# Beyond ATP, new roles of mitochondria

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Mitochondria, special double-membraned intracellular compartments or 'organelles', are popularly known as the 'powerhouses of the cell', as they generate the bulk of ATP used to fuel cellular biochemical reactions. Mitochondria are also well known for generating metabolites for the synthesis of macromolecules (e.g., carbohydrates, proteins, lipids and nucleic acids). In the mid-1990s, new evidence suggesting that mitochondria, beyond their canonical roles in bioenergetics and biosynthesis, can act as signalling organelles began to emerge, bringing a dramatic shift in our view of mitochondria's roles in controlling cell function. Over the next two and half decades, works from multiple groups have demonstrated how mitochondrial signalling can dictate diverse physiological and pathophysiological outcomes. In this article, we will briefly discuss different mechanisms by which mitochondria can communicate with cytosol and other organelles to regulate cell fate and function and exert paracrine effects. Our molecular understanding of mitochondrial communication with the rest of the cell, i.e. mitochondrial signalling, could reveal new therapeutic strategies to improve health and ameliorate diseases.

## Introduction

According to a leading endosymbiotic theory, more than 1.45 billion years ago, two prokaryotes – an archaeon and an α-proteobacterium – developed a mutually beneficial or 'symbiotic' biological relationship to support each other's nutritional requirements. Eventually, the archaeon acquired the α-proteobacterium that became the primordial mitochondrion. While the underlying mechanisms that explain the symbiosis between the α-proteobacteria and the archaea are not well understood, current data indicate that the exchange of metabolites between the host archaea and the endosymbiont α-proteobacteria forms the basis of this symbiosis. Over time, the endosymbiont passed most of its DNA to the host nucleus, keeping only some of its genes. To date, mitochondria are ubiquitous among all eukaryotic lineages, and continue to exchange metabolites with the cytosol constantly.

Richard Altmann, a German pathologist and histologist, first recognized the ubiquitous occurrence of these structures that looked like mitochondria and coined the name 'bioblasts' for them in 1890. In 1898, Carl Benda introduced the name 'mitochondria' (from Greek 'mitos': thread and 'chondros': granule) for these structures, referring to their appearance during spermatogenesis. Mitochondria are double-membrane-bound organelles with an outer membrane facing the cytosol and an inner membrane with cristae folds protruding into the matrix. The bulk of the electron transport chain (ETC) complexes localizes to the cristae, and tricarboxylic acid (TCA) cycle enzymes and mitochondrial genome localize to the matrix.

Extensive research over the next eight decades established two salient functions of mitochondria – production of ATP, the energy currency of the cells, and generation of biosynthetic intermediates (Figure 1). The citric acid cycle, also referred to as the TCA cycle, occurs in the mitochondrial matrix of eukaryotic cells, which can oxidize glycolysis-derived pyruvate, fatty acids, and amino acids and generate metabolic intermediates and reducing equivalents (e.g., NADH and $FADH_2$). These reducing equivalents feed electrons into the ETC, which pumps protons across the mitochondrial inner membrane to generate an electrochemical gradient that is necessary both to produce ATP through oxidative phosphorylation and to shuttle proteins into and out of the mitochondria. Mitochondria supply the bulk of the cellular ATPs and help cells maintain a high ATP/ADP ratio necessary to thermodynamically drive many biochemical reactions and, therefore, are popularly known as the 'powerhouses of the cell'. In addition, mitochondrial metabolic intermediates enter different biosynthetic pathways to generate many important macromolecules such as carbohydrates, lipids, proteins and nucleotides. Importantly, mitochondria are necessary for iron–sulphur cluster and heme synthesis. Our data, and that of others, point to the necessity of the biosynthetic functions of mitochondria to support the proliferation of cancer cells.

By 1990, many of the functional properties of mitochondria were discovered, and it was generally thought there was not much left to study in the field of



 

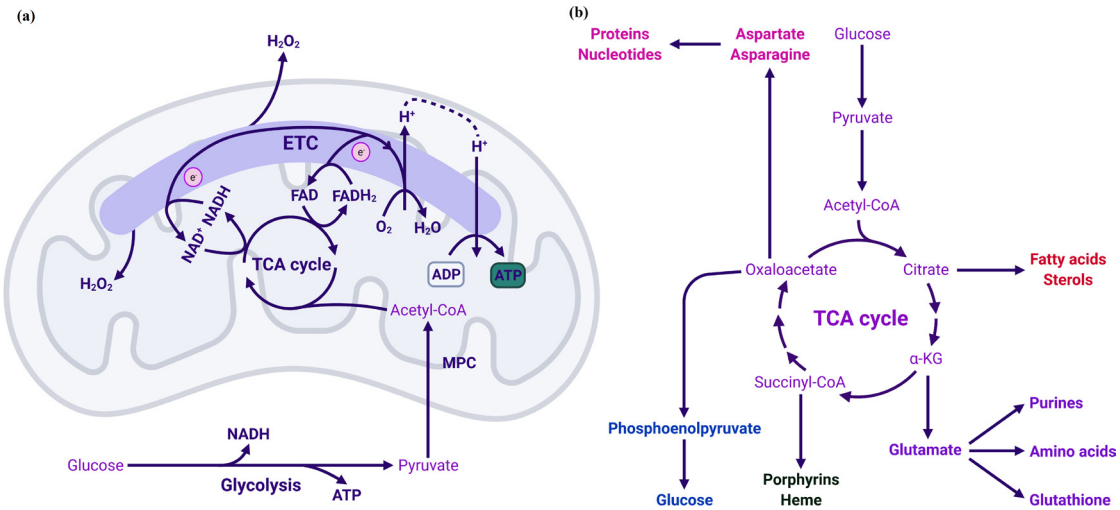


**Figure 1.** Mitochondria as bioenergetic and biosynthetic organelles. Mitochondria generate ATP through oxidative phosphorylation (a) and TCA cycle metabolites to support macromolecule synthesis for biomass production (b).

mitochondrial biochemistry. Much of the focus was on the burgeoning field of mitochondrial genetics. A key observation that showed mitochondria are not simply autonomous organelles but are responsive to cytosolic signals was by Rosario Rizzuto and colleagues, who observed that changes in cytosolic calcium levels could elicit changes in mitochondrial calcium. Subsequently, a 'breakthrough experiment' reported in 1996 by Xiaodong Wang's lab at Emory University School of Medicine demonstrated that mitochondria release cytochrome *c*, a key component of the ETC involved in oxidative phosphorylation, to induce a distinct form of programmed cell death, i.e., apoptosis. Thus, the fact that cytochrome *c* can act as a signalling molecule, beyond its role in the ETC, sparked a new interest among scientists in investigating other mitochondrial signalling mechanisms that dictate physiological and pathophysiological outcomes. Studies from multiple groups over the past two and half decades have firmly established mitochondria's roles as signalling organelles (Figure 2). The communication between mitochondria and cytosol can be both ways: the signal transduction

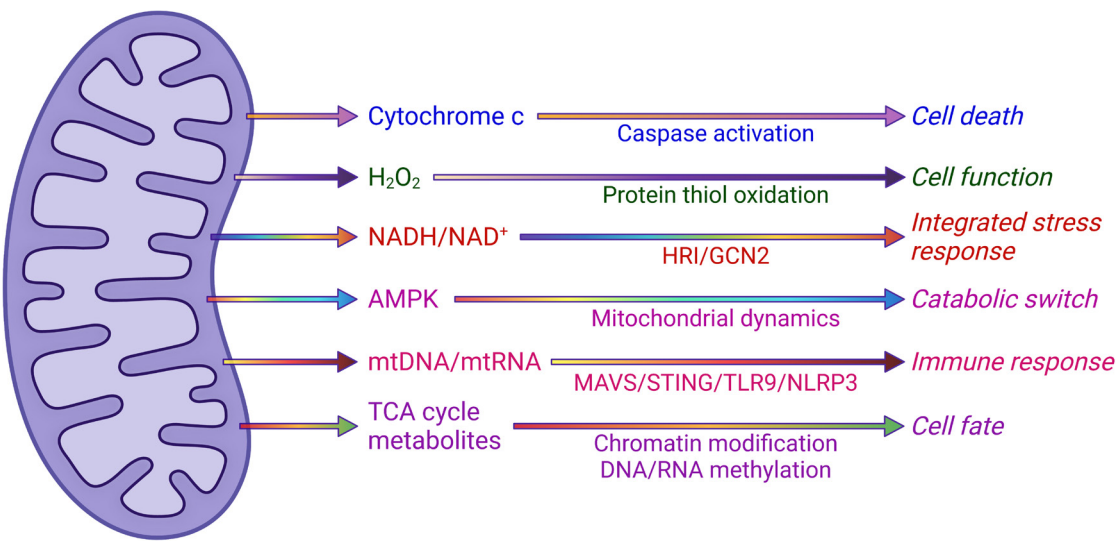


**Figure 2.** Mitochondria as signalling organelles. Mitochondrial reactive oxygen species (ROS), metabolites, nucleic acids, proteins and peptides, NADH/$NAD^+$ ratio and morphological dynamics can act as signals to regulate various cellular processes. AMPK: AMP-activated protein kinase; HRI: heme-regulated inhibitor; GCN2: general control nonderepressible 2; MAVS: mitochondrial antiviral-signalling protein; STING: stimulator of interferon genes; TLR9: toll-like receptor 9; NLRP3: NOD-, LRR- and pyrin domain-containing protein 3.

from mitochondria to cytosol is known as retrograde signalling, whereas the signal transduction from cytosol to mitochondria is known as anterograde signalling. In the following few sections, we will give a brief account of different retrograde signalling mechanisms by which mitochondria can communicate with the cell and thereby regulate physiological and pathophysiological outcomes.

## Mitochondrial reactive oxygen species (ROS)-mediated signalling

A by-product of cellular respiration is the generation of superoxide by the ETC. Immediately after its generation, superoxide dismutases (SODs) reduce superoxide into more stable and membrane-permeable $H_2O_2$ that leaks from mitochondria into the cytosol. $H_2O_2$ can serve as a signalling molecule by oxidizing specific sulphur-containing amino acids, i.e., cysteine and methionine, that are critical for the function, stability and subcellular localization of a protein. Inspired by Xiaodong Wang's cytochrome *c* finding, we showed in 1998 that the release of mitochondrial ROS is necessary for hypoxia-induced gene transcription. Subsequently, our ongoing works as well as others have demonstrated that mitochondria-generated $H_2O_2$ can control diverse physiological processes and diseases, including immunity, exercise, development, thermogenesis, oxygen sensing, cancer, ischemia-reperfusion, neurodegeneration and fibrosis. It is likely that $H_2O_2$ invokes beneficial physiological responses, whereas its ability to generate toxic hydroxyl radicals and lipid hydroperoxides triggers diseases. The specific cysteine or methionine targets of $H_2O_2$ within proteins, which are relevant for physiology or disease, are not yet fully deciphered.

## Mitochondrial metabolites as signalling molecules

Several mitochondrial metabolites can act as signalling molecules by regulating the activities of different cytosolic and nuclear enzymes. For instance, nucleic acid (i.e., DNA and RNA) methylation, and histone methylation and acetylation, which control gene expression, can be regulated by mitochondrial metabolites. Mitochondrial one-carbon metabolism contributes to the production of *S*-adenosyl methionine (SAM), a substrate for histone, DNA and RNA methyltransferases. The TCA cycle metabolite citrate can be exported into the cytosol and converted into acetyl-CoA, a substrate for histone acetylation. α-Ketoglutarate (α-KG), another TCA cycle metabolite, is a substrate for a wide range of dioxygenase enzymes that regulate different cellular functions. Some examples of α-KG-dependent dioxygenases include nucleic acid demethylases and Jumonji-C domain-containing histone demethylases that remove methyl groups from nucleic acids and histones, respectively, and prolyl hydroxylases that regulate hypoxic response. In contrast, succinate, fumarate and 2-hydroxyglutarate (2-HG) are competitive inhibitors of these dioxygenases. As a result, cells can employ the ratio of α-KG to these metabolites to communicate between mitochondria and cytosol/nucleus. How metabolites find their specific loci to modulate gene expression is not known.

## NADH/NAD$^+$ can relay signals from mitochondria

Mitochondrial complex I of the ETC regenerates NAD$^+$ through oxidizing NADH. Cells have different mechanisms of sensing a change in cellular as well as mitochondrial NADH/NAD$^+$ ratio. For instance, cells have a family of NAD$^+$-dependent deacylase enzymes, known as sirtuins, that remove various acyl groups (e.g., acetyl, succinyl, malonyl, glutaryl, or long-chain acyl groups) from proteins and thereby regulate their activities. Mammals have seven sirtuins (SIRT1–SIRT7) that localize in specific subcellular compartments. SIRT1 and SIRT2 localize in the cytosol or nucleus; SIRT6 and SIRT7 localize in the nucleus; and SIRT3, SIRT4 and SIRT5 localize in the mitochondrial matrix. An increase in NADH/NAD$^+$ ratio can inhibit nuclear SIRT1 to control fatty acid oxidation and amino acid catabolism during caloric restriction in mice. There is much interest in manipulating the NADH/NAD$^+$ ratio to improve health and ameliorate diseases.

NADH/NAD$^+$ can also directly dictate cell fate and function by regulating the L-2-HG/α-KG ratio. Hypoxia, acidity or impairment of mitochondrial ETC decreases NAD$^+$ regeneration and increases NADH/NAD$^+$, which induces an increase in L-2-HG levels. Mitochondrial and cytosolic malate dehydrogenases (MDH1 and MDH2, respectively) and lactate dehydrogenases (LDHA and LDHC) can use α-KG as a promiscuous substrate and NADH as a coenzyme to produce L-2-HG. Being structurally like α-KG, L-2-HG can competitively inhibit α-KG-dependent dioxygenases, including PHD2 (prolyl hydroxylase domain-2), nucleic acid demethylases and Jumonji C domain-containing histone demethylases. PHD2 is a key regulator of hypoxia-inducible factor (HIF)-dependent hypoxic response, and nucleic acid and histone demethylases regulate gene expression. We have demonstrated that an increase in mitochondrial NADH/NAD$^+$ triggers L-2-HG accumulation, causing an impairment of regulatory T cells (Tregs) concomitant with widespread autoimmunity due to DNA hypermethylation.



 

Mitochondrial NADH/$NAD^+$ ratio can also trigger the activation of the mammalian integrated stress response (ISR) through GCN2 (general control nonderepressible 2)-dependent phosphorylation of eIF2α (eukaryotic translation initiation factor two subunit α). This results in inhibition of global protein translation but enhances the translation of selective genes as an adaptive mechanism to counter metabolic stress. These include transcription factors (activating transcription factor 4 (ATF4) and ATF5) which increase the expression of genes involved in metabolism. An alternative mechanism involves an OMA1-DELE1-HRI pathway to trigger ISR. In response to mitochondrial stress such as ATP synthase inhibition or severe depolarization, OMA1, a protease localized in the mitochondrial inner membrane, cleaves DELE1 (DAP3-binding cell death enhancer 1), a protein also localized in the mitochondrial inner membrane. Cleaved DELE1 then leaves mitochondria and accumulates in the cytosol, where it physically interacts with HRI (heme-regulated inhibitor) and activates its eIF2α kinase activity to promote ATF4 translation downstream of eIF2α phosphorylation. ISR activation is linked to primary mitochondrial diseases. However, whether this is a beneficial or maladaptive response is not fully understood.

## Mitochondrial DNA and RNA can activate signalling cascades leading to immune response

Mitochondrial DNA (mtDNA) and RNA (mtRNA) can leak into the cytosol and induce immune response through different signalling cascades. Mitochondrial DNA, by mechanisms which are not yet fully defined, can leak into the cytosol where they act as damage-associated molecular patterns (DAMPs) to activate the cGAS-STING (cyclic GMP-AMP synthase-stimulator of interferon genes) pathway, which induces transcription of genes encoding type I interferons such as interferon-β (IFNβ), proinflammatory cytokines such as interleukin 6 (IL-6) and tumour necrosis factor (TNF). For example, herpes simplex virus infections can elicit mtDNA release to boost antiviral immune response due to increased IFNβ. As mitochondria are descendants of endosymbiotic bacteria and have a circular genome, mtDNA is subjected to bidirectional transcription, which generates highly unstable long mitochondrial dsRNAs (mtdsRNAs) consisting of heavy (H) and light (L) strand-encoded RNAs. Usually, RNA degradosome rapidly degrades the L-strand-encoded RNA. However, any defect in RNA degradosome formation leads to excessive cytosolic accumulation of mtdsRNAs, which resembles the dsRNA marker of viral replication and thereby triggers type I interferon response. Of note, mitochondrial antiviral-signalling protein (MAVS), which is primarily localized on the mitochondrial outer membrane, acts as a signalling hub for dsRNA-induced interferon-dependent immune response. Key unanswered questions include the following: (1) How is mtDNA or mtRNA released into the cytosol to find their respective immune receptors? (2) Why does MAVS require mitochondrial outer membrane localization for optimal function?

## Mitochondrial dynamics regulate cell fate and function

Mitochondria are highly dynamic organelles that continuously undergo opposing processes of fusion (the union of two mitochondria into one) and fission (the division of one mitochondrion into two), often referred to as 'mitochondrial dynamics'. Three large GTPases, mitofusin 1 (MFN1), mitofusin 2 (MFN2) and optical atrophy 1 (OPA1), orchestrate the mitochondrial fusion in mammalian cells. MFN1 and MFN2 localize to the mitochondrial outer membrane, and OPA1 localizes to the mitochondrial inner membrane. In contrast, the cytosolic protein dynamin-related protein 1 (DRP1) translocates to the outer mitochondrial membrane upon activation to trigger mammalian mitochondrial fission. Mitochondrial dynamics also encompass cristae remodeling, biogenesis and mitophagy, and have been linked to apoptosis as well as stem cell, neuronal cell, and T cell function. Mitochondrial dynamics change the mitochondrial size, shape, distribution and the relative mitochondrial volume occupied by the cristae vs matrix and thereby can regulate energy production, macromolecule synthesis, $Ca^{2+}$ signalling and redox and metabolite signalling in response to cellular stress or nutritional availability (Figure 3). For instance, when cells are deprived of nutrients, they activate AMPK (AMP-activated protein kinase), a crucial cellular energy sensor, due to elevated levels of AMP compared to ATP to promote fission and cause the destruction of defective mitochondria through mitophagy.

## Mitochondrial interaction with other organelles regulates cell fate and function

Mitochondria physically contact several organelles such as endoplasmic reticula (ER), lysosomes, Golgi apparatus, peroxisomes and lipid droplets (Figure 4). Mitochondria–ER contacts regulate $Ca^{2+}$ signalling, redox signalling, mitochondrial dynamics and quality control, lipid metabolism and unfolded protein response. Similarly, mitochondria–lysosome contacts regulate

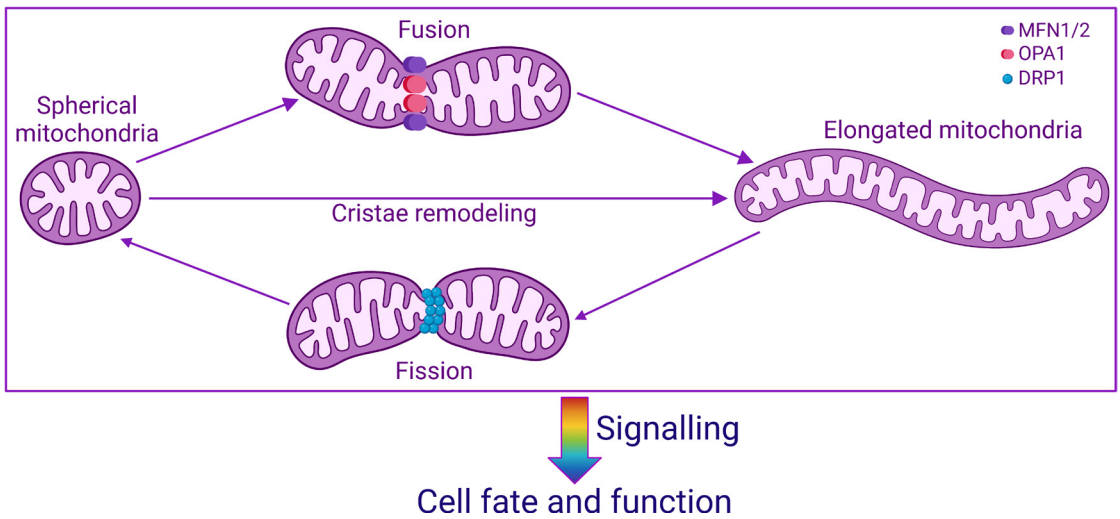


**Figure 3.** Mitochondrial dynamics dictate cell fate and function. Mitochondria are morphologically very dynamic. They can relay signals to other parts of the cells by changing their shapes through cristae remodeling as well as the opposing fusion and fission processes to dictate cell fate and function. MFN1/2: mitofusin 1/2; OPA1: optic atrophy-1; Drp1: dynamin-related protein 1.

mitochondrial and lysosomal dynamics and $Ca^{2+}$ signalling, and this is disrupted by mutations that are linked to Parkinson's disease. While there is evidence of physical contact between mitochondria and peroxisome, lipid droplets and Golgi apparatus, the cellular processes regulated by these interactions are yet to be fully deciphered. Moreover, the machinery that specifically tethers these organelles is not fully understood.

## Mitochondria-dependent paracrine signalling

Mitochondrial stress often induces cells to release soluble molecules, such as metabolites (e.g., succinate), proteins (FGF-21, GDF15) or peptides (MOTS-c, Humanin), that often act on other cells or tissues in a paracrine fashion to trigger a systemic response. These signalling molecules are also referred to as 'mitokines'. For instance, succinate, a TCA cycle metabolite that can act as an intracellular signalling molecule, can also act as an extracellular signalling molecule to regulate

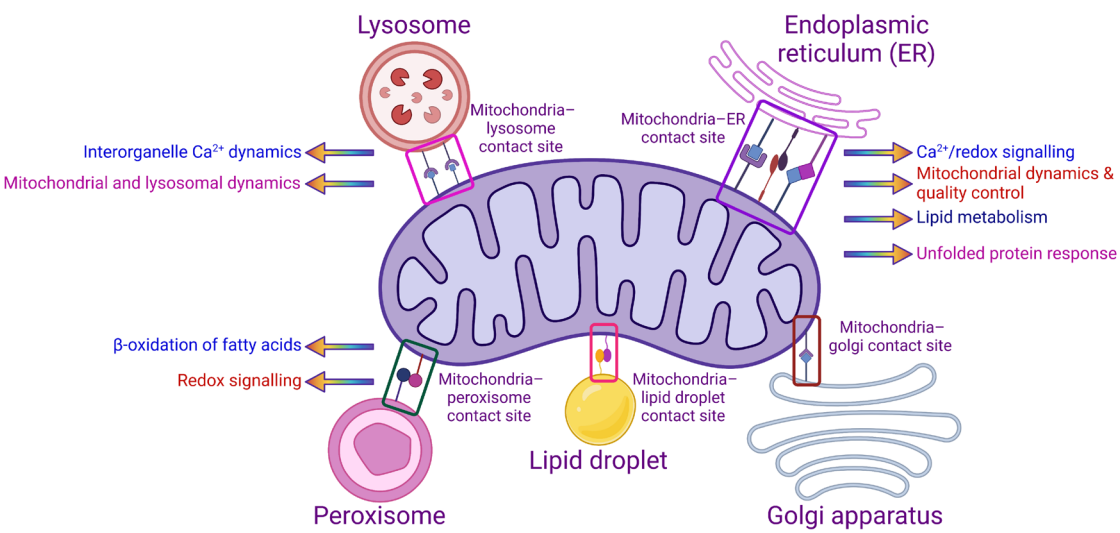


**Figure 4.** Mitochondria–organelles interactions regulate different cellular processes. Mitochondria can communicate with other cellular organelles by forming physical contacts to regulate various cellular processes.



 

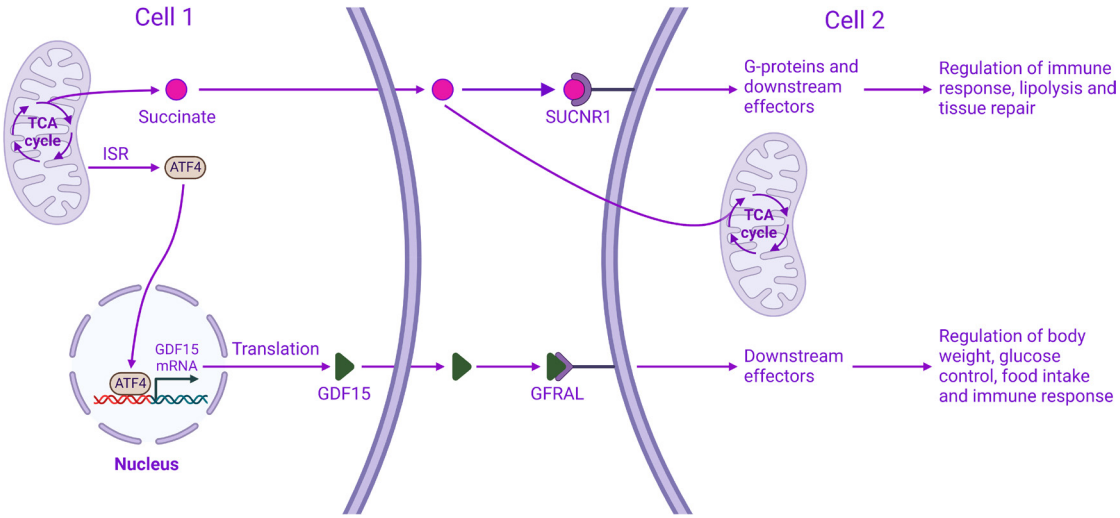


**Figure 5.** Mitochondrial signalling can systemically regulate physiological and pathological processes. Mitochondrial stress can induce a cell to release signalling molecules dubbed 'mitokines' (e.g., succinate and GDF15) that often act on other cells in a paracrine manner to dictate physiological and pathological outcomes. ISR: integrated stress response; ATF4: activating transcription factor 4; GDF15: growth differentiation factor 15; SUCNR1: succinate receptor 1; GFRAL: GDNF (glial cell line-derived neurotrophic factor) family receptor α-like.

immune response, lipolysis and tissue repair by binding to a G-protein-coupled receptor, SUCNR1 (succinate receptor 1), on target cells and activating G-proteins and downstream effectors (Figure 5). Similarly, GDF15 is released from cells in response to mitochondrial stress, binds to GFRAL (GDNF (glial cell line-derived neurotrophic factor) family receptor α-like) receptors on target cells and activates downstream effectors to regulate body weight, food intake, glucose metabolism and immune response (Figure 5).

## Conclusion

The past century established mitochondria as bioenergetic and biosynthetic organelles that support cell survival and biomass production, respectively. Much of the textbooks display the fate of carbon molecules through intermediary metabolism. Since the dawn of this century, much interest in mitochondria has been around their roles as signalling organelles in controlling physiology and diseases. Although the conceptual framework of mitochondria as signalling organelles has been established, there is a much-needed understanding of the detailed mechanisms. The hope is that new targets will be emerging to ameliorate diseases linked with mitochondria, such as Parkinson's disease, primary mitochondrial diseases and inflammation-associated diseases, as molecular details underlying mitochondria as signalling organelles paradigm are elucidated. ▪

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