate by isocitrate dehydrogenase (IDH1/2) via oxidative decarboxylation in the TCA cycle. α-KG is also an obligatory cosubstrate for 2-oxoglutarate-dependent dioxygenases (2-OGDDs), a large group of conserved

enzymes that catalyze hydroxylation reactions on different substrates, including proteins, lipids, nucleic acids, and intermediary metabolites [24]. In addition, α-KG has multiple functions in physiology via epigenetic regulations, because α-KG is a required substrate of some chromatin-modifying enzymes, including the chromatin-modifying Jumonji C (JmjC) domain-containing lysine demethylases (JMJDs), which are the major histone demethylases, and ten-eleven translocation (TETs) DNA demethylases involved in DNA demethylation [25]. It is important to note that the activity of JMJDs and TETs is dependent on the intracellular ratio of α-KG to succinate or other inhibitors, including fumarate or 2-hydroxyglutarate (2-HG) [26]. Thus, changes in the level of mitochondrial metabolite α-KG are capable of driving nuclear gene expression by affecting DNA and histone methylation profiles.

During the aging process, mitochondrial function is progressively impaired and metabolic flux in mitochondria declines, which exacerbates α-KG deficiency [27]. It has been reported that α-KG deficiency in progenitor stem cells increases with age. For example, the level of α-KG is reduced in the follicle fluids of aged humans, and supplementation with α-KG preserves ovarian function in mice [28]. Supplementation with α-KG has been shown to extend lifespan in *Caenorhabditis elegans*, *Drosophila*, and mice [29–31]. Studies in *C. elegans* showed that α-KG levels are significantly increased in long-lived mitochondrial mutants [32]. Interestingly, adding α-KG or I-2-hydroxyglutaratI(R)-2HG, a derivative of α-KG, extends the lifespan of *C. elegans* by inhibiting ATP synthase activity [29,33]. α-KG also extends lifespan in *Drosophila* by activating AMPK signaling and inhibiting the mTOR pathway [30]. Furthermore, supplementing α-KG in the form of a calcium salt (CaAKG) promoted a longer and healthier life associated with decreased levels of inflammatory cytokines in old mice [31].

These studies suggest that α-KG may be an ideal candidate for prolonged longevity studies in humans; however, the role of α-KG in the epigenetic modifications that occur during aging is still unclear. A recent study sponsored by the company Ponce de Leon Health showed a nearly 8-year reversal in the biological age of 42 individual humans taking Rejuvant, an α-KG based formulation, for 4–10 months [34]. Their conclusion was mainly based on the analysis of the DNA methylation clock, one of the well-established aging biomarkers. However, α-KG supplementation leads to both demethylation and hypermethylation of some CpG sites in the genome, suggesting that α-KG may have a broader effect on methylation-based aging, such as metabolic functions [34]. It is also worth noting that overexpression of JMJD-1.2/PHF8 and JMJD-3.1/JMJD3, the H3K27 demethylases that are potential substrates of α-KG, induces the expression of mitochondrial genes and extends lifespan in *C. elegans* [35]. It will be an interesting future research direction to examine the methylation landscape of chromatin to see whether the epigenetic clock of aging is slowed down or reversed in animals with α-KG supplementation.

NAD^+

NAD^+ and the reduced form, NAD^+ hydrogen (NADH), are crucial metabolites that are tightly connected with mitochondrial energy production. NAD^+ is a coenzyme for redox reactions and is essential for the central metabolic pathways: the TCA cycle, the ETC, glycolysis, and fatty acid β-oxidation. Changes in the cellular NAD^+/NADH ratio regulate the activity of three groups of enzymes, including class III histone deacetylase (sirtuins), cADP-ribose synthases (CD38), and poly ADP-ribose polymerases (PARPs), with subsequent effects on gene expression [36]. Studies from yeast, worms, and mice have shown that NAD^+ and sirtuins are linked to aging regulation. During aging, NAD^+ levels were found to be reduced across tissues in mice [36]. Brain NAD^+ levels also decline with age in humans [37]. The link between lifespan extension and supplementation with different NAD^+ precursors has been extensively explored in various

organisms [38–40]. A more comprehensive description of the role of NAD^+ in metabolism and aging has been reviewed elsewhere [41,42].

Sirtuins sense intracellular NAD^+ concentrations and transduce a signal via protein deacetylation. Sirtuins and their role in lifespan determination were originally reported in yeast [43]. It is worth noting that there have been controversial discussions about the role of Sir2 in aging in both worms and flies [44–46]. In particular, early successes in Sir2 overexpression-induced longevity were attributed to off-target effects of the transgenes and secondary mutations [44]. Mammals have seven sirtuins, with SIRT1 being the most similar to the yeast Sir2. Systematic overexpression of Sirt1 in mice prevented an age-related decline in metabolism but failed to extend lifespan [47,48]. PARP, another NAD^+-dependent enzyme, plays an active role in DNA repair, inflammation, and cell death and its effects on metabolism and aging as well as its competition with sirtuins are nicely summarized in recent reviews [49,50].

As a central metabolic regulator, NAD^+ helps sustain mitochondrial fitness and is essential for cellular homeostasis. NAD^+ can bolster mitochondrial function by enabling mitochondrial biogenesis and mitophagy in premature aging disease models [51]. Regenerating mitochondrial NAD^+ by bolstering mitochondrial complex I (MC1) activity is sufficient to increase the lifespan of mice with MC1 impairment in the brain [52]. Maintaining the NAD^+ level also sustains mito-nuclear communication during aging [53]. Genetic or pharmacological restoration of NAD^+ level increases lifespan in worms via activation of the **mitochondrial unfolded protein response (UPR^{mt})** by SIR-2.1 [54]. A recent study also showed that mitochondrial NAD^+ contributes to mitochondrial-to-nuclear communication by controlling nuclear ADP-ribosylation under H_2O_2-induced oxidative stress [55]. Thus, modulation of the NAD^+ levels to boost mito-nuclear communication can be viewed as a target to delay age-associated metabolic decline.

Methionine

Amino acids, traditionally viewed as the building blocks of proteins, can be catabolized into TCA cycle intermediates within mitochondria for energy production and they also play significant roles in signaling transduction [56]. From yeast to humans, the concentrations of free amino acids change with age and are altered in long-lived organisms [57]. In this section, we use methionine as an example to discuss the underlying mechanisms by which amino acids may influence the aging process.

Apart from its role in translational initiation, methionine is involved in multiple metabolic pathways, including the methionine cycle, the trans-sulfuration pathway, and polyamine biosynthesis [58]. Methionine is the major amino acid source of *S*-adenosyl-L-methionine (SAM), the primary donor of the methyl group for methylation [59]. In the cytosol, the folate cycle is coupled to the methionine cycle to generate SAM, which in turn is sustained by mitochondrial **one-carbon metabolism**. Cellular SAM levels determine the extent of histone methylation, in particular, H3K4me3 for the maintenance of defined cellular states [60]. Hence, mitochondrial metabolic fluctuation-induced changes in SAM levels may exert influence on histone and DNA methylations, which are critical for maintaining cellular homeostasis and regulating lifespan [61].

Methionine restriction extends the lifespan in many species [62]. It has been demonstrated that dietary restriction (DR) of methionine improves metabolic health and delays aging-related disease in mice [63]. In yeast, methionine restriction induces longevity via a retrograde response to change mitochondrial function [64]. In *C. elegans*, knocking down expression of *sams-1*, the gene encoding methionine adenosyltransferase (an enzyme that catalyzes the biosynthesis of SAM), promotes lifespan extension [65]. Several studies have suggested that methionine plays

a negative role in lifespan regulation [62,66]. However, long-lived flies contain higher levels of methionine [67]. Additionally, the reduction in fecundity caused by DR can be reversed by methionine supplementation, without compromising DR-induced longevity [68]. Studies in flies showed that methionine metabolism is reprogrammed during aging, accompanied by increased levels of *S*-adenosyl-homocysteine (SAH), which is converted from SAM. Suppression of dAhcyL1 (an SAH hydrolase) activity decreased the level of SAH and suppressed H3K4 trimethylation (H3K4me3), thus extending health and lifespan [67]. These data highlight the beneficial role of methionine in lifespan regulation, indicating that the flux in methionine metabolism could be more critical than the absolute level of methionine.

An exogenous supply of spermidine, a natural polyamine derived from methionine, can extend lifespan without adverse effects during life-long administration in eukaryotes ranging from yeast to mammals [69,70]. Mechanistically, spermidine treatment triggers histone H3 deacetylation by inhibiting HAT, which significantly upregulates autophagy-related transcripts and induces autophagy [69,71]. Because endogenous spermidine levels are negatively correlated with age and spermidine supplementation shows good safety in a recent clinical trial, a spermidine-rich diet may be promising to promote longevity and healthy aging. Taken together, these studies suggest that dietary interventions of amino acids could reshape mitochondrial-to-nuclear communication to influence cellular homeostasis and aging via epigenetic regulation, independent of their traditional roles in protein metabolism.

Mitochondrial-to-nuclear stress signals determine lifespan via epigenetic regulation

The metabolic status of the cell not only changes during aging but is also susceptible to environmental stimuli. Disturbances in mitochondria trigger stress signaling and communicate their functional state to the nucleus for adaptations. Distinct pathways are activated when mitochondrial function is compromised, such as loss of mtDNA, accumulation of mtDNA mutations, impaired respiration, disrupted mitochondrial protein homeostasis, and ROS production [3]. This signaling facilitates communication between mitochondria and the nucleus to alter gene expression, leading to metabolic adaptations and longevity [5]. Emerging studies have begun to reveal that chromatin modifications in response to mitochondrial perturbations facilitate mitochondrial-to-nuclear communication, thus leaving an epigenetic memory that may affect the aging process [72]. In this section, we discuss how mitochondrial stress signals modulate aging via epigenetic regulations.

Mitochondrial ROS (mtROS)

ROS are generated as a by-product of normal aerobic metabolism. For many decades, ROS were considered only to be 'toxic' products that cause oxidative damage to biomacromolecules, such as DNA, protein, and lipids, thus inducing the cellular oxidative stress response and accelerating aging. Redox signaling mediates cellular homeostasis by modifying the activity of transcription factors, metabolic enzymes, and epigenetic modifications [73]. However, the extent to which the oxidative stress response is involved in the aging process as a cause, a consequence, or a correlation, remains elusive.

The mitochondrion is a major source of ROS production, especially free radicals such as superoxide. Aging correlates with a decline in mitochondrial enzyme activity and increased ROS production. One study showed that stochastic bursts of superoxide production in mitochondria in day-3 adult *C. elegans* worms inversely correlates with lifespan, except for the long-lived mitochondrial mutant worms [74]. However, in the extremely long-lived naked mole rat, levels of ROS production are found to be similar to those in mice [75]. Once thought of merely as the destroyer of cellular homeostasis that causes oxidative damage and accelerated aging,

multiple studies suggest that mtROS can serve as important signaling molecules involved in aging, inflammation, and cancer regulation [76–78].

Studies in organisms from yeast to rodents have shown that mtROS signals can promote stress resistance and lifespan extension by respiration inhibition, caloric restriction, reduced TORC1 signaling, exposure to mild hypoxia, or temperature stress [79–81], but few studies have identified the signal through which the factors transmit information to elicit a highly specific transcriptional program. A hormetic mtROS signal extends yeast chronological lifespan by inactivating a JMJD histone H3K36 demethylase, Rph1p, via two DNA damage response kinases, thus enhancing binding of the silencing protein Sir3p and repressing subtelomeric transcription [82]. In *C. elegans*, elevated ROS levels during development increase stress resistance later in life and promote lifespan extension [83]. The early life ROS-mediated inactivation of the SET1/MLL histone methyltransferases leads to a reduction in global H3K4me3 levels, which causes improved redox homeostasis and, ultimately, increases longevity [83]. Therefore, it has become evident that mitochondrial dysfunction or mtROS signaling may influence lifespan and that the timing and levels of ROS generation are important factors.

The UPR^{mt}

Mitochondrial function is monitored by a series of quality control pathways that sense mitochondrial dysfunction and respond to cellular metabolic demands. These stress responses are initiated by signals produced within mitochondria and consequently induce a nuclear response that is aimed to protect mitochondrial function [18]. Although severe mitochondrial stress is detrimental, mild mitochondrial perturbation during development can have beneficial effects on the lifespan of organisms through epigenetic regulations [72].

In *C. elegans*, mitochondrial stresses caused by RNAi against the ETC components during development not only extend lifespan, but also elicit a transcriptional response known as the UPR^{mt}, which functions to resolve protein folding stress within mitochondria [84–86,115–117]. Intriguingly, activation of UPR^{mt} is required for mitochondrial stress-induced longevity, and the ability to activate the UPR^{mt} dramatically declines as animals mature and age [87,88]. The master regulator of the UPR^{mt} in *C. elegans* is the transcription factor ATFS-1, which has both a mitochondrial targeting sequence and a nuclear localization signal. When mitochondrial functions are compromised, the import efficiency of ATFS-1 is decreased and, instead, ATFS-1 accumulates in the nucleus to induce UPR^{mt} [118]. ATFS-1 itself represents a type of mitochondrial-to-nuclear communication for mitochondrial stress regulation. It is notable that severe mitochondrial stresses, such as inhibition of the mitochondrial import machinery, strongly induce the UPR^{mt} in *C. elegans*; however, these worms were short-lived [89]. In addition, animals with a gain-of-function allele of ATFS-1, the transcription factor for UPR^{mt}, are not long-lived, indicating that constitutive activation of the UPR^{mt} is not sufficient to induce lifespan extension [89].

Notably, there is only a small window of time during development in which the beneficial effects can occur, suggesting that mitochondrial dysfunction in early development can alter the epigenome landscape that adjusts organismal physiology to ultimately impact lifespan [86,88]. Indeed, mitochondrial stress causes widespread chromatin reorganization to induce the UPR^{mt} via multiple epigenetic factors. During mitochondrial stress, ATF7IP/LIN-65 accumulates in the nucleus, which requires the histone H3K9 methyltransferase MET-2/SETDB1 and the homeobox transcription factor DVE-1/SATB1 to promote chromatin compaction [90]. In line with this study, the JMJD histone demethylases JMJD-1.2/PHF8 and JMJD-3.1/JMJD3 are also required for UPR^{mt} activation and lifespan extension in response to mitochondrial stress [35]. However, mitochondrial stress-induced epigenetic changes are independent of

ATFS-1, indicating that other stress signals derived from mitochondria are required for chromatin reorganization.

Zhu *et al*. found that a decrease in acetyl-CoA levels resulting from mitochondrial stress functions as a signal for nucleosome remodeling and histone deacetylase (NuRD) complex-mediated chromatin reorganization and lifespan extension [91]. The histone deacetylase HDA-1, a component of the NuRD complex, coordinates with the chromatin organizer DVE-1 to regulate transcription of the UPR^{mt} in *C. elegans*. Its mammalian homologs HDAC1/2 also play a conserved role in modulating mitochondrial homeostasis [92]. The loss of NuRD components was sufficient to cause a progression of aging-related phenotypes [93]. Notably, mitochondrial stress can promote nuclear accumulation of NuRD subunits and overexpression of the NuRD complex components is sufficient to induce lifespan extension in *C. elegans* [91,92]. In addition, an acetyltransferase CBP-1/p300 positively regulates the UPR^{mt} and is required for mitochondrial stress-induced longevity [94].

It is interesting to speculate that NuRD complex-mediated histone deacetylation may coordinate with LIN-65/MET-2-mediated histone methylation to promote chromatin compaction, whereas JMJD histone demethylases and CBP-1 can maintain an open chromatin state at specific regions to promote induction of the UPR^{mt} (Figure 3). Whether alterations in the levels of other

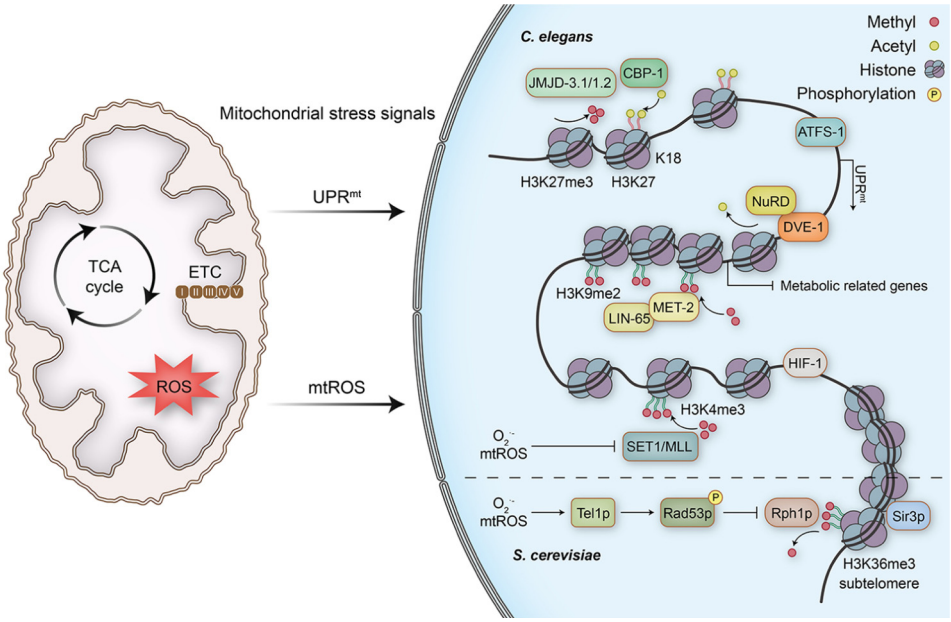

Figure 3. Mitochondrial-to-nuclear stress signaling modulates lifespan via epigenetic regulations. Mitochondrial stress leads to chromatin reorganization and global gene silencing. With the onset of mitochondrial stress, the decreased acetyl-CoA content derived from citrate induces nuclear accumulation of the nucleosome remodeling and histone deacetylase (NuRD) complex and the homeobox transcription factor (TF) DVE-1 to decrease histone acetylation and reorganize chromatin structure. The histone H3K9 methyl-transferase MET-2 and its cofactor LIN-65 also promote chromatin compaction and global gene silencing during mitochondrial stress. Concomitantly, two histone lysine demethylases that contain a Jumonji C domain (JMJDs), JMJD-1.2 and JMJD-3.1, and the acetyltransferase CBP-1 promote a relatively open chromatin state by removing methyl groups from H3K27me3 and adding acetyl groups to histones at the loci of the mitochondrial unfolded protein response (UPR^{mt}) genes to maintain a transcriptionally competent state for the TF ATFS-1 to activate transcription of UPR^{mt} genes. Mitochondria dysfunction can generate reactive oxygen species (ROS), which act as signaling molecules to modulate epigenetic marks such as Rph1p-mediated H3K36 demethylation, SET1 mediated H3K4me3 methylation. The ROS-mediated epigenetic changes in turn alter the expression of genes that regulate mitochondrial metabolism, to eventually regulate aging and longevity. Abbreviations: ETC, electron transport chain; TCA, tricarboxylic acid.

mitochondrial metabolites caused by mitochondrial perturbations can reshape epigenomes for lifespan regulation remains to be addressed in future studies. The UPR^{mt} was initially characterized in mammalian cells and the molecular mechanism of UPR^{mt} regulation has been extensively characterized in *C. elegans*. Whether the UPR^{mt} induction requires epigenetic regulation or whether mitochondrial dysfunction during development promotes longevity in mammals remains to be explored. The timing and extent of the stress response, or the other signaling pathways in addition to the UPR^{mt} following mitochondrial perturbations, are all essential factors in determining lifespan.

Mitochondrial stress not only signals to the nucleus within the cell, but can also be sensed between tissues and organs to coordinate the whole body to cope with locally sensed mitochondrial dysfunction, which is essential for organismal homeostasis and aging [18]. It will be crucial in future studies to explore the networks underlying systemic coordination of mitochondrial-to-nucleus stress signals for lifespan regulation (Box 1).

Concluding remarks

The studies reviewed here highlight the essential role played by mitochondrial-to-nuclear communication signals in the regulation of the aging process. External and internal metabolic cues can affect mitochondrial function, thus altering gene expression through epigenetic modifications [12]. Manipulation and restoration of some key mitochondrial metabolites to boost cellular metabolism and reverse age-associated epigenetic changes could be developed as therapeutic strategies to delay aging.

There is emerging evidence to indicate that mitochondrial metabolism is tightly regulated both spatially and temporally to elicit responses to nutrient availability and signaling cues [16]. Tissue-specific or even mitochondria-specific metabolome analysis will lead us to further understanding

Outstanding questions

How is the production of mitochondrial metabolites regulated both spatially and temporally to elicit epigenetic changes in response to mitochondrial dysfunction?

What are the specific epigenetic factors involved in mitochondrial-to-nuclear communications and how do they cooperate with transcription factors in response to various external and internal stimuli?

Do various mitochondrial metabolites act alone or in concert on the epigenome to regulate the aging process?

Are some organs or tissues more at risk than others in maintaining mitochondrial-to-nuclear communication during aging?

Can the intervention of mitochondrial-to-nuclear communications mimic the beneficial epigenetic changes to delay aging or alleviate age-onset diseases?

Box 1. Systemic control of mitochondrial-to-nuclear stress signaling for regulating lifespan

Mitochondrial stress signal from one tissue can induce a stress response in distal tissues via cell nonautonomous regulation, allowing organisms to better cope with localized mitochondrial stress during aging [88,119–121]. Muscle-specific knockout of mitochondrial cytochrome c oxidase (COX) causes a variety of disease phenotypes and shortens lifespan in mice [105]. T cells with a deficiency in mitochondrial transcription factor A (TFAM) show a chronic, induced inflammation accompanied by age, leading to multimorbidity and premature senescence [106]. At present, it is not known how the mitochondrial function in different tissues is coordinated with age.

In *C. elegans*, ETC impairment exclusively in neurons can activate the UPR^{mt} in a cell nonautonomous fashion in the intestine and increase lifespan [88]. It has been hypothesized that the secreted '**mitokine**' signal from tissues with mitochondrial dysfunction can function at a distance to elicit a mitochondrial stress response in distal tissues. A further study identified the retromer-dependent Wnt signaling function as the 'mitokine' signal that mediates the cell nonautonomous induction of the UPR^{mt}. Overexpression of the Wnt ligand EGL-20 specifically in the nervous system is sufficient to induce the UPR^{mt} in the intestine and promote lifespan extension in worms [107]. Surprisingly, neuronal mitochondrial stress can be sensed and reacted to by the mitochondria in the germline to promote the maternal inheritance of elevated mtDNA levels across multiple generations in a Wnt signaling-dependent manner. The transgenerational UPR^{mt} activation and elevated mtDNA levels enable the descendent worms to live longer and also confers increased stress tolerance, albeit with the trade-offs of delayed development and reduced fecundity [107,108].

One study using wild strains of *C. elegans* showed that natural variation in neuropeptide-mediated glia–neuron signaling modulates the rate of aging via SIR-2.1-mediated activation of the UPR^{mt} [109]. Disrupted mitochondrial function in muscles activates the UPR^{mt} and elicits an ImpL2 (insulin/IGF binding protein) signal to decrease global insulin/insulin-like growth factor signaling (IIS) and prolong lifespan in flies [110]. Systemic coordination of the mitochondrial stress response is also conserved in mammals. In mice, mitochondrial dysfunction in the hypothalamic POMC neurons leads to high-turnover metabolism and obesity and remodels adipose metabolism [111,112]. Patients with mitochondrial disorders who suffer from muscle weakness have excessive levels of fibroblast growth factor 21 (FGF-21) and growth differentiation factor 15 (GDF-15) in their serum [113,114]. It will also be important to understand which tissues/organs are responsible for coordinating the organismal prolongevity signals in higher organisms.

of metabolite-driven epigenetic regulations. How signal transduction in cell nonautonomous mitochondrial communication is orchestrated has not been explicitly addressed. The interplay between tissues and organs to coordinate the metabolic status of the body can also be used to develop therapeutic treatments for diseases.

Diet is known to have a significant influence on metabolism and lifespan. A well-established dietary intervention to promote longevity is DR. There is evidence to suggest that DR-induced lifespan extension could be mediated at the epigenetic level through alterations in the levels of metabolites to influence DNA methylation [95]. However, the DR-induced benefits cannot be effectively induced in late-life mice [96]. Mitochondrial stress during development causes widespread changes in chromatin structure to promote UPR^{mt}, perpetuating an early response that results in lifespan extension. The evidence indicates that, to some extent, epigenetic modifications are reversible and that chromatin is plastic. Thus, a suitable intervention involving nutrients in the diet could be a safe and effective way to modulate mitochondrial function and delay aging.

Despite these advances, our understanding of the interplay between mitochondrial-to-nuclear stress signaling remains limited (see Outstanding questions). It will be essential to determine how mitochondrial metabolites affect site-specific epigenetic modifications in a tissue-specific manner. Future studies will help us develop interventions on mitochondrial-to-nuclear communications to mimic the beneficial epigenetic changes for delaying aging or alleviating age-onset diseases.

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Declaration of interests

No interests are declared.

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