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Pleiotropic effects of mitochondria in aging

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Aging is typified by a progressive decline in mitochondrial activity and stress resilience. Here, we review how mitochondrial stress pathways have pleiotropic effects on cellular and systemic homeostasis, which can comprise protective or detrimental responses during aging. We describe recent evidence arguing that defects in these conserved adaptive pathways contribute to aging and age-related diseases. Signaling pathways regulating the mitochondrial unfolded protein response, mitochondrial membrane dynamics, and mitophagy are discussed, emphasizing how their failure contributes to heteroplasmy and de-regulation of key metabolites. Our current understanding of how these processes are controlled and interconnected explains how mitochondria can widely impact fundamental aspects of aging.

Mitochondria are evolutionarily derived from alphaproteobacteria that evolved in symbiosis within eukaryotic cells[1]. Although most alphaproteobacterial genes were transferred to the eukaryotic nucleus, mitochondria retained their genome to translate the remaining protein-coding genes within their DNA. This requires complex coordination of the transcription and translation from two genomes, and the import and processing of proteins into the mitochondria in an ever-changing cellular milieu[2,3]. Disruption of these finely controlled processes has been shown to impair cellular homeostasis. To cope with this downside of endosymbiosis, mitochondria have evolved multiple stress-response pathways. The mitochondrial stress-response (MSR) network contributes to the reconstitution of cellular homeostasis by preventing mitochondrial proteotoxicity and by redistributing and removing irreversibly damaged elements of the mitochondria[4]. In recent years, we have gained considerable insights into why a decline in the robustness of these MSR pathways contributes to cellular damage and organismal deterioration. This is underlined by our emerging understanding of how different types of mitochondrial defects are co-regulated and interact across cellular and systemic processes.

Here, we describe the pleiotropic effects of mitochondrial dysfunction in aging. We outline the major mitochondrial stress pathways, how their failure is interconnected with the expansion of mitochondrial DNA mutations and deregulated metabolism, and how this affects cellular and organismal homeostasis. We furthermore provide an integrated map of how combined mitochondrial defects impact several features of aging, suggesting conserved links that could potentially be harnessed to slow the aging process. We refer readers to other comprehensive reviews on topics not covered in depth here, such as cellular senescence, stem cell function, and reactive oxygen species (ROS)[5–7].

Mitochondrial stress responses in aging and longevity

Mitochondrial unfolded protein response. Appropriate handling and folding of proteins are essential, especially in mitochondria, whose proteome is encoded in both the nuclear and mitochondrial genomes. Mitochondrial protein homeostasis is ensured by an elaborate protein quality-control network composed of molecular chaperones and proteases[8] governed by the mitochondrial unfolded protein response (UPR^{mt}) (Fig. 1). Upon mitochondrial proteotoxic stress, the UPR^{mt} induces the expression of chaperones, proteases, and other stress-response genes, mediated by activating transcriptional factor associated with stress-1 (ATFS-1) in *Caenorhabditis elegans*, and activating transcription factor 4 (ATF4) along with ATF5 and DNA damage inducible transcript 3 (DDIT3, also known as CHOP) in mammals, to restore mitochondrial function and adapt to stress[9–12]. Several conditions that interfere with mitochondrial proteostasis, such as an increased load of unassembled, damaged, or unfolded proteins, play a substantial part in UPR^{mt} activation, with important implications in aging and longevity.

Disruption of most of the electron transport chain (ETC) subunits extends the lifespan in *C. elegans*, yeast, flies, and mice[13–18]. It is well established that activation of the MSR is a critical component of mitochondrial-stress-induced longevity. In worms, knockdown of oxidative phosphorylation (OXPHOS) complexes I, III, IV, and V, all encoded in both mitochondrial DNA (mtDNA) and nuclear DNA (nDNA), triggers the UPR^{mt} and extends lifespan[18], while disruption of complex II, which is encoded only by nDNA, does not affect longevity[19]. Consistent with these results, complex-IV-deficient mice also show activation of the UPR^{mt} and have a prolonged lifespan[14,20]. These data suggest that a mismatch between mtDNA- and nDNA-encoded ETC subunits, resulting in unassembled ETC components and the subsequent mitonuclear protein imbalance, is sufficient to drive the UPR^{mt} and lifespan extension in worms and mammals. In agreement, the reduced expression of *Mrps5*, which encodes a mitochondrial ribosomal protein that regulates the translation of mtDNA-encoded ETC genes, induces a mitonuclear imbalance resulting in activation of the UPR^{mt}, which correlates with an increased lifespan in the BXD mouse genetic reference population (GRP)[21]. In *C. elegans*, *mrps-5* RNA interference (RNAi) increased the lifespan by more than 50%, highlighting an evolutionarily conserved mechanism linking the UPR^{mt} to longevity[21]. In addition, pharmacologically inhibiting mitochondrial translation by using antibiotics that inhibit bacterial, and, hence, mitochondrial translation, such as doxycycline or chloramphenicol, induces the UPR^{mt} and extends the health span and lifespan across kingdoms of life, from animals (*C. elegans*)[21] to plants (*Arabidopsis thaliana*)[22,23]. Interestingly, as shown in yeast, worms, and mammals, such adaptive changes in mitochondrial translation can also affect cytosolic translation[24–26], suggesting that cross-compartment synchronization is essential to maintain protein homeostasis during mitochondrial stress.

Mitonuclear protein imbalance also contributes to the lifespan extension seen upon mitochondrial biogenesis. An increased protein-folding workload in the mitochondria can be perceived as proteostatic stress, resulting in activation of the UPR^{mt} (ref. [21]). This has been demonstrated by the lifespan-extending effect of resveratrol,

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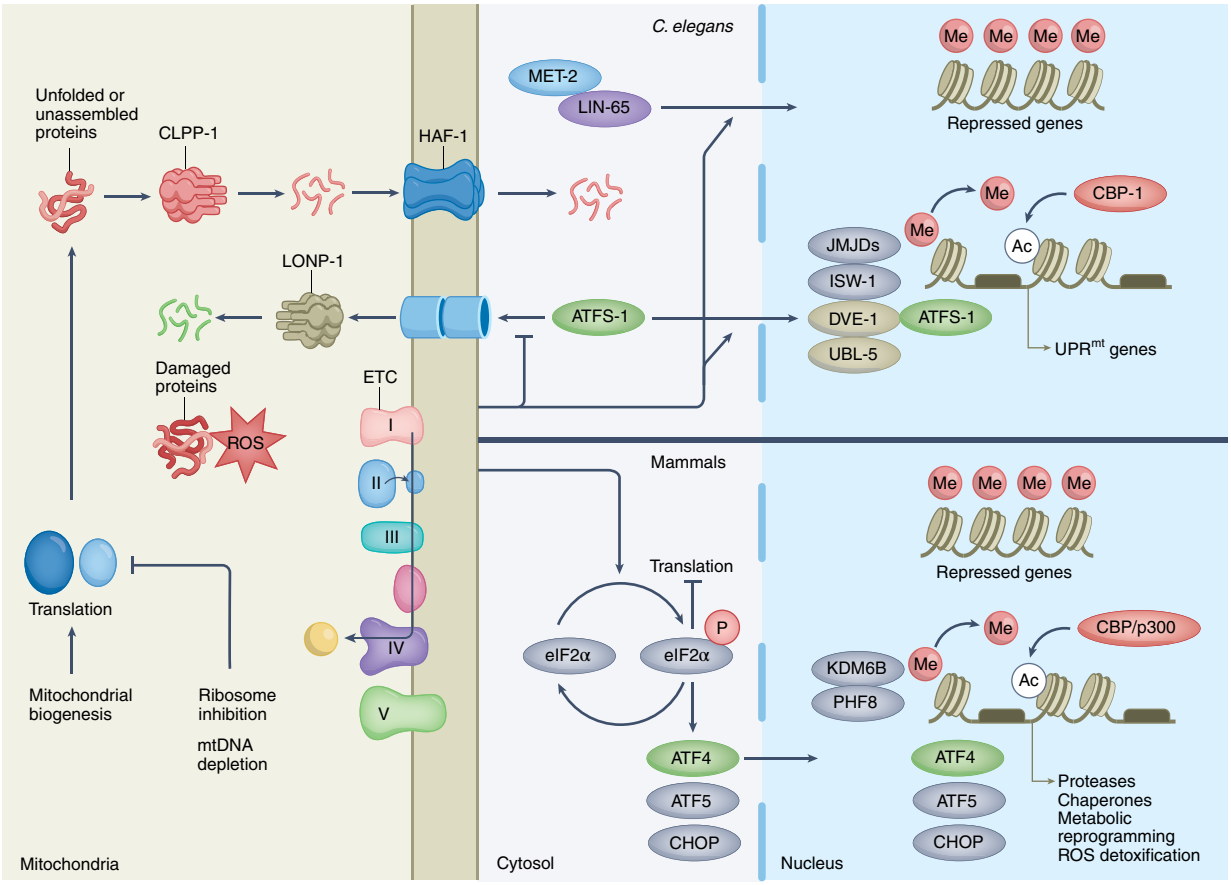

Fig. 1 | Main activation mechanisms of the UPR^{mt} in *C. elegans* and mammals. Stressors that induce proteotoxicity in the mitochondria such as accumulation of unassembled, unfolded, and damaged proteins can trigger the UPR^{mt}. The activation of the UPR^{mt} in *C. elegans* involves the digestion of unfolded or unassembled mitochondrial proteins by the mitochondrial matrix protease CLPP-1 and the transport of the fragmented peptides to the cytoplasm by HAF-1. Cytosolic accumulation of these mitochondrial peptides is at the heart of UPR^{mt} activation. ATFS-1 is a transcription factor that, under basal conditions, localizes to the mitochondria, where it is constantly degraded by the Lon protease 1 (LONP-1). During mitochondrial stress, mitochondrial protein import is limited by the cytosolic accumulation of mitochondrial peptides. ATFS-1 then can shuttle to the nucleus[12], where, in cooperation with other co-factors including DVE-1 and UBL-5, as well as epigenetic modulators including JMJDs and CBP-1 (refs. [247,248]), it orchestrates the expression of a broad set of genes involved in mitochondrial quality control and metabolism[12,252–255]. In mammals, several types of mitochondrial perturbations (such as oxidative or proteotoxic stress), trigger the ISR through the phosphorylation of translation initiation factor eIF2α, which shuts down global translation and favors the cap-independent translation of the ATF4 transcription factor, leading to the expression of UPR^{mt} and cytoprotective genes[9,49]. In addition to ATF4, at least two other transcription factors, ATF5 and CHOP, and several epigenetic modulators[247,248] are involved in UPR^{mt} activation[10,11]. The attenuation of general protein translation, together with the transcriptional induction of proteostasis genes, such as those encoding chaperones and proteases, thus antagonize cellular proteotoxicity.

a well-known inducer of mitochondrial biogenesis, which also triggers the UPR^{mt} in worms[21]. Likewise, genetic or pharmacological restoration of nicotinamide adenine dinucleotide (NAD^+) in *C. elegans* induces mitochondrial biogenesis and promotes longevity via induction of mitonuclear imbalance and UPR^{mt} (ref. [27]). These findings translate to mammals, as restoring NAD^+ levels by nicotinamide riboside (NR) administration in mice aged 24 months induces the UPR^{mt} and extends the lifespan[28]. However, when treatment with NAD^+ boosters was started in mice at 8 months of age, they were reported not to increase the lifespan[29].

There are specific spatiotemporal requirements for UPR^{mt} activation and longevity. In *C. elegans*, *cco-1* and *mrps-5* RNAi[21,30] and RNAi targeting other respiratory chain components[18], as well as doxycycline treatment, result in activation of the UPR^{mt} and promote longevity only when the perturbation occurs early in life, before the L3/L4 larval stage. This suggests the existence of a surveillance system that monitors mitochondrial activity early in life and establishes the rate of the aging process throughout adulthood through epigenetic modulation (Box 1). Moreover, the longevity effect of UPR^{mt} activation has been shown to be tissue-specific, as ETC disruption in neurons and intestine, but not in muscle, increases longevity in an UPR^{mt}-dependent manner[30]. UPR^{mt} activation by mitochondrial stress can also signal in a cell-nonautonomous manner to inform distant tissues of emanating mitochondrial stress. In worms, ETC inhibition or expression of toxic polyglutamine (polyQ) protein in neurons activates the UPR^{mt} in the intestine, suggesting the existence of extracellular signals that inform the whole organism of stress and prepare against it[30–32]. Accordingly, the Wnt/EGL-20 ligand of the Frizzled receptor is a signaling molecule secreted by neurons upon mitochondrial stress, which triggers the UPR^{mt} in peripheral tissues in the same organism[32] and across generations, conferring stress resistance and longevity in the descendants[33]. Interestingly, this transgenerational stress-protective inheritance by Wnt signaling is caused by increased mtDNA in the germline leading to a mitonuclear protein imbalance and UPR^{mt} (ref. [33]). Likewise, the increased lifespan observed in *Drosophila* upon mild mitochondrial disruption requires inter-organ cross-talk involving UPR^{mt} and insulin-like growth factor-binding protein 7, which

Box 1 | Epigenetic modulations in response to mitochondrial stress

Epigenetic mechanisms help to explain how environmental cues impinge on the regulation of the aging process[244]. Increasing evidence suggests that conserved mitochondria-to-nucleus stress-signaling pathways regulate aging through epigenetic modulation of nuclear gene expression. In yeast, mitochondrial stress induces longevity through trimethylation of H3 at K36 and requires the H3K36 demethylase Rph1 (ref. [245]). In *C. elegans*, the histone methyltransferase MET-2 and its nuclear cofactor LIN-65 cooperate to induce general chromatin compaction and transcriptional silencing[246]. Activation of specific UPR^{mt} genes then depends on the removal of transcription-repressive histone-methylation marks (for example, H3 trimethylated at K27 (H3K27Me3)) by the H3K27 demethylases JMJD-3.1 and JMJD-1.2 (or mammalian KDM6B and PHF8) and their subsequent replacement by transcriptionally active acetylation marks (for example, H3 acetylated at K27) by the acetyltransferase CBP-1 (or mammalian CBP and p300). This relays the mitochondrial stress signal, resulting in the selective transcriptional induction of diverse UPR^{mt} genes, mediated by the transcription factors ATFS-1 (or mammalian ATF4, ATF5 and CHOP) and DVE-1 (refs. [247,248]) (Fig. 1). Consistently, the H3K9 methyltransferase SET-6–BAZ-2 complex accelerates behavioral deterioration in *C. elegans* by repressing the expression of UPR^{mt} genes, thereby promoting the aging process[249]. Expression of KDM6B and PHF8, and of CBP and p300, also correlates with the expression of UPR^{mt} genes and extended lifespan in the mouse genetic reference populations, supporting a strong link between epigenetic remodeling and life extension[247,248]. How these epigenetic modulators sense mitochondrial stress remains largely unexplored. One possibility is that mitochondrial perturbations could alter the nuclear epigenome through mitochondrial-derived metabolites[221] (see 'Tricarboxylic acid cycle intermediates'). For instance, the level of mitochondrial-derived acetyl-CoA was found to be decreased upon mitochondrial perturbations, which can be sensed by the histone deacetylase complex (NuRD). The subsequent nuclear accumulation of the NuRD complex and DVE-1 could then reduce histone acetylation, reorganize chromatin structure, induce the UPR^{mt}, and enhance lifespan[229,250]. Likewise, the level of histone H4 acetylated at K5 decreases in normal aged worms and the short-lived *cbp-1* loss-of-function worms, which can be reversed by sodium butyrate, an HDAC inhibitor[251]. The MSR is thus controlled by epigenetic modulations to allow transcriptional regulation during aging and longevity.

systemically antagonizes insulin signaling[34]. In mice, growth differentiation factor 15 (GDF15) and fibroblast growth factor 21 (FGF21) were identified as UPR^{mt}-associated hormones promoting metabolic benefits, such as improved insulin sensitivity and protection against hepatic steatosis[35,36] as well as increased lifespan[37,38]. As these molecules are secreted during mitochondrial stress, they have often been termed 'mitokines'; however, as they are not directly released by mitochondria, the term 'metabokines' may be more appropriate.

In contrast to mild and acute (time-restricted) mitochondrial stress, which improves organismal homeostasis, chronic OXPHOS dysfunction is often detrimental. For example, several mouse models with mitochondrial defects have a reduced lifespan[39–41], and most of the human diseases associated with OXPHOS dysfunction are typified by protracted defects and hence are deleterious[42,43]. Circulating levels of FGF21 and GDF15 are substantially increased in mouse models of mitochondrial dysfunction[44,45] and in human mitochondrial disorders[45,46] and aging[47], which might represent an attempt of the organism to cope with the sustained stress. Moreover, the UPR^{mt} is, on the one hand, strongly induced in worm and mouse models of mtDNA deletion[44,48,49], whereas, on the other hand, it seems to be required for the propagation of mtDNA deletion in worms[48]. Collectively, these results suggest that prolonged activation of mitochondrial stress can be harmful, whereas precise, mild mitochondrial stress exerts a beneficial adaptive effect on organismal aging. This mitohormetic effect of the UPR^{mt} is in line with its temporal specificity, in which the UPR^{mt} needs to be inflicted in the larval stages to extend worm lifespan.

The UPR^{mt} pathway also modulates stem cell function in aging. In muscle stem cells of aged mice, restoring NAD^+ levels with NR activates the SIRT1-dependent UPR^{mt}, improving mitochondrial metabolism and attenuating senescence linked to increased lifespan[28]. Similarly, increasing NAD^+ levels by overexpressing NAMPT, the rate-limiting NAD^+ salvage enzyme, ameliorates cell senescence in aged mesenchymal stem cells[50]. The effects of NAD^+-induced UPR^{mt} signaling have also been shown to benefit muscular dystrophies, as it not only prevents MuSC senescence[28], but also attenuates skeletal muscle and heart deterioration in mouse models of muscle dystrophy[51]. Also, SIRT7, which is downregulated upon aging and controls the expression of several mitochondrial proteins in mice[52], improves the regenerative capacity of aged hematopoietic stem cells through a mechanism involving the activation of the UPR^{mt} (ref. [53]). Thus, the UPR^{mt} may be essential to maintain stem cell function during aging by preventing senescence.

Defects in how mitochondria sense and respond to stress are crucial for initiating organismal decline in aging. The UPR^{mt} emerged as an essential regulator of aging and longevity by synchronizing mitochondrial and nuclear genomes at the proteome level to maintain proper mitochondrial function upon stress. Suggestive of potential relevance in mammals, the UPR^{mt} network is active in mouse and human populations across multiple tissues[54] and is deregulated in several human age-related diseases, such as sarcopenia[55] and Alzheimer's disease[56,57].

Mitochondrial membrane dynamics. Mitochondria are dynamic organelles; they can be found as isolated organelles, fused in large networks, and even unequally distributed in the cytosol by organized mitochondrial transport and positioning[58]. Mitochondrial fusion and fission, termed mitochondrial membrane dynamics, are essential components of the MSR. Through dilution and segregation of damaged organelles, cells ensure homeostasis and survival upon stress[59,60]. Thus, cellular and organismal health relies on tight regulation of mitochondrial fission and fusion, and disruption in any of these pathways is linked to aging and several age-related diseases[59].

In mammals, fusion of the mitochondria is mediated by the GTPases mitofusin 1 (MFN1) and MFN2, which merge the outer mitochondrial membrane (OMM), and by optic atrophy 1 (OPA1), responsible for merging the inner mitochondrial membrane (IMM). Mitochondrial fission is predominantly orchestrated by the dynamin-related protein 1 (DRP1) GTPase in coordination with other OMM-associated receptors, such as mitochondrial fission factor (MFF) and mitochondrial fission 1 protein (FIS1)[61,62]. Both fission and fusion seem to facilitate the segregation and removal of dysfunctional mitochondria[63]. Moreover, DRP1 mediates two distinct types of mitochondrial fission: division at the midzone results in mitochondrial proliferation, whereas division in the periphery enables damaged material to be destined for mitophagy[64] (Fig. 2a).

The link between mitochondrial fission and fusion in aging was initially observed in lower organisms. In two fungal aging models, reducing mitochondrial fission by *Dnm1p* (homolog of mammalian *drp-1*) deletion enhances lifespan[65]. In worms, fragmentation of the mitochondrial network and swollen mitochondria are observed

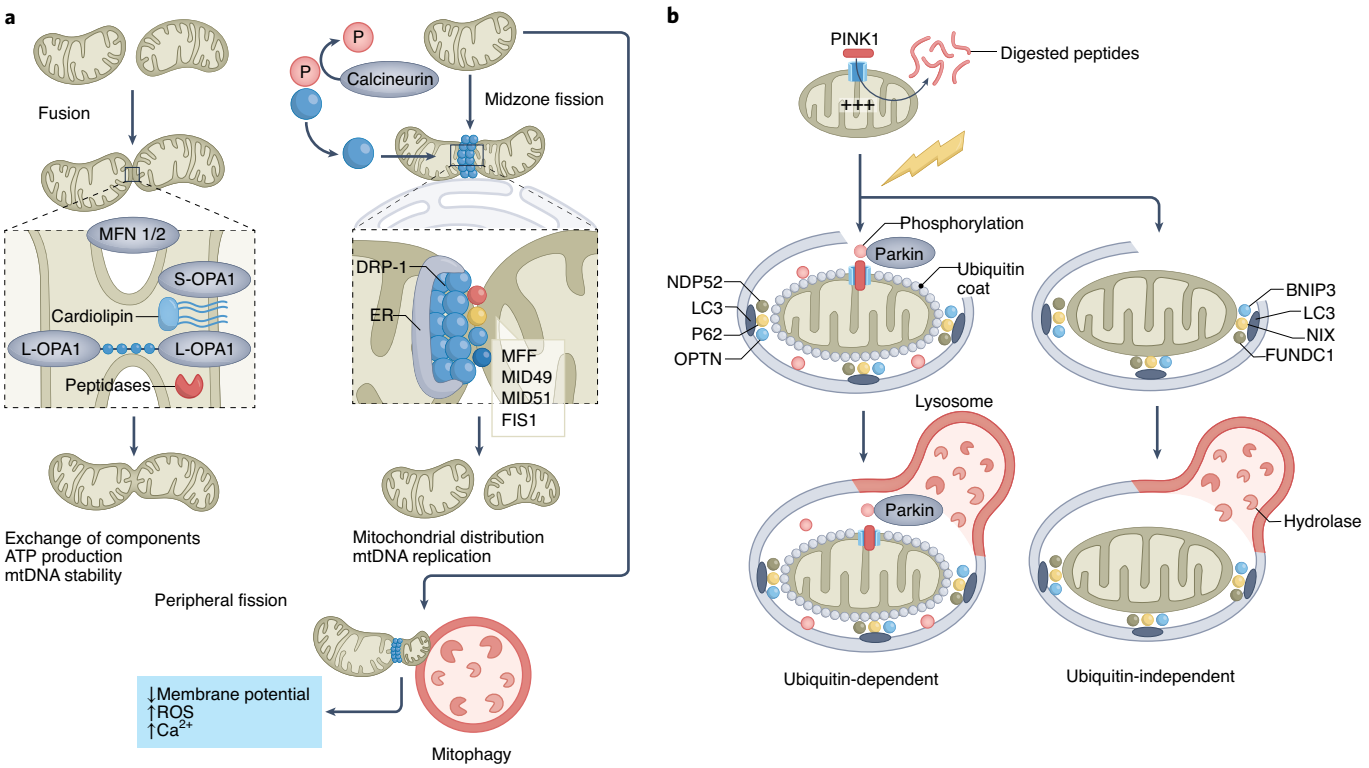

Fig. 2 | Mitochondrial membrane dynamics and mitophagy. a, In mammals, fusion of mitochondria is regulated by two mitofusins (MFN1 and MFN2), proteins of the dynamin-related family of large GTPases, located in the OMM, and OPA1, located at the IMM. Fusion initiates with the docking of two MFN1 proteins in the mitochondria, inducing conformational changes that drive GTP hydrolysis with subsequent fusion of the mitochondrial OMMs. At the IMM, unprocessed OPA1, known as long OPA1 (L-OPA1), is cleaved by the peptidases OMA1 and YME1L to form the short form of OPA1 (S-OPA1), which, in association with cardiolipin, facilitates the fusion of the adjacent IMM following OPA1-dependent GTP hydrolysis. Fission is predominantly orchestrated by the dynamin-related protein 1 (DRP1). When dephosphorylated by calcineurin at Ser637, DRP1 translocates from the cytosol to the mitochondrial surface. There, DRP1 binds to its OMM receptors mitochondrial fission factor (MFF), mitochondrial dynamics protein of 49 kDa (MID49), MID51, and mitochondrial fission 1 protein (FIS1). DRP1 then oligomerizes and induces GTP-hydrolysis-membrane constriction. DRP1 also mediates mitochondrial peripheral fission, enabling damaged material to be destined for mitophagy. **b**, PINK1, a mitochondrial serine/threonine-protein kinase, senses impaired mitochondria and signals to the cytosolic E3 ligase parkin. Under basal conditions, PINK1 is imported to the mitochondria by TOM and TIM translocases, leading to the proteolytic cleavage of PINK1 by mitochondrial proteases. Upon stress, the IMM depolarizes and inhibits protein import. Uncleaved PINK1 hence accumulates in the OMM and activates parkin through direct phosphorylation of the parkin Ub-like (UBL) domain or through the phosphorylation of ubiquitin. Activated parkin additionally ubiquitinates multiple substrates in the OMM to recruit autophagy receptors, including p62, optineurin (OPTN), and NDP52, which facilitate the recruitment of LC3 and engulfment of impaired mitochondria by autophagosomes. Ubiquitin-independent mitophagy is regulated by the recruitment of autophagy receptors, such as BNIP3, NIX, and FUNDC1, to the mitochondrial membrane. These receptor proteins then recruit LC3, enabling the engulfment of mitochondria by the autophagosomes.

with aging[21,66,67], and diverse longevity pathways are associated with increased mitochondrial fusion[68]. Accordingly, inhibition of mitochondrial fusion abrogates the lifespan extension in long-lived mutant worms[66,68]. Aging manifests itself differently in distinct tissues, as in the case of germline cells, which connect generations and are essentially immortal. In worms, these cells avoid transmitting damage to the next generation by an invigoration of proteostasis, requiring a switch from fragmented to an elongated, fused mitochondrial network[69].

However, mitochondrial fusion is not always analogous to lifespan extension. In *Drosophila*, reduction in mitofusion levels caused by overexpression of parkin, an E3 ubiquitin ligase involved in the ubiquitin–proteasome system and mitophagy, attenuates mitochondrial fusion and triggers mitochondrial fission, leading to an increase in multiple markers of mitochondrial activity and lifespan extension[70]. Alternatively, triggering mitochondrial fission through upregulation of *Drp1* in the midlife of flies preserves mitochondrial respiratory function and prolongs lifespan[71]. Moreover, reducing mitochondrial translation by *mrps-5* RNAi in *C. elegans* extends the lifespan while triggering mitochondrial fragmentation[21]. In this setting, altering fission or fusion synergizes with reduced mitochondrial translation to prolong the worm's lifespan[72]. Simultaneous ablation of both mitochondrial fission and fusion produces opposing phenotypes in yeast and *C. elegans*. In yeast, this double ablation shortens the lifespan[73], whereas in *C. elegans*, it extends the lifespan by increasing the homeostasis, fatty acid oxidation, and peroxisomal function in the mitochondrial network[74].

In mice, both fission and fusion are impaired with age. Aged mice demonstrate reduced DRP1 activity and alterations in mitochondrial morphology in several tissues, including skeletal muscle, neurons, and oocytes[75,76]. Interestingly, both muscle-specific DRP1 overexpression or DRP1 knockdown in 18-month-old mice causes muscle atrophy[77]. Recently, it has been shown that the RNA-binding protein pumilio 2 (PUM2) increases with age in worms, mice, and humans[67]. PUM2 prevents *Mff* translation, suggesting a potential mechanism by which mitochondrial fission is impaired in aging[67]. Finally, ablation of both DRP1-mediated fission and MFN-mediated fusion in mice accelerates mitochondrial senescence in the heart[78].

These findings suggest that mitochondrial fission and fusion critically contribute to aging when not properly balanced.

Mitochondrial fusion is essential for maintaining mtDNA stability by diluting mtDNA mutations. Expansion of mtDNA mutations has been linked to age-associated mitochondrial decline in several species (see 'mtDNA integrity in aging'). This notion was extensively studied using proofreading-deficient POLG mutator mice. This mouse model was engineered to contain proofreading-deficient mitochondrial DNA polymerase (POLG) through substitution of an alanine residue for aspartate on the POLG catalytic subunit (p.D257A), resulting in accumulation of mutated mtDNA and consequently accelerated aging[39,40]. Interestingly, while the mutator mouse survives into adulthood, crossing this strain with knocked out *Mfn1* results in mitochondrial dysfunction and embryonic lethality[79]. Of note, people with OPA1 mutations present mtDNA instability as indicated by multiple mtDNA deletions[80,81]. These findings suggest that mitochondrial fusion is essential for diluting mutated mtDNA. However, it cannot be ruled out that the effect of membrane dynamics on mtDNA propagation may have tissue and temporal specificity. Indeed, fragmentation of the mitochondria is essential for removing mutant mtDNA in germline tissues of *Drosophila*, providing evidence for a fission-based selection against deleterious mtDNA mutations[82], which seems opposite to the beneficial effects of fusion in the *C. elegans* germline[69].

Mitophagy. When mitochondrial stress accumulates to levels that exceed the capacity of stress responses, autophagy of the mitochondria, termed mitophagy, takes place. Among all mitochondrial quality-control systems, mitophagy is the only one that mediates the turnover of the whole organelle, thus avoiding cellular damage and apoptosis. In higher eukaryotes, mitophagy operates in different cell types and tissues through ubiquitin-dependent and ubiquitin-independent pathways (Fig. 2b). The ubiquitin-dependent mechanism is mediated by the PINK1–parkin axis, which ubiquitinates multiple substrates to recruit autophagy receptors. Ubiquitin-independent mitophagy is regulated by direct recruitment of autophagy receptors, such as BNIP3, NIX, and FUNDC1. Both pathways culminate in the engulfment of mitochondria by autophagosomes[83].

There is accumulating evidence that mitophagy affects aging and the lifespan in different organisms[84]. In *C. elegans*, mitophagy is required for lifespan extension of several long-lived mutants, including worms with reduced insulin–IGF-1 signaling or impaired mitochondrial function and mutants subjected to caloric restriction[85]. Furthermore, moderate mitochondrial deficiency and hypoxia response promote longevity in worms in a mitophagy-dependent manner[86]. In line with these results, deficiency in *dct-1* and *bec-1*, both key autophagy genes, recapitulates the effect of aging on mitochondrial mass in young adult worms[85]. In *Drosophila*, parkin null mutants exhibit reduced lifespan and locomotor defects driven by muscle degeneration[87], whereas parkin overexpression reduces proteotoxicity and extends lifespan[70]. Consistent with these findings, the longevity effect of mitochondrial perturbation in flies involves systemic repression of insulin signaling, facilitating mitophagy by enhancing lysosome biogenesis[34].

Mitophagy decline has been observed in several tissues in mice upon aging. In a transgenic mouse strain expressing the fluorescent mitophagy reporter mt-Keima, a decrease of ~70% in mitophagy was observed in the hippocampus of 21-month-old mice compared with that in young 3-month-old mice[88]. On a similar note, loss of parkin in POLG mutator mice causes a massive loss of dopaminergic neurons by 1 year of age, suggesting that Parkin prevents neuronal deterioration following mitochondrial mutagenesis[89]. Mitophagy also declines in the mouse heart upon aging, contributing to OXPHOS dysfunction and heart failure[90]. Moreover, defective mitophagy was observed in muscle-specific stem cells isolated from aged mice and humans[91]. This was associated with mitochondrial dysfunction and senescence, which can be restored by re-establishing mitophagy[91]. In accordance with these findings, boosting mitophagy improves OXPHOS function in aged worms and mice[67,92] and in a muscular-dystrophy mouse model, typified by accelerated muscle degradation[93]. Like in lower organisms, hypoxia also promotes mitophagy in mammals[94], in which it has been shown to protect against mitochondrial toxicity and extend the lifespan in a genetic mouse model of mitochondrial disease[95]. Consistent with these results, hypoxic preconditioning attenuates ischemia and reperfusion injury through mitophagy in mice[96]. Mitophagy thus might represent a conserved strategy to maintain mitochondrial quality in hypoxic conditions.

A variety of mitophagy modulators have been shown to mitigate the effects of aging. Urolithin A (UA), a gut-microbiome-derived natural compound, induces mitophagy both in vitro and in vivo following oral administration in mice and humans[92,97]. In *C. elegans*, UA prevents the accumulation of dysfunctional mitochondria with age and extends lifespan[92]. These effects translate to rodents: UA improved muscle health in two mouse models of age-related muscle decline[92] and a mouse model of muscular dystrophy, resulting in an increased survival rate[93]. Furthermore, UA treatment lowered protein aggregation and prevented cognitive impairment in animal models of Alzheimer's disease[98]. The positive effects on mitochondrial health upon oral consumption of UA and its favorable safety profile[97] have recently been documented in humans[99]. Other classes of compounds, such as actinonin, spermidine, and NAD^+ enhancers, also exert their beneficial effects in models of aging and age-related disease through the enhancement of mitophagy[56,98,100–103].

Mitophagy in inflammaging. Defective mitophagy has emerged as a central contributor to inflammation, which may underlie the age-dependent increase in low-grade inflammation, termed inflammaging. Parkin-deficient mice challenged with immunogenic stressors, such as low-dose lipopolysaccharide (LPS), develop Parkinson's-disease-like symptoms, including loss of dopaminergic neurons and motor defects[104]. These phenotypes were also observed in aged *Parkin*$^{-/-}$ mutator mice[105] and *Pink1*$^{-/-}$ mice infected with Gram-negative bacteria[106]. Interestingly, PINK1 and parkin were shown to repress mitochondrial antigen presentation delivered by mitochondrial-derived vesicles, thus suppressing an immune response provoked by inflammation[107].

Independently of pathogen infection, immune responses can be triggered by intracellular molecules from senescent or dying cells, termed damage-associated molecular patterns (DAMPs)[108,109]. mtDNA release is a potent DAMP, activating both intracellular and extracellular immune pathways. When released in the cytosol, mtDNA stimulates the NLRP3 inflammasome, resulting in IL-1β and IL-18 secretion and apoptosis. Additionally, cells can sense mtDNA in the cytosol through the cyclic GMP–AMP synthase (cGAS), which is activated by double-stranded DNA, leading to the production of 2′3′ cyclic GMP–AMP (cGAMP), a second messenger molecule and agonist of the stimulator of interferon genes (STING). Mitophagy can prevent inflammation by promoting mtDNA clearance from damaged mitochondria, thus preventing cytosolic mtDNA release and subsequent STING1 activation[105]. Consistent with these data, mitophagy restrains inflammasome activation in macrophages by reducing cytosolic accumulation of mtDNA[110,111]. Furthermore, mitophagy flux is involved in the inflammatory responses mediated by IRGM1, a master regulator of type I interferon[112], and can attenuate inflammation by directly restraining NLRP3-inflammasome overactivation in macrophages[111]. These findings support a role for mitophagy in restraining innate immune pathways. However, in acute inflammatory conditions, such as sepsis, mitophagy can exert opposite effects. For example, in a mouse model of sepsis caused by polymicrobial infection, pharmacological inhibition of mitophagy promotes macrophage activation favoring bactericidal clearance, leading to a higher survival rate[113]. Accordingly, mitochondrial

modulators that promote mitophagy led to immunoparalysis, a secondary immune suppression in sepsis, counteracting the removal of infectious agents and worsening survival[113].

Circulating mtDNA increases gradually with age and correlates with serum inflammatory markers[114], suggesting a causal role of extracellular mtDNA in age-related innate immune activation. One proposed explanation is that age-related failure of mitochondrial quality control could increase the release of mitochondrial-derived DAMPs; for instance, mitochondrial fusion seems to regulate TLR9-mediated NF-κB activation in skeletal muscle of mice through mtDNA[115]. However, recent findings have challenged this notion, showing that a large portion of cell-free mtDNA in the blood is contained within whole mitochondria and does not circulate as naked DNA[116]. Additionally, cell-free mtDNA varies in response to common physiological stressors, such as exercise and psychological stress, suggesting that not all forms of cell-free mtDNA are pro-inflammatory[117].

Integration of cross-compartment MSR pathways. The coordination of several MSR pathways is critical for resolving cellular stress. This is exemplified by the fact that mitochondrial membrane dynamics and mitophagy occur in conditions of UPR^{mt} activation. Both increased fission[21,118,119] and fusion[27] have been observed when UPR^{mt} is activated. Whether fission or fusion occurs presumably depends on the type and strength of the inflicting stress, thus all contributing to the proteostatic capacity and protection against spreading of damaged macromolecules and organelles. Increased mitophagy has been detected in mammalian cells and flies overexpressing mutant forms of EndoG[120] or OTC-Δ (refs. [118,119,121]), and upon RNAi depletion of *ND75* (ref. [34]), which encodes an ETC component, all conditions that strongly induce the UPR^{mt}. Moreover, several autophagy genes are downstream targets of the master UPR^{mt} regulator, ATFS-1, in *C. elegans*[122]. Cellular homeostasis under mitochondrial stress is hence maintained by the integrated coordination of MSR pathways. The UPR^{mt} and presumably other interconnected protein stress responses act as the first line of defense against proteotoxicity. When the stress level overcomes the capacity of mitochondrial proteostasis, mitochondrial membrane dynamics and mitophagy come into action to redistribute and remove irreversibly damaged elements of the mitochondrial network. Inability to induce or coordinate these MSR pathways contributes to OXPHOS dysfunction and the decline in whole-organism physiology.

It is now evident that perturbations in the mitochondria can lead to broad cellular adaptations integrating cytosolic proteostatic responses, as shown by the recently identified mitochondrial to cytosolic stress pathways in yeast and worms[123–128]. These pathways restore cytosolic proteostasis by decreasing protein synthesis and/or increasing protein folding and degradation, highlighting the existence of an integrated, rather than a compartment-specific, proteostatic adaptation to mitochondrial stress. This view is supported by the integrated stress response (ISR) in mammals[9]. Several mitochondrial insults trigger the ISR, which induces a global response to restore cellular homeostasis by attenuating cytosolic translation and expression of cytoprotective genes. The ISR interconnects several proteostasis pathways, such as the UPR in the endoplasmic reticulum and the UPR^{mt}, by shared signaling effectors, including eIF2α and ATF4 (refs. [9,129]). Consistent with a functional link of mitochondrial to cytosolic proteostasis, pharmacological or genetic ablation of mitochondrial ribosomes attenuates cytosolic translation in worms and mammalian models[9,25], induces UPR^{mt}, and promotes health- and lifespan extension in several organisms[21–23] (Fig. 3a). Lipid signaling might have a coordinating role in these cross-modal stress pathways. Silencing of carnitine palmitoyltransferase (*CPT*) or mitochondrial chaperone *mtHSP70* (*hsp-6* in *C. elegans*) in worms triggers both the UPR^{mt} and the heat shock response (HSR), resulting in a chaperone-mediated reduction in cytosolic proteotoxicity[123]. The underlying mechanism of this adaptation involves attenuation of ceramide biosynthesis[123] (Fig. 3b). In line with these observations, pharmacological ceramide depletion by myriocin, a high-affinity inhibitor of the ceramide de novo biosynthesis pathway, promotes lifespan extension in yeast[130] and *C. elegans*[131]. This seems to involve global proteostasis remodeling through translation attenuation and improved mitochondrial homeostasis[132], common signatures of lifespan extension. By studying how yeast cells eliminate protein aggregates in the cytosol, another cross-compartment proteostasis pathway was recently revealed, wherein mitochondria can import and degrade misfolded cytosolic proteins, a phenomenon termed mitochondria as a guardian in cytosol (MAGIC)[133]. Further studies are needed to determine to what extent these pathways are conserved in vertebrates. One recent study, suggesting that this is indeed the case, has found that fine-tuning of mitochondrial and cytosolic translation is required for sustained killing of virally infected cells by cytotoxic T cells[134].

From all this, it becomes clear that mitochondria use MSR pathways to adapt themselves and the cellular milieu to stressful situations. Thus, impairment in these MSR pathways contributes to mitochondrial dysfunction and organismal aging. Conversely, depending on the type and intensity of the stress, the MSR can lead to beneficial cellular adaptations. This finely controlled mechanism, termed mitohormesis, is proposed to protect organisms from a decline in mitochondrial function that commonly occurs during aging and to extend the lifespan across species (Fig. 3c).

mtDNA integrity in aging

mtDNA encodes only 13 OXPHOS proteins in mammals, yet it is essential for mitochondrial homeostasis. Unlike the nuclear genome, the mtDNA is replicated continuously and independently of the cell cycle. Given the inefficient mtDNA repair system, it will inevitably lead to the introduction of base errors over time. Thus, mtDNA of somatic cells is prone to accumulate mutations throughout the lifetime of an organism, which progressively leads to increasing levels of heteroplasmy, that is, the coexistence of intact and mutant mtDNA copies in the same cell. Above a certain threshold, heteroplasmy of mtDNA mutations translates into detrimental physiological consequences driving aging and disease[135,136], including impairments to glucose metabolism and cognition[137] and lifespan shortening[138], in mice.

When mtDNA mutations occur in germline cells, they can be maternally transmitted as polymorphisms to the next generation, conferring sequence variability within species and allowing their subgroup classification as haplotypes. During evolution, mtDNA lineages, or haplogroups, emerged from the segregation of these different mtDNA sequences due to migration flow. Although enrichment of certain haplotypes might have helped our ancestors to adapt their physiology to different environmental conditions[139], meta-analysis studies have reported the association of some haplotypes with several pathological conditions, such as Alzheimer's disease[140], multiple sclerosis[141], and type 2 diabetes[142]. Conversely, other haplotypes have been associated with physiological benefits, including increased longevity in Haplogroup D among the Japanese population[143] and in Haplogroup J among the European population[144], although other factors, such as environment and ethnic background, could also explain this phenotype[145].

Cytoplasmic hybrid (cybrid) cell lines and conplastic organismal models carrying different mtDNA variants under the same nuclear background have been extensively used to study the cellular and physiological impact of mtDNA–nDNA (in)compatibility, overcoming some limitations in association studies. In flies, mitonuclear matching has been shown to modulate lifespan[146], and these epistatic interactions are further modified by diet[147]. Moreover, mitonuclear mismatch can have detrimental effects on mitochondrial metabolism and ROS metabolism during *Drosophila* aging[148] and

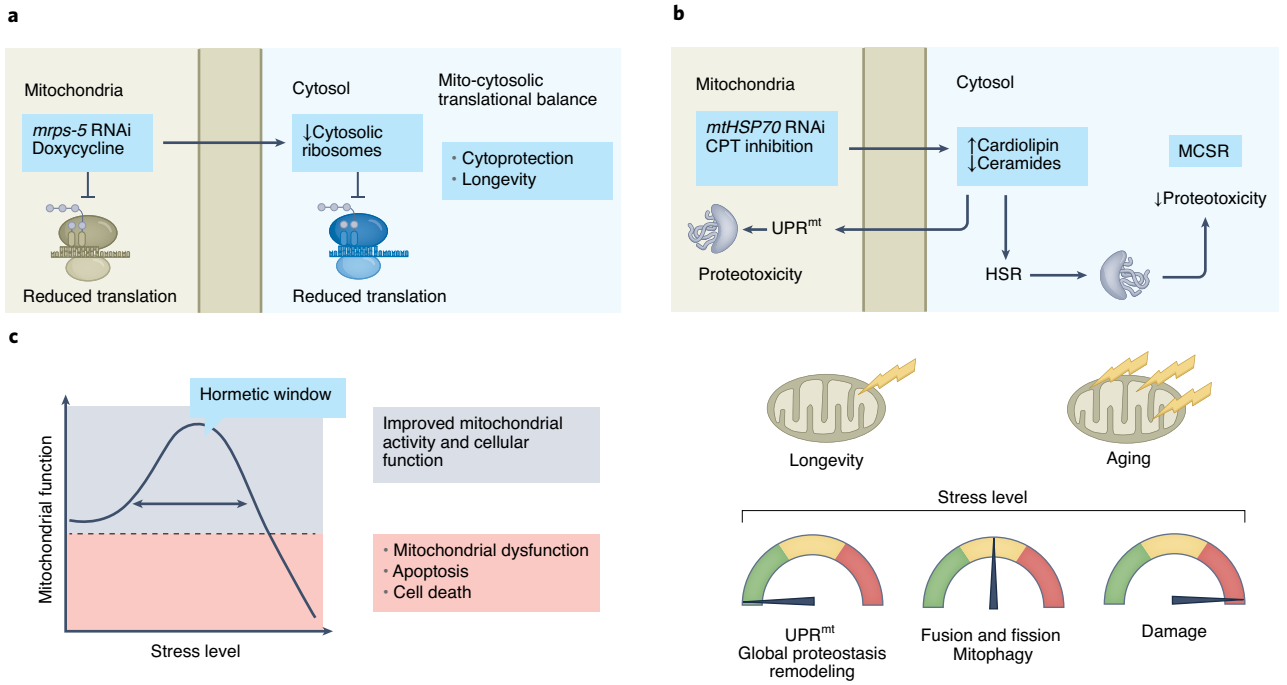

Fig. 3 | MSR contribution to the reconstitution of cellular homeostasis. a, Inhibition of mitochondrial translation by *mrps-5* RNAi or pharmacologically using doxycycline not only blunts mitochondrial translation, but also inhibits cytosolic translation and promotes lifespan extension in *C. elegans*. **b**, In *C. elegans*, inhibition of CPT or silencing the mitochondrial chaperone *mtHSP70* (*hsp-6* in *C. elegans*) triggers both the UPR^{mt} and the cytosolic HSR through a mechanism involving the attenuation of ceramide biosynthesis, termed mitochondrial to cytosolic stress response (MCSR). **c**, Stress signals induce mitohormesis through activation of the MSR to improve mitochondrial and cellular function. Moderate stress activates the UPR^{mt} to prevent proteotoxicity; if the stress exceeds the capacity of the UPR^{mt}, membrane dynamics and mitophagy are activated; sustained exposure to stress that exceeds the overall MSR capacity leads to cellular damage and organismal decline. When the amount of stress does not exceed the capacity of the MSR, the adaptive biological response leads to an improvement in cellular health and organismal lifespan.

can affect their fitness[149]. Interestingly, a study using cybrid mouse cell lines engineered to contain different mtDNA haplotypes in an identical nuclear background showed that strain-specific mtDNA variants, such as NZB mtDNA, lead to increased ROS levels, which act as a signal for mitochondrial biogenesis[150]. It was later observed that conplastic mice with NZB mtDNA on the C57BL/6 nuclear background present tissue-specific reorganization of mitochondrial supercomplexes, improved ETC capacity, and improved overall energy metabolism, resulting in healthspan and lifespan extension[151] (Fig. 4a). Like in the cybrid cells, this phenotype seems to involve a mitohormetic response, as these conplastic mice show a mild increase in ROS levels[151], which presumably primed these beneficial adaptations. As described, a mismatch between mtDNA and nDNA can have profound consequences in physiology, which may also underpin the multivariable pathological characteristics of germline-transmitted mtDNA mutations. Owing to this haploid nature of mtDNA, high mutation rates in the mtDNA are counteracted by conserved selective mechanisms, such as bottleneck effects and purifying selection, to attenuate the transmission of deleterious levels of mutated mtDNA[152–155]. It has recently been shown that cells with defective mitochondria can be selectively eliminated by cell competition during early development in mice as another step of purifying selection[156]. Despite these protective mechanisms, heteroplasmy is often inherited, is shaped by selective forces under nuclear control in the mammalian germline[157], and can affect aging. Supporting this notion, another conplastic mouse strain harboring AKR/J mtDNA under a C57BL/6 nuclear background demonstrates impairment in multiple metabolic pathways, resulting in a shorter lifespan than that of wild-type C57BL/6 mice with low levels of heteroplasmy[138]. Furthermore, mtDNA mutations in the maternal germline can be transmitted to mice with a wild-type nDNA background and shorten their lifespan[158,159]. These findings indicate that the rate of aging may be set early in life, with germline-transmitted mtDNA mutations potentially having profound lifelong consequences. With the advance of mitochondrial gene-editing techniques[160], the precise manipulation of mtDNA variants may uncover how mutations in the germline affect aging and longevity.

The frequency of mtDNA mutations, be they point mutations or large-scale deletions, increases with age in humans and animal models[161–165] (Fig. 4b). Deletions of mtDNA are characterized by the loss of single or multiple portions of the mitochondrial genome, which can cause both multisystemic and tissue-specific diseases[42,166] Currently, it is not firmly defined whether these mutations are causal or correlative with aging, but strong indications suggest that they can contribute to OXPHOS dysfunction and some aging phenotypes[167,168]. This hypothesis originated from analysis of the different 'mutator' mouse strains[39,40]. The homozygous mice accumulate both point mutations and deletions in the mtDNA associated with reduced lifespan and accelerated aging, as manifested by sarcopenia, cardiomyopathy, loss of bone mass, and thymic involution[39,40,169,170]. Notably, over 300 human diseases are associated with *POLG* mutations, many of which manifest symptoms of age-related diseases[171].

Conversely, although homozygous *Polg*$^{mut/mut}$ mice age prematurely, heterozygous *Polg*$^{mut/+}$ mice seem to age normally regardless of their 500-fold higher mtDNA mutation load, suggesting that mtDNA mutation load does not define lifespan, at least up to a certain level[172]. This might also be true for large mtDNA deletions, as twinkle transgenic mice with multiple large mtDNA deletions do not show a reduction in the lifespan or a premature-aging phenotype[173]. These studies hence suggest that heteroplasmy must reach a certain level, known as the biochemical threshold[174], to

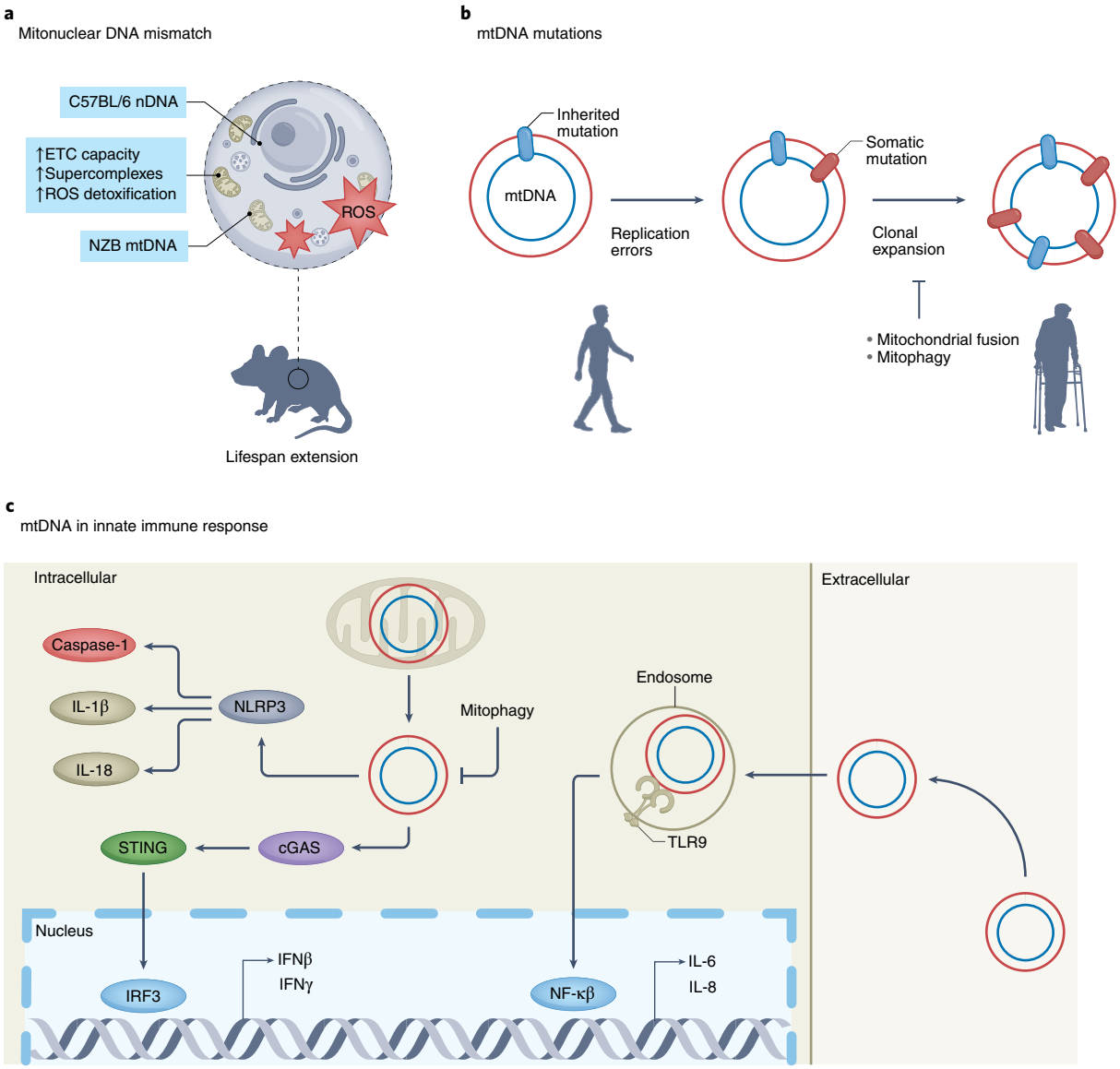

Fig. 4 | Potential mtDNA actions in aging. a, The rate of aging is influenced by mitonuclear DNA matching. Mitochondrial mismatch in conplastic mice carrying NZB mtDNA under a C57BL/6 nuclear background induces ROS production, which acts as a mitohormetic signal to improve mitochondrial homeostasis, leading to lifespan extension. **b**, mtDNA mutations accumulate during aging by replication errors and clonal expansion of both inherited and somatic mutations. Mitochondrial fusion and mitophagy are crucial pathways involved in mtDNA integrity by diluting and eliminating mutated DNA. **c**, mtDNA acts as a potent DAMP, activating intracellular and extracellular pathways. In the cytosol, mtDNA stimulates the NLRP3 inflammasome, promoting IL-1β and IL-18 secretion and apoptosis by caspase-1 activation. Additionally, mtDNA is sensed by cGAS, which activates STING. This pathway triggers the IRF3 transcription factor, leading to the expression of type I IFN genes. Circulating mtDNA can elicit an immune response by activating TLR9 in endosomes, leading to NF-κB-mediated expression of IL-6 and IL-8.

affect OXPHOS and organismal aging. Yet, in human tissues such as skeletal muscle, mtDNA mutations rarely reach this threshold, probably because these deletions form and clonally expand within individual muscle fibers, underlying heterogeneity across the tissue. Focal regions adjacent to myonuclei seem to be hotspots where the heteroplasmy originates before spreading across the muscle fiber[175], providing evidence for a cell-specific local proliferative advantage of mutant mtDNA. These cell-specific defects could then trigger cell-nonautonomous signaling events, contributing indirectly to organismal aging.

The underlying mechanisms that govern heteroplasmy in somatic cells are largely unknown. However, it has been proposed that mutant genomes have a selective advantage over non-mutant ones, resulting in their accumulation[176]. For unknown reasons, the induction of the UPR^{mt} in *C. elegans* seems to accelerate the accumulation of mutated mtDNA copies over intact ones[48,177]. In heteroplasmic worms, the UPR^{mt} regulator ATFS-1 binds preferentially to mutated mtDNA and promotes the binding of POLG through a mechanism involving the mitochondrial protease LONP-1, suggesting that ATFS-1 stability is a key process in the maintenance of heteroplasmy[178]. Conversely, mitochondrial fusion and mitophagy are essential for maintaining mtDNA integrity by diluting and eliminating mutated mtDNA, respectively[79,80,179,180] (Fig. 4b). Finally, mtDNA release is also a potent DAMP, activating both intracellular and extracellular immune pathways that could affect heteroplasmy (Fig. 4c). Thus a decline in MSR fitness can affect the age-related expansion of mtDNA mutations which may also have tissue[181,182] and cellular specificity[182,183].

Mitochondrial metabolites and aging

Nicotinamide adenine dinucleotide. NAD^+ is a cofactor involved in multiple metabolic reactions. It also serves as a substrate for many NAD^+-consuming enzymes, such as the poly-ADP-ribose polymerase (PARP), the cyclic ADP-ribose synthase CD38, SARM1, and sirtuins, a family of seven protein deacylases localized in the nucleus (SIRT1, SIRT6, and SIRT7), cytosol (SIRT2), and mitochondria (SIRT3–SIRT5). Decline in NAD^+ levels impairs the activity of sirtuins, which are important modulators of mitochondrial homeostasis and aging[184–187].

In multiple model organisms, NAD^+ levels decline upon aging[27,188], presumably due to reduced expression of nicotinamide phosphoribosyl (NAMPT), the rate-limiting enzyme for NAD^+ synthesis[189], or by increased expression and activity of NAD^+-consuming enzymes, such as PARP[27] and CD38 (refs. [190–192]). In line with this view, the age-dependent decline in NAD^+, which compromises mitochondrial homeostasis, can be recovered through administration of NAD^+ precursors or PARP inhibition in *C. elegans* and mice[27,188]. Moreover, restoration of NAD^+ levels with NR supplementation enhances lifespan in mice and improves mitochondrial and stem cell function[28]. These observations corroborate the protection from OXPHOS defects in mice with genetic or pharmacological ablation of PARP activity[193,194], or mice treated with NMN[195] or NR[196]. Deletor mice containing a mutation in *Twinkle*, which encodes a mitochondrial replicative helicase, display reduced levels of NAD^+, mitochondrial impairment, and progressive muscle myopathy; treatment with NR slows early and late-stage disease progression by restoring mitochondrial function[197]. In agreement, both NR administration and PARP inhibition improve ETC function and exercise intolerance in *Sco2*-knock out/knock in mice, another model of mitochondrial disease[198]. Moreover, in mouse models of ataxia telangiectasia, an autosomal disorder characterized by progressive neurodegeneration and cerebellar ataxia, increasing NAD^+ levels delays the accelerated-aging phenotype, including MSR decline, and extends the lifespan[103]. Similarly, PARP inhibition or NAD^+ supplementation rescues mitochondrial dysfunction and premature aging in a mouse model of Cockayne syndrome[199] and restores mitochondrial abnormalities in xeroderma pigmentosum group A (XPA)-deficient cells and worms, which prevents the attenuation in lifespan[200]. NAD^+ depletion is also observed in people with Werner syndrome and invertebrate models of the disease, a human premature aging disease caused by mutations in the Werner DNA helicase gene[102]. Restoring NAD^+ in *C. elegans* and *Drosophila* Werner syndrome models delays the accelerated-aging phenotype, including stem cell dysfunction, and extends the lifespan[102]. These findings suggest that boosting NAD^+ levels prevents mitochondrial dysfunction in not only aging, but also rare genetically determined mitochondrial diseases and DNA-repair disorders known to accelerate the aging process.

Additional findings have demonstrated the importance of NAD^+ in the immune system. Increasing NAD^+ levels can benefit several inflammatory conditions in mouse models of aging[28,188], ataxia-telangiectasia autoimmunity[103], and muscular dystrophy[51]. In older humans, NR administration for only 21 days was sufficient to reduce circulating inflammatory cytokines[201]. However, it is unclear whether this anti-inflammatory effect is secondary to the physiological benefits of NAD^+ or perhaps is more probably caused by direct programming of immune cells. NAD^+ levels decline in immune cells upon aging, and boosting NAD^+ levels restores the age-related decrease in OXPHOS and immune function in macrophages from older humans and mice[202]. Interestingly, pro-inflammatory M1-like, but not naive or M2, macrophages express high levels of the NAD-consuming enzyme CD38, induced by cytokines released from senescent cells[190,191]. These M1-like macrophages accumulate in tissues such as visceral white adipose tissue and liver during aging, thereby reducing global tissue NAD^+ levels[191], suggesting that senescent cells promote tissue NAD^+ reduction via activation of macrophages.

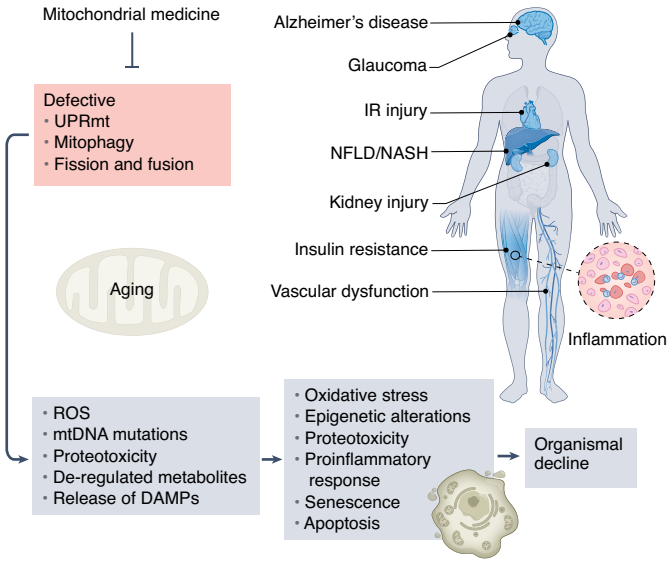

Fig. 5 | Pleiotropic effects of mitochondria in aging. Mitochondrial alterations in aging initiate with a decline in MSR pathways leading to the accumulation of mtDNA mutations, release of damaged toxic mitochondrial material (for example, DAMPs), mtROS generation, proteotoxicity, and deregulated metabolites (TCA intermediates, NAD^+). These alterations have a broad detrimental effect on cellular homeostasis and, through a complex signaling mechanism (involving mitokines, metabolites, and more), contribute to systemic organismal decline and the onset of several age-related diseases. Pharmacological modulation of the MSR, such as through the use of NAD+ enhancers or mitophagy inducers, can be effective strategies to prevent aging-related cellular and organismal decline. IR injury, ischemia–reperfusion injury; NFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis.

Collectively, these findings suggest that a decrease in systemic NAD^+ levels is a crucial driver of organismal decline in aging. This is further supported by the overarching therapeutic effect of NAD^+ boosters in several animal models of common age-related conditions, ranging from diabetes and obesity[195,196], non-alcoholic fatty liver disease[203], kidney injury[204–206], impaired muscle function and sarcopenia[51,188,196], glaucoma[207], ischemia–reperfusion injury[208,209], vascular dysfunction[210], to cognitive decline[56,103,211,212]. Taken together, the health benefits and the prevention of age-associated MSR and OXPHOS decline support the use of NAD^+ boosters as therapy for some of these age-related diseases. The efficacy of NAD replenishment was recently illustrated in the setting of human acute kidney injury[206] and COVID-19 (ref. [213]).

Tricarboxylic acid cycle intermediates. Tricarboxylic acid cycle (TCA) metabolites are by-products of energy metabolism with essential roles in cellular homeostasis, fueling anabolic reactions and adjusting metabolic pathways through signaling cascades or allosteric modulation of key enzymes. There is increasing evidence that TCA metabolites are essential mediators of cellular signaling by their actions in chromatin modifications, DNA methylation, and post-translational protein modifications[214]. For instance, citrate leads to the cytosolic production of acetyl-CoA that fuels histone and protein acetylation through acetyltransferases[215,216], thus modulating gene expression[217–220]. In this regard, impaired mitochondrial metabolism affects epigenetics by restricting the production of TCA intermediates. Indeed, genetic ablation of the ETC impairs histone acetylation, which can be restored by reconstitution of TCA

Box 2 | Key questions on the pleiotropic effects of mitochondria in aging

Although the link between mitochondrial homeostasis and aging is well established, several important questions need to be answered to allow a clear mechanistic level to the understanding of this connection.

- What are the molecular mechanisms underlying the cross-modal effect of the MSR on stress in other cellular compartments? Are there common signaling effectors that could explain their co-decline in aging?
- How does aging affect mitochondrial communication with other cellular compartments and organelles?
- What MCSRs are conserved in mammalian aging? What are the signaling molecules involved in these responses?
- How do mutations in mitochondrial DNA in the germline shape aging and longevity? What are the underlying mechanisms that govern heteroplasmy in somatic cells? Is there tissue and cellular specificity?
- How is mtDNA released in the cytosol and in the circulation during aging, and how does it signal?
- We should also understand better age-related differences in mitochondrial function at the subcellular, cellular, tissular, and organismal levels. A better definition and characterization of the various mitokines or metabokines is required.
- Can we design strategies that safely prevent or revert age-induced changes in mitochondrial metabolites that mediate aging pathways?
- How can we harness mitohormesis to improve healthspan and increase longevity? What is the safe threshold to induce mild mitochondrial stress in aging, and at what stage of the lifespan?

function in human cells[221]. Moreover, DNA- and histone-methylation status are both regulated by 2-oxoglutarate-dependent dioxygenases (2-OGDO), such as ten-eleven translocation (TET) hydroxylases and histone demethylases that use α-ketoglutarate (α-KG) and oxygen as co-substrates for oxidation of target molecules[214]. Succinate and fumarate are potent inhibitors of these 2-OGDO enzymes[222], underscoring the tight control of the chromatin epigenetic landscape by the TCA.

Changes in nutritional pathways and epigenetic state are crucial to aging and are affected by TCA metabolites. In *C. elegans* and *Drosophila*, administration of α-KG extends lifespan through a mechanism involving the inhibition of the target of rapamycin (TOR)[223,224]. Fumarate and malate, when administered to worms, extend lifespan, which is associated with the induction of the glyoxylate shunt, an extra-mitochondrial pathway of energy production, mild mitochondrial uncoupling, and expression of the longevity regulators DAF-16 and SIR-2.1 (refs. [225,226]). Conversely, the accumulation of succinate causes an opposite effect in worms and flies: succinate dehydrogenase (SDH) mutants display increased ROS levels and accelerated aging[227,228]. Acetyl-CoA seems to be an essential mitochondrial signal regulating the rate of aging in *C. elegans*. Upon mitochondrial stress early in life, levels of acetyl-CoA decrease, resulting in the nuclear accumulation of the histone deacetylase complex (NuRD), allowing epigenetic and transcriptional remodeling for lifespan extension in worms[229].

α-KG levels decline upon mammalian aging[230,231] and correlate with alterations in the epigenetic landscape in several tissues, such as the brain[232], adipose tissue[231], and bones[233]. Not surprisingly, replenishment of α-KG levels attenuates several age-related disorders. Increasing the levels of α-KG ameliorates age-related osteoporosis in aged mice by reducing accumulation of histone H3 trimethylated at K9 and H3 trimethylated at K27 at the promoters of the osteogenesis-related genes *Bmp2*, *Bmp4*, and *Nanog*, improving bone marrow mesenchymal stromal and stem cell function[233]. Moreover, restoration of α-KG in middle-aged mice increases DNA demethylation at the promoter of the transcriptional regulator of brown adipocytes *Prdm16*, which induces brown adipocyte genes and prevents age-associated obesity[231]. Increasing α-KG levels furthermore restores age-related redox alterations[234], reduces inflammation[235], delays fertility decline[236], and extends the lifespan[235] in mice (as in flies and worms—see above), suggesting that α-KG is a potent signaling metabolic intermediate involved in mammalian aging, presumably by epigenetic modulation. Also, an elevated α-KG:succinate ratio is involved in the maintenance and differentiation of pluripotency of embryonic stem cells[237,238] and modulates the differentiation of germ cells[239] by DNA and histone demethylation. Similar to what happens in *C. elegans*, accumulation of succinate by reduced SDH activity in mammals, as observed during aging[240,241], can counteract the epigenetic actions of α-KG by inhibiting 2-OGDO demethylases[237,238], thus contributing to age-related epigenetic alterations[232] and diseases. Fumarate has recently been shown to act as a terminal electron acceptor in the mammalian ETC under conditions of hypoxia, yet its connection with aging remains to be established in mammals[242].

Conclusion and perspectives

Work over recent years has uncovered the impressive ability of the mitochondria to maintain homeostasis in a variety of stressful situations. The importance of this adaptive response is underlined by our increasing understanding of how defects in these mitochondrial responses are intimately associated with aging. Mitochondria have a pleiotropic effect on aging, which can comprise protective or maladaptive responses. The nature of their response will depend on how mitochondria can sustain their MSR pathways within the ever-changing cellular milieu that is exposing them constantly to various levels of stress (Fig. 5). In recent years, we have gained considerable insight into how age-related processes are intimately wired to different types of MSR. Still, the functional interactions of these pathways and their implication in aging remain largely unexplored (Box 2). Identifying shared molecular signals of stress responses will be crucial to shed light on how MSR dysfunction contributes to proteostasis collapse during the aging process[243]. For instance, NAD^+ gradually declines during aging and seems to integrate many of these stress responses. Other signaling molecules, such as bioactive lipids and mitochondrial metabolites, may warrant more attention, as they also seem to have a role in cross-compartment stress communication. On the same note, mitochondrial DNA has evolved as a critical signaling factor in cellular homeostasis. Given some unique features of mitochondria, such as their haploid inheritance, constant replication rates, and inefficient DNA-repair system, mtDNA is prone to accumulating mutations throughout life, leading to a progressive increase in heteroplasmy. Moreover, the rate of aging may be set early in life by germline-transmitted mtDNA mutations. Yet, the underlying mechanisms in mammals are poorly known. Future studies should focus on the involvement of MSR in regulating the clonal expansion of both somatic and inherited mtDNA mutations, as observed in model organisms[69,82]. Under these circumstances, how damaged mitochondria or components of the mitochondria, including mtDNA, are released during aging and whether this signals cellular and systemic inflammation are important questions that should be addressed. Hopefully, unveiling the pleiotropic effects of mitochondrial dysfunction will allow us to better understand fundamental aspects of how mitochondria have a commanding role in the aging clock.

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Author contributions

T.L. and J.A. conceived the project, drafted the first version, and revised the manuscript. T.Y.L. and A.M. contributed to the conception and writing of the manuscript.

Competing interests

J.A. is a consultant to Mitobridge/Astellas, Amazentis, NOV Metapharma, and Metro Biotech. The other authors have no competing interests.

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