Review

EMBO reports

Systemic effects of mitochondrial stress

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Abstract

Multicellular organisms are complex biological systems, composed of specialized tissues that require coordination of the metabolic and fitness state of each component. In the cells composing the tissues, one central organelle is the mitochondrion, a compartment essential for many energetic and fundamental biological processes. Beyond serving these functions, mitochondria have emerged as signaling hubs in biological systems, capable of inducing changes to the cell they are in, to cells in distal tissues through secreted factors, and to overall animal physiology. Here, we describe our current understanding of these communication mechanisms in the context of mitochondrial stress. We focus on cellular mechanisms that deal with perturbations to the mitochondrial proteome and outline recent advances in understanding how local perturbations can affect distal tissues and animal physiology in model organisms. Finally, we discuss recent findings of these responses associated with metabolic and age-associated diseases in mammalian systems, and how they may be employed as diagnostic and therapeutic tools.

Keywords aging; mitochondria; stress
Subject Categories Membrane & Trafficking; Metabolism
DOI 10.15252/embr.202050094 | Received 23 January 2020 | Revised 30 March 2020 | Accepted 17 April 2020 | Published online 24 May 2020
EMBO Reports (2020) 21: e50094

See the Glossary for abbreviations used in this article.

Introduction

Mitochondria are key organelles in cells, prominent for their role in producing the basic unit of cellular energy, ATP, through the process of oxidative phosphorylation (OXPHOS). In addition to OXPHOS, they serve many other important cellular roles: They sequester cellular calcium; generate and detoxify reactive oxygen species; house the synthesis of phospholipid, steroids, quinone, and heme; house fatty acid breakdown through β-oxidation; and serve as a metabolic platform for the TCA and urea cycles [1].

Mitochondria are intriguing organelles not only for the myriad functions they serve, but also for their unique and intricate structure. The mitochondrion can be divided into four subcompartments, defined by the two membranes it is composed of: the outer membrane (OM) and inner membrane (IM), and two aqueous spaces, the intermembrane spaces (IMS), and the mitochondrial matrix [2,3]. These distinct sub-compartments serve as platforms for different processes. For example, the respiratory complexes of OXPHOS localize to the invaginated inner membrane (cristae) [4]. The various compartments, together, house an estimated total of over a thousand distinct proteins [5–7], with a mix of proteins from bacterial and eukaryotic origins. Recently, spatially resolved proteomics revealed that the human mitochondrial matrix alone is home for almost 500 proteins [8], with dozens of proteins newly identified as localized to the organelle. It has also been established that different organisms, tissues, and cellular states (such as stress, see below) have unique signatures of protein localization to mitochondria. Furthermore, mitochondria harbor their own genome (mitochondrial DNA, mtDNA), which, in humans, is a 16kB circular molecule, encoding thirteen electron transport chain (ETC) proteins, 22 tRNAs, and two rRNAs [9]. The majority of the proteins within the organelle, therefore, are of nuclear origin and hence must be translated in the cytosol and then imported through specialized machineries: the translocase of the outer membrane (TOM) and translocase of the inner membrane (TIM) complexes, which span their respective membranes allowing for protein import and sorting [10,11]. Import into the mitochondrial matrix of some proteins also depends on the electrochemical potential of the mitochondria [12].

In addition to serving multiple key functions for the cell, mitochondria have also emerged as fascinating signaling hubs that can control not only cellular programs via interaction with other compartments (for example, with the endoplasmic reticulum, reviewed elsewhere [13]), but also affect distal tissues to dictate organismal health by mechanisms we are only starting to understand. The intercellular effects of mitochondria and their underlying mechanisms are the focus of this Review.

Mitochondria in disease and aging

Mitochondria have been implicated in many diseases, with defects in hundreds of genes implicated in mitochondrial biology causing pathologies [14,15]. These defects span mutations in the mitochondrial genome itself [16], in nuclear genes encoding mitochondrial components [17], and in genes belonging to different functional classes, such as mtDNA replication machinery, mitochondria fission and fusion, OXPHOS, or biosynthesis of iron–sulfur clusters [18,19]. Furthermore, mitochondria have been placed in the center of aging

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Glossary

ATP	adenosine triphosphate
CNS	central nervous system
DAMP	damage-associated molecular pattern
DCV	dense core vesicles
DNA	deoxyribonucleic acid
ER	endoplasmic reticulum
ER-SURF	endoplasmic reticulum surface-mediated protein targeting
ETC	electron transport chain
IM	inner membrane
IMS	intermembrane space
ISR	integrated stress response
mitoCPR	mitochondrial compromised protein import response
mPOS	mitochondrial precursor overaccumulation stress
mtDNA	mitochondrial DNA
MTS	mitochondrial targeting sequence
OM	outer membrane
OXPHOS	oxidative phosphorylation
ROS	reactive oxygen species
TCA	tricarboxylic acid cycle
TIM	translocase of the inner membrane
TOM	translocase of the outer membrane
UPRam	unfolded protein response activated by mistargeting of proteins
UPRmt	unfolded protein response of mitochondria
WAT	white adipose tissue

studies for over 70 years. Mitochondrial dysfunction was shown to increase with age in both model systems and humans [20,21], and mtDNA mutations have been shown to increase with age [22].

The connection between mitochondria and aging was hypothesized in two main theories. In 1956, Denham Harman put forward the hypothesis that the accumulation of oxidative damage generated by reactive oxygen species is the key driver of aging [23]. This hypothesis implicated mitochondria in aging, as the main source of ROS is the ETC within the mitochondria. Since then, many studies have supported or contradicted the theory [24,25], emphasizing that the relationship between ROS and longevity is more complex. Interestingly, an alternative theory, "the rate of living" [26,27], suggests that an organism has a finite number of breaths, so that metabolic rate inversely correlates with life span. This theory is supported by many studies showing that decreasing mitochondrial function can alter longevity; for example, mutating OXPHOS genes increases organismal life span [28–31]. This complexity in the connection between ROS, mitochondrial activity, and longevity was further deepened, as ROS have also been proposed to serve as a signaling molecule that may have beneficial effects by inducing an adaptive response that will counteract stress [32]. The concept, named mitohormesis, describes a delicate balance, where low levels of mitochondrial stress may be beneficial by inducing an adaptive program that increases life span and health span [33–36].

Maintaining homeostasis in mitochondria

Mitochondria harbor over a thousand distinct proteins that are dedicated to carrying out the diverse set of mitochondrial functions. These proteins must be imported into the mitochondria, acquire, and maintain a proper confirmation, as unfolding or misfolding may cause loss of function of the protein and/or trigger protein aggregation. Accordingly, several mitochondrial quality control mechanisms exist ensuring homeostasis within the organelle (Fig 1), with dedicated pathways monitoring the integrity of the proteins within mitochondria, e.g., protein homeostasis. Not surprisingly, the mechanisms of protein homeostasis and aging are connected and shared, thus making mitochondrial quality controls an intriguing aspect in our understanding of aging [37].

The disruption of mitochondrial protein homeostasis may be caused by a variety of signals that can trigger a cellular response, by perturbing the internal status of the organelle. For example, loss of coordination in gene expression between the mitochondria and the nucleus can trigger such a cellular response [38], as certain subunits of the ETC are encoded in the nuclear genome, while some are encoded by the mitochondrial genome. If the expression of ETC proteins will not be coordinated, the assembly of ETC complexes may fail, lead to accumulation of unassembled subunits, and, in turn, result in impaired oxidative phosphorylation and ROS production [39–41]. Other signals that can propagate a response are mitochondrial protein aggregation [42,43], protein misfolding [44], disturbances to mitochondrial translation [45,46] or DNA replication [47], disruptions to mitochondrial membrane potential, and failure in mitochondrial import [48,49]. Intriguingly, direct comparison of the transcriptional responses to drugs targeting different mitochondrial processes revealed little overlap, with activation or inhibition of hundreds of genes being specific to a particular stress [46].

The preliminary stage for a mitochondrial protein encoded in the nucleus is to get into the mitochondria and undergo import, a process which on its own can have detrimental effects if functionally impaired [50,51]. The response to mitochondrial import defects is elicited by several pathways, which may be some-what overlapping, that have been mapped in yeast. These include two pathways that protect the cell against import stress: mPOS (mitochondrial precursor overaccumulation stress) and UPRam (unfolded protein response activated by mistargeting of proteins) which inhibit protein synthesis in the cytosol [52,53]. mPOS also induces the integrated stress response, which has been implicated in mitochondrial stress response in mammals (see below). UPRam facilitates degradation of the mislocalized mitochondrial proteins that are not imported. An additional pathway, mitoCPR, protects the mitochondria from the import stress: Two proteins Cis1p and Msp1p clear stalled proteins from the mitochondrial import translocase by promoting the degradation of unimported proteins [54]. In addition to these pathways, the ER surface can capture and salvage mitochondrial precursor proteins ("ER-SURF") and reroute them to the mitochondria [55]. The existence of these import stress-related pathways in higher organisms is not clear, although some overlap with the ISR may exist.

In the case of protein aggregation or misfolding within the mitochondria, one of the most extreme solutions is eliminating the defective organelle by mitophagy (mitochondrial autophagy) [56]. In healthy mitochondria, the levels of PINK1 are low as its import into mitochondria exposes a degron for cytosolic proteasomal degradation. When mitochondria are defective, for example, by accumulation of unfolded proteins, PINK1 accumulates on the OM and recruits Parkin to ubiquitinate the organelle via its E3 ubiquitin ligase activity [57,58]. The elimination of the organelle can then be

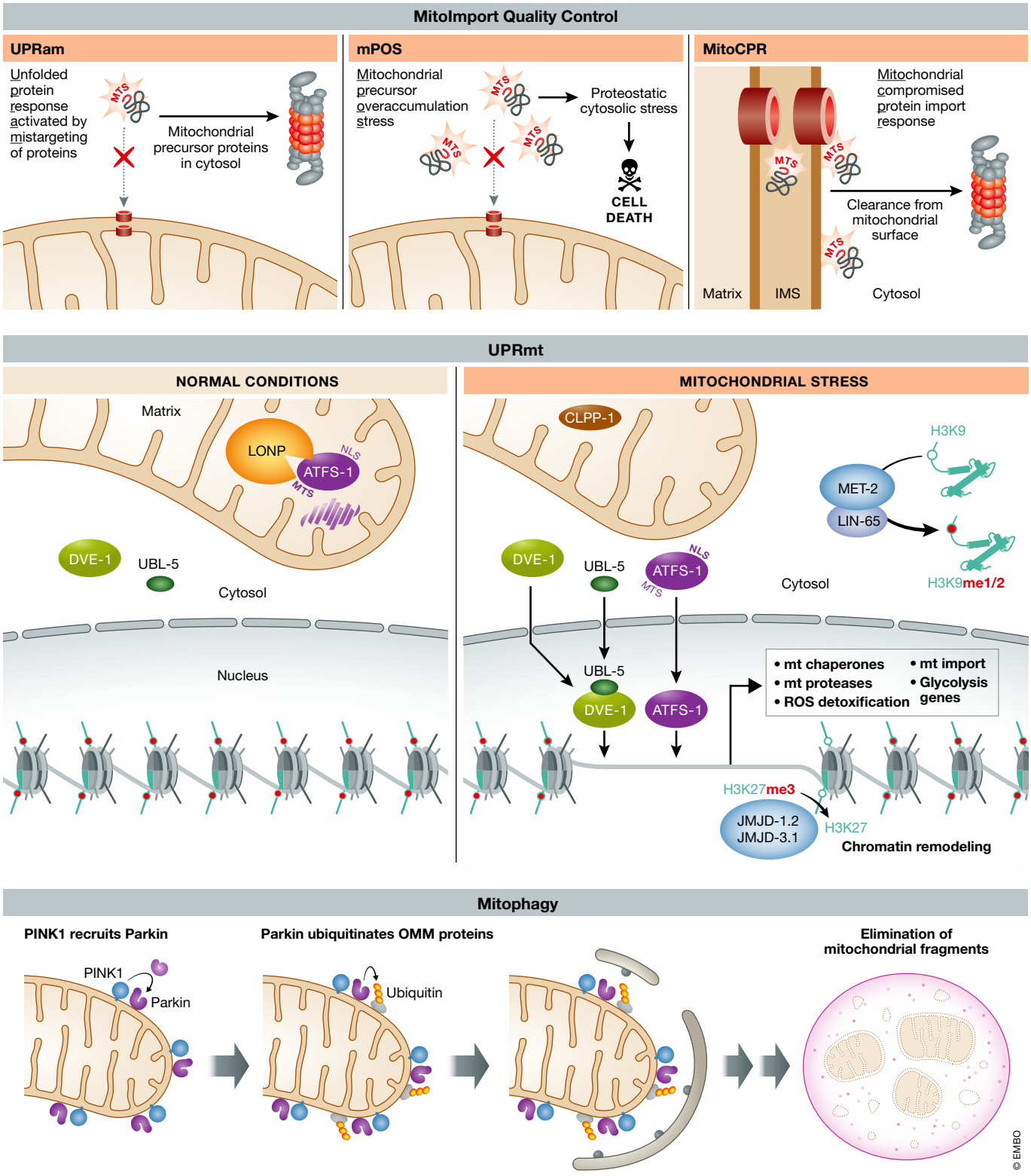

Figure 1. Pathways protecting mitochondria.

Several cellular pathways may be employed to protect mitochondria against stress, protecting the organelle at different levels. See text for details.

achieved by mitochondrial fission to generate small mitochondrial fragments that can be eliminated, in a process that requires the dynamin-related protein Drp1 [59,60]. In addition to PINK1/Parkin, many other proteins participate in mitophagy. For example, polyubiquitinated substrates on the mitochondria can recruit the LC3 adapters p62, OPTN, NDP52, TAX1BP1, and NBR1, which, in turn, can recruit the phagophore to the mitochondria [61]. Furthermore, damaged mitochondria can be recognized in a ubiquitin-independent manner by recognition by LC3 adapters [62,63].

In certain situations, it may be beneficial to fix the damage, rather than eliminating the organelle. Similar to other compartments [64,65], mitochondria have evolved myriad mechanisms to protect themselves against perturbations that may causes detrimental effects. These mechanisms sense a shift in the equilibrium between unfolded proteins and protein chaperones, and a tailored response is induced in an attempt to alleviate the disequilibrium. These perturbations have been studied extensively by both genetic and pharmacological means. For example, changing the stoichiometry between mitochondrial protein complexes by knockdown of genes encoding subunits of the ETC, or inhibiting the activity of one of the complexes in the ETC, can cause accumulation of orphan protein subunits and also generate ROS leakage. In addition, perturbations to mitochondrial translation, or knocking down the mitochondrial protein protease *spg-7* can disrupt mitochondrial function as well [66]. Other perturbations, for example, to the mtDNA, can also elicit detrimental effects, as shown by mutating the mitochondrial DNA helicase Twinkle, or using a mutated mtDNA genome [67,68].

As a first line of defense, mitochondria can employ protein chaperones and proteases that reside within the different compartments of the organelle. These proteins can refold or degrade misfolded or unfolded proteins, and alleviate the proteotoxic stress. For example, the *LONP* and *ClpP* proteases reside in the mitochondrial matrix [69] and can degrade misfolded proteins, while the mtHsp70 and the Hsp60/10 complexes can be employed to refold proteins [70]. Interestingly, mitochondrial chaperones, proteases, and other molecules aimed to alleviate the stress are part of a coordinated response. This response is part of the mitochondrial protein quality control program termed the UPRmt. The UPRmt has been shown to induce a transcriptional program aimed to alleviate the mitochondrial stress and is in the center of this Review.

At the molecular level, the UPRmt is best characterized in the nematode *Caenorhabditis elegans*. In nematodes, the regulation of the UPRmt is wired in the cellular localization of the transcription factor ATFS-1, which has two localization signals—to the nucleus, and to the mitochondria. Under normal conditions, ATFS-1 localizes to mitochondria through its mitochondrial localization signal, where the protein is degraded by the mitochondrial protease LONP-1. However, under conditions of stress, ATFS-1 is no longer imported into mitochondria, allowing for another domain within the protein, the nuclear localization signal, to dominate sending the transcription factor into the nucleus, where it can bind target genes and activate a coordinated transcriptional response [71]. This response includes, for example, the upregulation of mitochondrial chaperones, such as *hsp-60* and *hsp-6*, homologs of the mammalian mtHsp60 and mtHsp70 [72]. In addition to the upregulation of mitochondrial chaperones, quality control proteases, xenobiotic response pathway components, and glycolysis genes are also induced [71,73]. When mitochondria are stressed, unfolded matrix mitochondrial proteins are cleaved by the protease CLPP-1 into peptides. These peptides are then exported by the ABC transporter HAF-1 and diffuse to the cytosol [74,75]. By an unknown mechanism, these peptides alter mitochondrial import and, subsequently, can affect ATFS-1 localization to mitochondria. Furthermore, recent work suggests that the MTS of ATFS-1 acts as a sensor of mitochondrial import [76], as it has a low net charge and is sensitive to changes in mitochondrial membrane potential.

Genetic perturbations of genes involved in mitochondrial translation, mitochondrial import, ETC, TCA cycle, lipid metabolism, and iron–sulfur cluster biogenesis have all been shown to activate the UPRmt [76]. Intriguingly, some perturbations to non-proteome entities (e.g., perturbations to mtDNA, ROS production) can cause activation of the UPRmt, despite not being a direct insult to the proteome, presumably challenging the definition of the response a protein-centered response [68]. However, these perturbations can cause secondary effects that can damage the proteome of mitochondria, and thus activating the UPRmt. Furthermore, it is important to note that not all impairments to mitochondrial processes cause UPRmt activation. For example, perturbation to mitochondrial Ca^{2+} homeostasis does not induce the UPRmt [77]. Interestingly, it has been proposed that mitochondrial import is crucial in activating the UPRmt directly; however, it is possible that a decrease in ATP levels due to ETC dysfunction is the cause of mitochondrial stress, as many mitochondrial chaperones require ATP for their activity [78].

The transcriptional response of UPRmt can only be explained partially by ATFS-1; that is, only ~50% of genes depend on ATFS-1 [71]. In addition to ATFS-1 translocation from mitochondria to the nucleus, two parallel pathways that can affect the mitochondrial health have been described. First, the transcription factor DVE-1 translocates to the nucleus upon mitochondrial stress, forming a complex with the small ubiquitin-like protein UBL-5 [72,79]. This requires the activity of the protease, CLPP-1. The transcriptional changes that occur in response to mitochondrial stress are also accompanied by widespread changes to the nuclear chromatin landscape. These alterations have been shown by our group to depend on the repressive histone methyltransferase MET-2/SETDB1 [80] and its co-factor LIN-65, which are required to methylate H3K9, and are induced during mitochondrial stress [81]. These alterations to the chromatin landscape are required for DVE-1 translocation to the nucleus. In addition to H3K9 methylation, other histone modifications have also been implicated in UPRmt in nematodes. Overexpressing the histone demethylases, *jmjd-1.2*/PHF8 and *jmjd-3.1*/JMJD3, recapitulated the response to mitochondrial stress, and their expression was also required for the life span extension by mitochondrial ETC perturbation [82]. These genes were suggested to regulate UPRmt through reducing H3K27me3 from genes induced in the UPRmt program. The information of mitochondrial stress may also persist to the next generation, as mutating *wdr-5.1*, a component of H3K4 methylation, or disrupting the DNA modification N6-methyladenosine, abrogates the inheritance of adaptation to mitochondrial stress [83]. Moreover, work in yeast described chromatin changes that are dependent on the Rph1p-induced H3K36 demethylation in response to mitochondrial oxidative stress and subsequent chronological longevity [84]. In addition to the DVE-1/UBL-5 pathway, the GCN-2 kinase also responds to mitochondrial stress and phosphorylates eIF2α, to attenuate protein translation and decrease the protein load on the mitochondria [85].

In mammalian cells, several studies tackled the identification of the pathway homologous to nematodes. The works that discovered UPRmt in mammalian cells precede those in *C. elegans*. However, the molecular details of the pathway have not yet been completely elucidated. Early works showed that a deletion of mtDNA in mammalian cells can induce the expression of the nuclear-encoded mitochondrial chaperonins HSPD1 (HSP-60/CPN60) and HSPE1 (CPN10) [86], serving as the conceptual basis that a transcriptional program encoded in the nucleus can respond to mitochondrial perturbations. Inducing mitochondrial proteotoxic stress by expressing a mutated unfolded orthenine transcarbamylase (OTC) in mammalian cells also induced these mitochondrial chaperones, along with the chaperone, DNAJA3, and peptidase CLPP, but not cytoplasmic or ER chaperones [87]. This work mapped a binding element for the ISR-associated C/EBP homologous protein (CHOP) in the mitochondrial chaperonin promoter, implicating it in the response. Supporting the idea of the UPRmt, proteomic data in mice showed that the expression of the nematode homologs of the mitochondrial chaperones Hspd1, Hspe1, Hspa9, proteases Clpp, Lonp1, and the transcriptional regulator Ubl5 are also coordinated in mice [88], and deleting SURF1, a protein required for the assembly of the cytochrome c oxidase (Complex IV), is capable of inducing the homologs of the canonical nematode UPRmt genes in skeletal muscle [89]. The Harper group found that exposing HeLa cells to chemical inhibitors of matrix proteins HSP90/TRAP18 or LONP induces the expression of proteins involved in mitochondrial protein folding and reduced mitochondrial proteins translation [90].

In the search of the master regulator of the UPRmt, a sequence homolog of the nematode ATFS-1 was identified—the mammalian ATF5, a transcription factor induced under conditions of stress [91]. Expressing ATF5 in worms lacking ATFS-1 was able to rescue the UPRmt, making ATF5 a compelling candidate master regulator of UPRmt in mammals. Moreover, ATF5 was required to induce the expression of mitochondrial chaperones, such as HSP60 and mtHSP70, in HEK293T cells [92]. Nonetheless, despite numerous indications that ATF5 has a role in UPRmt in mammals, it is not clear whether it is the dominant regulator of the response. In a recent study by the Auwerx group, the response to different drugs targeting mitochondrial processes, such as mitochondrial translation, OXPHOS, and mitochondrial import, was dominated by ATF4, a transcription factor regulating the integrated stress response [46]. In their experimental setup, mitochondrial stress activated metabolic pathways of amino acid biosynthesis, promoted synthesis of certain lipids, regulated the pool of GSH, and inhibited mitochondrial translation and OXPHOS complexes, but did not induce canonical UPRmt genes such as HSP60. While it is clear that ATF4 and the ISR have a role in mitochondrial functions, such as ATP-dependent respiration, < 20% of genes upregulated in response to mitochondrial stress have the ATF4-associated DNA motif (5′-TTGCATGACG-3′), which is also shared with other bZIP-transcription factors such as DDIT3/CHOP. Additional motifs found in genes responsive to mitochondrial stress have been termed mitochondrial unfolded protein response element 1 and 2 (MURE1 & 2), and span ~20% of predicted mitochondrial genes; however, their corresponding transcription factors have not been identified [93]. The triggering of mitochondrial stress resulted not only in ATF4-dependent effects, but also in ATF4-independent effects, such as inhibition of the expression of mitochondrial ribosomal proteins. How ISR-related responses, and other mitochondria-specific responses, possibly coordinate, cooperate, and interact with each other is unknown. Thus, it is still not clear how the UPRmt is regulated in mammals. It is possible that some of the discrepancies may be attributed to a complex, layered response. The nature of the perturbation can cause a differential response, as exemplified in the induction of stress specifically in the IMS of MCF7 cells, which activates the estrogen receptor and upregulates NRF1 and the IMS protease HTRA2. Moreover, mitochondria may be dysregulated in transformed cell lines which were used in many of these studies. Furthermore, cell culture studies typically use high-glucose media which decreases the necessity for OXPHOS, and the tissue-context, such as oxygen availability or tissue rigidness, may not be biologically representative in these experimental settings. Of note, as some of the perturbations to mitochondrial functions can stimulate ROS production, the response of mitochondrial stress may be mixed with that of oxidative stress (Reviewed in ref. [94]), a conundrum that future works may tackle.

While studies showed that ATF5 nuclear target genes are induced following mitochondrial stress and that overexpressing ATF5 allows detecting it in mitochondria, it has not been directly shown that the regulation of ATF5 is similar to that of ATFS-1 upon stress. Peculiarly, ATF5 has also been described to have an anti-apoptotic role by inducing BCL-2 transcription [95], connecting, as previously established, mitochondrial regulation to apoptotic cell death. Moreover, examining the conservation of the role of the CLPP protease also suggests differences between the invertebrate and vertebrate UPRmt. In mice lacking the CLPP protease in heart and skeletal muscle, in normal conditions or a mitochondrial stress model, the UPRmt signaling was not altered [96]. Furthermore, directly testing the requirement of CLPP to induce UPRmt in HEK293 cells suggests that CLPP may not be a driving force in the mechanism of UPRmt and may only be a target of it, along with decreasing mitochondrial protein synthesis.

An additional mechanism proposed to help alleviate mitochondrial stress is mediated by the cytoplasmic kinase GCN-2; however, this mechanism may contrast the activity of UPRmt [85,97]. When dysfunctional mitochondria produce ROS, GCN-2 phosphorylates the translation initiation factor eIF2α, causing a decrease in cytosolic translation. The shutoff of translation reduces the load of mitochondrial protein import and may also affect the import of genes involved in UPRmt which must be imported into the organelle to carry out their function.

UPRmt intercellular signaling

The energetic demands of specific cells or tissues are variable. Different tissues may have differences in mitochondrial number, activity levels, protein composition, and morphology [98]. For example, different tissues in rats have differences in mtDNA levels per cell, with particularly high mtDNA levels in heart and skeletal muscle [99]. Accordingly, the activity of the UPRmt also differs between tissues.

In the last decade, the nematode *C. elegans* has been an extremely valuable tool to studying these tissue-specific differences, due to its genetic-tractability, transparency, and the mapping of tissue-

specific promoters. In a series of studies described below, mitochondrial stress was induced in specific tissues, and the effects on distal tissues and organismal physiology were examined. In a study from our group in 2011 [100], the effects of triggering mitochondrial stress in specific tissues was directly tested in *C. elegans*. Harnessing the genetic tools available in nematodes allowed generating transgenic worms that harbor an inverted repeat hairpin (HP), directed toward a specific gene, which is expressed under various tissue-specific promoters. In this experimental setup, the mitochondrial gene, *cox-5b/cco-1*, was knocked down in different tissues. Due to the intimate connection between mitochondrial activity and life span [31], the effects on longevity were tested. Interestingly, triggering mitochondrial stress in the intestine and in neurons caused an increase in life span, while in the muscle it caused the inverse, underlining that the cellular context of the mitochondrial stress is crucial. Surprisingly, knocking down *cox-5b/cco-1* in neurons was able to activate the UPRmt in other tissues, as observed using a fluorescent protein regulated by the mitochondrial chaperone promoter, *hsp-6*. The expression of this fluorescent reporter was localized to the intestine, suggesting that a signal from the nervous system travels to this distal tissue in a phenomenon termed the UPRmt cell non-autonomous response, where perturbing mitochondrial functions in specific tissues has systemic effects. These results implied the existence of the "mitokine", a molecule that signals mitochondrial stress between tissue and can promote survival. Of note, similar phenomena have been observed for the UPR of the endoplasmic reticulum and the HSR, though their mechanisms and effects are distinct [101,102]. In nematodes, both the intestine and the sensory neurons are in direct contact with the environment, and it is possible these cells are more sensitive to sensing and responding to stressors that may affect mitochondria.

Similar observations of systemic effects were detected when perturbing mitochondria by means other than knockdown of ETC components, such as expressing the ROS-producing fluorescent protein KillerRed in neurons, localized to either the IM or OM [103], or the aggregation-prone polyglutamine (Q40) protein [104]. Interestingly, not every mitochondrial perturbation induces a systemic response: Uncoupling the electron transport from ATP production by expressing the uncoupling protein UCP-4 (localized to the IM) in neurons was not able to elicit a response in the intestine [103], again underscoring the differences between perturbations. Further experiments deleting *spg-7* in specific subsets of neurons showed that the sensory neurons ASK, AWA, and AWC, and the interneuron AIA, are capable of eliciting the distal response. These neurons form chemical synapses or gap junctions with the AIA interneuron, suggesting the AIA neuron may be a key entity in the signaling to the periphery, but does not play a role in the longevity phenotype as it is not able to extend life span [103].

The identification of the mitokine molecule(s) has focused on neuropeptides, as components of the dense core vesicles (DCV) release, such as *unc-31* (calcium activator protein for secretion), were shown to be required for to distal signaling [104]. Further work overexpressing the neuropeptide *flp-2* in neurons was capable of inducing peripheral UPRmt, but was not required for KillerRed induced cell non-autonomous UPRmt, and did not extend life span [103]. *flp-2* is indeed expressed in the AIA interneuron and was shown to act downstream and be required for the ASK, AWA, and AWC neurons to induce UPRmt distally. However, FLP-2 acts only locally within the nervous system and stimulates other long-range signals. Interestingly, different models of UPRmt, including deletion of specific genes in the nervous system or expressing Q40, required serotonin, suggesting it may be a key signal of the systemic UPRmt [104]. However, serotonin does not seem to act alone.

Recent work by our laboratory performed a genetic screen to uncover the mitokine(s) and the required signaling pathways [105]. First, in the neurons, the retromer complex was implicated in the signaling of Q40-induced mitochondrial stress. The retromer complex regulates the recycling and retrieval of protein cargo from endosomes to trans-Golgi or the cell surface. One of the receptors recycled by this complex is a transmembrane protein, MIG-14, which is known to bind Wnt and be required for its secretion from the cell [106]. Once Wnt is release, MIG-14 is recycled by the retromer complex to the Golgi, to be available for further Wnt binding and secretion. Testing for different Wnt ligands, EGL-20 (Wnt16b in humans) was revealed to be necessary and sufficient to induce the UPRmt from neurons to the intestine, where it is perceived by the Wnt-Frizzled receptor and signaled through the β-catenin pathway [105] (Fig 2). Additional work found that the GPCR FSHR-1 is required within the nervous system (but not in the intestine) to regulate mitochondrial mass in the intestine [107]. However, this seems to be a distinct pathway from the "mitokine" signaling, as it was found to be expendable for mtUPR neurons-to-intestine communication.

It is interesting to note that the cell non-autonomous phenomenon is not confined to the neuronal-intestine axis. Knocking down the cytocome c gene, *cyc-2.1,* in the germline also activates the intestinal UPRmt and AMPK pathway, and can also prolong life span. This work postulated that an additional molecule, a germline-produced mitokine, also exists [108]. An additional tissue capable of signaling mitochondrial stress is muscle. A mild stress in muscle mitochondria of flies stimulates the secretion of an insulin-antagonizing peptide, *ImpL2* (*IGFBP7* in humans), which causes a systemic repression of insulin signaling [109]. Moreover, impairing mitochondrial function in muscles of flies via a Complex I perturbation also perturbs the function of fat tissues via the secreted TGF-β ligand, Actβ [110]. Of note, different groups induced mitochondrial stress responses using different tools or genetic perturbations (for example, knocking down an ETC component versus overexpressing an uncoupling protein), likely causing slightly different effects on the perturbed cell.

Systemic responses to mitochondrial stress in mammals

While the genetic and experimental tools in nematodes are valuable to study the cell non-autonomous responses, it is intriguing to ask whether cell non-autonomous responses to mitochondrial stress are conserved in mammalian systems and whether they can modulate aging in more complex animals (Fig 3).

One early observation that serves as a strong indication for such a system was in mice, where a secreted factor from muscle was found to cause systemic metabolic improvements, with mice exhibiting increased glucose tolerance and insulin sensitivity [111]. Some of the early works focused on skeletal muscle, since mitochondrial function in this tissue has been shown to be crucial for systemic energy homeostasis, and have been

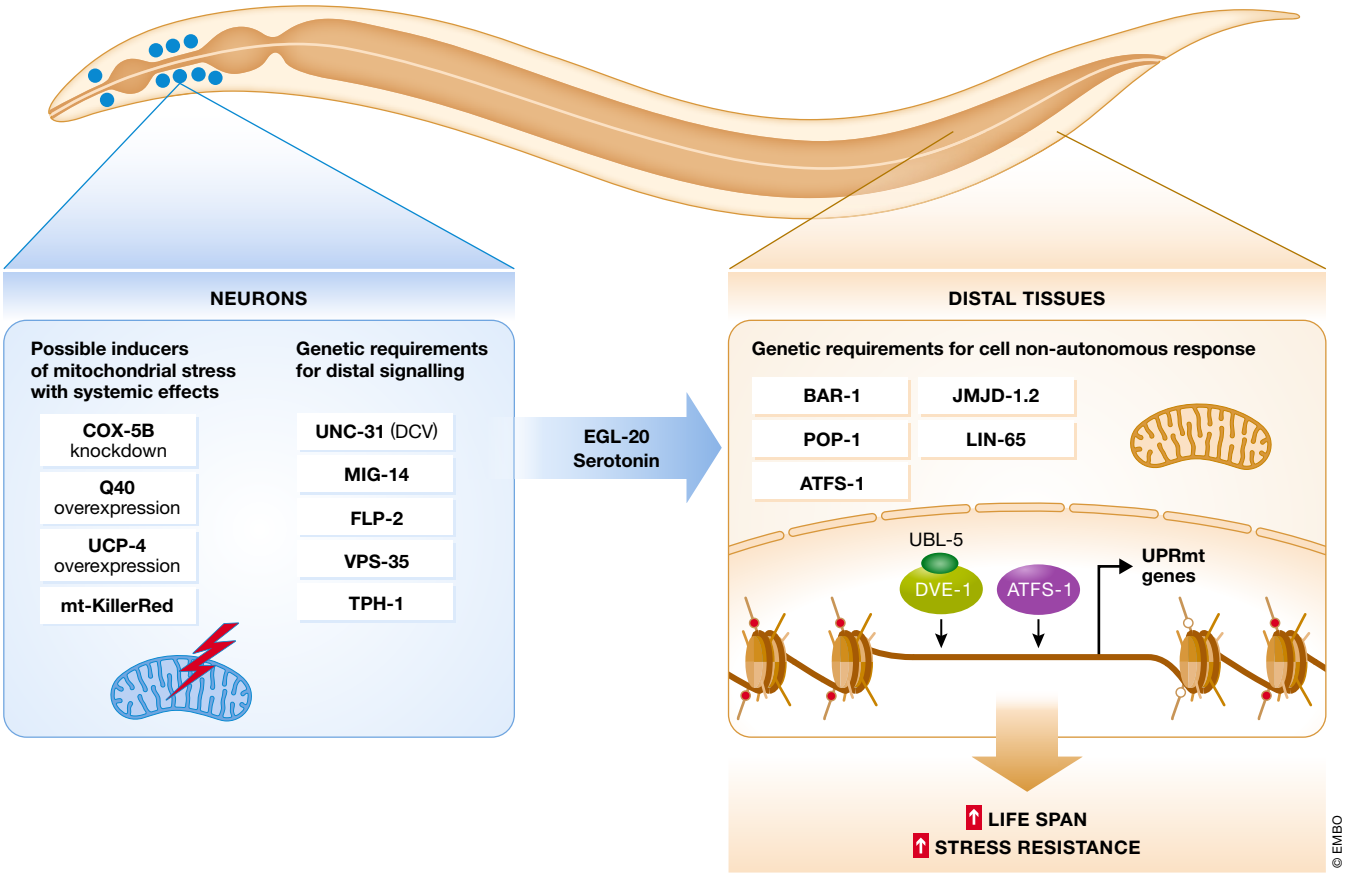

Figure 2. Cell non-autonomous regulation of mitochondrial stress in *Caenorhabditis elegans*.
The communication between the nervous system and the periphery, and the genetic components important for this communication in *C. elegans*, are shown.

implicated in type II diabetes [21]. Intriguingly, a study in which the autophagy gene Atg7 was deleted specifically in skeletal muscles, showed an increase in mitochondrial dysfunction in the tissue, and subsequently caused an increase in the protein, FGF21. Physiologically, FGF21 was shown to be induced by fasting and, in turn, induce ketogenesis and lipolysis [112]. The induction of FGF21 was dependent on ATF4 and was shown to improve energy expenditure by browning of white adipose tissue (WAT) and to increase glucose tolerance and insulin sensitivity, making mice leaner and more resistant to a high fat diet [113]. A different perturbation to mitochondrial dynamics, by depleting the mitochondrial fusion protein Opa1 from muscle tissues (induced depletion in 5-month-old mice, or constitutively) also triggered FGF21 secretion but showed severe systemic effects, including weight loss, induced systemic inflammatory response, and a variety aging phenotypes and premature death. Interestingly, these effects were largely dependent on FGF21, as deleting Opa1 and FGF21 simultaneously in muscle tissues reverted many of the effects [114,115]. In a different study, the depletion of Opa1 earlier in life, at 4-week-old mice, was only partial and resulted in beneficial effects when challenged, including attenuation of age-induced weight gain and glucose intolerance, prevention of diet induced obesity and insulin resistance, again, dependent on FGF21 [116]. These contradictory effects underline the importance of considering the age, the extent of the depletion, and context of the perturbations.

Directly perturbing mitochondria by expressing the uncoupling protein 1 (UCP1) in skeletal muscle also induced the ISR and FGF21, and WAT browning was dependent on FGF21, despite no whole-body metabolic adaptations [117,118]. FGF21 is normally expressed in the liver, but upon stress conditions can also be expressed in other tissues [119]. Other perturbations to mitochondria, such as mutating the mitochondrial DNA polymerase POLG (in the whole organism), also involve FGF21. Interestingly, in the mice POLG model, which serves as a model system for progeria, young mice actually benefit from increased mitochondrial activity and improvement to their metabolism, and become resistant to obesity when challenged with a high fat diet, a phenomenon that at least partially depends on FGF21 [120]. Remarkably, overexpression of FGF21 in hepatocytes is sufficient to increase life span of mice [121].

Since FGF21 elicited effects on distal tissues, with liver and adipose tissue as its main targets, it was functionally defined as a mammalian "mitokine"; however, it was also defined as "myokine",

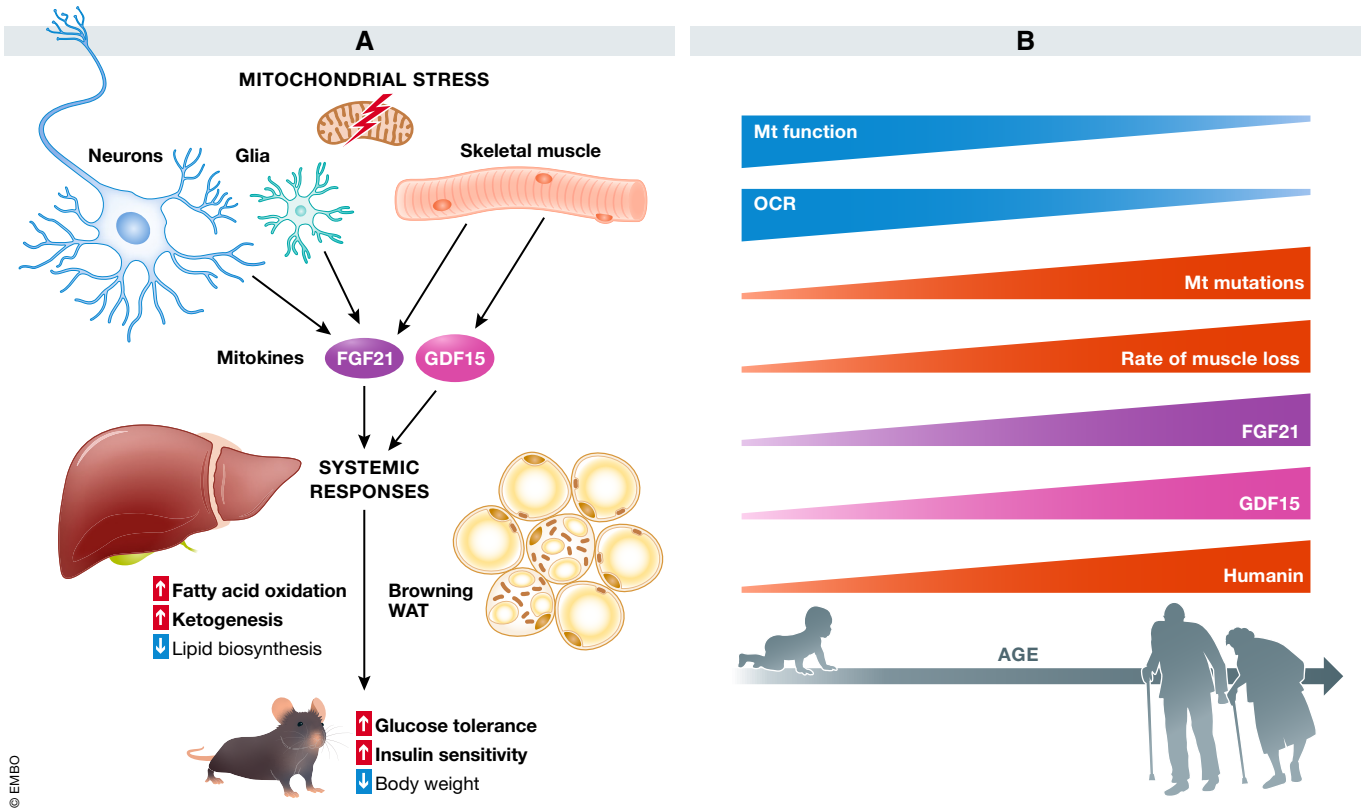

Figure 3. Cell non-autonomous regulation of mitochondrial stress in mammals.
(A) The signaling tissues, the mitokines, and the effects on the receiving tissues are noted. (B) The age-associated change in mitochondrial phenotypes, and the levels of different mitochondrially associated proteins, is shown.

as it originates from muscle tissues. It is important to note that other "myokines" have been defined that are not induced by mitochondrial stress, such as Fndc5/irsin [122]. However, the induction of FGF21 is not constricted to skeletal muscle. Similar to *C. elegans*, when mitochondrial stress was induced in forebrain neurons of mice, using a depletion of the mitochondrial fission gene Drp1, the ISR was induced, and subsequently FGF21 was produced in the brain [123]. Interestingly, FGF21 was also expressed in the brains of prion-inoculated mice, and mice carrying the FTD-associated tau mutation. Administration of FGF21 to mice reduced body weight, affected the liver, and activated MAPK signaling and gluconeogenesis, placing the liver as a main regulatory hub for FGF21 effects [124–126].

In light of the intriguing results from mice, FGF21 has also been studied in humans. Interestingly, insulin was found to induce the expression of FGF-21 in human skeletal muscle and increase the levels of FGF-21 circulating in the blood. Furthermore, muscular FGF21 expression was associated with hyperinsulinemia [127]. As a diagnostic tool, FGF21 seemed to be predictive of muscle mitochondrial pathologies in a quantitative manner (e.g., the more FGF21, the more severe the pathology) [128]. However, its use as a biomarker may be confounded by other factors, as it is regulated by circadian and nutritional factors, and modulated by dietary manipulations as produced by the liver. Overall, administrating FGF21 in humans improved plasma lipids and decreased body weight, but failed to improve glucose metabolism as seen in mice (see ref. [129] review on FGF21 in human studies).

In an interesting work by the Suomalainen group [130], both the disease and molecular signatures of the development of mitochondrially stressed mice was characterized. These mice, termed the Deletor mice, have a dominant mutation in Twinkle, the replicative helicase of mtDNA, causing accumulation of mtDNA deletions in skeletal muscle and the heart. This work revealed a temporal order for the mitochondrial stress response on the transcriptional level, which coincides with the severity of the disease. In the 1st ISRmt stage (< 12 months), FGF21, GDF15, and ATF5 genes (among others) are induced. Later, in the 2nd metabolic ISRmt stage, ATF3&4, mTORC1, transsulfuration, and serine biogenesis are induced, in an FGF-21-dependent manner. Finally, a mild upregulation of the UPRmt occurs, as measured by the induction of mitochondrial chaperones in mice of 2 years of age (Fig 4). Their findings show that FGF21 affects the muscle tissues itself in an autocrine fashion, and also other tissues in an endocrine fashion. Strikingly, this work also showed that muscle-derived FGF21 has effects on the brain, as it causes an increase in glucose uptake in the brain, and mitochondrial proliferation in the dorsal hippocampus. Thus, the reverse communication, from the periphery to the brain, is also signaled by the mitokines in mammals. When the temporal

order was examined in myoblasts, in response to the mitochondrial translational drug, actinonin, a similar order was observed; however, some differences were noticed—e.g., mitochondrial HSPs were induced.

As analogous experiments to those performed in nematodes, several groups perturbed mitochondrial function within the nervous system and tested for systemic effects. The Brüning group induced a defect in OXPHOS by deleting AIF, a caspase-independent inducer of apoptosis that also acts as an assembly factor for the ETC Complex, in the hypothalamic POMC neurons of mice [131]. While this defect was previously studied by whole-animal mutation, when introduced only in POMC neurons, this defect caused only a mild defect to OXPHOS and was able to protect mice from defects in glucose metabolism in obese mice. Furthermore, it caused cell non-autonomous effects, improving leptin and insulin sensitivity, increased thermogenesis, and improved glucose metabolism in obesity.

Other perturbations in other CNS cell types have also been shown to elicit systemic effects. Deleting the uncoupling protein 2, Ucp2, in microglia of mice causes a significantly lower body weight, increased energy expenditure and O_2 consumption, and CO_2 production [132]. Of note, these mice also decreased their food consumption during the dark phase. In another line of work, perturbation to mtDNA in neurons or astrocytes by deleting Twinkle caused late-stage neurological deterioration [133]. Surprisingly, the perturbation in astrocytes, the most abundant cell type in the brain, led to early weight loss (not due to reduced food consumption), severe early-onset progressive neurological disease, inflammation, and spongiotic encephalopathy. Remarkably, mitochondrial perturbations to astrocytes in other models yielded different phenotypes. For example, inducing an ETC defect by deleting the complex IV assembly factor Cox10 in glia (GLAST driving Cre) increased glycolyic metabolism of cells, without showing signs of glial pathology [134]. In addition, an astrocyte-specific deletion of the mitochondria AAA protease, Afg3l2, caused a cell non-autonomous effect to neurons, as it caused morphological degeneration and changed the electrophysiological properties of Purkinje neurons [135].

Interestingly, a study using mice with a skeletal muscle deficiency in the mitoribosomal subunit Crif1 revealed an additional factor that is secreted: growth differentiation factor 15 (GDF15) [136]. Originally, GDF15 was found as a cytokine produced by macrophages, bearing similarity to the TGF-β superfamily [137], and later shown to be produced also by cardiomyocytes, and controlling body growth [138]. GDF15 induction was dependent on CHOP, was secreted upon the mitochondrial stress, and promoted oxidative functions and lipolysis in the liver and adipose tissues, with mice showing, again, lower body weight, resistance to obesity, and improved insulin sensitivity. The administration of GDF15 to mice promoted similar effects [139], and prolonged life span when over-expressed [140]. In addition, GDF15 was shown to regulate appetite [141] and more recently to promote daytime-restricted anorexia [142] and was also implicated in cancer [143], with patients with metastasis having higher levels of circulating GDF15 [144].

In the last decade, additional circulating mitochondria-related molecules have been described. For example, mitochondrial damage-associated molecular patterns (DAMPs) are released to mitochondria and have a role in inflammation [145], and their dysregulated function has also been implicated in disease [146]. Remarkably, these DAMPs include components of the ETC, TCA metabolites, mtDNA transcriptional machinery proteins, and mtDNA itself. An additional peptide molecule derived from the mitochondrial genome is Humanin which has described roles in apoptosis, inflammation, metabolism, and stress, and is able to suppress phenotypes of different conditions such as Alzheimer's disease and stroke [147,148].

Currently, it is not clear whether the pathway identified in *C. elegans*, the Wnt signaling pathway, or Wnt16b specifically, has similar mitochondria-related effects in mammalians. In contrast,

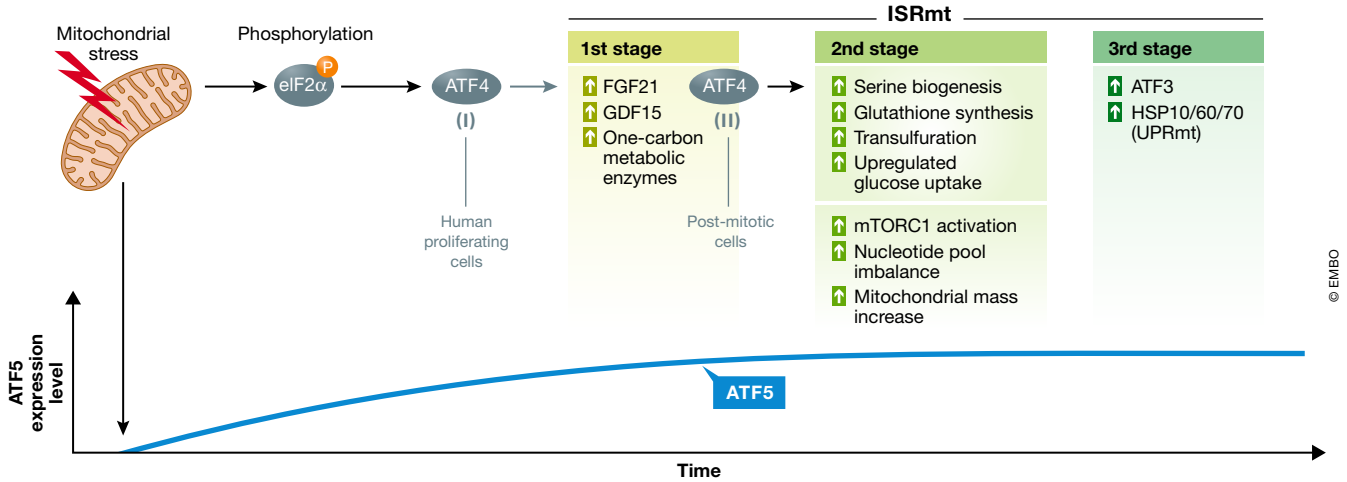

Figure 4. The temporal order for the mitochondrial stress response on the transcriptional level in a mitochondrial disease model (as defined in ref. [130]).
Note that in human proliferating cells, ATF4 induction precedes the 1st stage of ISRmt (I). In post-mitotic cells, the induction of ATF3 and ATF4 was dependent on FGF21 (II), so that induction of ATF4 does not precede 1st stage ISRmt in this model. ATF5 induction is independent of ATF4. Of note, in a separate study [46], a reduction in mitochondrial ribosomal proteins, and a decrease in mitochondrial translation, in response to mitochondrial stress in HeLa cells, was also described.

In need of answers

1 How do the different mitochondrial QC mechanisms (mtUPR, mtCPR, mitophagy, etc.) relate to one another? Do they work together, in parallel, or does every perturbation have a specialized tailored response?
2 How does the integrated stress response communicate or interact with mitochondrial stress pathways?
3 How is the mitochondrial stress response in mammals sensed?
4 Does the Wnt pathway also control mitochondrial systemic effects in mammals?
5 Can peripheral tissues (e.g., the intestine or muscle) signal to the nervous system in events of local mitochondrial stress? How?
6 Do the different molecules identified as "mitokines" in mammals act additively or synergistically?
7 What are the physiological conditions, and in what life-stage are they most significant, in which the mitochondria signal to other tissues?

serotonin was recently implicated as beneficially regulating neuronal mitochondrial biogenesis [149] and in rescuing the mitochondrial dysfunction observed in a neurodevelopmental syndrome models [150,151]. While these studies suggest a beneficial effect of serotonin on mitochondria, overexpressing the serotonin 5-HT2B receptor in hearts was previously shown to cause abnormal mitochondrial function [152]. Interestingly, the levels of GDF15 were the most correlative with age in a study examining the plasma proteome of healthy individuals [153], and FGF21, humanin, and GDF15 all showed highest levels in centenarians [154].

Concluding remarks

The responses to mitochondrial perturbations, both on the cellular level and intercellular level, can take many different forms. The type of the stressor, the context (e.g., localization within the organelle), and the tissue in which the stress occurs can dictate whether a certain response will be mounted. Remarkably, disrupting mitochondrial function in some tissues has systemic, often beneficial, effects, possibly setting the stage for therapeutic opportunities. Future efforts will continue to map the differences and commonalities between the different stressors and the responses to these stressors and perhaps delineate a set of rules for us to understand how, and perhaps why, certain tissues are more susceptible to mitochondrial stresses. The research advances outlined here make it clear there is not only one mitokine species, but a "mitokine network" of several signaling molecules acting in concert. Uncovering this "mitokine network" in both model organisms and humans will shed light on how mitochondria act as a cellular and organismal hub, that is mis-regulated in metabolic and age-associated diseases. Thus, it is crucial that we understand the basic principles of the regulation of mitochondrial proteostasis, both intracellularly and intercellularly. Beyond our fundamental understanding of possible defects and the tailored responses to them, it is possible that activating the regulatory pathways protecting mitochondria, such as chaperones or proteases, will have beneficial effects in patients by targeting therapeutics to the organelle. As noted, mitochondrial dysfunction is associated with many diseases, including heart, muscle, liver, and neurodegenerative diseases. Mitochondrial chaperones have also been shown to be important in cancer [155,156]. For example, mislocalized Hsp90 to the mitochondria contributes to tumorigenesis and regulates tumor cell metabolism [157].

Many mitochondrially targeted therapeutics, based on new druggable entities, are under investigation and undergoing or completed clinical trials. For example, MitoQ, a mitochondria-targeted antioxidant, has been tested for its beneficial effects in clinical trials. MitoQ is a molecule that accumulates in mitochondria where it can reduce mitochondrial ROS [158] and was shown to improve vascular endothelial function in healthy adults [159] and decrease liver damage in hepatitis C patients [160]. While the benefits of some of these drugs remains controversial, and the mechanism and cellular pathways activated by many of the tested drugs is unknown, some of the drugs showing beneficial effects may be potentially inducing the UPRmt. Moreover, the observed beneficial clinical effects may be a result of UPRmt activation. For example, metformin [161] has been suggested to function, at least partially, through ETC inhibition (which is capable, as noted, to trigger UPRmt activation in *C. elegans*), and are used as treatments for type II diabetes. Metformin targets the ETC Complex I, and by that decreases NADH oxidation and oxygen consumption rates [162,163]. Additionally, metformin has been shown to induce high levels of GDF15, further implicating the drug in regulating systemic mitochondrial homeostasis [164]. Furthermore, the use of "mitokines" as potential biomarkers of mitochondrial diseases may be reporting on specific mitochondrial dysfunctions that occur in these patients and can help with designing patient-tailored treatments [165]. Many of the clinical trials [166], together with preclinical trials on beneficial effects of "mitokines" [167], highlight the importance of understanding the canonical pathways protecting mitochondria, and the critical players involved in these pathways. It is crucial that we elucidate the key regulators activating these pathways, in both model organisms and humans, and how these pathways are interacting with each other to ensure mitochondrial, cellular, and organismal homeostasis.

Acknowledgements

We thank Dr. Hope Henderson, Dr. Jenni Durieux, and Dr. Ryo Higuchi-Sanabria for feedback on the manuscript. R.BZ is supported by the Larry L. Hillblom Foundation.

Conflict of interest

The authors declare that they have no conflict of interest.

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