Cell Communication and Signaling

REVIEW

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Small but mighty: mitochondrial DNA at the centre of retrograde signalling

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Abstract

Mitochondria form highly dynamic and interconnected networks that continuously communicate with the cytoplasm and the nucleus to maintain cellular homeostasis and coordinate adaptive responses to stress. This bidirectional communication, known as mito-nuclear crosstalk, is essential for regulating metabolism, redox balance, immune activation, and cell fate decisions. While retrograde signalling has traditionally been viewed as a consequence of metabolic or oxidative perturbations, mounting evidence positions mitochondrial DNA (mtDNA) as a central and active regulator of these signalling pathways. Beyond encoding essential subunits of the electron transport chain, mtDNA functions as a signalling hub that conveys information about mitochondrial functional status to the nucleus. Perturbations in mtDNA integrity, copy number, or expression initiate retrograde responses through metabolic rewiring, alterations in redox and calcium signalling, and activation of stress-responsive transcriptional programmes. In addition, mtDNA-derived products, including mitochondrial-derived non-coding RNAs (mt-ncRNAs) and mitochondrial-derived peptides (MDPs), have emerged as key messengers that shuttle between cellular compartments, reshape nuclear gene expression, and influence cellular and systemic responses to stress. These molecules participate in diverse processes, ranging from mitochondrial biogenesis and quality control to innate immune activation and epigenetic regulation. This review synthesises current knowledge on mtDNA-driven retrograde signalling, highlighting both classical and emerging mechanisms by which the mitochondrial genome communicates with the nucleus. We discuss how mtDNA instability, defective repair, and altered mitochondrial dynamics trigger signalling cascades involving metabolic sensors, calcium fluxes, and innate immune pathways. We further examine the growing evidence supporting regulatory roles for mt-ncRNAs, including small RNAs, long non-coding RNAs, double-stranded RNAs, and circular RNAs, as well as MDPs such as Humanin, SHLPs, and MOTS-c, in coordinating adaptive nuclear responses.

By integrating these diverse signalling modalities, this review highlights mtDNA as an integral and active signalling platform that coordinates mitochondrial stress sensing with nuclear adaptive responses.

Keywords Mitochondria, Mitochondrial DNA, Mitochondrial-derived Peptides, Mitochondrial-derived non-coding RNAs, Mito-nuclear crosstalk, Retrograde signalling

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Introduction

Mitochondrial structure and function

Mitochondria are crucial intracellular organelles that play a central role in metabolism, cell death, growth, and, most importantly, energy production in the form of ATP. This places them at the core of cellular integrity, function, and survival [1]. They have a unique double-lipid-bilayer structure, with two internal compartments separated by two membranes: the outer mitochondrial membrane (OMM) and the inner mitochondrial membrane (IMM), with the intermembrane space (IMS) separating them [2]. The OMM separates the mitochondria from the cytosol and is permeable to ions and small molecules due to the presence of porin proteins, such as the voltage-dependent anion channel (VDAC), the primary transporter of ions, nucleotides, and metabolites [3]. Larger proteins need to be transported through translocases (i.e., TOM complex) [4]. The IMS is an aqueous space located between the OMM and the IMM, facilitating the exchange of molecules and ions between the cytoplasm and the matrix [5]. The IMM is impermeable to most ions and small molecules, allowing only selective passage, thereby generating a membrane potential vital for ATP production. The IMM proteins can facilitate electron transport chain (ETC) redox reactions, ATP synthase, and specific transport proteins to regulate the passage of metabolites into the mitochondrial matrix. There are two distinct zones within the IMM. The inner boundary membrane is the region in close contact with the OMM and is the site of protein translocation [6]. The second region forms finger-like projections called cristae that invaginate into the organelle's matrix. At the tips of these projections, the respiratory chain complexes and the F_1F_0-ATP synthase that produces ATP via oxidative phosphorylation (OXPHOS) are located [7]. This arrangement creates the characteristic curvature, typical of mitochondria. The innermost compartment is called the matrix. Surrounded by the IMM, it is the compartment where enzymes involved in the Krebs cycle are located, lipid oxidation and synthesis take place, and mitochondrial DNA (mtDNA) is housed, along with mitochondrial ribosomes needed for the translation of mitochondrial messenger RNA into peptides. Most mitochondrial proteins reside in the matrix, making this compartment the primary site for mitochondrial biochemical processes, including replication, repair, recombination, transcription, and translation [8].

Mitochondrial DNA

The fact that mitochondria contain their own DNA, distinct from nuclear DNA, further distinguishes them as a unique organelle. One of the main rationales for the existence of their own DNA, along with their double membrane, is the endosymbiotic theory, which posits that alphaproteobacterial-like organisms were engulfed during early eukaryotic evolution, creating a symbiotic relationship [9]. It is thought that the last eukaryotic common ancestor (LECA) lived approximately 1.8 billion years ago, and contained an aerobic organelle similar to mitochondria [10]. This organelle played a role in OXPHOS via the ETC and ATP synthase, much like mitochondria as we know them today [11]. A key distinction is that the mitochondrial genome of LECA likely encoded at least 69 proteins, including those for the ETC, ATP synthase, ribosome components, and some proteins involved in protein translocation and heme maturation. In contrast, modern mammalian mtDNA encodes only 13 proteins, all of which play a role in the ETC [12]. This suggests that, over evolutionary time, many genes have been lost or transferred to the nucleus through endosymbiont gene transfer [13]. The reason mitochondria in aerobic organisms retain a portion of their own DNA remains unclear. However, prevailing theories suggest that gene retention may be due to the high hydrophobicity of the retained proteins, making traversing the mitochondrial membranes a significant barrier, and/or to local synthesis of proteins involved in redox reactions, which may have provided an evolutionary advantage [14]. In addition, transferring all mitochondrial genes to the nucleus might have been disadvantageous or inefficient for the cell [15]. Therefore, the retention of mtDNA is almost always selectively beneficial, and successful transfer can only occur when mitochondrial mutation rates are high in small populations [14].

Despite its small size, ranging from 15.7 to 19.5 Kb in mammals, present-day mtDNA is remarkably efficient, encoding 13 OXPHOS subunits, 22 transfer RNAs (tRNAs), and two ribosomal RNAs (rRNAs). Maintaining its integrity is crucial for mitochondrial activity and energy production [12], and like its nuclear counterpart, it is susceptible to oxidative damage [16]. Moreover, emerging evidence suggests that mtDNA is not merely a passive target of injury but an active participant in signalling to the nucleus, initiating diverse retrograde responses to communicate its genomic state, transcriptional activity, and translation products [17]. This crosstalk between the mitochondria and the nucleus is known as the mito-nuclear crosstalk.

Mito-nuclear crosstalk

Communication between the nucleus and mitochondria is bidirectional, facilitated by the continuous exchange of molecules, proteins, metabolites, and nucleic acids, all of which coordinate in response to stress, metabolic alterations, and cell survival [18]. The nucleus can exert functional control over mitochondria via anterograde signalling [19]. Mitochondria are highly dynamic organelles, and through their biosynthetic and bioenergetic

roles, they can sense stress, respond accordingly, and communicate their functional status back to the nucleus via a retrograde signalling pathway, reprogramming gene expression [20]. Retrograde signalling can be initiated by many factors, but the key initiators are metabolic and oxidative stress, immune activation, UV radiation, and mtDNA damage. The signal can be transmitted to the nucleus as an increase in Ca^{2+}, a change in AMP/ATP or NAD^+/NADH ratios, and an imbalance of reactive oxygen species (ROS) formation [21, 22]. More recently, the discovery of mtDNA-derived products, such as mtDNA fragments, mitochondrial non-coding RNAs (mt-ncRNAs) and mitochondrial-derived peptides (MDPs), have also been shown to change the nuclear landscape [23–25] (Fig. 1).

In this review, we explore the emerging role of the mitochondrial genome as a central initiator of retrograde signalling, emphasising both active and passive molecular mechanisms that mediate this communication. We examine how mtDNA triggers signalling pathways, the involvement of its non-coding RNA transcripts, and the function of peptides derived from mtDNA expression, summarised in Table 1. By integrating these aspects, this review offers new insights into how mtDNA serves as a pivotal regulator of retrograde signalling, deepening our understanding of its contribution to cellular homeostasis, disease progression, and the ageing process.

Mitochondrial DNA retrograde signalling

The coordination of anterograde and retrograde signalling is essential for preserving mtDNA integrity [130, 131]. As mtDNA encodes 13 vital proteins of the ETC, it relies heavily on the nucleus for the transcription of proteins involved in mtDNA repair, replication, and transcription. Therefore, mitochondria must communicate their functional status to the nucleus via retrograde

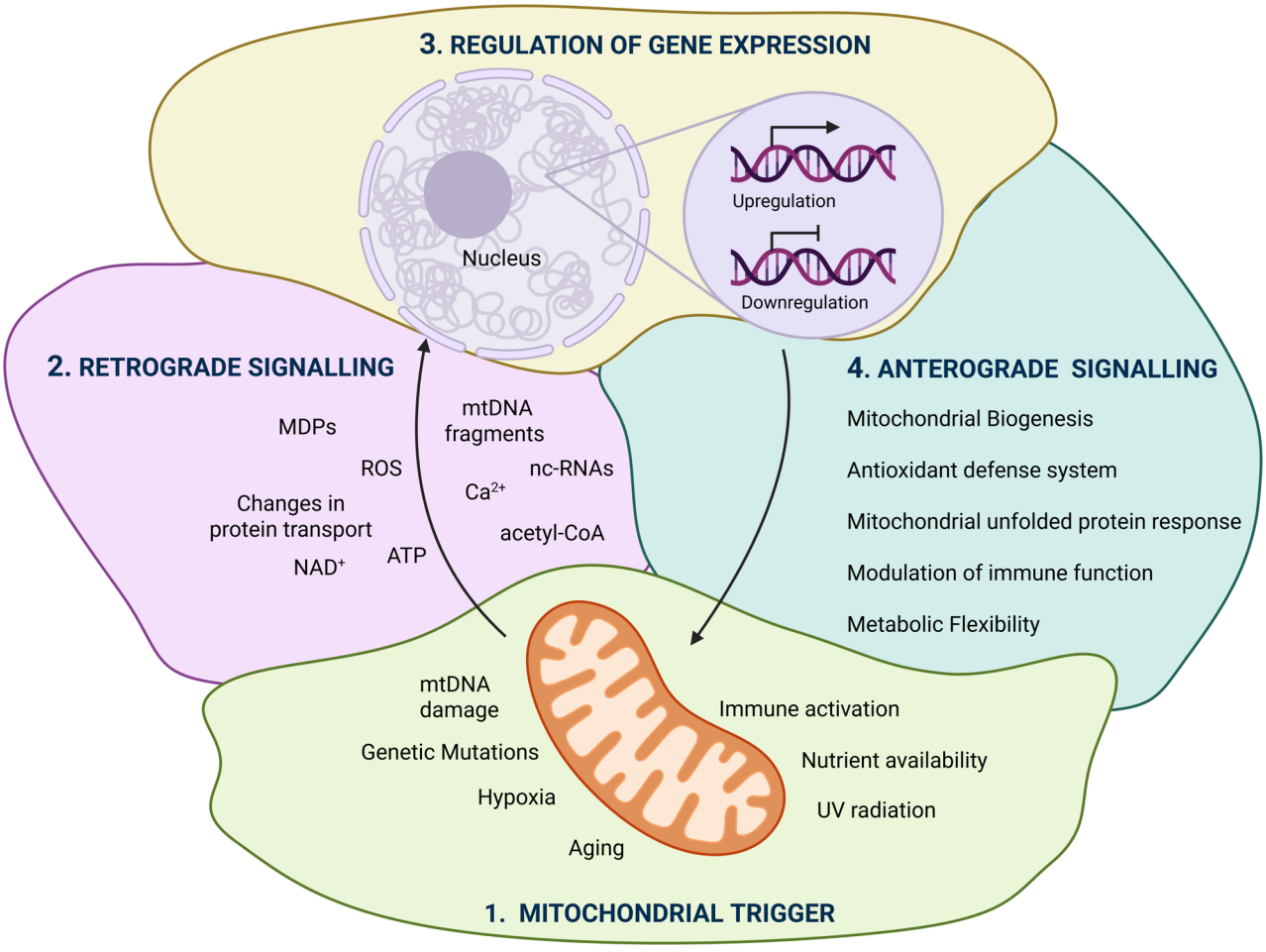

Fig. 1 Mito-nuclear crosstalk pathways: Mito-nuclear communication is bidirectional. (1) Mitochondrial triggers, including mtDNA damage and genetic mutations, alter mitochondrial function and lead to the (2) release of signalling molecules such as ATP, ROS, Ca^{2+}, acetyl-CoA, mtDNA fragments, MDPs, and non-coding RNAs. These signals engage retrograde signalling, transmitting mitochondrial status to the nucleus. In response, (3) the nucleus adjusts gene expression, resulting in (4) anterograde signalling that regulates mitochondrial biogenesis, antioxidant defence, the mitochondrial unfolded protein response, immune modulation, and metabolic flexibility. Together, these processes form a dynamic feedback loop that maintains mitochondrial function and, in turn, cellular homeostasis

Table 1 The table summarises the three main triggers of mitochondrial retrograde signalling discussed in the text, together with the corresponding initiating stimulus or effector, the activated pathway, and the resulting cellular response

	Initiation	Pathway activation	Cellular response	References
Mitochondrial DNA	Metabolic and oxidative stress	a. AMP/ATP AMPK activation b. ROS stabilises HIF-1α c.Ca^{2+} activates CaMK and calcineurin d. PDC translocates to the nucleus	a. PGC1α-mediated antioxidant defence b. NRF1/2, JNK/ERK mediated mitochondrial biogenesis, glycolysis, cell survival c. NFAT, NF-κB, CREB d. Histone acetylation, cell cycle regulation	[26–41]
	Genetic and structural instability	a. Oxidative DNA damage b. DNA stress activates AMPK c. Cytoplasmic mtDNA activates cGAS/STING d. mtDNA translocates to nucleus	a. Recruits APE1, Rad51, PARP1 b. Promotion of mitochondrial biogenesis genes by FOXO & PGC1α c. Proinflammatory signalling d. Genomic instability	[42–58]
	Mitochondrial membrane potential	a. Fe^{2+} accumulation induces ROS b. Sustained Ca^{2+} and mPTP opening	a. mtDNA damage and mitophagy b. Facilitate mtDNA release activation of cGAS/STING	[59–68]
	Quality control defects	a. Fission triggers mtDNA release b. Perturbed mtDNA c. ATFS-1 activation of UPR^{mt} d. TFAM interacts with autophagy proteins	a. NF-κB activation b. Induces UPR^{mt} and mitophagy c. inhibits mtDNA OXPHOS genes d. Overload activates cGAS/STING	[69–79]
Mitochondrial-derived non-coding RNA	Small non-coding RNA	a. Mito-ncRNA-805 increases with cigarette smoke b. Small mtRNAs bind AGO2 c. SmithRNAs modify H3 methylation	a. Upregulates mitochondrial bioenergetic genes b. Alter gene expression c. Epigenetic changes	[80–84]
	Long non-coding RNA	a. LncCytb shuttles between mitochondria & nucleus in hepatocellular carcinoma b. MDL1 binds cytoplasmic p53 c. Ca-ncRNA interacts with gene promoters;	a. Modulates splicing factors to enhance glycolysis b. Prevents nuclear entry c. Regulated by glucose and TNF-α	[85–92]
	Double-stranded RNA	a. Escapes mitochondria b. Binds to PKR	a. Activates interferon and proinflammatory cytokines by RIG-I/MDA5 b. eIF2α phosphorylation and translation regulation	[93–100]
	Circular RNA	a. Meccind1/5 upregulated b. sponges miRNAs c. Meccind5 binds hnRNPs; d. MC-cox2 expression regulated by PGC1α;	a. Hepatocellular carcinoma & increase mtDNA copy number b. Regulate metabolism c. Regulate RNA binding proteins d. Inhibits mPTP, reducing oxidative stress	[101–103]
	tRNA	a. mtDNA mutations b. tRNA fragments bind AGO proteins c. mt-tRNAGlu downregulation d. Activates MDA5	a. Disrupted OXPHOS, UPR^mt b. Modulate gene expression c. Affects pyruvate carrier, lactate secretion d. NF-κB, TNF, interferon, IL-6	[104–108]
Mitochondrial Derived Peptides	Humanin	a. Binds BAX/BAK b. Extracellularly binds FPR2, gp130/WSX1/CNTFR	a. Prevents OMM permeabilisation b. Activates PI3K/AKT, reduces oxidative stress, and improves insulin sensitivity	[109–115]
	SHLP-2	a. Localises with complex I b. Interacts with BAX c. Activates NRF2 d. Extracellularly binds CXCR7	a. Reduces oxidative stress b. Prevents OMM permeabilisation c. Reduces ROS in osteoblasts d. Activates ERK/STAT3, improves insulin sensitivity	[116–124]
	MOT-c	a. Translocates to nucleus under AMPK activation b. SIRT1/PGC1α modulation	a. Activates NRF/ARE pathways b. Regulates MOTS-c production in skeletal muscle	[125–129]

signalling pathways to reprogram nuclear gene expression and maintain mtDNA integrity. These pathways are often initiated by metabolic and oxidative stress cues, such as increased Ca^{2+}, ROS, or changes in NAD^+/NADH or AMP/ATP ratios [21, 22, 42, 132]. Here, we discuss how mtDNA can initiate retrograde signalling by: (1) inducing metabolic stress; (2) deficient repair capabilities; (3) altering mitochondrial membrane potential; and (4) disrupting quality control mechanisms.

Metabolic and oxidative stress

Mitochondria serve as the central hubs of cellular metabolism, but electron leakage from ETC complexes during OXPHOS can be accepted by oxygen to form superoxides known as ROS [133]. If not effectively neutralised by antioxidants, ROS can accumulate, leading to oxidative stress [134]. Oxidative stress and ROS accumulation are induced by mtDNA damage, leading to impaired function of ETC proteins, reduced ATP production, and

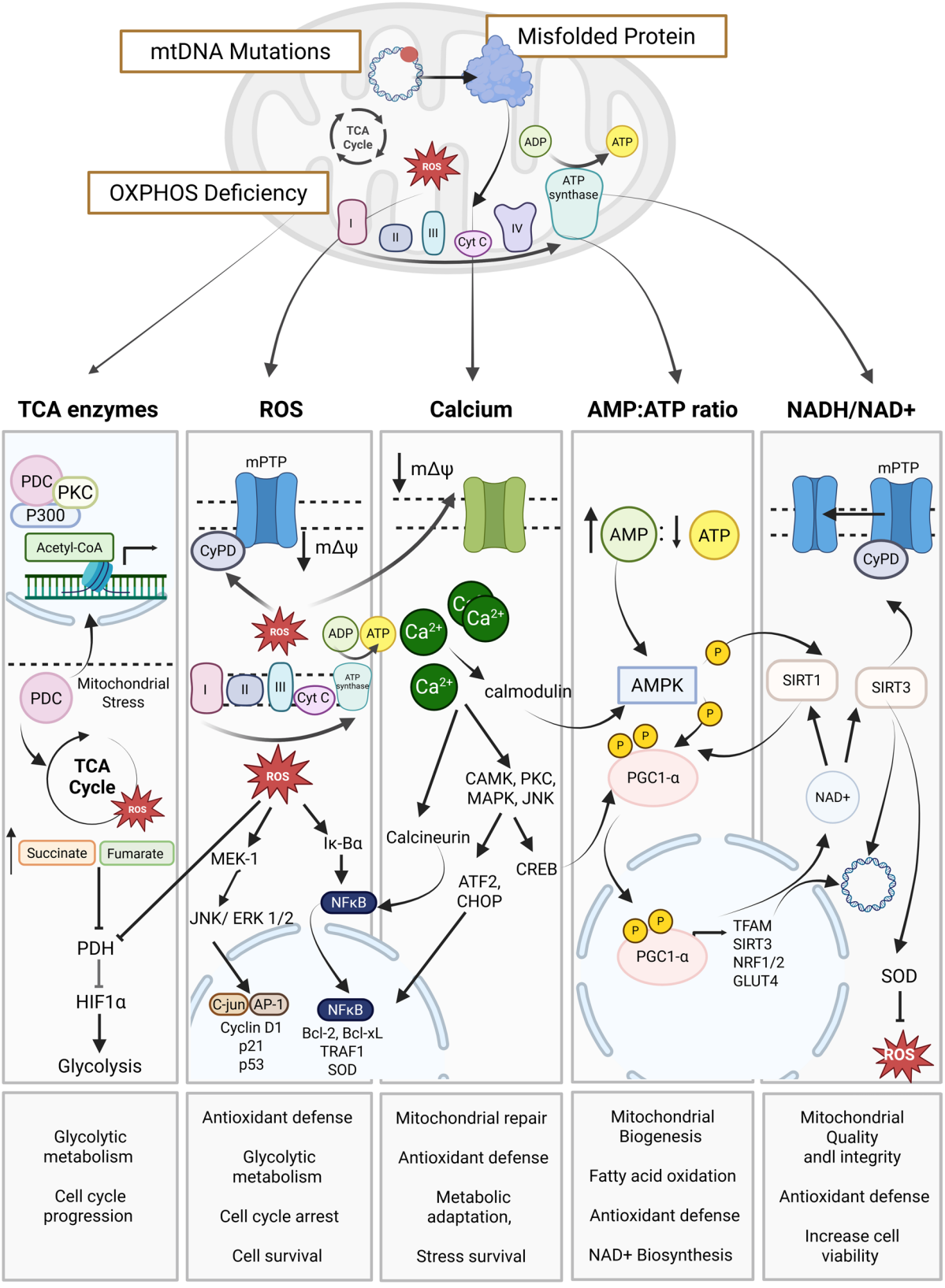

Fig. 2 (See legend on next page.)

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Fig. 2 mtDNA-Driven Metabolic Retrograde Signalling. Mutations in mtDNA can cause the production of dysfunctional or misfolded proteins. When these proteins are incorporated into or degraded within the cell, they impair OXPHOS and ETC functions, reducing metabolic capacity. These metabolic disturbances cause fluctuations in AMP/ATP and NAD^+/NADH ratios, affect Ca^{2+} handling, increase ROS levels, and alter the proposed activity of TCA enzymes. All of these changes can trigger retrograde signalling through sensor proteins such as AMPK, SIRT, and calmodulin, initiating cascades that lead to genetic alterations and promote mitochondrial biogenesis and metabolic shifts. Additionally, retrograde signalling can be directly activated, for example, by PDC, which can translocate to the nucleus to influence acetylation and epigenetic regulation

metabolic stress [135]. Therefore, mitochondria have to activate adaptive metabolic and signalling responses to preserve energy homeostasis (Fig. 2) [136–138]. This metabolic adaptability is a phenomenon named the Warburg effect, commonly observed in cancer cells, in which metabolism can shift from oxidative phosphorylation to glycolysis even in the presence of oxygen [139–141].

Alterations in mitochondrial metabolic activity can be directly sensed by altered AMP/ATP ratios. A rise in AMP and a reduction in ATP are sensed by AMP-activated protein kinase (AMPK), which, in turn, phosphorylates PGC-1α, promoting transcription of genes involved in mitochondrial biogenesis, mtDNA repair, and antioxidant defence [26]. Altered ATP production often coincides with reduced NAD^+ availability, a key cofactor for redox enzymes [27]. NAD^+ levels regulate the recruitment of nuclear-encoded mtDNA repair proteins such as APE1, TFAM, and EXOG1 to mitochondria under stress [28, 42]. Low NAD+ levels impair this process, increasing mtDNA's susceptibility to mutations and consequently mitochondrial dysfunction [29]. The imbalance can also activate SIRT1/3, promoting closure of the mitochondrial permeability transition pore (mPTP) and activation of antioxidant enzyme superoxide dismutase (SOD). As a consequence, an imbalance in the NADH/NAD^+ ratio promotes quality control and integrity of mtDNA, antioxidant defence and cell viability [30].

ROS can directly modulate transcription factors such as HIF-1α, NRF1/NRF2, and kinases JNK/ERK, that regulate genes involved in mitochondrial biogenesis, glycolytic metabolism and cell survival [138]. Moreover, ROS can oxidise channels, including mPTP and calcium channels, thereby affecting the ability to maintain a membrane potential and altering calcium homeostasis [31]. Changes in calcium homeostasis are sensed by calcium-sensitive kinases/phosphatases, such as CaMK and calcineurin, which activate NFAT, Nf-κB, and CREB [32]; as a pleiotropic transcription factor, CREB regulates genes involved in inflammatory signalling and metabolic control [33], suggesting that calcium-dependent CREB activation may plausibly contribute to coordinated shifts in inflammatory tone and cellular metabolic programming. It has been proposed that Ca^{2+} plays a pivotal role in coordinating pathways activated by ATP/ADP, NADH/NAD^+ and changes in ROS levels [27]. This is unsurprising as Ca^{2+} can simultaneously increase NADH generation, drive ATP synthesis, and alter the rate of ROS formation, making it a single, rapid, integrative signal that coordinates the cell's metabolic and redox responses to changing energy demand [34, 35]. Not only do metabolic intermediates initiate retrograde signalling, but the metabolic enzymes themselves also play key roles in shaping the epigenetic landscape. Changes in these enzymes' concentration or under metabolic rewiring [36] have been investigated recently by Marques et al.. The authors show how mtDNA mutations using a macrophage and mouse model altered the TCA enzymes, with a significant increase in α-KG, fumarate and malate levels in response to mtDNA perturbations, supporting further the mtDNA ability to influence gene expression through TCA metabolites [37].

Metabolic enzymes involved in energy production can initiate retrograde signalling in response to damaged mtDNA by eliciting their own moonlighting functions. Both cytoplasmic pyruvate kinase (PKM2) and mitochondrial pyruvate dehydrogenase complex (PDC) can translocate to the nucleus and perform their non-canonical function by forming a complex with p300, to locally produce acetyl-CoA to acetylate histones, altering the epigenetic landscape [38, 39, 141] and upregulating the expression of phosphorylated Rb, E2F, Cdk2 and cyclin A. These changes induce a progression from the G1 to the S phase of the cell cycle. Nuclear translocation of PDC can be triggered by mitochondrial stress, as shown by Suntendra et al., who observed decreased mitochondrial PDC and a corresponding increase in nuclear PDC following rotenone treatment, a complex I inhibitor known to induce oxidative stress [39, 40]. This suggests that, beyond its central role in supplying acetyl-CoA to the citric acid cycle, PDC activity mediates the connection between mitochondrial metabolism and nuclear regulation under oxidative stress. Similarly, dysregulation of other tricarboxylic acid (TCA) cycle enzymes can influence retrograde signalling pathways, potentially under conditions of mtDNA damage and mitochondrial stress. Disruptions in the TCA cycle can generate oncometabolites, such as 2-hydroxyglutarate, succinate, and fumarate. The accumulation of oncometabolites has been shown to disrupt redox balance in cancer cells, suggesting a plausible cycle of heightened ROS production coupled with a self-sustaining cycle of mtDNA damage [36]. Metabolic enzymes can directly sense nutrient supply and demand. Succinate and fumarate accumulation resulting from mutations in TCA enzymes, succinate

dehydrogenase and fumarate hydratase, respectively, inhibit Pyruvate Dehydrogenase (PDH), thereby releasing HIF-1α from inhibition and stabilising and promoting glycolysis. Therefore, when ROS is increased, metabolism can be favoured via the aerobic glycolytic pathway over oxidative phosphorylation, thereby reinforcing the Warburg effect [41]. PDH substrates are also inhibited by increased ROS levels. An example is the α-Ketoglutarate (α-KG), a substrate for chromatin-modifying dioxygenases such as JMJD/KDM and TET DNA demethylases. Inhibition of α-KG further modulates the epigenetic landscape, reprogramming nuclear gene expression to adapt to metabolic stress [36]. A recent mouse model using a mtDNA mutator $Polg^{m}$ found a reduction in α-KG in oocytes and depletion of DNA methylation but when α-KG was supplemented DNA methylation state and transcriptional activity was restored [142]. An important study highlighting how mtDNA mutation rate can control epigenetic reprogramming and gene expression through mitochondrial metabolites. We anticipate that further studies will elucidate additional roles of metabolic enzymes in retrograde signalling.

The communication of oxidative stress from the mitochondria is increasingly recognised to extend beyond cellular boundaries, altering the microenvironment and even spanning different tissues. An increase in ROS production appears to regulate metabolic flexibility in immune cells within the tumour microenvironment (TME). Retrograde signalling pathways involving Ca^{2+}/calcineurin, AMPK, and SIRT1 can suppress glycolysis and promote OXPHOS, facilitating M1-to-M2 macrophage polarisation. PI3K/Akt signalling and tumour suppressor p53 also influence macrophage metabolism and phenotype, though p53's role is context-dependent, sometimes promoting M1-like and other times M2-like activation. Collectively, these studies elucidate how ROS can influence immune cell function and metabolic plasticity outside the cell and within the TME [141].

Genetic and structural instability

Damage to mtDNA results in metabolic flexibility aimed at maintaining cellular energy balance. While enhanced metabolic flexibility can initially support cell survival under stress, excessive oxidative damage can further compromise mtDNA stability. Efficient mtDNA repair is therefore crucial for maintaining mitochondrial stability and homeostasis. However, when repair mechanisms fail, and mtDNA instability persists, retrograde signalling pathways activate to communicate mtDNA instability to the nucleus.

mtDNA is susceptible to damage, for example, by ROS, which can result in mutations; oxidation is the most abundant form of damage, with 8-oxo-dG the most prevalent oxidised form [143, 144]. It's been suggested that mtDNA mutations further exacerbate ROS production, as mutations in mtDNA have been found to correlate with increased ROS production, especially in cancer cells. It is possible to hypothesise the occurrence of a vicious cycle between mitochondrial dysfunction and increased ROS, resulting in a deeper state of mitochondrial dysfunction; however, demonstrating this causality is particularly challenging [145–148]. Shabalina et al. recently revisited this debate and assessed how mtDNA mutations affect ROS production, showing that the way you measure ROS greatly affects the results. Under traditional assay conditions (succinate without rotenone), ROS production appeared lower compared to wild type. However, there was a reduced membrane potential caused by a feedback inhibition of succinate oxidation and reduced oxidative capacity, limiting reverse electron transport, which normally drives high ROS production. In contrast with forward electron flow maintained (e.g., pyruvate/malate or fatty acid substrates), ROS production was higher in mutator mitochondria; these conditions reflect a more normal cellular metabolism. This does not definitively prove the ROS–mtDNA self-fueling cycle hypothesis but shows that, under physiologically relevant conditions, mtDNA mutations can indeed promote increased ROS production [149]. This work enhances the hypothesis and further endorses its viability as a plausible mechanism in mitochondrial ageing.

Mutations in mtDNA are not only disruptive to metabolism but also reprogram intracellular signalling pathways. Approximately 1 in 4,300 people are affected by mtDNA mutations, which can lead to mitochondrial disorders [150]. Specific mutations in mtDNA can activate pathways such as the integrated stress response (ISR) or the constitutive activation of the PI3K-Akt-mTORC1 axis, linked to NADH: NAD+ imbalance, increased ROS production, and heightened glycolytic flux, as exemplified by the m.3243 A > G point mutation [151]. For more details on how pathogenic mitochondrial DNA mutations influence cellular physiology, Chung et al. review [152] offers a comprehensive overview on how these mutations rewire multiple signalling and metabolic pathways, including stress responses, mitochondrial quality control, while the review by Darfarin et al. comprehensively covers the molecular mechanisms and signalling pathways of the mitonuclear crosstalk in maintaining mtDNA integrity [42].

Mitochondria employ several mechanisms to counteract the accumulation of mtDNA mutations, including maintaining a high genome copy number, organising DNA into protective nucleoids, undergoing dynamic fission–fusion cycles, and activating repair pathways; all of which depend on coordinated regulation of gene expression [42].

The decrease in mtDNA stability initiates two retrograde signalling pathways to mediate the regulation of mtDNA repair and stability: (1) the oxidative damage-driven pathway, in which lesions generated by ROS activate APE1, PARP1 and RAD51, and (2) the DNA stress pathway triggered by mtDNA instability and replication stress, initiating p38, AMPK, and CAMKII. Under oxidative stress conditions, cytosolic-based repair proteins APE1 and RAD51 translocate to the mitochondria in response to mtDNA lesions to initiate repair [42–44]. PARP1 is one of the earliest proteins to localise to mitochondria under oxidative stress, although it negatively regulates mtDNA repair by binding to proteins in the repair pathway and metabolic enzymes, resulting in replication impairment and depletion of NAD^+ [45]. PARP1-mediated NAD^+ depletion becomes a major regulator of mtDNA stability, as low NAD^+ impairs the recruitment of APE1 and TFAM [29]. The balance of PARP1 activity and NAD^+ availability defines a central mechanism that preserves mtDNA integrity during metabolic and oxidative stress.

The DNA stress pathway relies on AMPK oxidation; under high oxidative stress, AMPK is phosphorylated and activated [153]. Once phosphorylated, AMPK can activate transcription factors such as FOXO3 and PGC-1α, and translocate to the nucleus to bind to antioxidant and autophagy promoters [46]. FOXO3 forms a complex with SIRT1 under oxidative stress, leading to deacetylation and increased transcriptional activity, including genes involved in cell cycle arrest, antioxidant SOD2, and mitochondrial biogenesis [47, 48]. Not only can AMPK/SIRT control FOXO3 transcriptional activity, but it can also promote PGC-1α activity. PGC-1α, a master regulator of mitochondrial biogenesis, enhances its co-activator function to promote the transcription of NRF1/NRF2, directly activating genes of the ETC [42]. This cascade drives the anterograde response of OGG1, NTG1 and, most importantly, TFAM [42, 154, 155].

Transcription factor A (TFAM) is a key player in mtDNA transcription and replication [156], and is also involved in nucleoid compaction, protection from ROS, and the regulation of mtDNA copy number. Increased TFAM levels increase mtDNA nucleoids and reduce gene expression, leading to deficient oxidative phosphorylation (OXPHOS) [157]. On the contrary, when TFAM is reduced, mtDNA is not properly packaged, leading to mitochondrial release of mitochondrial damage-associated molecular patterns (mtDAMPs). A well-characterised mtDAMP is the mtDNA itself [158]. Because mtDNA has archaeal-like features, it triggers an immune response [49], where the released mtDNA binds to cyclic GMP-AMP synthase (cGAS), a cytoplasmic DNA sensor, which then activates and converts ATP and GTP into cGAMP [50]. This secondary messenger binds to stimulator of interferon gene (STING), causing it to dimerise and translocate from the endoplasmic reticulum (ER) to the Golgi-ER intermediate compartment and the Golgi apparatus [51]. The dimerised STING recruits TBK1 and IKK, which directly phosphorylate IRF3, promoting its activation and, in addition, attracts NF-κB, leading to their translocation to the nucleus, where they induce the expression of pro-inflammatory molecules like IFN-1, TNF, and IL-6, thus initiating a systemic immune response (Fig. 3) [52–55]. While this pathway typically defends against infections, overactivation by mtDNA release can contribute to organ injury and inflammation. This mechanism must be finely tuned to trigger only a retrograde signal, without compromising the physiology of the whole cell [159]. STING activity extends beyond the canonical induction of interferon signalling and can induce autophagosome biogenesis. Upon activation by cGAMP, STING translocates from the endoplasmic reticulum to ER–Golgi intermediate compartments, which serve as membrane platforms for LC3 lipidation and autophagosome formation [51].

In recent years, a new emerging field of research investigating the nuclear transfer of mtDNA has emerged. mtDNA released from mitochondria can, not only trigger an inflammatory response, but it can also move to the nucleus and integrate into the genome through non-homologous end joining as nuclear mitochondrial DNA segments (NUMTs). This process contributes to genomic instability and changes in gene regulation, potentially impacting chromatin structure [56]. NUMTs are now recognised as dynamic signalling elements that may be intensified in ageing tissues, heightening inflammation and affecting telomere stability and cellular ageing [57]. The buildup of NUMTs has been linked to ageing, cancer, and mitochondrial disorders, especially as increased mtDNA release and faulty quality control can raise the chance of nuclear incorporation [58]. The increase in NUMTs and genome instability may increase the probability of further mitochondrial disorders. As next-generation sequencing improves, insights into specific NUMTs and heteroplasmic mtDNA will guide us with a greater understanding of their implications in disease and ageing.

To prevent an over-release of mtDNA, mitochondria can isolate the damaged molecules through fission-fusion. The first line of action is linked to the changes in the AMP/ATP ratio. Decreased ATP levels promote fission, leading to degradation of the compromised mitochondrial region [160, 161]. Conversely, when ATP levels increase, mitochondria undergo fusion events to optimise energy efficiency and maintain cellular health by distributing the electrochemical gradient evenly across the fused mitochondria [162, 163]. These events are facilitated by the nucleus by providing proteins such as Drp1, MTFP1, and Fis1 for fission, and OPA1, Mfn1,

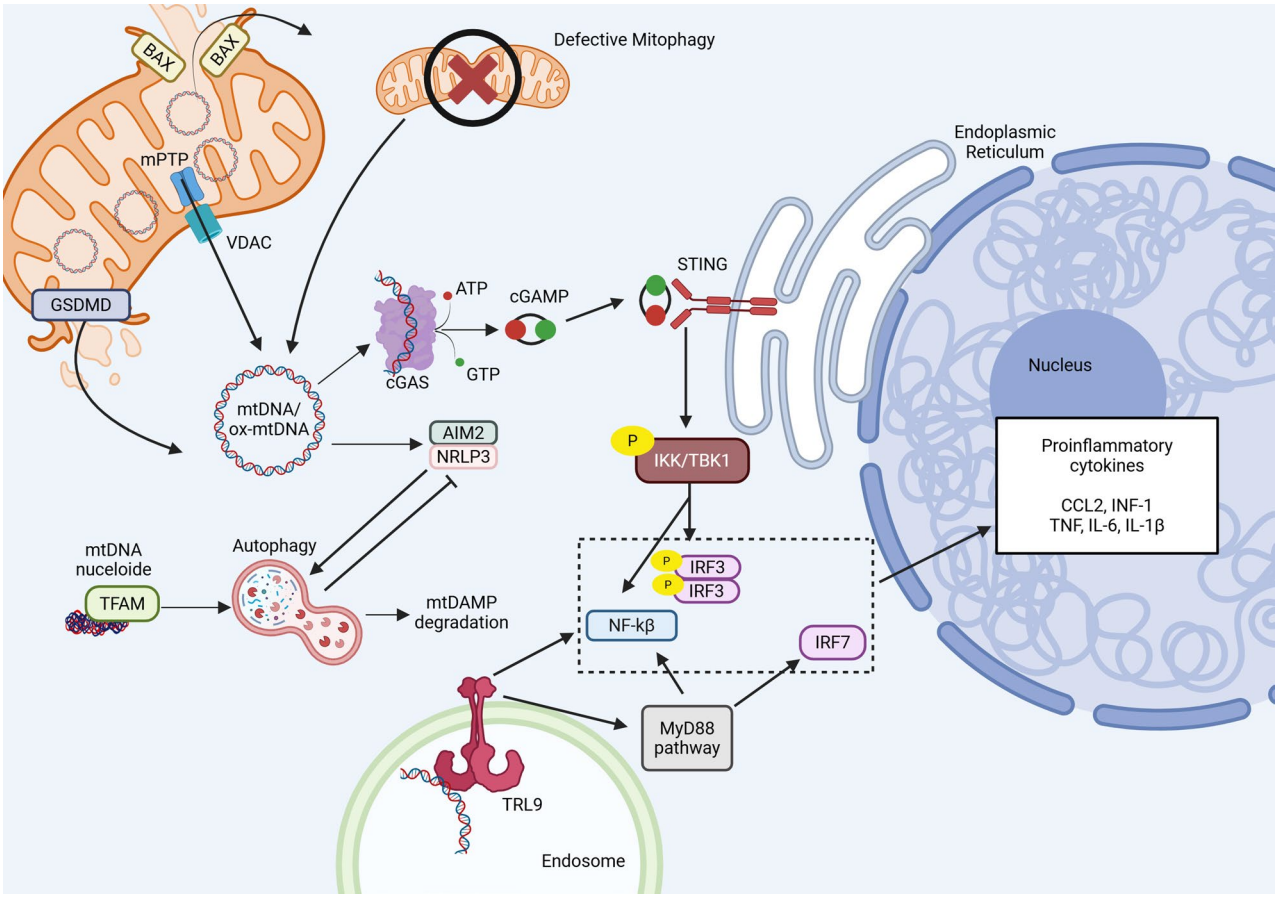

Fig. 3 mtDNA retrograde signalling. mtDNA is located in the mitochondrial matrix, but under triggers such as metabolic disorders, defective mitophagy, ROS, and genetic replication errors, it can be released into the cytoplasm, where it can initiate inflammatory pathways, particularly the STING pathway, inducing pro-inflammatory cytokine expression. Co-leakage with TFAM leads to LC3-mediated autophagy to clear cytoplasmic mtDNA. Autophagy itself can inhibit NRPL3, thereby activating TRL9 and amplifying proinflammatory cytokines. Released mtDNA can enter the nucleus and integrate into the genome, generating an unstable nuclear genome

and Mfn2 for fusion [162]. Not only do these proteins aid in mitochondrial fusion and fission, but it has been shown that an overexpression of MTFP1 can directly influence mtDNA levels, decreasing the mtDNA copy number, whereas silencing or knocking out MTFP1 increases it [161].

Mitochondrial membrane potential

Fission-fusion dynamics not only compartmentalise mtDNA but also help to maintain the mitochondrial membrane potential. When the membrane potential collapses, mitochondrial function deteriorates, activating retrograde signalling aimed at restoring the ETC activity [137, 164, 165]. This is critical, as the IMM holds the ETC, where complexes I, III, and IV act as proton pumps, generating a proton gradient that is harnessed to produce ATP [59, 165]. Damaged mtDNA affects ETC, thereby also disrupting the ability to maintain the mitochondrial membrane potential. The proton gradient drives ion transport, creating an inward driving force for cations and an outward driving force for anions. This loss of the gradient can occur through natural ageing and/or mitochondrial disease [55]. Either way, the loss of the membrane potential results in a loss of ion specificity, leading to an accumulation of cations within the mitochondria, in particular the lesser-spoken-of Fe^{2+} and Ca^{2+} [59], which can initiate retrograde signalling.

One mechanism inducing the retrograde signalling to the nucleus is Fe^{2+} accumulation. Fe^{2+} is an essential cofactor in the electron transport chain, in enzyme catalysis, and DNA replication and repair [59]. An overload of Fe^{2+} leads to loss of mitochondrial membrane potential itself, exacerbating the already unstable condition of mitochondria. Moreover, excess Fe^{2+} increases ROS production and triggers cell death, linking iron overload disorders with impaired mtDNA and respiratory dysfunction [60]. Excessive Fe^{2+} can also generate iron-catalysed radicals that directly damage mtDNA, impairing mitochondrial function [61]. In the presence of mutations of divalent metal transporter (DMT-1), its transport

machinery, high levels of Fe^{2+} can also initiate the apoptotic pathway, leading to mtDNA leakage and activation of the cGAS-STING pathway [62]. Recently, Picca et al. found that when iron homeostasis was perturbed, it not only altered mtDNA stability but also Pink1 levels, linking iron overload to activation of mitophagy [63].

Fe^{2+} is not the only cation affected by the loss of the membrane potential, but also Ca^{2+}. Loss of membrane potential can lead to or be generated by sustained mPTP opening, leading to dysregulation of Ca^{2+} [166]. Sustained channel opening can be triggered by elevated Ca^{2+} levels and ROS, which can oxidise mitochondrial membrane lipids and critical thiol residues on proteins involved in mPTP regulation, such as CypD and ANT, thereby lowering the threshold for pore opening [64]. ROS can further modulate Ca^{2+}, and the interplay between ROS and Ca^{2+} is essential for PARP activation [167, 168]. Shevstova et al. showed that ROS and mitochondrial Ca^{2+} are also responsible for PARP activation and for sequestration of mitochondrial calcium uptake, thereby protecting cells against mitochondrial depolarisation and cell death [65]. Indeed, as the membrane potential decreases, the propensity to open the pore increases, creating a self-feeding cycle of membrane depolarisation, permeabilisation [169]. Nakagawa et al. showed that sustained opening of the mPTP triggers mitochondrial depolarisation in platelets, resulting in swelling, dysfunction, and clearance [64, 66]. Sustained opening of the mPTP may also allow for the passage of mtDNA fragments [67]. Yu et al. supported this hypothesis, showing that TDP-43, a DNA/RNA binding protein, triggers mtDNA release via mPTP to activate cGAS/STING in Amyotrophic Lateral Sclerosis [68].

Not only can the IMM become depolarised, but the OMM can also polarise during apoptosis or stress, thereby initiating retrograde signalling through the release of the Apoptosis-Inducing Factor (AIF) [170]. Where AIF can now translocate to the nucleus, bind to nuclear DNA and associated proteins such as CypA, and reorganise chromatin, leading to large-scale fragmentation and caspase-independent cell death [69, 171]. In parallel, the primary apoptotic effectors Bcl-2, BAX, and BAK undergo conformational activation and oligomerisation on the OMM, forming macropores that allow the passage of proteins, cytochrome c, and, under certain conditions, mtDNA fragments [172].

Quality control defects

Depolarisation of the mitochondrial membrane is also beneficial in the context of mtDNA mutations. To rescue mitochondria and prevent apoptosis, mitochondria can undergo fission to compartmentalise damage. The Mitochondrial Fission Process 1 protein (MTFP1) has been shown to mediate mitochondrial quality control and to be intimately associated with mtDNA homeostasis [161]. Mitochondrial fission has been shown to release mtDNA, activate the NF-κB signalling pathway through binding to TLR9 in endosomes [70], and promote the biosynthesis of chemokines such as CCL2, thereby polarising macrophages into an inflammatory phenotype [173]. Fission also helps prevent the reintegration of dysfunctional mitochondria.

The depolarisation of the dysfunctional mitochondrial membrane leads to mitophagy mediated by Pink1 [174]. Under physiological conditions, Pink1 is imported into the mitochondria and degraded by E3 ubiquitin ligases, and the loss of membrane potential prevents import and allows for OMM accumulation, promoting its autophosphorylation and leading to the recruitment of Parkin, which ubiquitinates OMM proteins, marking the mitochondria for recognition by autophagy receptors such as SQSTM1/p62 [175]. This process drives the interaction between ubiquitinated mitochondria and autophagosomes, leading to their degradation [176]. Although mitophagy serves as a quality-control mechanism, it is not entirely efficient; consequently, mtDNA mutations can persist, allowing the accumulation of dysfunctional mitochondria. In PolgA mutator mice, which accumulate mtDNA mutations due to defective proofreading, resulting in aberrant activation of mTOR and degradation of autophagy initiator ULK1, suppressing autophagy, allowing dysfunctional mitochondria to persist [177, 178]. Conversely, studies using mt-Keima reporter mice have shown that mitophagy levels vary by tissue and can be modulated by mitochondrial stress, indicating that mtDNA defects may, under specific contexts, influence mitophagy activity [179].

Mitophagy is not only inducible by loss of membrane potential but can also be induced by the protein unfolded response (UPR^{mt}). As Suntandy et al. showed, mtDNA perturbation can drive the UPR^{mt} response. Not only can mtDNA directly activate the UPR^{mt} response, but it can also do so indirectly by releasing mitochondrial precursor proteins (mtProt) and ROS, which oxidise the cytosolic HSP40 protein DNAJA1, leading to enhanced recruitment of cytosolic HSP70 to c-mtProt. Consequently, HSP70 releases HSF1, which translocates to the nucleus and activates transcription of UPR^{mt} genes [71]. Further, the study by Michaelis et al. showed that upon UPR^{mt}, the pre-sequence translocase-associated motor complex dissociates from the protein import machinery, resulting in decreased protein import and mitophagy induction. They demonstrated that reduced protein import is sufficient to activate mitophagy without membrane depolarisation [72]. Together, these studies suggest a mechanism where mtDNA perturbations initiate the UPR^{mt}, driving mitophagy and changes in genes expression.

The UPR^{mt} system is mainly known as a stress response pathway activated in response to mtDNA defects to maintain mitochondrial integrity [146]. Using *C. elegans*, Nargund et al. demonstrated that the transcription factor ATFS-1 is crucial for activating the UPR^{mt} response [73]. ATFS-1 has both a nuclear localisation sequence and a mitochondrial targeting sequence. Under normal conditions, ATFS-1 is imported into mitochondria and quickly degraded by the protease Lon peptidase 1. During mitochondrial stress, impaired protein import causes ATFS-1 to accumulate in the nucleus, where it promotes the expression of various genes and inhibits mtDNA-encoded OXPHOS genes. It also encourages the assembly of NADH ubiquinone oxidoreductase factors to support ETC complex activity. ATFS-1 thus limits further transcription of OXPHOS genes while preserving existing complexes, optimising respiration during stress [74]. In a paper published in 2023, Dai et al. showed that ATFS-1 holds the ability to shift the balance toward mtDNA repair by disrupting mitochondrial pre-initiation transcription complex assembly [75]. Moreover, this transcriptional inhibition reduces age-related mtDNA damage, thereby improving cellular longevity, suggesting that ATFS-1 serves as a key regulator of mitochondrial genome expression and maintenance [75]. Paradoxically, Lin et al. found that in *C. elegans*, ATFS-1 not only accumulates in the nucleus upon mitochondrial stress, but also accumulates within dysfunctional mitochondria, preferentially associating with and promoting replication of deleterious mtDNA. Inhibiting UPR^{mt} reduced the proportion of mutant mtDNA, demonstrating that this stress response pathway can actively sustain harmful mitochondrial genomes, potentially contributing to a vicious cycle of further mtDNA damage and stronger retrograde signalling pathways [76]. Subsequent transcriptomic and proteomic studies revealed strong conservation of UPR^{mt} in mammals, with ATF5 being the homolog of ATFS-1 [180]. ATF5 responds to mitochondrial dysfunction by inducing chaperones, proteases, and metabolic regulators to restore mitochondrial function, and it has been shown to be upregulated in many cancers [146]. Yang et al. found that using a POLG mutator mice with progressive mtDNA mutation burdens leads to NAD^+ depletion and triggers the UPR^{mt} in an ATF5-dependent manner, suggesting a clear pathway of activation [181].

As highlighted above, mtDNA is susceptible to damage, and a key function of mitophagy is the elimination of mitochondria containing damaged or mutated mtDNA. To rescue mtDNA before initiating mitophagy, mtDNA is compacted into nucleoids with quality-control proteases [182–184]. Under oxidative and inflammatory stress, mitochondrial nucleoids undergo significant alterations in structure, localisation, and function, profoundly impacting cellular homeostasis [77, 185, 186]. Nucleoids are proposed to escape through the mPTP opening [187–189]. BAX and BAK allow herniation of the inner mitochondrial membrane through the outer membrane, potentially facilitating the release of nucleoids and other mitochondrial components into the cytoplasm [172, 190]. Recent research has also highlighted the role of gasdermin (GSDM) proteins, particularly GSDMD and GSDME, in mitochondrial membrane permeabilisation and potential nucleoid release [191].

The release of nucleoids means TFAM and mtDNA are simultaneously released into the cytoplasm. TFAM-mediated clearance of nucleoids in the cytoplasm via the autolysosomal pathway has recently emerged as a key quality-control mechanism. When mtDNA is released into the cytoplasm, TFAM interacts with autophagy proteins, such as microtubule-associated protein 1 light chain 3B (LC3B) [192], recruiting autophagosomes to engulf damaged mtDNA and associated nucleoids. Lysosomes ensure the degradation of damaged nucleoid components, thereby limiting inflammation by preventing the accumulation of cytoplasmic mtDNA [78]. Autophagy dysfunction, such as the knockout of key autophagy genes or inhibition of lysosomal function, leads to increased cytoplasmic accumulation of TFAM and mtDNA [77]. This accumulation can activate the cGAS-STING pathway, exacerbating inflammation and potentially contributing to various pathological conditions [78]. This selective autophagic clearance of cytoplasmic TFAM–mtDNA complexes represents an important quality-control mechanism that limits inappropriate immune activation and preserves cellular homeostasis by preventing persistent nucleoid-derived signals from engaging inflammatory pathways such as cGAS-STING [79].

mtDNA can initiate retrograde signalling through a multitude of mechanisms, either directly by escaping into the cytoplasm and activating the innate immune system, or indirectly by accumulating damage that affects the ECT and its metabolites, thereby altering metabolite homeostasis and initiating retrograde signalling. Additionally, RNA transcripts derived from mtDNA can also serve as retrograde signalling molecules.

Retrograde signalling through non-coding mitochondrial RNA

In recent decades, a compelling field of research has emerged around RNA sequences once dismissed as non-functional simply because they cannot be translated into proteins. These so-called "junk" RNAs are now recognised as key regulators of cellular physiology. Among them, mitochondrial non-coding RNAs (ncRNAs) have gained particular attention. In addition to the 22 tRNAs essential for mitochondrial translation, mitochondria

also produce a diverse and expanding collection of small non-coding RNAs (sncRNAs), long non-coding RNAs (lncRNAs), double-stranded RNAs, and circular RNAs (circRNAs) [193, 194], which partake in retrograde signalling, summarised in Fig. 4. For the purpose of this review, mtDNA-derived ncRNAs are of particular interest due to their well-documented involvement in mitochondrial retrograde signalling.

Small non-coding RNAs

sncRNAs are a diverse group of RNA molecules, shorter than 200 nucleotides in length, that regulate gene expression, genome stability, or cellular processes, including well-known classes such as microRNAs (miRNAs) and small interfering RNAs (siRNAs) [195]. Most research focuses on the sub-group of miRNAs, classified as between 21 and 25 nucleotides. In 2011, Mercer et *al.* completed a deep mitochondrial transcriptome analysis of eight cell lines and identified and annotated 31 novel snRNA derived from 17 loci across the mtDNA, with 84% localising to tRNA genes [196]. This discovery was further supported by sequencing of ncRNA in HEK293 and HeLa cell lines, where 4 miRNAs (hsa-miR-4461, hsa-miR-4463, hsa-miR-4484, and hsa-miR-4485) and 24 putative novel miRNAs were also assigned to a mitochondrial origin, matching the genome at the positions corresponding to 16 S rRNA, tRNA, and subunits of complex I [197].

Later, sncRNAs have been shown to respond to mitochondrial stress and initiate retrograde signalling. Blumental-Perry et al. demonstrated that a tissue-specific sncRNA, mito-ncR-805, was upregulated in response to cigarette smoke in a mouse model of chronic obstructive pulmonary disease. Under stress conditions, mito-ncR-805 undergoes spatial and temporal dynamics by re-localising from the mitochondria to the nucleus, thereby promoting changes in 14 nuclear-mitochondrial genes involved in mitochondrial bioenergetics, including NDUFB7 and ATP6V, which are essential subunits involved in ATP generation, thereby promoting

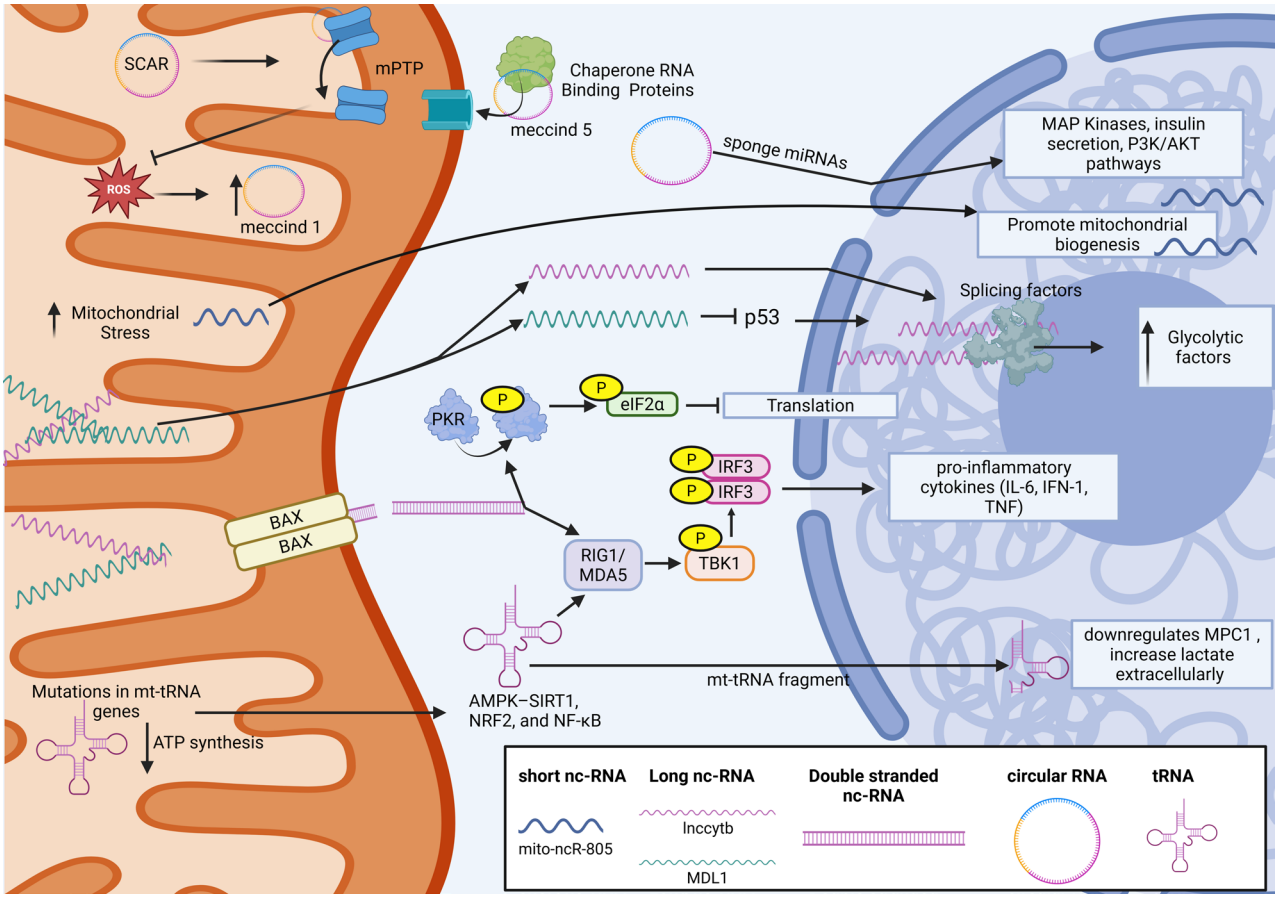

Fig. 4 mt-ncRNA retrograde signalling. Mitochondrial-derived ncRNAs originate from mtDNA and initiate retrograde signalling indirectly by altering cell homeostasis: modulating miRNAs, preventing global protein synthesis, inhibiting p53, chaperoning proteins into the mitochondria to maintain homeostasis, and modulating ATP production and Ca^{2+} handling. They also directly initiate retrograde signalling by translocating into the nucleus to modulate splicing factors that promote mitochondrial bioenergetics and metabolism. As well as activate cytosolic signalling, including MAPK and RIG-I like receptor pathways, resulting in a proinflammatory environment

mitochondrial bioenergetics [80]. Interestingly, they found that mito-ncR-805 accumulates in mitochondrial granules, which have previously been described as centres for post-transcriptional mRNA processing, suggesting that it may be stored serving as a "stress-response hub". Furthermore, the authors hypothesise that mito-ncR-805 accumulation in mitochondrial granules could coordinate with activation of the DELE1–eIF2α pathway to signal mitochondrial dysfunction to the cytosol and nucleus via the ISR [80, 81]. The authors then go on to suggest that an export mechanism in the repurposing of the TOM-TIM translocase, in which, under mitochondrial stress, there is an increase in co-localisation with TOM20 [80]. Supported by this article, which found an enrichment of RNA-binding proteins near TOM20, supporting the idea that TOM20 could possibly be repurposed for RNA export [198, 199].

To further show the regulatory role of mt-snRNA, Pozzi & Dowling demonstrated that sm-mtRNAs physically associate with a canonical nuclear-encoded RNA-regulatory protein, Argonaute 2 (AGO2). They identified a particular snRNAs, encoded within the mitochondrial tRNA for methionine, whose sequence and expression are conserved across vertebrates, implying evolutionary conservation of this RNA species. In effect, these data support a model in which mitochondrial-derived RNAs could serve as messengers in retrograde signalling.

However, the authors acknowledge several major unanswered questions: how binding to AGO2 alters gene expression, it remains unclear how these small mt-RNAs act to modulate target nuclear transcripts, and under what physiological or cellular conditions such regulation would occur [82]. Also unresolved are the mechanisms by which mitochondrial RNAs exit mitochondria and how frequently such trafficking happens in vivo.

Recently, a class of mitochondria-specific ncRNAs called smithRNAs that are highly transcribed with a main role of regulating nuclear transcripts have been described. They were identified in the manila clam *Ruditapes philippinarum* and have since been proposed in other species; however, to date, evidence for their presence and function is primarily based on RNA sequencing data [200, 201]. smithRNAs are transcribed from the mitochondrial genome, and have been reported to regulate nuclear targets. The complementarity of a small region of the sncRNA with the 3' UTR of target messengers was shown to be a good predictor to identify the smithRNAs target genes [82]. Two smithRNAs, M_smithRNA106t and 145t, have been demonstrated to partake in retrograde signalling, as when M_smithRNA106t is injected into clams, there is a significant reduction of histone H3 methylation levels, while injected M_smithRNA145t leads to significantly increased histone H3 acetylation [83]. Plazzi et al. concluded that smithRNAs are likely widespread and may evolve fairly easily *de novo* from mitochondrial transcripts, providing a common route for mitochondrial regulation of nuclear genes and thereby contributing to mito-nuclear co-adaptation or incompatibility [84, 202].

Mitochondrial long non-coding RNA

SncRNAs are widely documented in terms of their role and involvement within the cell, but more recently, longer non-coding transcripts dubbed long non-coding RNAs (lncRNAs) have been identified and have shown a role within retrograde signalling.

LncRNAs are longer than 200 nucleotides, and as most of the genome is transcribed, high-throughput sequencing has revealed tens of thousands of these lncRNAs, far outnumbering protein-coding genes [203]. Many discovered lncRNAs have gone unannotated, due to their low expression levels, cell or tissue-specific expression, poor evolutionary sequence conservation, and/or inefficient splicing or low stability, yet they are believed to play functional roles in controlling gene expression, structural functions, and signal transduction [204, 205]. It has been known that nuclear lncRNAs play an important role in anterograde signalling [206], but it appears that mitochondrial lncRNAs can also regulate retrograde signalling. Mitochondrial lncRNAs can be divided into three categories: (1) transcripts from individual mitochondrial genes, such as lncND5, lncND6, and lncCytb; (2) chimeric transcripts combining sequences from multiple mtDNA regions; and (3) predicted or computationally identified lncRNAs, referred to as putative mitochondrial lncRNAs [207, 208].

As mentioned heavily before, the passage of nucleotides is not easy, and, like mitochondrial DNA, it cannot easily pass out due to mitochondrial impermeability; it could be released through mitophagy intermediates, voltage-dependent anion channel (VDAC), facilitated by mPTP, and BAX/BAK pores during apoptosis or mtDNA damage, summarised nicely in a review by Giordano et al. [209]. Nowadays, the most accepted mechanisms of release involve the formation of BAX/BAK pores, in which oligomerisation at the OMM forms a large pore, facilitating the release of ncRNA and mtDNA. This mechanism was recently validated by Victorelli et al., who demonstrated that when BAX/BAK oligomerisation was inhibited, the level of cytoplasmic mtRNA reduced [210].

Although the mechanism of lncRNA release is not yet clear, several research groups have focused on the mechanism of action of mitochondrial lncRNAs in retro-signalling. Recently, lncCytb, MDL1AS, and lncND5 have been reported to shuttle between mitochondria and the nucleus. The shuttling of lncCytb enables interactions with splicing factors, thereby enhancing glycolysis and mediating pre-mRNA processing and mRNA maturation

of glycolysis-related genes [86]. Not surprisingly, the change in subcellular localisation of lncRNAs can be initiated by hypoxia or hypoglycaemia through IDH1, a common feature of mitochondrial disorders [87]. The implications of lncCytb extend further in hepatocellular carcinoma disease and have been reported to shuttle between the nucleus and mitochondria [88]. Additionally, mitochondrial ncRNA MDL1 has been shown to bind cytoplasmic p53, modulating its subcellular localisation and preventing its nuclear entry and transcriptional control. It may mediate crosstalk between mitochondria and the nucleus [85].

Sriram et al. reported lncRNAs associated with nuclear chromatin and termed mt-caRNAs. Predominantly located in gene promoters and in human epithelial cells, the mtRNA–chromatin attachment levels change in response to cellular stress induced by high glucose and tumour necrosis factor alpha (TNFα). A knockdown of a subset of sense-strand–derived non-coding mitochondrial RNAs caused marked suppression of antiviral pathways and type I interferon responses [89]. The first sense and antisense mt-caRNAs were SncmtRNAs and ASncmtRNAs, and found to be associated preferentially with heterochromatin, and the sense RNA expression was strongly correlated with cell proliferation [90]; antisense RNA revealed tumourigenic properties [91]. Therefore, mt-caRNAs are an example of RNA-mediated mitochondrial retrograde response that acts through transcriptional regulation [92].

Double-stranded RNA

Double-stranded RNA (dsRNA) is most commonly associated with constituting some viral genomes; therefore, nearly all organisms have the capability of recognising dsRNA and mounting a response, the primary aim of which is to mitigate the potential infection [93]. In 2018, Dhir et al. discovered in vivo mitochondria-encoded dsRNAs (mt-dsRNAs) in HeLa cells using J2 antibody, which specifically recognises dsRNAs, and noted that the mitochondria generate 99% of cellular dsRNA [94]. mt-dsRNAs are highly unstable molecules whose half-life and production are under strict surveillance by the mitochondrial degradosome, which includes SUV3 and PNPase, making investigation hard. However, loss of either SUV3 or PNPase leads to massive accumulation of mt-dsRNAs [94, 95]. mt-dsRNAs are able to escape into the cytoplasm in a PNPase-dependent manner, where the endogenous dsRNAs trigger the same receptors that have evolved to detect viral dsRNAs [93, 94]. The release of mt-dsRNAs into the cytoplasm depends on BAX/BAK pores, which resemble the reported release of other mtDAMPs into the cytosol during apoptosis, mitochondrial dynamics or mtDNA breaks [96, 97, 172]. Mt-dsRNAs have been postulated as key agonists of the innate immune response, by binding to MDA5 and less so RIG-1 leading to the expression of type-1 interferons and proinflammatory cytokines [98]. One group also showed that protein kinase RNA-activated (PKR), a central kinase involved in mRNA translation, transcriptional control, proliferation and apoptosis, can bind to mt-dsRNA to play a regulatory role [99]. mt-dsRNA binding promotes PKR phosphorylation and activation, which then leads to phosphorylation of downstream targets such as eIF2α, inhibiting translation and signalling within the cell [100]. Their immunogenic nature also suggests that their accumulation might be associated with autoimmune disorders [98].

Mitochondrial tRNAs as mediators of retrograde signalling

Although traditionally viewed as static adaptors in mitochondrial translation, mitochondrial tRNAs (mt-tRNAs) are increasingly recognised as active participants in retrograde signalling. Mutations in mt-tRNA genes, such as *MT-TL1* and *MT-TK*, disrupt OXPHOS and precipitate mitochondrial stress, leading to altered redox balance, reduced ATP synthesis, and activation of integrated stress response, including UPR^{mt}, altering the nuclear landscape [104]. These transcriptional responses promote mitochondrial biogenesis, antioxidant defence, and metabolic adaptation, linking mt-tRNA dysfunction directly to nuclear reprogramming in mitochondrial disorders such as MELAS and MERRF [211].

Beyond genetic mutations, stress-induced cleavage of mt-tRNAs leads to the formation of mitochondrial tRNA-derived fragments (mt-tRFs). Also mitochondrial dysfunction can affect mt-tRF production, via their impact on mtDNA transcription, which produces mitochondrial tRNA-derived fragments that can translocate to the cytosol, interact with argonaute proteins, and modulate nuclear gene expression [105]. Meseguer et al. found that mt-tRF derived from mt-tRNAGlu (UUC) is upregulated in MELAS cybrid cells. This specific mt-tRF directly interacts to downregulate the mitochondrial pyruvate carrier protein 1 (MPC1) mRNA, leading to extracellular lactate accumulation [106], thereby providing an RNA-mediated retrograde signalling route.

Dysregulation of mt-tRNA processing enzymes (e.g., RNase P, ELAC2, TRNT1) further amplifies mitochondrial stress and nuclear transcriptional responses. Moreover, oxidised or misfolded mt-tRNAs escaping into the cytoplasm may engage innate immune sensors such as RIG-I or MDA5, recruiting kinases TBK1/IKK. RIG1 and MDA5 activate and phosphorylate IRF3/7 to promote IFN-β expression. Additionally, MDA5 can also activate NF-κB, inducing TNF, IFN-β and IL-6 that translocate to the nucleus, triggering interferon and inflammatory pathways reminiscent of the mt-dsRNA and mtDNA response [55, 107, 108]. Collectively, these findings

position mt-tRNAs and their derivatives as multifaceted messengers that translate mitochondrial dysfunction into nuclear and immune responses, bridging mitochondrial stress signalling as another retrograde signalling pathway.

Circular RNA

mtDNA contains inverted repeat sequences implicated in various DNA recombination and repair processes [212]. The complementary base-pairing of inverted repeats can form hairpin structures, which may drive the circularisation of mitochondrial RNA molecules, thereby contributing to the formation of mitochondrial circular RNAs (mt-circRNA) [213]. Recent studies have suggested that mt-circRNAs play a role in maintaining mitochondrial function. Moreover, the aberrant expression of diverse mt-circRNAs has been observed across multiple cancer cell types [214].

Sanadgol et al. showed that mt-circRNA exhibits dynamic distribution both inside and outside the mitochondria, and may shuttle between these compartments. Interestingly, two of the mt-circRNAs they were studying, circmntnd5 and meccind1, demonstrated, through in silico studies, the potential to act as sponges to regulate miRNAs through their unique back-splice junction sequences. Interestingly, these miRNAs (has-miR-5186, 6888-5p, 8081, 924, 672-5p) are predominantly associated with insulin secretion, proteoglycans, and the MAPK signalling pathways. mc-COX2 and circMNTND5 may also exert epigenetic regulation on RNA biogenesis, potentially through the antagonism of key proteins, AGO1/2, EIF4A3, and DGCR8 [215]. This finding is particularly significant in light of the complex interplay between RNA biogenesis and mt-circRNAs. In another publication, Liu et al. aided in identifying mt-circRNAs and their ability to facilitate the entry of nuclear-encoded proteins (e.g., TOMM40, PNPase) into mitochondria by acting as chaperones [101].

Other mt-circRNAs have been mainly characterised in the pathological context. Meccind1 and meccind5, for example, have been shown to be upregulated in hepatocellular carcinoma [101]. The expression level of meccind 1 increases under environmental stressors such as UV and H_2O_2 exposure, and it is positively associated with mtDNA copy number; by contrast, meccind 5 binds to heterogeneous nuclear ribonucleoproteins, RNA-binding proteins with diverse roles in RNA metabolism and regulation, and promotes their mitochondrial importation [24].

Zhao et al. found that the mitochondrial circRNA mc-COX2 (SCAR) binds to ATP5B, inhibiting the mPTP and thereby reducing oxidative stress. Mitochondrial-targeted delivery of SCAR via mitochondria-targeting nanoparticle mitigated inflammation and liver injury in a nonalcoholic steatohepatitis (NASH) mouse model, highlighting its therapeutic potential. Moreover, fibroblasts from NASH patients exhibit reduced SCAR expression, resulting in increased mitochondrial permeability and ROS production. SCAR plays a role not only in NASH, but also in chronic lymphocytic leukaemia (CLL). In CLL, SCAR is highly enriched in plasma exosomes, and its levels correlate with disease progression and prognosis. This change in SCAR level is mediated by PGC-1α in response to lipid-induced endoplasmic reticulum stress [102]. These findings suggest that SCAR/mc-COX2 may act in retrograde signalling, linking once more, mitochondrial function to nuclear and cellular stress responses [102, 103].

While circRNA is typically non-coding RNA, one group assessed protein-coding potential and found that mecciND1 lacked predicted proteins, while SCAR/mc-COX2, mecciND5, and circMTND5 contained multiple open reading frames (ORFs), many showing similarity to human proteins such as immunoglobulin heavy chain, RyR2, ZCCHC14, and TARM1, highlighting a potential for diverse functional interactions. From the three ORFs of mecciND5, one lacked similarity with human proteins, while two others showed similarity with IgH. The diversity in predicted proteins and their associations with important proteins adds an intriguing layer to these RNAs and potential as retrograde signalling molecules [215].

Retrograde signalling through mitochondrial-derived peptides

Small open reading frames in circular mitochondrial DNA can encode multiple microproteins, known as mitochondrial-derived peptides (MDPs). MDPs are secretory proteins that can interact with diverse extracellular processes [109, 216]. MDPs have garnered considerable attention as potential neuroprotective agents due to their ability to modulate mitochondrial function, reduce oxidative stress, and suppress neuroinflammation [217]. It is unknown whether mitochondrial open reading frame mRNAs are exported intact and translated in the cytosol, or whether translation occurs in the mitochondrion and peptides are exported later on [125]. Nevertheless, studies detecting these peptides in the cytoplasm, the nucleus, and even in serum seem to support the mitochondrial export theory [125].

MDPs can be secreted from mitochondria via mitochondrial derived vesicles (MDVs), exosomes, and the natural degradation of the mitochondrial membrane during mitophagy. Work on MDVs (mito-vesicles) shows that mitochondria can bud small vesicles that traffic specific cargo like, mtRNA, mtDNA, proteins to lysosomes, or be secreted as extracellular vesicles. MDVs are up to 10-fold smaller than mitochondria, have a narrower intermembrane space (~6 nm), and lack several

mitochondrial structures and components normally present in mitochondria, including *cristae* and ribosomes. They may include the OMM, IMM, and matrix, and can be targeted to become multivesicular bodies (MVBs) [218]. MDVs might be released into the extracellular space upon the fusion of MVBs with the plasma membrane [219]. This selective packaging and release process can involve proteins such as SNX9 and OPA1, which facilitate the incorporation of specific cargo, including peptides and mtDNA. Mitochondrial stress further stimulates the release of molecules, such as mtDNA and other damage-associated molecular patterns, which exhibit strong proinflammatory activity. The selectively packaged components targeted for degradation or secretion make MDV a rapid-response mechanism to mitigate mitochondrial stress [220–222].

Metabolites hold the ability to regulate MDV formation: (1) fumarate accumulation, induced MDV formation and release to the cytosol, mtDNA and mtRNA initiating cGAS/STING and RIG-1 signalling inducing inflammation [223]; (2) the ketone body β-hydroxybutyrate (BHB) [224], whose concentration increases during prolonged fasting can also drive a post-translational modification of SNX9, a key regulator of MDV formation, enhances SNX9's interaction with proteins of the IMM and matrix, promoting the formation of MDVs that preferentially sequester and remove damaged mitochondrial components [225]. Previous studies have demonstrated that BHB can enhance mitochondrial function by preventing hyperexcitability, restoring mitochondrial membrane potential, reducing ROS, and improving ATP production [224, 226]. The convergence of metabolites and MDVs biogenesis marks a new frontier in mitochondrial biology. Several publications showed that MDVs are a flexible and evolving system. This opens an area of research that is likely to produce interesting results and offer further insights into how mitochondria facilitate retrograde signalling, tightly regulating mitochondrial biogenesis and cellular function, particularly in the context of MDVs for controlling MDP retrograde signalling [227].

When released, MDPs can initiate retrograde signalling to alter mitochondrial biogenesis in response to stressors. Indeed, control of MDP expression can modulate apoptosis, metabolism, and the oxidative stress response. To date, eight MDPs have been characterised: Humanin, Small Humanin-Like Proteins (SHLPs 1–6), and Mitochondrial Open Reading Frame of the 12S rRNA-c (MOTS-c) (Fig. 5) [109].

Humanin

Humanin was the first biologically active MDP to be discovered, encoded by the mitochondrial 16 S rRNA gene (MT-RNR2) and possessing anti-apoptotic and neuroprotective effects [109, 110], improving insulin sensitivity and preventing oxidative stress damage caused by ischemia/reperfusion [111]. Humanin peptide can be either 21-amino acids in the mitochondria or 24-amino acids in the cytoplasm [112, 113]. Whether it is synthesised inside or outside the mitochondria is still unknown, but both are bioactive and inhibit apoptosis in many diseases, including cancer. It is still unclear which is more common, suggesting unappreciated complexity [111, 228, 229]. When translated in the mitochondria, it produces a 21-amino acid peptide that can bind both intracellularly and to cell membrane receptors like Aβ17–28, FPRL-1/2, CNTF, IGFBP-3, BAX, and tBid [111], enabling interactions with pro-death proteins such as BAX and BAK to block mitochondrial membrane permeabilisation [111].

Humanin engages with extracellular receptors, such as the gp130/WSX1/CNTFR complex and formyl peptide receptor 2 (FPR2), activating survival pathways, including the PI3K/AKT pathway, and suppressing oxidative stress [113]. By activating the PI3K/AKT pathway, it stimulates mitochondrial biogenesis, thereby restoring electron transport chain efficiency and reducing ROS production. Its ability to modulate mitochondrial-nuclear crosstalk, as evidenced by nuclear-encoded Humanin-like genes (MTRNR2L1–MTRNR2L13), suggests a broader role in cellular stress adaptation, though these nuclear variants remain poorly understood [114]. Humanin has been shown to mitigate insulin resistance, reduce oxidative stress, and regulate autophagy in diabetes models, suggesting a role in maintaining metabolic homeostasis [115].

SHLPs

The MT-RNR2 gene also encodes SHLPs, a family of homologous MDPs [144]. These peptides, named SHLP1 through SHLP6, are 20–38 amino acids long and serve as crucial retrograde signalling molecules, promoting communication between mitochondria and the nucleus while affecting cellular processes related to metabolism, apoptosis, and ageing [116]. The functions and believed role of the SHLPs are nicely described in a review by Miller et al. [109]. Among the SHLPs, SHLP2 is the most studied due to its strong neuroprotective effects, particularly in Parkinson's disease.

SHLP2's cytoprotective role and metabolic effects have been investigated in in vitro studies showing that SHLP2 preserves OXPHOS subunit levels by localising to/associating with mitochondrial complex I subunits [117], enhancing mitochondrial respiration and mitochondrial abundance [118]. More recently, in Parkinson's models, a variant of SHLP2 was shown to bind to complex I showing enhanced protection against mitochondrial dysfunction [119]. Another study suggested that SHLP2 regulates apoptosis by interacting with BAX, preventing OMM permeabilisation and subsequent cytochrome

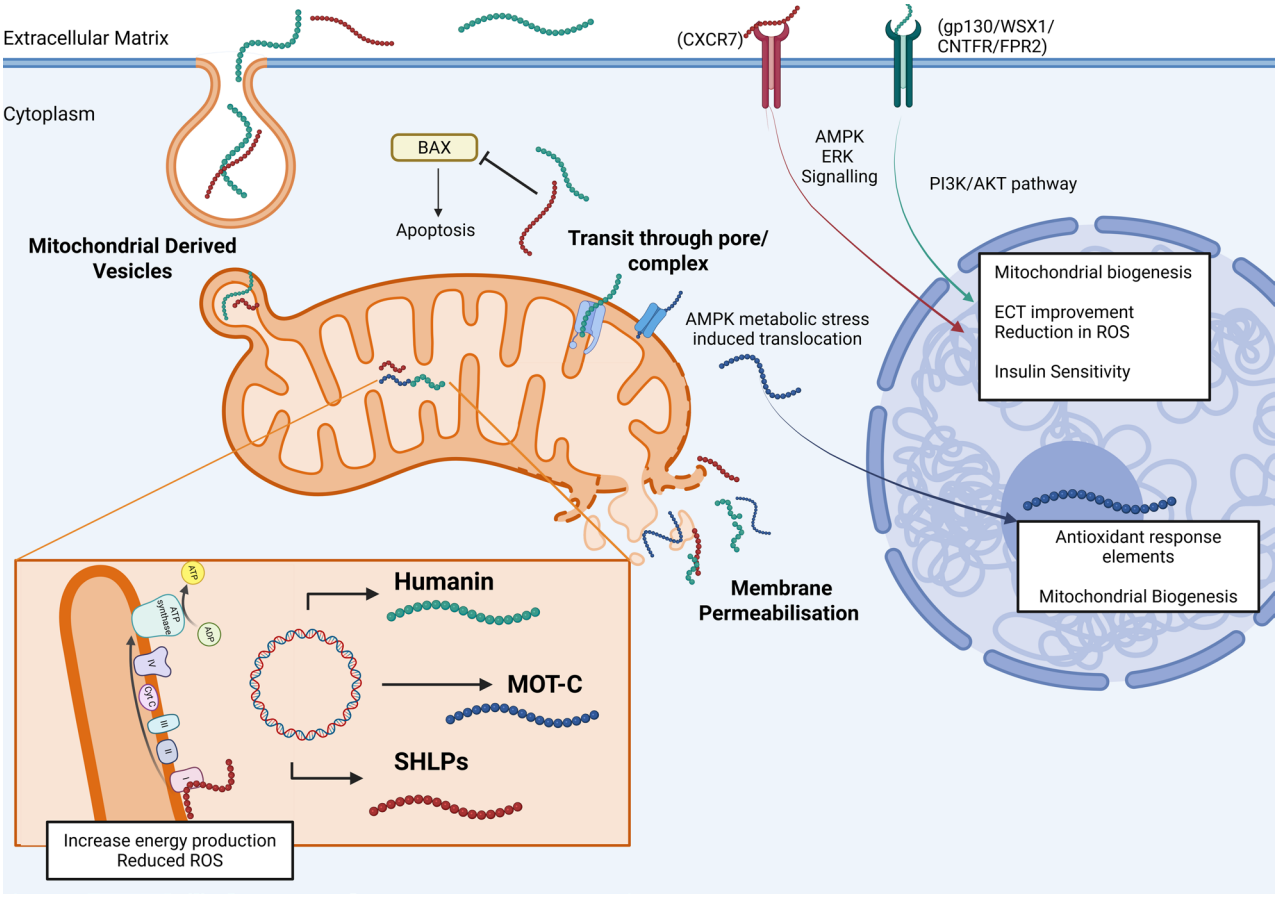

Fig. 5 Mitochondrial-derived peptides in retrograde signalling. mtDNA contains short open reading frames that produce small peptides. These peptides can exit the mitochondria via various pathways, including membrane permeabilisation, fission-fusion mechanisms, vesicular transport, and transport complexes such as VDAC and BAK/BAX allowing these peptides to initiate retrograde signalling by either translocating to the nucleus to modulate mitochondrial biogenesis or can exit the cell by budding into MDVs, and being released to bind to extracellular receptors that initiate signalling pathways such as AMPK, ERK and PI3K pathways that modulate mitochondrial biogenesis, ETC improvement and metabolic alterations. Additionally, MDPs can indirectly initiate retrograde signalling by inhibiting BAK/BAX proteins or binding to complex I of the ETC, thereby affecting mitochondrial homeostasis and altering metabolites that can trigger retrograde responses.

c release, and inhibiting caspase–3–dependent apoptosis [120, 121]. Recently, a pivotal discovery demonstrated that SHLP2 modulates AMPK/ERK signalling in pre-osteoblastic cells to mitigate oxidative stress in the presence of hydrogen peroxide by increasing the expression of antioxidants SOD1/SOD2 and decreasing pro-apoptotic markers BAX and cytochrome c. Not only did SHLP2 mitigate cell death, but it also restored osteogenic functions, as evidenced by the expression of RUNX2 and OSX, demonstrating restoration of osteogenic activity [122]. ERK pathway activation was further demonstrated: when SHLP2 was systemically administered in mice, it bound to CXCR7 in hypothalamic neurons, activated ERK signalling, and subsequently phosphorylated and activated transcription factors that influence energy balance and insulin sensitivity [123, 124]. Collectively, this establishes SHLP2 as a multifaceted regulator of metabolism and oxidative stress, acting as a crucial signal of mitochondrial status.

MOTS-c

MOTS-c (Mitochondrial Open Reading Frame of the 12 S rRNA-c) is an MDP encoded by the mitochondrial 12 S rRNA gene with a role in regulating metabolic processes, maintaining mitochondrial homeostasis, and promoting cellular resilience under stress conditions [126]. Interestingly, circulating levels of MOTS-c decline with age and are associated with reduced mitochondrial efficiency and increased susceptibility to metabolic dysregulation [126]. MOTS-c is unique among mitochondrial-derived peptides as it primarily resides in the mitochondria but, under certain stress conditions, such as metabolic stress, translocates to the nucleus, where it interacts with transcription factors [125, 127, 128], like NRF2 activating ATF1/ATF7, enabling it to regulate mitochondrial biogenesis, ensuring that cells maintain sufficient energy production during metabolic challenges [109]. This nuclear translocation is shown to be AMPK-dependent under metabolic stress and MOTS-c possesses the ability

to bind to NRF2-target genes that contain antioxidant response elements (AREs) and regulate the expression of genes involved in mitochondrial protection, modulating both mitochondrial and nuclear function, highlighting its role as a retrograde signalling molecule, providing an essential link between mitochondrial health and cellular metabolism [125]. All reveal a complex regulatory mechanism of mitochondrial function. However, the mechanism by which MOTS-c mRNA/protein transits from mitochondria to the cytoplasm and nucleus remains unresolved. How MOTS-c selectively regulates specific nuclear genes (beyond ARE-containing genes) remains unclear. In addition to also containing tissue-specific activation, one study found that the SIRT1-PGC-1α pathway also mediates the production and/or secretion of MOTS-c in skeletal muscle [129].

MOTS-c represents a paradigm shift in our understanding of mitochondrial retrograde signalling, revealing that mitochondria are not merely energy producers but active regulators of nuclear gene expression and cellular adaptation. By translocating to the nucleus under stress to modulate antioxidant and metabolic pathways, MOTS-c bridges mitochondrial function with systemic homeostasis [125].

Conclusions

mtDNA plays an emerging and multifaceted role in retrograde signalling, not only as a signalling molecule itself but also through its derived RNA transcripts and peptides, indicating that they are key mediators of inter-organelle communication, metabolic adaptation, and stress responses. Current evidence indicates that mtDNA-driven retrograde signalling contributes to diverse cellular processes, including metabolic reprogramming, modulation of the cell cycle, and activation of innate immune pathways such as the inflammasome.

Despite these advances, many fundamental questions remain unanswered. How are mtDNA, mitochondrial RNAs, and peptides selectively released under stress conditions, and what regulatory mechanisms control their synthesis, stability, and transport? The spatial control of their transcription and translation, whether occurring inside or outside the mitochondria, remains poorly defined, as does the machinery governing their export. While the mPTP, BAX/BAK macropores, and other membrane permeabilisation events are hypothesised to mediate this release, the very existence and nature of some of these channels remain debated.

MDPs are emerging as key modulators of cellular resilience, but the mechanisms governing their expression, processing, and translocation remain largely unresolved. Current evidence suggests that mitochondrial stress and metabolic alterations can induce MDPs production. Notably, one study reported that increased MOTS-c expression correlates with changes in mtDNA methylation during cellular senescence, highlighting the possibility of epigenomic regulation within mitochondria [230]. It is also well recognised that mitochondrial RNA metabolism is tightly regulated, and features such as tRNA modifications, ribosome biogenesis, and unique aspects of mitochondrial translation could provide additional layers of control over MDPs synthesis, although direct experimental confirmation is still lacking. Furthermore, the mitochondrial genome is known to contain multiple nested short open reading frames (sORFs) within its polycistronic transcripts, raising the hypothesis that previously unannotated ORFs may be conditionally translated under stress. While ribosome-profiling studies have hinted at the existence of such novel mitochondrial microproteins, their functional and physiological significance remains to be established [231]. Continued research into these regulatory mechanisms will be essential to fully elucidate how MDPs are generated and how they exert their diverse roles in health, aging, and disease.

However, several key questions remain about mt-ncRNAs. It is still unclear how these RNAs are synthesised, how their biogenesis, processing, and degradation are regulated, which factors control their expression, turnover, and tissue-specific distribution, and how they respond to intracellular and intercellular signals [24].

Elucidating how these molecules traverse mitochondrial membranes, whether through transient permeabilisation, vesicular transport, or yet-undiscovered translocases, will be critical for understanding the full scope of mitochondrial–nuclear communication. The ongoing research into MDPs and non-coding RNAs will deepen our understanding of the intricate regulatory networks that connect mitochondrial function with nuclear gene expression, ultimately offering new insights into cellular homeostasis and potential therapeutic targets in metabolic, inflammatory, and degenerative diseases.

Abbreviations

AGO	Argonaute
AIF	Apoptosis-Inducing Factor
AMPK	AMP-Activated Protein Kinase
ARE	Antioxidant Response Element
BAX	Bcl-2-Associated X Protein
BHB	Beta-Hydroxybutyrate
cGAS	Cyclic GMP-AMP synthase
CHOP	C/EBP Homologous Protein
circRNA	Circular RNA
CLL	Chronic Lymphocytic Leukemia
dsRNA	Double stranded RNA
DELE1	Death Ligand Signal-Enhancing Factor 1
ECT	Electron Transport Chain
ER	Endoplasmic Reticulum
Fpr2	Formyl Peptide Receptor 2
GADMD	Gasdermin D
IMM	Inner Mitochondrial Membrane
IMS	Inter Membrane Space
ISR	Integrated Stress Response
α-KG	α-Ketoglutarate

LC3B	Microtubule-associated protein 1 light chain 3 beta
LECA	Last Eukaryotic Common Ancestor
lnc-RNA	Long Non-Coding RNA
MDP	Mitochondrial Derived Peptide
MDV	Mitochondrial-Derived Vesicles
miRNA	Micro RNA
MPC1	Mitochondrial Pyruvate Carrier 1
mPTP	Mitochondrial Permeability Transition Pore
MTFP1	Mitochondrial Fission Process 1
mtProt	Mitochondrial Protease
mt-ncRNA	Mitochondrial Non-Coding RNA
MOTS-c	Mitochondrial Open Reading Frame of the 12S rRNA-c
MSR	Mitochondrial Stress Response
mt-circRNAs	Mitochondrial Circular RNAs
mtDAMPs	Mitochondrial Damage-Associated Molecular Patterns
mtDNA	Mitochondrial DNA
mt-dsRNAs	Mitochondrial Double-Stranded RNAs
mt-tFRs	Mitochondrial tRNA-Derived Fragments
mt-tRNA	Mitochondrial tRNAs
MVB	Multivesicular Bodies
NASH	Non-Alcoholic Steatohepatitis
ncRNA	Non-Coding RNA
NUMTs	Nuclear Mitochondrial DNA Segments
OMM	Outer Mitochondrial Membrane
ORF	Open Reading Frame
OXPHOS	Oxidative Phosphorylation
PDC	Pyruvate Dehydrogenase Complex
PKM2	Pyruvate Kinase
ROS	Reactive Oxygen Species
rRNA	Ribosomal RNA
SCAR	Steatohepatitis-associated circRNA ATP5B Regulator
SHLPs	Small Humanin-Like Proteins
siRNA	Small Interfering RNA
sncRNAs	Small Non-Coding RNAs
SOD	Superoxide Dismutase
STING	Stimulator of Interferon Genes
TCA	Tricarboxylic Acid Cycle
TFAM	Transcription Factor A
TME	Tumour Microenvironment
TNFα	Tumor Necrosis Factor Alpha
tRNA	Transfer RNA
UPR^{mt}	Mitochondrial Unfolded Protein Response
VDAC	Voltage-Dependent Anion Channel

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E.H. conceived the review, drafted the manuscript and prepared the images. V.B. contributed to critical revision and intellectual input. C.V. conceived the review, supervised the work, and revised the manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

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The authors declare no competing interests.

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