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Review

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Mitochondrial cross-compartmental signalling to maintain proteostasis and longevity

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Lifespan in eukaryotic species can be prolonged by shifting from cellular states favouring growth to those favouring maintenance and stress resistance. For instance, perturbations in mitochondrial oxidative phosphorylation (OXPHOS) can shift cells into this latter state and extend lifespan. Because mitochondria rely on proteins synthesized from nuclear as well as mitochondrial DNA, they need to constantly send and receive messages from other compartments of the cell in order to function properly and maintain homeostasis, and lifespan extension is often dependent on this cross-compartmental signalling. Here, we describe the mechanisms of bi-directional mitochondrial cross-compartmental signalling resulting in proteostasis and longevity. These proteostasis mechanisms are highly context-dependent, governed by the origin and extent of stress. Furthermore, we discuss the translatability of these mechanisms and explore therapeutic developments, such as the antibiotic studies targeting mitochondria or mitochondria-derived peptides as therapies for age-related diseases such as neurodegeneration and cancer.

This article is part of the theme issue 'Retrograde signalling from endosymbiotic organelles'.

1. Introduction

As energy suppliers of the cell, mitochondria are responsible for making ATP through oxidative phosphorylation (OXPHOS). The OXPHOS system comprises five multi-subunit complexes that consist of both nuclear (nDNA) and mitochondrial DNA (mtDNA)-encoded proteins. Although 99% of all mitochondrial proteins are transcribed from nDNA and translated in the cytosol, eukaryotic cells are still dependent on the additional 13 mitochondrial proteins transcribed and translated in the mitochondrion itself. In order to tune these two genomes and allow them to cooperate to maintain the stoichiometry of mitochondria- and cytosol-translated OXPHOS proteins, several retrograde signalling pathways have evolved. When there is a disturbance in this stoichiometry, the cell has learned to cope with this by activation of many proteostasis pathways.

It has been known for many years that lowering the expression of nDNA-encoded proteins in one of the OXPHOS complexes I, III, IV or V using RNAi extends lifespan in *Caenorhabditis elegans* [1,2]. The same is true in *Drosophila melanogaster*, whose lifespan was extended when treated with RNA interference (RNAi) against OXPHOS genes [3]. This lifespan extension, however, was not consistently correlated with increased resistance to the free-radical generator paraquat, suggesting that it cannot simply be attributed to a mitohormetic mechanism that is induced by reactive oxygen species (ROS) as a byproduct of OXPHOS inhibition [3]. This is in line with observations that mitochondrial production of ROS is insufficient to determine longevity [4]. Instead, several signalling pathways were identified that relay stress in or at mitochondria to other parts of the cell and thereby initiate adaptive responses that can alleviate stress. Indeed, in recent years, many reports have shown mitochondrial retrograde signalling mechanisms to be

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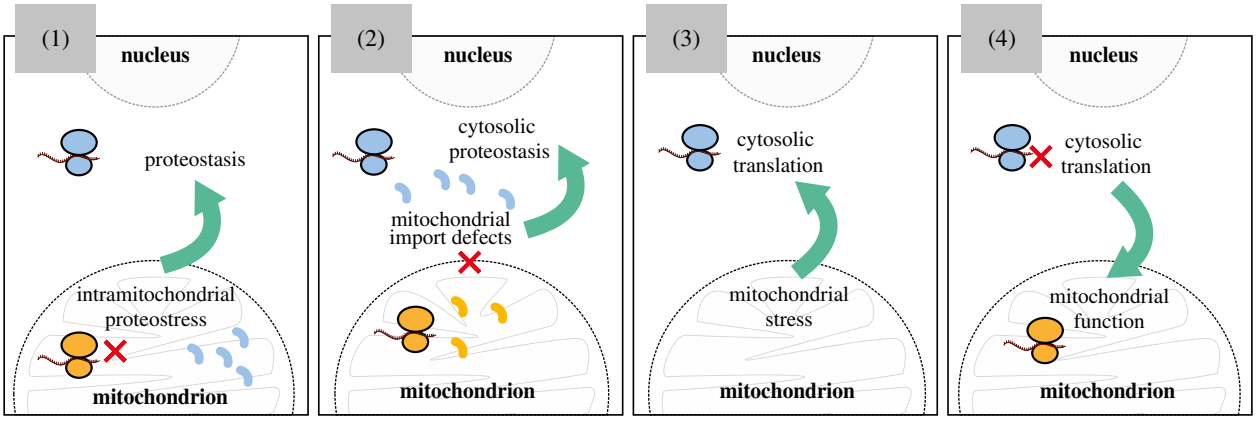

Figure 1. Mitochondrial cross-compartmental proteostasis signalling routes. (1) Unfolded proteins inside the mitochondria induce both cytosolic and mitochondrial responses to restore proteostasis and ensure proper OXPHOS assembly. (2) When mitochondrial import is disrupted, cytosolic proteostasis pathways are activated in order to cope with accumulation of mitochondrial precursor proteins in the cytosol. (3) Mitochondrial (bioenergetic) stress reduces cytosolic protein synthesis and activates proteostasis while maintaining specific translation of mitochondrial proteins, in order to cope with both energy deficits and mitochondrial dysfunction. (4) Inhibition of cytosolic translation itself affects mitochondrial function via several pathways, resulting in bi-directional mitochondrial cross-compartmental proteostasis signalling.

important for longevity induced by mitochondrial perturbations. Both mitochondrial dysfunction and loss of proteostasis are classified as hallmarks of ageing [5]. Although described as two individual processes, we now know they are tightly linked in the context of ageing. Here, we review these mitochondrial cross-compartmental signalling pathways that ensure proteostasis and promote longevity, and discuss their potential therapeutic application in age-related diseases.

2. Mechanisms of mitochondrial retrograde proteostasis signalling in longevity

Mitochondrial cross-compartmental proteostasis signalling pathways can be divided into four main routes, which can be distinguished based on the site where proteostasis stress originates as well as the nature of this stress (figure 1). In brief, we distinguish (figure 1, 1) communication from mitonuclear protein imbalance originating from within mitochondria as well as (figure 1, 2) mitochondrial import defects, both of which induce cellular proteostasis. Moreover, (figure 1, 3) mitochondrial (bioenergetic) stress can affect cytosolic protein synthesis and (figure 1, 4) inhibition of cytosolic protein synthesis also affects mitochondrial function. These four routes, and how they play a role in longevity, will be described below.

(a) Communicating disturbed intramitochondrial protein homeostasis

Dysfunctional mitochondria activate stress-responsive pathways in order to restore mitochondrial function and promote cellular survival. A well-described example of this signalling in the context of mitochondrial protein-folding homeostasis is the unfolded protein response in the mitochondria (UPR^{mt}), which is a similar but distinct regulatory mechanism compared with the unfolded protein response in the endoplasmic reticulum (ER) (UPR^{ER}). The UPR^{mt} is activated by unfolded proteins in the mitochondria and ultimately activates dedicated nDNA-encoded chaperones such as heat shock protein (HSP) 70 and HSP60 [6]. The UPR^{mt} in *C. elegans* is dependent on the cleavage of unfolded proteins by the mitochondrial CLPP-1 protease into smaller peptides [7]. These peptides are exported into the cytosol by the HAF-1 transporter and are proposed to activate the transcription factor ATFS-1 (figure 2, panel 3) [8]. ATFS-1 then translocates to the nucleus and induces the expression of *hsp-6* and *hsp-60* (homologues of HSP70 and HSP60), together with cofactors ubiquitin-like protein 5 (UBL-5) and DVE-1 [7,9]. In mammalian cells, it is the transcription factor ATF5 that mediates the UPR^{mt} as it localizes to the nucleus to induce transcription of genes that promote mitochondrial proteostasis mechanisms (figure 2, panel 4) [10]. These mechanisms include proteins that aid proper protein folding, protein complex assembly and mitochondrial quality control to ultimately restore mitochondrial proteostasis. Underscoring the importance of this signalling, the lifespan extension induced by perturbations in OXPHOS complexes is dependent on the UPR^{mt}. For instance, the lifespan extension in *cco-1* RNAi-treated worms with perturbed OXPHOS complex IV was prevented when the UPR^{mt} cofactor *ubl-5* was silenced [11]. Interestingly, this pathway is also involved in other ageing conditions such as senescence. During ageing in mammals, senescent cells accumulate in various tissues and organs, and these cells have decreased mitochondrial functioning [12]. Senescent hepatocytes showed compromised UPR^{mt} despite mitochondrial dysfunction, and overexpression of CLPP inhibited senescence by restoring mitohormesis [13].

The proper assembly of complete OXPHOS complexes is also dependent on mitochondrial protein synthesis, carried out by mitochondrial ribosomes. Inhibiting mitochondrial ribosomes either pharmacologically or genetically extended lifespan in a conserved manner [14]. The knockdown of mitochondrial ribosomal protein (MRP) genes using RNAi in *C. elegans* caused longevity, reduced basal respiration, delayed development and lowered ATP content as a result of reduced mitochondrial amount or activity [14]. Pharmacological inhibition of mitochondrial translation using antibiotics such as doxycycline and chloramphenicol similarly induced longevity [14]. The inhibition of mitochondrial ribosomes causes a stoichiometric imbalance between mtDNA- and nDNA-encoded OXPHOS proteins. This mitonuclear protein imbalance is a trigger for the activation of UPR^{mt}, which is required for the lifespan extension as RNAi of either *haf-1* or *ubl-5* reduced the lifespan extension after inhibition of mitochondrial translation [14]. Although the active reduction in mitochondrial translation has not been shown to extend lifespan in mammals, the use of forward genetics in the BXD mouse genetic reference

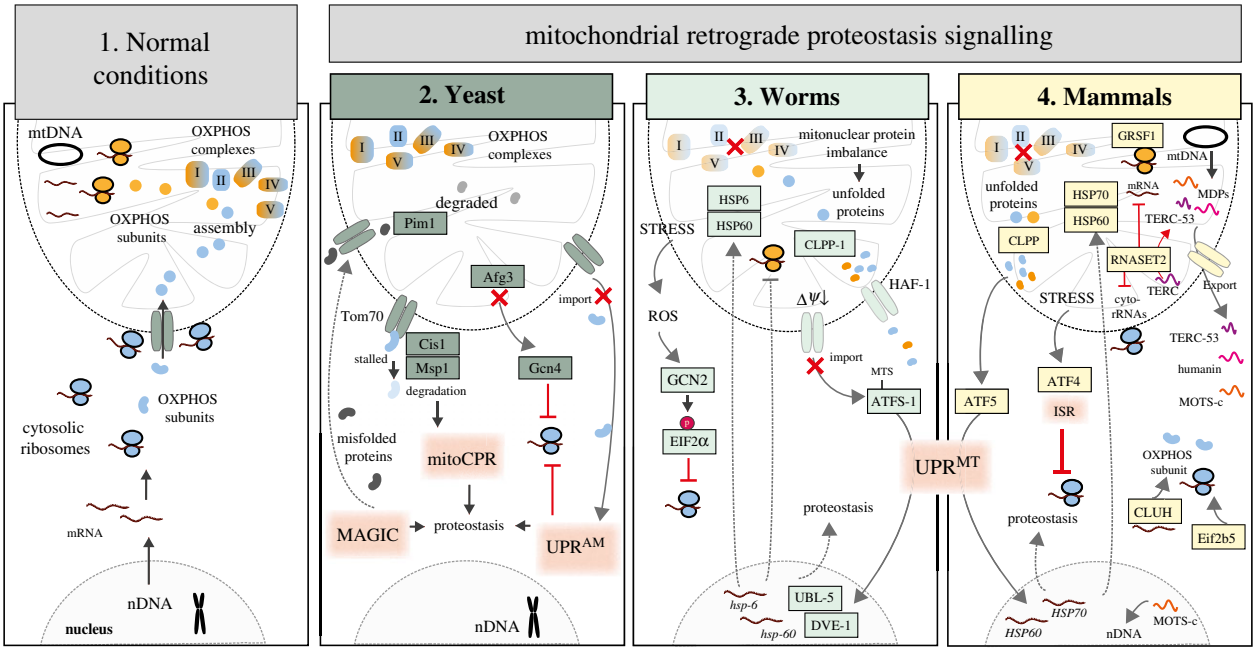

Figure 2. Mechanisms of mitochondrial cross-compartmental signalling in different models. Panel 1: OXPHOS complexes need to be properly assembled, and consist of proteins that are synthesized in the cytosol from nDNA as well as in the mitochondria from mtDNA, a process that is highly conserved across species. The mitochondrial preproteins synthesized in the cytosol need to be imported into the mitochondria where they meet the mtDNA-encoded subunits and are assembled into OXPHOS complexes. Panel 2: in yeast, a defect in mitochondrial import machineries leads to the accumulation of mitochondrial precursor proteins in the cytosol, which activate a proteostatic response termed UPR^{am} (activated by mistargeted mitochondrial proteins), resulting in the inhibition of cytosolic protein synthesis and increased proteasomal degradation. Another process, termed mitoCPR (compromised protein import response), activated by accumulated precursors on the mitochondrial surface restores proteostasis by clearing these stalled proteins from the import channels by binding of Cis1 to Tom70, recruiting Msp1 which targets them for proteosomal degradation. Finally, aggregation-prone misfolded proteins are imported into mitochondria for degradation through LON protease, a proteostasis process named MAGIC (mitochondria as guardian in cytosol). In the mitochondrial AAA protease *afg3*Δ mutant, Gcn4 is activated, which in turn decreases cytosolic translation and extends lifespan. Panel 3: in *C. elegans*, a mitomitonuclear protein imbalance leads to unfolded proteins in the mitochondria, which are cleaved by CLPP-1 into smaller peptides. These peptides are exported by HAF-1, which then causes translocation of ATFS-1 to the nucleus, and initiates the process called the unfolded protein response in mitochondria (UPR^{mt}). A decrease in membrane potential also induces UPR^{mt} by reduction in protein import leading to the translocation of ATFS-1. When ATFS-1 localizes to the nucleus, together with cofactors UBL-5 and DVE-1, it causes expression of *hsp-6* and *hsp-60*, which then go to the mitochondria to restore proteostasis. Mitochondrial stress can also signal via ROS, which activates the kinase GCN-2, which then phosphorylates EIF-2α, leading to repressed cytosolic translation. Panel 4: in mammalian cells, unfolded proteins within mitochondria are also cleaved by CLPP into peptides, which activate the UPR^{mt} through the transcription factor ATF5 localizing to the nucleus. There, HSP60 and HSP70 are transcribed and are imported into the mitochondria. Another key regulator of the mitochondrial stress response is ATF4, which reduces global cytosolic translation and activates expression of cytoprotective genes by activation of the ISR. The RNA-binding protein CLUH and eukaryotic initiation factor Eif2b5 regulate expression of nDNA-encoded mitochondrial proteins, and CLUH can also localize to the mitochondrial outer membrane. The multifunctional protein RNASET2 inside the mitochondria degrades both cytosolic rRNAs and mitochondrial mRNAs and processes TERC into TERC-53. TERC-53 together with mitochondria-derived peptides such as humanin and MOTS-c can be exported outside the mitochondria, where they communicate to the rest of the cell. Lastly, the mitochondrial RNA-binding protein GRSF-1 regulates mitochondrial translation.

population does hint towards a role for MRP genes, and the OXPHOS system it translates, in mammalian longevity [14]. The lifespan of BXD mouse strains ranges from approximately 365 to 900 days [15]. Based on a negative correlation between MRP gene expression and lifespan in these mice, it was deduced that a 50% reduction in MRP expression corresponded with an approximately 250 day lifespan extension [14].

While inhibition of mitochondrial translation induces the UPR^{mt}, the reverse is also true, as UPR^{mt} itself slows down mitochondrial translation and pre-RNA processing in mitochondria [16]. To induce UPR^{mt}, HeLa cells were treated with either CDDO, an inhibitor of matrix protease LON, or a specific inhibitor of the matrix HSP90 chaperone TRAP1 (gamitrinib-triphenylphosphonium (GTPP)), which cause mitochondrial protein misfolding [16]. Transcriptomics and proteomics revealed that such acute activation of UPR^{mt}, besides the expected upregulation of mitochondrial chaperonins, reduced pre-RNA processing and led to rapid, but reversible, translational inhibition of mtDNA-encoded proteins [16].

Another level of communication occurs through mitochondria-derived peptides (MDPs) encoded by short open reading frames in the mtDNA, which were discovered to communicate to other parts of the cell (figure 2, panel 4). A typical example is humanin. The circulating levels decline with age in mice and humans [17], and humanin expression had neuroprotective effects in human cells with different variants of familial Alzheimer's disease genes [18]. Moreover, supplementation of humanin in male Wistar rats had cardioprotective effects [19]. Another mtDNA-encoded peptide, named MOTS-c, localizes to the nucleus in response to metabolic stress and binds chromatin to regulate nuclear gene expression [20].

(b) Proteostasis of mitochondrial proteins following import defects

Under normal conditions, mitochondrial (pre)proteins are synthesized in the cytosol and are imported into the mitochondria by complex machineries, including translocases of the outer

mitochondrial membrane (TOM) and translocases of the inner mitochondrial membrane (TIM) proteins (reviewed in [21]) (figure 2, panel 1). A defect in mitochondrial import machineries, for instance, in the yeast temperature-sensitive mutant defective for mitochondrial protein import *mia40-4int*, leads to the accumulation of mitochondrial precursor proteins in the cytosol, which can activate a proteostatic response [22] (figure 2, panel 2). This response, termed UPR^{am}, is activated by mistargeted mitochondrial proteins and results in the inhibition of cytosolic protein synthesis and increased proteasomal degradation [22]. This communication activated by mitochondrial dysfunction via unfolded proteins promotes proteostasis and thereby may provide another explanation for the beneficial effects of mild mitochondrial dysfunction on ageing.

Another process activated upon impaired mitochondrial protein import in yeast was termed mitoCPR (compromised protein import response), and was activated by accumulated precursors on the mitochondrial surface (figure 2, panel 2) [23]. MitoCPR restored proteostasis by clearing stalled proteins from the import channels. Accumulating precursors induced the expression of mitochondrial outer membrane localized Cis1, which binds to Tom70. Cis1, in turn, recruited the ATPase Msp1 to remove stalled precursors and target them for proteosomal degradation [23]. Finally, in yeast, it was shown that aggregation-prone mitochondrial and cytosolic misfolded proteins were imported into mitochondria for degradation through LON protease Pim1 [24]. Indeed, protein aggregates that form under heat shock interact with mitochondrial import complexes and enter the intermembrane space and matrix [24]. This mitochondria-mediated proteostasis mechanism was named MAGIC (mitochondria as guardian in cytosol) [24]. A potential link between MAGIC and mitophagy was proposed [25], as mitochondrial foci harbouring misfolded proteins within the mitochondrial matrix were selectively removed in a process that involved mitochondrial fission mediated by Drp1 [26].

In *C. elegans*, compromised mitochondrial import was linked to the activation of UPR^{mt} [27]. Most conditions inducing UPR^{mt} have in common that they decrease mitochondrial membrane potential, which in turn reduces protein import [27]. Therefore, it was hypothesized that it is this reduced protein import that is responsible for UPR^{mt} activation [27]. The weak mitochondrial targeting sequence (MTS) of ATFS-1 is proposed to act as a sensor driving this mechanism as it localizes to the nucleus when mitochondrial protein import is compromised [27].

(c) Cytosolic proteostasis induced by mitochondrial stress

In addition to the various UPRs that are activated upon mitochondrial protein-folding stress, there are other signalling pathways that contribute to the maintenance of cellular and mitochondrial proteostasis in response to other kinds of mitochondrial stressors. Energy in the form of ATP is produced in the mitochondria, and it has been estimated that more than 50% of this energy is used for ribosome biogenesis and mRNA translation [28–30]. Yet, the rhythmicity of protein synthesis is not directly coupled to ATP-level oscillations in hepatocytes [31]. Likewise, in an *Escherichia coli* cell-free platform that mimics the intracellular, energy-limited environment, protein synthesis continued even at low ATP levels, actually enabling cell adaptation during energy limitation [32]. This suggests that ATP availability does not directly control protein synthesis rates, although it does not rule out possible other signals from the mitochondria controlling cytosolic protein synthesis.

It is not unexpected that mitochondrial stress controls proteostasis in the cytosol, since 99% of all mitochondrial proteins are synthesized in the cytosol and require careful tuning with the mitochondrial proteome [33,34]. Long-lived *C. elegans* mutants with dysfunctional mitochondria, such as the ubiquinone synthesis mutant *clk-1(qm30)* and the complex III mutant *isp-1(qm150)*, attenuate cytosolic translation in a manner that is distinct from the UPR^{mt} [33]. Unlike the UPR^{mt}, this communication required ROS production, which in turn activated the general control non-derepressible 2 kinase (GCN-2), one of the four dedicated kinases responsible for phosphorylating the α subunit of eukaryotic initiation factor 2 (eIF2α) (figure 2, panel 3). When phosphorylated, eIF2α represses global cytosolic translation while still preferentially translating transcripts involved in stress responses [35]. The lifespan extension in the mitochondrial mutants was dependent on GCN2, again pointing out that mitochondrial retrograde signalling plays an important role in longevity. Remarkably, other reports showed that the lifespan extension in these mutants was dependent on the UPR^{mt} [36]. Most likely, mitochondria communicate via multiple parallel routes to ultimately promote cytoprotection and longevity.

Another mitochondrial stress signalling pathway acts via the transcription factor ATF4, which was identified in mammals as a key regulator of the mitochondrial stress response [37]. Following treatment of mammalian cells with four different kinds of mitochondrial stressors, a multiomics approach revealed that ATF4 activates expression of cytoprotective genes, and reprogrammes metabolism by activation of the integrated stress response (ISR) [37] (figure 2, panel 4). Although ATF4 can be activated by phospho-eIF2α [38], its activation from these mitochondrial stressors was not dependent on GCN2 or any of the other eIF2α kinases [37]. Activation of ATF4 independently of GCN2 or phospho-eIF2α has been reported before in the context of methionine-deficient cells [39]. These reports suggest that there is an alternative distinct pathway from mitochondrial stress towards ATF4. Interestingly, overexpression of the ATF4 homologue in yeast, Gcn4, reduced protein synthesis capacity and extended lifespan [40]. Gcn4 directly repressed the expression of cytosolic ribosomal proteins and thereby reduced polyribosome formation. In yeast, the mitochondrial AAA protease Afg3 was identified as a mitochondrial determinant of cytoplasmic mRNA translation [41]. Besides decreased cytosolic translation, *afg3*Δ mutants also showed lifespan extension, both of which were dependent on Gcn4. In *C. elegans*, RNAi of the Afg3 homologue *spg-7* also extended lifespan, but was dependent on the UPR^{mt} as *spg-7* RNAi did not extend lifespan in the *atfs-1(tm4919)* mutant [42].

Taken all together, stressed mitochondria can activate parallel and complementary protective signalling pathways in order to maintain cytosolic proteostasis. The mechanistic similarities and differences between these pathways remain to be identified.

(d) Cytosolic translation controls mitochondrial function

In addition to the mitochondrial signals affecting cytosolic proteostasis (described in §2a–c), communication also occurs in the reverse direction. The mitochondrial cross-compartmental communication works in a feedback loop as it was shown

that mitochondrial ribosomes, synthesizing the mtDNA-encoded proteins, in turn adapt their activity to the influx of nDNA-encoded proteins [43]. Moreover, a synchronization between cytosolic and mitochondrial translation has been reported in yeast, where cytosolic translation controls mitochondrial translation in a unidirectional manner [44]. There was however, also in yeast, evidence for bi-directional translation synchronization. Mitochondrial translation efficiency was shown to communicate to the rest of the cell and control cytosolic proteostasis via transcription factors Msn2/4 and affect chronological lifespan [45].

The specific localization of cytosolic ribosomes and mRNAs at the outer mitochondrial membrane is important for mitochondrial function and proteostasis. Cytosolic ribosomes were shown to localize to the surface of mitochondria and interact with the TOM complex [46]. This suggests that mRNAs encoding mitochondrial proteins are locally translated at the surface of mitochondria and directly imported into the mitochondria [46,47]. The mitochondrial outer membrane receptor OM14 acts as a receptor for cytosolic ribosomes, which facilitated co-translational mitochondrial import in yeast [48]. In mammalian cells, cytosolic translation occurring on the outside of mitochondria is different since the rRNAs that are associated with the mitochondrial outer membrane have very different decay patterns from ER or cytosolic rRNAs [49]. Surprisingly, the selective degradation of cytosolic rRNAs on the outer membrane was carried out inside mitochondria, by mitochondrial intermembrane space RNase T2 (RNASET2). This RNASET2 also acts as a mitochondrial RNA degradation enzyme [50] and is important for RNA trafficking, as it can process an imported RNA component of mammalian telomerase TERC to a shorter form, TERC-53 [51]. This shorter form is exported back to the cytosol in response to changes in mitochondrial function, to communicate to other cellular compartments [51], and was found to regulate senescence [52]. These parallel actions of RNASET2 regulate cytosol-to-mitochondria proteostasis in a concerted manner. Although its full involvement in longevity is to be established, it is worth noting that *C. elegans* mutants of RNST-2, the orthologue of RNASET2, show accumulation of rRNA and ribosomal proteins and are short-lived [53].

A second example of localized cross-organellar proteostasis regulation involves the RNA-binding protein CLUH in mammalian cells [54]. This cytosolic RNA-binding protein controls the stability and translation of target mRNAs encoding mitochondrial proteins [55] through interaction with ribosomes at the mitochondrial outer membrane [56]. The loss of the *D. melanogaster* orthologue *clu* led to a shortened lifespan [57]. In mammalian cells, CLUH also localizes close to mitochondria and around tyrosinated tubulin, suggesting a role in regulating transport or translation of target transcripts close to mitochondria [54]. Besides RNA-binding proteins, there are also specific translation initiation factors such as eIF2B that can regulate mitochondrial function. Mice carrying a mutation in *Eif2b5* have an imbalance in the stoichiometry of OXPHOS proteins and of MRPs [58], and RNAi of *eIF-2Bε*, an orthologue of *Eif2b5*, in *C. elegans* resulted in lifespan extension [59].

Once OXPHOS proteins are synthesized, they need to be assembled into OXPHOS complexes. Assembly factors responsible for the assembly of a certain OXPHOS complex selectively engaged with ribosomes containing mRNAs that code for proteins of that complex [60]. It was even shown in yeast that one of the MRPs, Mrpl35, also functions as an assembly factor for OXPHOS complex IV [61]. An RNA-binding protein specific for mitochondria, GRSF1, localizes to RNA granules, where it regulates mitochondrial translation and ribosome assembly [62]. Interestingly, age-associated methylation changes revealed hypermethylation of GRSF1 during ageing [63]. Moreover, knockdown of GRSF1 in human primary fibroblasts induced cellular senescence, which was negatively correlated with lifespan and healthspan [63]. Together, this suggests that age-dependent hypermethylation of GRSF1 reduces its expression, which could contribute to cellular senescence during ageing [63].

As described in the previous sections, mitochondria both send and receive signals in order to balance (OXPHOS) protein homeostasis. Altogether, many communication pathways between mitochondria and other parts of the cell exist in order to balance the synthesis, import and assembly of mitochondrial proteins. Ultimately, these pathways induce proteostasis in both the mitochondria and the cytosol and play a crucial role in longevity.

3. Therapeutic developments for age-related diseases

Ageing affects all part of the body and is a major risk factor for most chronic diseases. Interventions that extend lifespan often have beneficial effect on age-related diseases. It is evident that mitochondrial proteostasis and communication between mitochondria and other parts of the cell are involved in the regulation of lifespan in lower organisms. This provides a rational basis to investigate interventions targeted at maintaining cross-compartmental proteostasis in the context of age-related disease (figure 3).

(a) Neurodegenerative diseases

Ageing is the major risk factor for neurodegenerative diseases, marked by the accumulation of unfolded and aggregated proteins. In Alzheimer's disease (AD), the most common form of dementia, proteotoxicity, comes from amyloid-β (Aβ) aggregation. In the brain of AD patients, OXHPOS proteins were downregulated and mitochondrial import pathways were disrupted [64]. Also, in both familial and sporadic AD, UPR^{mt} genes were upregulated [65]. Moreover, in a *C. elegans* model expressing human amyloid-β to mimic AD, it was shown that inhibiting the UPR^{mt} aggravates the paralysis phenotype [64], suggesting the importance of this pathway in AD development and prevention. Indeed, activating UPR^{mt} by inhibiting mitochondrial translation genetically or pharmacologically using the antibiotic doxycycline restored fitness and delayed disease progression in these worms. The same results were achieved when inducing UPR^{mt} by NAD^+-boosting compounds in *C. elegans* or in transgenic mice with AD [64]. The emerging role of the UPR^{mt} in neurodegenerative diseases has prompted clinical trials with doxycycline treatment in patients with amyloidosis (clinicaltrials.gov; NCT01677286 and NCT03474458). Similar to UPR^{mt}, the expression of the mtDNA-translated peptide, humanin, is also associated with AD [66]. Humanin administration had neuroprotective effects *in vitro* in human cell culture models and improved cognition *in vivo* in aged mice [67].

Parkinson's disease (PD) is another well-studied neurodegenerative disease, in which proteotoxicity is thought to arise

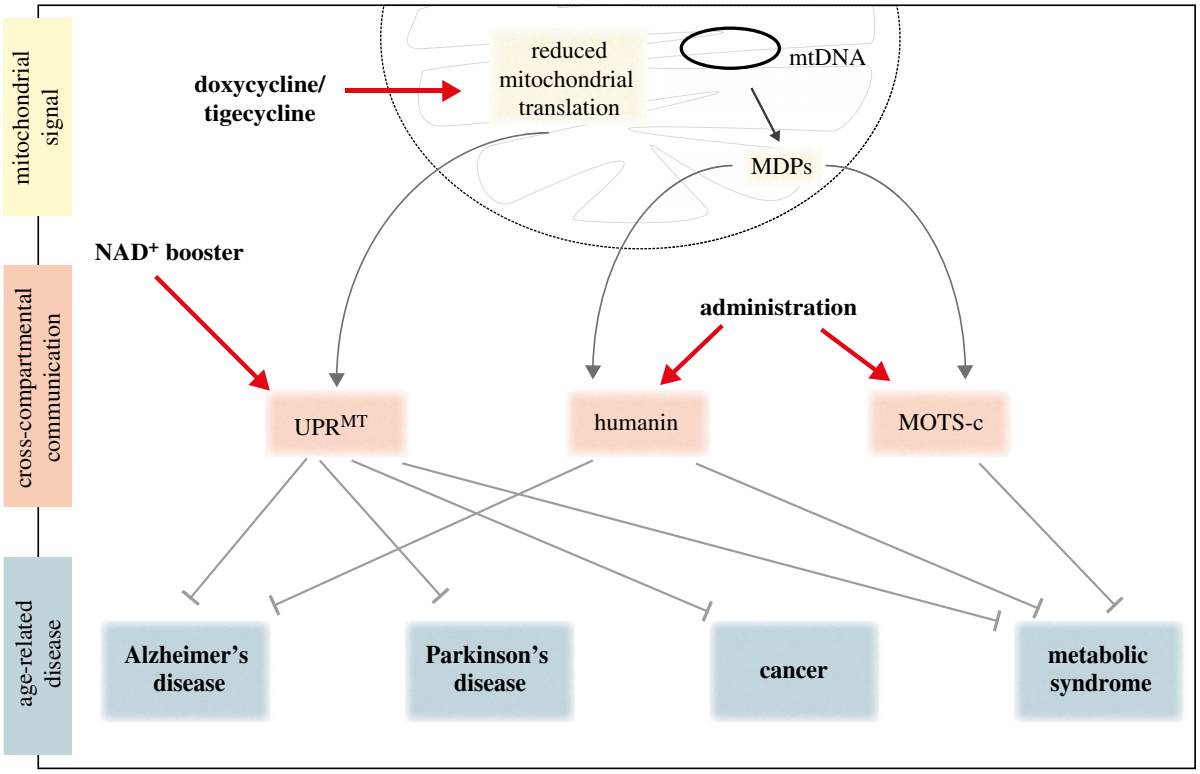

Figure 3. Examples exploiting mitochondrial cross-compartmental signalling to treat age-related diseases. Overview of activating mitochondrial signals (yellow) such as reducing mitochondrial translation or mtDNA-derived MDPs that lead to cross-compartmental communication (red). Activation of these signals and/or communication pathways can inhibit age-related diseases (blue) such as Alzheimer's disease, Parkinson's disease, cancer and metabolic syndrome.

from α-synuclein aggregation [68]. Almost 30 years ago, it was discovered that there are OXPHOS defects in PD [69]. Moreover, gene expression profiling of multiple regions in post-mortem PD brain compared with healthy controls identified mitochondrial ribosomal protein S6 (MRPS6) as a differentially expressed gene that may be involved in the pathophysiology of PD [70]. In line with this involvement of the mitoribosome in PD, doxycycline administration resulted in neuroprotection in a neurotoxin 6-hydroxydopamine (6-OHDA) Parkinson model [71]. In addition to mitochondrial translation, mitochondrial protein import dysfunction was shown to play a role in the pathogenesis in PD. TOM40 was significantly reduced in the brain of PD patients and in α-synuclein transgenic mice, and overexpression of Tom40 in α-synuclein transgenic mouse brains ameliorated energy deficits as well as oxidative burden [72]. Although the mechanistic basis of this interaction needs further study, it was shown that α-synuclein can bind to TOM20, which inhibits mitochondrial protein import in PD [73]. Similarly, in human post-mortem PD tissue, TIM23 and TOM20 protein levels were decreased [74]. In cells treated with MPP+, the active metabolite of Parkinsonian neurotoxin MPTP, which inhibits OXPHOS complex I, a dose-dependent decrease in TIM23 and TOM20 was observed. Interestingly, MPP+-treated cells exhibited a transcriptional activation of *CLPX* and *HSP9*, which was not observed at the protein level. This suggests that import defects might impair the mitochondrial translocation of chaperones and the activation of the cytoprotective UPR^{mt} [74]. It still needs to be determined if activation of the UPR^{mt} indeed could be a possible treatment option for PD.

(b) Cancer

Cancer, often seen as an age-related disease, is one of the most common causes of death worldwide [75]. Cancer cells require a high amount of energy to proliferate, and some cancer types have upregulated expression of OXPHOS proteins to fulfil this need for energy [76]. Also, the expression of MRPs is often changed in cancer. For instance, melanomas have an overexpression of most MRPs, and MRPL10, MRPL38 and MRPS27 are overexpressed in most other cancer types [77]. Several studies showed that the inhibition of mitochondrial translation using the antibiotic tigecycline prevented the growth of a variety of cancer types, including lymphoma, leukaemia and renal cell carcinoma [78–80]. This treatment caused prolonged survival in mice with lymphoma and human leukaemia [78,79]. Furthermore, doxycycline treatment prevented the progression of tumour growth in mice with human breast cancer, glioblastoma or cervical cancer [81–83]. Normal proliferating cells were not affected, since sensitivity to tigecycline was induced by the proto-oncogenic transcriptional factor Myc [78]. In line with this, oxygen consumption rate was increased in leukaemia cells and tigecycline rescued this increase, resulting in lower oxygen consumption, while it did not affect the oxygen consumption of healthy haematopoietic cells [79]. These effects are not specific for tigecycline, as the antibiotics linezolid and chloramphenicol, also targeting the mitoribosome but through other mechanisms, had similar though less potent effects to tigecycline [78]. The use of antibiotics was not only successful to treat cancer cells, but such mitochondrial translation inhibitors also reduced the growth of many different cancer stem cells lines [79,84,85].

Since the inhibition of mitochondrial translation activates the UPR^{mt} in lower organisms, this activation could be involved in the elimination of cancer cells. Indeed, activating UPR^{mt} in human tumour cells using inhibitors of Hsp90 chaperones selectively targeted to mitochondria, led to the activation of autophagy and regulated the protein-folding

environment in mitochondria [86]. This in turn enhanced tumour cell apoptosis initiated by death receptor ligation, which inhibited intracranial glioblastoma growth in mice [86].

(c) Metabolic syndrome and cardiovascular diseases

Metabolic diseases including type 2 diabetes mellitus (T2DM) and obesity are closely associated with the alteration of OXPHOS gene expression [87]. T2DM caused by decreased insulin action is a metabolic feature of ageing. Moreover, there is a wealth of data linking mitochondrial dysfunction to the development of T2DM [88]. In line with this, the UPR^{mt} marker HSP60 was reduced in the hypothalamus of both obese mice and diabetic mice and humans, which was associated with mitochondrial dysfunction [89]. Interestingly, a low-dose doxycycline treatment reversed the diabetes phenotype of *db/db* mice, a model for T2DM with leptin deficiency [90]. Another mechanism linking mitochondrial proteostasis to metabolic syndrome is the mtDNA-translated peptide MOTS-c. MOTS-c was shown to promote metabolic homeostasis and reduce obesity and age-dependent and high-fat-diet-induced insulin resistance in mice [91]. Furthermore, circulating MOTS-c plasma levels were associated with insulin sensitivity in lean individuals [92] and downregulated in patients with coronary endothelial dysfunction [93], which are both age-related diseases. The other MDP, humanin, also regulates insulin action, and infusion of humanin improved overall insulin sensitivity in mice [17].

Age is also a major risk factor for the development of cardiovascular diseases, and mitochondria play a central role in this process [94]. Mitochondrial dysfunction in heart failure conditions can be reversed by the activation of UPR^{mt} [95]. Indeed, activating the UPR^{mt} through supplementation with NAD^+-booster nicotinamide riboside led to a cardioprotective effect and reduced cardiomyocyte death and contractile dysfunction in mice [95]. Interestingly, there was also evidence in myocardial tissue from patients with aortic stenosis that UPR^{mt} activation correlates with reduced tissue cardiomyocyte death and fibrosis and lower plasma levels of biomarkers of cardiac damage and dysfunction [95].

4. Conclusion and perspectives

Understanding the cross-compartmental mitochondrial signalling mechanisms reversing or slowing the ageing process brings great promise to treat or prevent age-related disease. Aside from longevity, the evolution of mitochondrial cross-compartmental signalling is crucial for cellular functioning in general. It is postulated that owing predominantly to uniparental transmission and independent replication of DNA molecules, mtDNA is mutated much faster compared with nDNA [96]. To accommodate this, nDNA should be prepared for mitochondrial changes and capable of keeping the mitonuclear collaboration functional. In the most extreme case, speciation can occur as a result of this, for instance, when nuclear-encoded proteins and mitchondrial RNAs fail to bind [97]. This emphasizes the importance of mitonuclear communication and the necessity for the organism's viability to evolve mitochondrial cross-compartmental signalling.

When it comes to implementing specific treatments, it seems that inducing a UPR^{mt} with, for example, antibiotics or NAD^+ boosters is a promising method to treat or prevent age-related diseases. In many cases, it remains to be established, though, whether or not the activation of UPR^{mt} is causally involved in the pathophysiology and/or treatment of age-related diseases. Other players involved in mitochondrial cross-compartmental signalling could also be promising, although they have not yet been linked to longevity. For instance, silencing the microRNA-382 induced mitonuclear protein imbalance by downregulation of MRPs and this activated UPR^{mt} in muscle cells [98]. More non-coding RNAs are being identified, which also have a modifying role in cross-compartmental proteostasis and could be explored as possible targets to treat age-related diseases [99].

Other novel therapeutic targets include the mRNA-binding proteins, such as GRSF1, RNASET2 and CLUH, since these were shown to be important in mitochondrial cross-compartmental communication and possibly longevity. For example, a recently described candidate is the RNA-binding c6orf203, which is involved in regulating mitochondrial translation [100]. Another mRNA-binding protein is Pumilio2 (PUM2), which is induced upon ageing and acts as a negative regulator of lifespan [101]. PUM2 inhibits the translation of the mitochondrial fission factor (Mff) and thereby mediates mitochondrial homeostasis [101]. It would be interesting to explore the effect of c6orf203, Pumilio2 and other mRNA-binding proteins in ageing/age-related diseases.

A major challenge in the translatability of these potential treatments for ageing and age-related diseases is the dosing. While modest downregulation of genes coding for OXPHOS proteins or MRPs using RNAi results in longevity, mutants of these same genes in worms, flies or mice are often lethal. In humans, mutations in OXPHOS proteins or proteins involved in mitochondrial translation lead to metabolic diseases often causing severe symptoms, including cardiomyopathy, hearing loss and brain abnormalities [102–104]. In the context of possible treatments, the aim would be to reduce but not fully deplete these proteins in order to activate the cytoprotective cross-compartmental signalling without leading to complete defects in mitochondrial function. In addition, the more we know on the precise mechanisms of mitochondrial compartmental signalling, the more we could exploit these for possible treatments.

Excitingly, the first clinical trials have been initiated exploiting mitochondrial cross-compartmental signalling to target age-related diseases, for example using low-dose antibiotics inhibiting mitochondrial translation. It is still to be proven if these treatments, and possibly other unexplored treatments, turn out to be successful. With the human population getting older, and the last years of life often being accompanied by age-related diseases, therapeutic approaches that could prevent these diseases would have a huge impact.

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