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Mechanisms of Mitochondrial Respiratory Adaptation

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Abstract

Mitochondrial energetic adaptations encompass a plethora of conserved processes that maintain cell and organismal fitness and survival in the changing environment by adjusting respiratory capacity of mitochondria. These mitochondrial responses are governed by general principles of regulatory biology exemplified by changes in gene expression, protein translation, protein complex formation, transmembrane transport, enzymatic activities and metabolite levels. These changes can promote mitochondrial biogenesis and membrane dynamics that in turn support mitochondrial respiration. Main regulatory components of mitochondrial energetic adaptation include the transcription coactivator PGC1α and associated transcription factors, mTOR and ER stress signalling, TOM70-dependent mitochondrial protein import, cristae remodelling protein complexes MICOS and OPA1, lipid remodelling, and the assembly and metabolite-dependent regulation of respiratory complexes. These adaptive molecular and structural mechanisms increase respiration to maintain basic processes specific to cell types and tissues. Failure to execute these regulatory responses cause cell damage and inflammation or senescence, compromising cell survival and the ability to adapt to energetically demanding conditions. Thus, mitochondrial adaptive cellular processes are important for physiological responses including to nutrient availability, temperature, and physical activity, and their failure leads to pathologies associated with mitochondrial dysfunction such as metabolic and age-associated diseases and cancer.

Introduction

All biological processes are subject to general regulatory principles that include transcription, translation, post-translational and metabolite-dependent activity that define adaptive (acute or chronic) responses. These dynamic responses in turn are sustained and/or attenuated through feedback or feed forward mechanisms that ultimately dictate cellular health and fitness. Functional adaptations of mitochondria do not escape these principles. Carbohydrates, amino acids, and lipids are imported into the cell and delivered to mitochondria, where biosynthetic reactions occur and ATP is generated using the respiratory

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chain in a process referred to as oxidative phosphorylation (OXPHOS). Consequently, mechanisms of crosstalk between the mitochondria and nucleus or mitochondria and cytosol evolved to adapt cells, tissues, and organisms to energetically demanding conditions present during physiological or disease-associated responses.

Mitochondrial respiration is supported by the assembly of respiratory complexes(Box 1), and is coupled to the breakdown of metabolic fuels, generation of biosynthetic intermediates, mitochondrial redox, and metabolite import/export[12]. Mitochondrial respiratory complexes I-V are multimeric protein complexes that reside in inner mitochondrial membrane invaginations termed cristae **[G]**. For complexes I, III, IV, and V, assembly requires the prior synthesis of subunits encoded by both the nuclear and mitochondrial genomes. The proton gradient across the inner mitochondrial membrane generated by the proton pumping activity of complexes I, III, and IV is paired to phosphorylation of ADP to ATP at complex V[3]. In thermogenic brown and beige adipose tissues **[G]**, complex V is not highly represented and instead, futile cycles of electron transport[4,5] dissipate the proton gradient to promote thermogenesis rather than ATP production[6,7]. In this regard, brown adipose tissue mitochondria are very unique and also exhibit extremely high mitochondrial content and cristae density, and are characterized by expression of uncoupling protein 1 **[G]**[6]. A byproduct of normal respiratory metabolism exacerbated under certain stimuli such as mitochondrial dysfunction, ischemia-reperfusion events[8,9], and cold exposure in brown adipose tissue[10], among others, are reactive oxygen species (ROS). These species contribute to signalling events from the mitochondria to the cytosol or nucleus but, if unchecked, may also damage cellular structures including DNA. Aside from their catabolic functions, mitochondria are central regulators of cell death[11], govern cellular Ca^{2+} levels through sequestration and release[12], and synthesize certain molecules such as fatty acids, amino acids, nucleotides, haem **[G]**, and iron-sulfur clusters **[G]**[13]. Mitochondria are therefore fundamental to cellular metabolism and physiology, and not surprisingly, are downstream targets for cellular responses and adaptations that control their activities.

Cristae provide proper spatial distribution to the respiratory chain complexes[1]. These structures originate from inner mitochondrial membrane protuberances and elongate inside the mitochondrial matrix[14], which is mediated by a multi-subunit complex in the inner mitochondrial membrane known as the mitochondrial contact site and cristae organizing system (MICOS)[14]. Mitochondrial dynamin like GTPase OPA1 (refs.[15,16]) and phospholipids[17,18] transported from other organelles or synthesized within mitochondria also participate in cristae biogenesis. The dynamic remodelling of cristae architecture[19] adapts to cellular metabolic requirements and synchronizes with the respiratory chain to increase the respiratory capacity of cells[20,21]. The control of mitochondrial cristae dynamics is exerted by the endoplasmic reticulum (ER), which senses cellular metabolic and energetic fluctuations and triggers downstream stress responses[21]. Physical contacts known as mitochondrial–ER contacts (MERCs) create a unique environment of protein–protein interactions to regulate cristae formation in addition to other essential processes of mitochondrial dynamics such as fission, fusion or mitophagy[22]. ER stress **[G]** promotes cristae formation through processes that coordinate mitochondrial protein import[21], lipid

synthesis[18], and mitochondrial respiratory complex assembly[20] to sustain mitochondrial function, cell adaptation and survival.

Cold exposure, dietary nutrients, or physical activity are sensed at the organismal and cellular level activating hormonal, metabolite, or protein signalling that control mitochondrial-linked gene transcription and mRNA translation to enhance respiratory capacity (Fig. 1). This is coupled with the assembly and activity of new, functional respiratory complexes within mitochondrial cristae. In some cases, such as cold exposure in brown adipocytes[23,24] or exercise in skeletal muscle[25,26], this adaptation is linked with mitochondrial biogenesis that involves the synthesis and incorporation of new mtDNA, protein, and membrane into pre-existing mitochondria, thereby increasing mitochondrial mass. Mechanisms of cross-compartment translational control also exist to synchronize synthesis of nuclear-encoded respiratory chain subunits with mitochondrial-encoded subunits to ensure proper stoichiometry of respiratory complexes and preserve mitochondrial protein homeostasis[27–31]. Membrane synthesis, membrane dynamics (fission and fusion) further contribute to the organization of mitochondrial membranes and the embedded protein complexes.

In this Review, we discuss the different regulatory mechanisms that modulate mitochondrial respiration to facilitate cellular adaptation to different metabolic and growth conditions. In particular, we focus on transcriptional, translational, post-translational and metabolite control mechanisms that promote mitochondrial respiratory chain assembly and cristae biogenesis under physiological conditions of high energy demand. Throughout, we highlight the main cellular components that execute these regulatory mechanisms and their effects on cellular and organismal physiology.

Transcriptional regulation mechanisms

Except for a few mitochondrial proteins and RNAs encoded by the mitochondrial DNA, the rest of proteins in this organelle are synthesized from mRNAs produced from the nuclear DNA[32]. Activation of transcriptional programs supply mitochondrial proteins that increase mitochondrial biogenesis and respiratory capacity[33]. The most studied transcriptional axis that controls mitochondrial respiratory adaptation in mammals are the gene expression networks centred on PGC1α coactivator, which are active in different energy-demanding conditions such as cold[23], physical activity[34,35], perinatal cardiac adaptation[36], brain dopaminergic activity[37,38], or kidney energetic demands[39], among others (Fig. 2). How exactly PGC1α is able to sense the different signals required for these various responses is not known. One model is that PGC1α-interacting factors activated by specific stimuli provide context-dependent, cellular adaptations including mitochondrial biogenesis and upregulation of large sets of nuclear-encoded mitochondrial genes involved in specific metabolic, energetic, and structural functions[40,41]. In addition, PGC1α itself is regulated at the mRNA and protein level (see following section) and by several post-translational modifications such as phosphorylation and acetylation that occur in response to the different signals[42].

PGC1α is highly expressed in energy-demanding tissues such as liver, brown adipose tissue, kidney, heart or skeletal muscle[23]. It associates with transcription complexes linked to different sets of processes regulating mitochondrial function including mitochondrial DNA replication[43], transcription[23], translation[44], OXPHOS[24], haem biosynthesis[45], iron-sulfur clusters biogenesis[33], TCA cycle[46], protein import[47,48], and metabolite trafficking[49]. The mechanism of action of PGC1α is through physical interaction with transcription factors (including nuclear respiratory factors (NRF1 and NRF2)[24], oestrogen-related receptors (ERRs)[50], Yin Yang 1 (YY1)[51], and peroxisome proliferator-activated receptors (PPARs)) that bind to promoter regions and activate mitochondrial genes promoting mitochondrial biogenesis (Fig. 2)[23]. The magnitude of PGC1α-mediated gene activation depends on additional recruitment of chromatin remodelling factors including the p300/CBP acetyl transferase[52] or the mediator complex **[G]**[53]. Of note, although PGC1α is an important component of stress-induced mitochondrial biogenesis, in some cases, the response might not fully require this coactivator but rather other isoforms or family members such as PGC1β[54]. In addition, a novel mechanism of PGC1α to regulate gene expression, outside of transcription: PGC1α is able to directly bind to intronic regions of a subset of mRNAs encoding metabolic genes (including mitochondrial ATP transporter *Slc25a25*) via its RNA binding domains[49,55].

In response to different external stimuli, cellular signalling transduction pathways are used to coordinate transcriptional, translational, and post-translational processes that increase mitochondrial biogenesis and respiration. Exposure to lower temperatures in brown/beige fat or physical activity in skeletal muscle exemplify potent signals that stimulate mitochondrial biogenesis and respiratory adaption[56–58]. Cold promotes secretion of catecholamines **[G]** that activate cAMP signalling to stimulate mitochondrial biogenesis[42], which involves the activity of energy-sensing pathways centred on mTOR **[G]**[59] and AMPK **[G]**[60]. These signal transduction pathways control, among other factors, transcriptional activity of PGC1α and mitochondrial adaptation in low temperature environments. Another signal downstream of cAMP is the ER stress kinase, PERK, that controls mitochondrial protein import and cristae formation[21,61]. Cessation of adrenergic signalling and cAMP deactivates the mitochondrial biogenesis program and promotes mitophagy signals to eliminate mitochondrial mass[62]. Some of the pathways that induce mitochondrial adaptation during exercise in the muscle are similar to those conferred by cold such as AMPK or cAMP[63–65]; another major pathway involves Ca^{2+} signalling[35,66].

Translational regulation

Mitochondria require stringent regulation of cytosolic mRNA translation and subsequent protein import to dynamically respond to energetic and environmental stimuli. There are multiple points of regulation of translation of mitochondrial proteins including translation initiation, targeting of mRNAs to different subcellular locations for protein synthesis, and, for the components of the respiratory chain, synchronized co-translational assembly of nuclear-encoded and mitochondria-encoded subunits into respiratory complexes (Fig. 3). In this section, we describe how these translational control mechanisms communicate with metabolic stimuli to adapt mitochondrial respiration to the needs of the cell.

mTORC1–4E-BP1 regulates translation of nuclear-encoded mitochondrial mRNAs

Many metabolic signals including insulin–PI3K–AKT signalling, ATP sensing through AMPK[67,68], and amino acid levels[69,70] regulate the nutrient sensor mTORC1, enabling mTORC1 to efficiently integrate metabolic information to control mitochondrial respiratory function. mTORC1 promotes translation of the majority of nuclear-encoded mRNAs through regulation of mRNA 5′ cap-binding protein eIF4E (eukaryotic initiation factor 4E) that recognizes the m7GpppN cap structure at the 5’ end of eukaryotic mRNAs[71]. mTORC1 phosphorylates eukaryotic initiation factor 4E binding proteins (4E-BP1/2)[72] to prevent their inhibitory action towards eIF4E and the assembly of the eIF4F translation initiation complex on mRNAs (reviewed in[73]). Along with its role in translation, mTORC1 also phosphorylates chromatin modifier YY1 that interacts with PGC1α and targets it to mitochondrial gene promoters[47,51], syncing transcriptional and translational mitochondrial biogenesis programs.

Cap-dependent translation is intrinsically linked to intracellular energy availability and mTORC1 activity. During energetic surplus, mTORC1 promotes eIF4E binding to nuclear-encoded mRNAs through phosphorylation of 4E-BP1/2. Although most nuclear-encoded mRNAs require eIF4E for cap-dependent translation, certain mRNA classes defined by their 5’ UTR sequences and structures are particularly sensitive to eIF4E cap binding[74–78]. A class of these mRNAs contain short 5’ UTRs (<30 nucleotides), often with Translation Initiator of Short 5′ UTR (TISU) elements **[G]** that function both in transcription, through interaction with YY1, and translation [REF]. Nuclear-encoded mitochondrial mRNAs are enriched with TISU elements such as respiratory complex subunits *NDUFS4, NDUFS6, UQCRC1, ATP5O*, and numerous mitochondrial ribosomal proteins[76–78]. Presence of TISU enables efficient translation initiation under translational stress from glucose deprivation or AMPK agonism[79–81], which mildly affect 4EBP1/2 phosphorylation. This facilitates mitochondrial ATP production during periods of energetic insufficiency. However, potent inhibition of 4E-BP1/2 phosphorylation with mTORC1 inhibitors impairs translation of mitochondrial mRNAs with short 5’ UTRs (TISU and non-TISU)[78] and decreases mitochondrial respiration, TCA metabolism, ATP turnover, mtDNA content, and mitochondrial mass[76]. Intriguingly, cell survival and mitochondrial network integrity is preserved under these conditions through decreased mTORC1-dependent translation of the mitochondrial fission factor MTFP1[82]. Overall, mTORC1 regulated protein synthesis integrates cellular energy status with dynamic and rapid regulation of mitochondrial respiration and ATP production through selective translation of nuclear-encoded mitochondrial genes.

Translation of PGC1α through alternative open reading frames

An additional layer of translational regulation of mitochondrial function involves repressive upstream open reading frames (uORFs) in the *PPARGC1A* mRNA (encoding PGC1α)[83]. uORFs are a major class of genetic element within the 5’ UTR of mRNAs that regulate translation initiation of the main coding sequence. In fact, ~ 50% of human transcripts contain at least one uORF, indicating selective pressure for maintaining these elements[84,85]. Translational control mediated by uORFs is primarily evident in stress responsive conditions, where the eIF2·GTP-Met-tRNA^{Met} ternary complex necessary for initiation of translation becomes depleted, permitting ribosomal scanning downstream of

the inhibitory uORF. *PPARGC1A* mRNA from humans to flies contain uORF(s) that limits PGC1α protein levels[83]. Intriguingly, this element and regulatory paradigm is absent from blue fin tuna *PPARGC1A* sequence[83], consistent with its high oxidative muscle fibre and mitochondrial content. Mice containing a *PPARGC1A* uORF^{TAA} mutation show increased tolerance to acute kidney injury (AKI)[83], which is associated with PGC1α suppression[86,87]. Future research is needed to establish whether uORF translational control of PGC1α is a primary mechanism to promote mitochondrial adaptation under physiological stimuli (e.g. exercise) or stress conditions that lead to reduced translation initiation ternary complex levels.

Role of RNA granules in translation control of mitochondrial transcripts

Other post-transcriptional regulatory mechanisms occur in distinct, membraneless organelles termed RNA granules to adapt mitochondrial respiratory function to metabolic stimuli. Clustered mitochondria homologue (CLUH) is a cytosolic RNA-binding protein that localizes to RNA granules to preserve translational capacity of target mRNAs required under nutrient deprivation that affect respiratory chain function, TCA metabolism, fatty acid oxidation, and amino acid catabolism[88–90]. This is supported by CLUH interaction with ribosomal proteins, translation factors, and RNA-binding proteins G3BP1 and G3BP2, as well as its proximal localization to the mitochondrial outer membrane where it may facilitate co-translational import into mitochondria[111]. CLUH also acts as an regulator of PINK1-Parkin mitophagy pathway **[G]** contributing to mitochondrial network remodelling and health[112]. Interestingly, mTORC1 is negatively regulated by CLUH within RNA granules, establishing a model where CLUH positively and negatively regulates specific mitochondrial transcripts (such as TFAM and mitoribosomal components)to adapt to nutrient limitation during fasting[88]. *Cluh* mRNA was also found to be directly bound by PGC1α during glucagon stimulation in hepatocytes to promote *Cluh* expression[49]. This provides a novel layer to PGC1α nuclear function outside classical transcriptional regulation that may connect cytosolic translation of nuclear-encoded mitochondrial transcripts through modulating *CLUH* mRNA stability or processing during fasting[49].

Brown adipose tissue also employs unique mechanisms to regulate mitochondrial gene expression using FAM195A, a recently identified RNA-binding protein that contains an extended disordered low-complexity domain[93]. These types of proteins are intrinsically disordered and facilitate phase separation within RNA granules[94]. FAM195A was found to stabilize mRNAs essential for oxidation of branched-chain amino acids **[G]** (BCAAs) to drive thermogenesis in brown adipose tissue[93]. Accordingly, knockout mice exhibit whitening of brown adipose tissue, increased sensitivity to cold, and defects in BCAA oxidation signified by increased plasma levels of leucine, valine, and isoleucine[93]. These initial observations indicate that one mode of translational regulation of mitochondrial mRNAs is through stabilization within RNA granules, emphasizing the need to further study these organelles and their connection to the translational machinery.

Mitochondrial protein translation regulation in respiratory chain assembly

The mitochondrial translation machinery evolved distinctly from its cytosolic counterpart owing to its bacterial origin and subsists to synthesize 13 polypeptides (8 in yeast)

that remain encoded in the mtDNA. A synchrony between the mitochondrial and nuclear genomes and surveillance of the mitochondrial proteome via intercompartmental communication and signalling pathways contributes to respiratory chain formation. One approach is through nuclear encoded auxiliary factors that stimulate translation initiation and progression. In yeast, translational activators specific for certain mitochondrial-encoded mRNAs promote translational capacity in mitochondria. For example, there are at least seven translational regulators of complex IV subunits CO1, CO2, and CO3[95]. Translation of mitochondrial mRNAs is predominantly regulated in a positive manner through interaction with the mitochondrial ribosome and localization to specific sites on the inner membrane[95]. Metabolic cues associated with mitochondrial biogenesis such as a switch in nutrient source away from glucose feedback to translational activators to facilitate the efficient assembly of respiratory complexes[95]. Furthermore, translational rather than transcriptional programs are synchronized between the cytosol and mitochondria where intercompartmental communication is unidirectional from the cytosol to mitochondria[27]. This enables yeast mitochondrial translation to swiftly coordinate with levels of synthesis of nuclear-encoded respiratory chain subunits.

Rapid communication between translational machineries does not occur in mammalian mitochondria[28]. Mitochondrial translational activators are largely missing and mitochondrial RNAs also lack 5’ UTRs present in yeast, requiring a different mode of translational regulation[96]. TACO1 is the only known regulator of mitochondrial translation and facilitates the translation of mitochondrial CO1 synthesis through binding to various regions of the coding region[97,98]. Mitochondrial protein homeostasis in higher eukaryotes is also challenged by the fact that translation of subunits within the multimeric respiratory complexes does not occur in stoichiometric balance[28,99]; there is a 2–32-fold variance in subunit synthesis rates for dual origin (nuclear and mitochondrial genomes) respiratory chain complexes[28]. Despite this, average protein synthesis for each respiratory complex is highly correlated across compartments to conceivably avoid proteotoxic stress in the mitochondria[28]. One mediator of this interaction is the mitochondrial RNA binding protein LRPPRC[28]. In *LRPPRC* knockout cells, cytosolic and mitochondrial translation are anti-correlated, which induces protein quality control pathways such as the unfolded protein response **[G]** (UPR) of the ER[28]. Metabolic stimuli such as growth signalling or nutrient deprivation regulate LRPPRC[100,101], suggesting that metabolic signals may establish translational coordination between compartments to drive respiratory chain assembly. For example, mTORC1 controls LRPPRC protein levels in cells[101], while the mitochondrial NAD^+-dependent deacetylase SIRT3 targets LRPPRC to promote respiratory chain function in fasted livers[100].

Translational synchrony occurs on a longer timescale in higher eukaryotes and hinges on the mitochondrial translation machinery sensing the import and assembly of nuclear-encoded respiratory factors and adjusting mitochondrial translation rate accordingly[28,95]. This mode of feedback regulation allows for fine-tuning of respiratory complex formation and mitigates protein folding stress from unassembled protein subunits. Aside from LRPPRC, assembly factors C12ORF62, MITRAC12, and MITRAC15 adjust synthesis of mitochondrial-encoded CO1 and ND2 with import of nuclear-encoded polypeptides[29–31,102].

TIM21, a component of the mitochondrial protein translocase also mediates the assembly of pre-sequence-containing subunits into early assembly intermediates upon their import into mitochondria[31]. Although different mechanisms regulate cross-compartment protein synthesis in yeast and humans, it will be important to further define these processes and upstream factors controlling their communication to enhance mitochondrial respiration.

Post-translational mechanisms

Transcriptional and translation control is energetically costly and time-consuming for cells. In order to provide flexibility to environmental cues, cells developed strategies to regulate mitochondrial protein activities which include post-translational modifications (PTMs) and protein-protein interactions. These mechanisms can control mitochondrial protein import, respiratory chain assembly and rapidly adapt mitochondrial function to cellular requirements during acute energetic stress, in contrast to prolonged transcriptional and translational programs. Communication through signalling cascades between the ER and mitochondria including PTM regulation of OMM proteins and Ca^{2+} influx to regulate TCA enzymes[103] also fine-tunes mitochondrial function to cellular demands. In this section, we discuss different mechanisms by which mitochondrial function can be modulated through post-translational mechanisms (Fig. 4).

Respiratory chain complex regulation by PTMs

PTMs incorporate chemical groups to amino acid side chains that modulate the activity and assembly of proteins and protein complexes. The respiratory machinery of mitochondria comprises several complexes with multiple subunits that are subject to different modifications[104]. The role of these modifications and their regulation needs to further study, but their stoichiometries are high enough to be detected by cryogenic electron microscopy[105] highlighting their potential importance in regulating OXPHOS activity. In this subsection we summarize the most relevant modifications on OXPHOS complexes and their impact on mitochondrial respiration.

Phosphorylation is most widely studied modification across the proteome. More than 90% of mitochondrial proteins contain at least one known phosphorylation site[104]. Phosphorylation is dependent on OXPHOS activity which yields the ATP necessary for kinase reactions[106]. Respiratory complexes are phosphorylated at multiple sites with distinct results on activity and assembly. Protein Kinase A (PKA) has been postulated as a major kinase for different mitochondrial proteins[107–111]. Phosphorylation of respiratory complex I (NDUFA1, NDUFS4 and NDUFB11) by PKA increases the assembly and function of this complex[107,108,112]. PKA also phosphorylates respiratory complex IV subunit COX4I1 on serine 58 which is particularly relevant for more efficient electron flux under conditions where cells are reliant on OXPHOS activity[109,110]. Phosphorylated Ser58 in COX4I1 prevents binding and allosteric inhibition by ATP, and toggles between activation (phosphorylated) and inactivation (non-phosphorylated) of the ETC[110]. Under hypoxic conditions, PKA can inhibit complex IV activity by phosphorylation on Ser40 to prevent excessive ROS production[113]. Other kinases also regulate the function of different OXPHOS subunits. The cyclin B1–Cdk1 complex phosphorylates several components of complex

I including NDUFA12 (on Thr120 and Thr142), NDUFB6 (Thr5, Ser29 and Ser55), NDUFS2 (Ser364), NDUFV1 (Thr383), and NDUFV3 (Ser105) which promotes complex I activity during mitosis[114]. PINK1 phosphorylates NDUFA10 on Ser250 phosphorylation to augment complex I activity[115], and mitochondrial c-Src kinase phosphorylates Tyr215 of succinate dehydrogenase complex II subunit SDHA which is required for electron transfer and prevention of ROS production[116]. Fgr tyrosine kinase-dependent phosphorylation of Tyr604 increases complex II activity and compensates for complex I inhibition[117,118]. While the landscape of protein phosphorylation in the OXPHOS complexes is abundant, further research is necessary to determine the responsible kinases and understand their implications in mitochondrial respiratory function.

Acetylation is the addition of acetyl groups to lysine amino groups. This process can be regulated by acetyl-transferases, but due to the high concentrations of acetyl-CoA inside mitochondria (>1 mM) and slightly alkaline conditions (pH 8.0) in the matrix, mitochondrial proteins can spontaneously be acetylated[119]. The general regulation of protein acetylation has been previously reviewed[120] and here we focus on the impact on respiratory function. Deacetylase sirtuin 3 (SIRT3) counteracts the effects of acetylation on multiple mitochondrial complexes including the OXPHOS components[121]. Depletion of SIRT3 has revealed decreased activity of complex I *in vivo* and *in vitro* and associates with poor recovery of hearts after ischemia[122–125]. Similarly, complexes II and V are deacetylated by SIRT3 to increase their activities[125,126]. Of note, most of these works manipulate SIRT3 which deeply alters mitochondrial function[127] and therefore it is necessary to uncouple the OXPHOS specific effects from the general mitochondrial dysfunction.

Cysteine oxidation from ROS is an important regulatory modification on cellular and specifically, mitochondrial proteins[128]. In brown adipocytes, UCP1 dissipates the proton gradient across the mitochondrial inner membrane. Under conditions of elevated glucose, fatty acid, or branch chain amino acid oxidation, UCP1 Cys253 becomes oxidized, further increasing its activity, mitochondrial respiration, and thermogenic capacity in this tissue[10]. Abolition of Cys253 oxidation results in obesity and inflammation[129]. Another example of ROS-dependent respiratory regulation is the oxidation of Cys39 in ND3 of complex I[130–132]. Oxidation of this residue prevents ROS production due to reverse electron transfer (i.e: reductive activity of complex I from ubiquinol to NADH formation) during ischemia.

Mitochondrial protein import and respiratory control

Mitochondrial biogenesis relies on the existence of mitochondrial import machineries that are responsible for the import of most nuclear-encoded mitochondrial proteins. Consequently, precursor protein import is a critical and highly regulated process that ensures the proper assembly of membrane protein complexes required for cristae formation and the respiratory chain biogenesis. The TOM complex at the outer mitochondrial membrane is the main entry gate for protein import[133]. The main core of the TOM complex (also known as General Import Pore, GIP) consists of subunits TOM40, TOM22, and the small components TOM5, TOM6, and TOM7. TOM40 has a distinct β-barrel structure as opposed to the α-helical structure of the other subunits and interacts with N-terminal positively charged fragments of precursor proteins to facilitate their import into the intermembrane

space[134–136]. Aside from the GIP, the TOM complex also includes TOM20 and TOM70 receptors that recognize and anchor precursor proteins prior to their import through the TOM40 pore.

TOM70 and TOM20 receptors are essential to recognize and drive precursors to the GIP. TOM20 participates in the recognition of mitochondrial precursors that contain a pre-sequence motif ($\phi\chi\chi\phi\phi$, where ϕ is hydrophobic residue and χ is any amino acid) while TOM70 has been classically associated with the import of mitochondrial ion and metabolite carriers[137–141]. Further studies have expanded the range of TOM70 substrates showing the ability of the receptor to bind non-canonical substrates[142] including hydrophobic[140,141] and aggregation-prone proteins[143], and proteins with internal mitochondrial targeting sequence-like signals **[G]** (iMTS-L)[144]. Moreover, TOM70 and TOM20 can participate in the import of identical substrates in a sequential manner where TOM70 transfers to TOM20 before reaching the GIP[145], demonstrating a crosstalk between the TOM components to regulate mitochondrial protein import. This may explain the compensatory effects on protein import of TOM70 substrates when TOM70 is absent[143,145], preventing the identification of bona fide substrates. Mutations in the receptor TOM70 negatively affect the assembly of respiratory complexes showing direct connection between this receptor and mitochondrial respiration[146] although the specific mechanism on complex assembly is unclear. The delivery of precursors from TOM70 to the GIP is still under debate. Direct interactions with TOM70 and mitochondrial precursors have been shown *in vitro*[147] and may be mediated by Cys-Cys disulfide bonds[148]. However, since TOM70 interacts with heat shock proteins 70 (HSP70) and 90 (HSP90)[140,141], direct delivery of mitochondrial protein precursors from these chaperones to the GIP has been proposed similar to the SEC translocon **[G]** systems in the ER[149]. Transient interactions between the chaperone clamps of TOM70 and the EEVD domain of HSPs increase the concentrations of chaperone–precursor complexes at the gate of the GIP to drive import into mitochondria[140,149]. The involvement of TOM70 in immune responses[150,151] and Ca^{2+} signalling[152] expand the functions of the receptor beyond protein import and suggests a central role in cellular signalling.

Regulation of TOM complex activity is essential to ensure proper mitochondrial function[153]. PTMs control TOM complex activity. Initial studies in yeast established that phosphorylation events control both the biogenesis the of TOM complex and precursor protein import through cytosolic kinases Ck2 and Pka[137]. Ck2 phosphorylates the receptors Tom22 and Tom20, which promotes biogenesis of the TOM complex[137]. In contrast, Pka-driven phosphorylation of Tom70 impairs the binding and import of, at the very least, classical yeast Tom70 substrates AAC carrier **[G]** and PiC carrier **[G]**[137]. In mammals, an interplay between O-GlcNAcylation and phosphorylation at Ser94 of TOM70 controls mitochondrial protein import that is linked to cristae formation during cold stress in brown adipocytes[21]. Upon norepinephrine (NE) or cold stress, activation of the ER kinase PERK triggers a post-translational modification cascade that results in increased TOM70-dependent precursor protein import. More specifically, PERK-activated protein O-GlcNAc transferase (OGT) directly and indirectly stimulates precursor protein import through glycosylation of TOM70 and CK2, respectively, driving mitochondrial cristae formation and cellular respiration in brown adipocytes[21]. CK2-driven phosphorylation negatively affects

TOM70 activity and is prevented by competition with *O*-GlcNAc[21,154,155] occurring on the same site and by OGT-mediated inhibition of CK2 activity[155]. Previous studies found that CK2 negatively controls brown adipocyte function and mitochondrial biogenesis[156] and inhibits PERK activities[157] critical for ER–mitochondrial communication[158], reinforcing the importance of this signalling network on mitochondrial function. Given these results, PTMs of the TOM complex are a conserved method to regulate precursor protein import, however, the specific PTMs and cytosolic enzymes involved adapted to different cellular functions and needs.

Mitochondrial protein import functions in concert with numerous ATP-dependent chaperones and AAA+ proteases **[G]** that ensure proper import, processing, and stabilization of protein substrates[159]. The mitochondrial matrix chaperones HSP60 and mitochondrial HSP70 (mtHSP70) fold precursor proteins to avoid protein aggregation[159]. TIM23-dependent protein import across the mitochondrial inner membrane and subsequent protein folding is mediated by mtHSP70 (ref.[159]). In conjunction with mtHSP70, the matrix protease LONP acts as a chaperone to promote protein incorporation into the inner membrane and matrix independent of its protease function[160–162]. LONP anti-aggregation activity acts towards numerous substrates including subunits of the respiratory chain, mitochondrial ribosome[160,161], and the OXA1Linsertase of the inner membrane[161]. Mitochondrial proteases are also downstream targets of cellular signalling pathways. In response to hypoxia or serum starvation in cancer cells, LONP is phosphorylated by AKT **[G]** to enhance its protease activity and limit accumulation of unfolded respiratory chain subunits[163]. The *i*-AAA protease YME1L is hyperactivated under hypoxic or glutamine starvation conditions, degrading mitochondrial protein translocases and lipid transfer proteins to limit mitochondrial biogenesis[164]. Altogether, the tight control of the import machineries and processing of mitochondrial protein precursors by proteases[165] is a limiting step in the regulation of all mitochondrial processes and a quality control[166] mechanism that responds to cellular stressors such as hypoxia, fasting, or cold.

Assembly of mitochondrial respiratory protein complexes and supercomplexes

During assembly of the respiratory chain, intermediate subcomplexes and a growing number of accessory proteins progressively interact to form intact respiratory complexes over the time course of hours[167,168]. Once assembled, respiratory complexes dynamically form higher order assemblies termed supercomplexes in response to metabolic pressures. Complex I predominantly exists in complex with other respiratory complexes including a dimer of complex III (I+III_2; subscript denotes stoichiometry) or in conjunction with complex III and IV (SC I+III_2+IV); complex III exists as an obligate dimer and in complex with complex IV (SC III_2+$IV_{1–2}$); complex IV exists mainly as a monomer, but to a lesser extent as dimers and multimers[169–171]. The co-existence of isolated complexes and their super-assemblies is known as the plasticity model of the electron transport chain[172,173], which asserts a functional role of varied assemblies on electron transport. Consistent with this scenario, stoichiometry of isolated and superassembled respiratory chain complexes is regulated in cells under the physiological stimuli of exercise in muscle[170,174] and fasting in liver[175].

The physiological role of supercomplexes has been heavily debated with the main models contending reduced ROS production[176,177], CoQ and CytC substrate channelling **[G]**[175,177], enhanced kinetics[178–181], respiratory chain complex stability[182–184] and assembly[185], and diminished protein aggregation in the protein-rich inner mitochondrial membrane[186]. Despite this, metabolic and physiological conditions that modify stoichiometries of supercomplexes to isolated complexes are consistent with increased efficiency or quantity of mitochondrial respiration. Supercomplexes are elevated in proliferative cells in response to changes in nutrient conditions or metabolic constraints[18,20,187]. Cells facing ER stress from glucose deprivation upregulated the PERK–eIF2α–ATF4 pathway to transcriptionally induce supercomplex assembly factor 1 (SCAF1), which acts a bridge between $CIII_2$ and IV to promote supercomplex III_2+IV (a.k.a. Q-respirasome) formation[178,188,189] as well as particular I+III_2+IV supercomplexes (also known as N-respirasomes)[188,189,187]. This resulted in enhanced cellular respiration, ATP production, and proliferation[20]. Increased N-respirasome assembly driven by lipid remodelling in the mitochondria (see also next section) also supports growth and proliferation of cancer cells under nucleotide limitation[18]. Furthermore, supercomplexes promote tumour growth under oxygen limited conditions[190,191] and the proliferation and viability of acute myeloid leukaemia cells *in vivo*[192]. These findings point to a model where supercomplexes provide metabolic flexibility under metabolically demanding conditions to fuel proliferation and highlight the therapeutic potential of targeting supercomplexes or their assembly factors in cancer. Proof of this model was acquired in a recent study of the yeast Q-respirasome, where mutations in the complex III-subunit Cor1 that specifically impaired supercomplex assembly, limited the diffusion of cytochrome *c* between complex III and IV and decreased coupled respiration with NADH and succinate as electron donors[180]. This led to a competitive disadvantage of mutant strains cultured in nutrient conditions that require mitochondrial respiration, which was rescued through cytochrome *c* overexpression[180]. Consistent with this, cryo-EM kinetic studies established that electron transfer between complex III and complex IV in the yeast Q-respirasome involves 2D diffusion of positively charged cytochrome *c* across the negatively charged surface of the supercomplex, in contrast to bulk exchange with solvent (3D diffusion)[179]. This charged surface is conserved in the mammalian Q-respirasome and may explain the enhanced respiratory activity of superassembled complex III and IV[178].

Supercomplexes control metabolic activity under energetically demanding conditions in organismal physiology as well. In humans and mice, endurance exercise training causes a redistribution of respiratory complexes into supercomplexes that correlates with increased mitochondrial respiration[170,174]. This appears to be functionally relevant since wild-type SCAF1 male and female mice exhibit higher top speeds in exercise performance compared to their mutant or knockout counterparts[177]. SCAF1 in zebrafish also promotes growth, increases female fecundity, and prevents fat deposition[193], while in mice it facilitates bodyweight maintenance of male mice under severe intermittent fasting[177]. Thus, respirasomes appear to increase the efficiency of mitochondrial respiration using certain substrates, a subtle phenotype that becomes apparent under specific metabolic or physiological states. However, they can also be selected against to optimize mitochondrial respiration. For example, N-respirasomes are reduced when certain fuel sources with low NADH/FAD electron ratios are preferred such as fatty acids in liver during fasting[175].

Mitochondrial membrane remodelling

Mitochondrial membranes represent an intricate network of protein-protein and protein-lipid interactions that dictates mitochondrial morphology and functions such as respiration[18,20], TCA cycle activity, and metabolite trafficking[17]. The composition of mitochondrial membranes is subject to continuous change and altered by protein translation[28,95] and lipid synthesis rates[18], which depend on metabolic cues. We previously discussed the importance of protein translation and mitochondrial protein translocation in sustaining mitochondrial respiratory function. Here, we discuss the regulatory mechanisms that control mitochondrial membrane morphology and dynamics and consider how these processes impact respiratory function of mitochondri**a**.

Regulation of mitochondrial membrane dynamics

Mitochondria are highly dynamic structures that undergo continuous cycles of membrane fusion and fission to expand or contract the mitochondrial network. These opposing events maintain mitochondrial network homeostasis and adjust mitochondrial function to different cellular conditions such as nutrients, oxidative stress, or other internal/external signals. In general, membrane fission events help recycle damaged mitochondria via mitophagy, while fusion events distribute mitochondrial contents including proteins and mtDNA to prevent accumulation of pathological molecules[22]. Altogether, these events safeguard the health of the mitochondrial network and ensure optimal respiratory capacity. As such, an imbalance in mitochondrial membrane dynamics leads to cellular dysfunction and pathology, in particular affecting the nervous system, perhaps reflecting the high dependency of neurons on mitochondrial respiration[194]. Membrane fission and fusion events are controlled by dynamin-like GTPase proteins and their accessory factors, which are excellently reviewed elsewhere[22,195]. Mitochondrial fission requires PTM-mediated dynamin-related protein 1 (DRP1) localization to mitochondria and occurs at sites where ER tubules constrict mitochondria[196]. During glucose or glutamine starvation or ischemic conditions, PKA-mediated phosphorylation of DRP1 prevents its localization to mitochondria and leads to unopposed mitochondrial fusion, cristae abundance, maintenance of ATP levels, and cell viability[197–199]. Cell proliferation and oncogenic transformation also stimulates mitochondrial fusion, respiration, and ATP output[200]. Mitochondrial fragmentation is also associated with increased respiration in certain physiological contexts such as thermogenic/adrenergic stimulation of brown adipocytes[201], exercise in cardiac tissue[202], and immune activation of B and T cells[203,204]. Thus, general rules about the relation between mitochondria fission/fusion dynamics and enhanced respiratory rates may not exist. Instead, mitochondria uniquely adapt their morphology to different cellular stimuli in order to promote mitochondrial respiration.

Independent of general length and connectivity changes to the mitochondrial network, the mitochondrial inner membrane undergoes structural rearrangements forming cristae. Cristae are invaginations of the inner mitochondrial membrane that harbour the respiratory complexes[1] and sustain respiratory super complex assembly and function[205]. Cristae are a structural platform that allows proper distribution and function of respiratory complexes to maximize respiratory capacity[20,205]. For instance, cold stimulation in brown adipocytes

stimulates cristae formation to increase respiratory function without affecting levels of OXPHOS components[21,206]. MICOS, OPA1 and complex V dimers along with specific lipid composition shape cristae architecture. The MICOS complex consists of two distinct subcomplexes, MIC60 and MIC10, that provide spatial organization for cristae[14,207]. OPA1 also plays a role in the stabilization of cristae junctions, tying in cristae dynamics with the formation of respiratory supercomplexes[14–16]. Under low nutrient conditions, associations between OPA1 and mitochondrial solute carriers modulate OPA1 function which can regulate complex V assembly and cristae function and morphology, independently of its fusion-related GTPase activity[208]. Cristae formation is initiated at the cristae junction in a process where the MIC60 subcomplex promotes the invagination of the inner mitochondrial membrane[14]. This process is also favoured by the stabilization of the mitochondrial membranes by interactions between the MICOS and outer mitochondrial membrane components such as SAM50 (ref.[209]). Later, the MIC10 subcomplex promotes cristae elongation[14]. Despite the predominant role of each MICOS subcomplex in different aspects of cristae biogenesis, both interact and are not independent entities. For example, loss of MIC60, MIC19, MIC13 or MIC10 is sufficient to cause dramatic defects in cristae density and morphology[14,210,211]. The coordination of the MICOS subcomplexes relies on the MICOS component MIC19, which bridges MIC60 and MIC10 subcomplexes in yeast[212]. Similarly, mammalian MIC19 is a limiting component[4] that supports cristae biogenesis through the stabilization of inner and outer mitochondrial membrane interactions[209]. Respiratory complex V abundance is correlated with cristae formation[213–215]. Complex V dimers increase the flexibility of cristae curvature[216] at the edge of cristae along with mitochondrial lipids such as cardiolipin[217]. However, lower levels of complex V do not necessarily imply reduced cristae density. An example of this is brown adipose tissue, where complex V is clearly underrepresented (owing to prevalence of uncoupled respiration in this tissue)[218] and dense and highly functional cristae are still formed[21].

The role of the MICOS subcomplexes and OPA1 in cristae formation are extensively reviewed[219], however, regulation upstream of MICOS components is not well defined. The key player is the ER, which communicates with the mitochondria directly via membrane contact sites, known as MERCs to regulate various aspects of mitochondrial dynamics (Box 2). In proliferative cells, ER stress kinase PERK controls cristae formation during nutrient stress conditions through an unknown mechanism[20]. In post-mitotic brown adipocytes, the PERK–OGT–TOM70 axis (see subsection Mitochondrial protein import and respiratory control) controls mitochondrial import of MIC19 and cristae formation[21]. Consistent with a coordination between mitochondrial protein import and cristae biogenesis, TOM70 co-localizes with MICOS complexes at the cristae junction[19]. MICOS subunits contain a Cys-X_9-Cys twin motif (CHCH domain) that increases protein instability and aggregation propensity[210,220]. This cysteine motif may justify the requirement of the TOM70 pathway as a chaperone to assist the recognition[148] and import of MIC19 (refs.[21,221]). Interestingly, loss of MIC19 can be compensated by overexpression MIC25 (ref.[209]), a highly similar MICOS component[222] with lower endogenous expression levels. Compared to other MICOS components, MIC19 import seems insensitive to membrane potential fluctuations[21,221,223] and therefore explains its pivotal role in cristae organization and stability especially under

stress conditions such as norepinephrine stimulation where membrane potential decreases with increased respiration.

MICOS components are also controlled by PTMs. PKA-dependent phosphorylation of MIC60 downregulates PINK1 and subsequent recruitment of Parkin to the mitochondria, negatively regulating mitophagy/mitochondrial clearance[111]. In *Drosophila*, PINK1 mediates the phosphorylation of MIC60 and sustains cristae formation through MIC60 oligomerization which positively controls cristae formation and neuronal function[224]. Together, these connections of MIC60 with PINK1 and Parkin[111,224] hint at a connection between cristae formation and mitochondrial function that prevents oxidative stress via efficient respiration and mitophagy. PINK1 phosphorylation sites in *Drosophila* seem to be conserved across species[224], while PKA-dependent sites appear to be exclusive of mammals[111]. The presence of different phosphorylation sites might be a consequence of divergent evolutionary adaptive responses, however, the interplay between PKA and PINK1 indicates a quality control mechanism that dictates inner mitochondrial membrane dynamics through post-translational control of the MICOS components.

Cristae biogenesis requires a balance between protein–protein interactions and lipid incorporation into cristae membranes. The balance and contribution of these processes is an open question. Mitochondrial–ER contacts establish a node of communication, which would allow for lipid traffic to feed mitochondrial membrane formation, yet the contribution of this lipid transfer at membrane contact sites as well as proteins involved in this process are largely unknown. In yeast, the ERMES system consisting of Mmm1, Mdm10, Mdm12, and Mdm34 is the primary candidate for tethering non-vesicular transfer of lipids between ER and mitochondria[225]. In mammals, VPS13D has been identified as a lipid transfer protein at ER–mitochondria contacts[226]. Unlike molecule diffusion, the involvement of enzymes in lipid trafficking denotes specificity and efficiency for these processes. On the mitochondrial side, the MICOS complex is implicated in mitochondrial lipid uptake. MIC26 and MIC27 which are members of the MIC10 subcomplex participate in the trafficking of incoming lipids from the ER across the mitochondrial membranes, which could support cristae formation and respiratory function[227,228]. Loss of MIC26 or MIC27 has little effect on cristae biogenesis as opposed to point deletions of MIC60, MIC19, MIC13, or MIC10 downregulation[14,210]. However double deletion of MIC26 and MIC27 negatively impacts cristae formation and respiratory complex formation perhaps due to a compensatory expression of one of the subunits when the other is downregulated[227]. The study of mitochondrial–ER protein interactions as well as MICOS assemblies and lipid uptake by mitochondria is fundamental to understand cristae formation and dynamics, and in extension, to understand the regulation of mitochondrial respiratory function.

Mitochondrial lipid composition and remodelling

Membranes are composed of different phospholipid species together with other membrane lipids such as sphingolipids **[G]** and sterols. The physiochemical properties of membranes including viscosity, permeability, curvature, thickness, tension, and lateral compartmentalization control protein dynamics and signalling cascades. Phospholipids contain a phosphate headgroup modified by choline, ethanolamine, or other organic

molecules and fatty acid chains with varying degrees of length and saturation that are connected through a glycerol backbone. Cylindrical phospholipids like phosphatidylcholine form bilayers and create liquid crystalline lamellar phases, while others that possess a small headgroup in comparison to their acyl chains like phosphatidylethanolamine are conical and produce non-bilayer structures such as inverted hexagonal phases[229–231]. Lipid composition differs considerably across organelles, but is fairly consistent for a particular organelle across cell types, highlighting the importance of phospholipid ratios[231]. Mitochondrial membrane composition is tailored to each mitochondrial compartment to enable membrane shape and the function of protein machineries within these locations. The conical lipids cardiolipin and phosphatidylethanolamine in the mitochondrial inner membrane control the assembly, stability, and activity of respiratory chain complexes, and promote the higher order assembly of mitochondrial supercomplexes that alter the efficiency of the electron transport chain[232–235]. Phospholipid synthesis, transport, remodelling, and degradation are all means to alter mitochondrial phospholipid composition in response to physiological stimuli such as cold exposure or other metabolic stressors[17,18,236–239].

The connection between membrane composition and metabolic stimuli or growth signalling pathways in eukaryotes is accepted, but not highly explored. Initial observations of yeast cultured on a non-fermentable carbon source such as lactate found that levels of mitochondrial phosphatidylethanolamine and cardiolipin increased to aid respiratory chain assembly[240,241]. In mammals, thermogenic capacity is modulated through activation of beige and brown adipose depots and requires changes to mitochondrial phospholipid composition to support increased electron transport activity and consumption of metabolic substrates including glucose, lipids, and BCAAs[17]. Mice housed at 5°C displayed specific induction of proteins involved in phospholipid metabolism that caused dramatic accumulation of both phosphatidylglycerol, a precursor in cardiolipin synthesis, and nascent cardiolipin species[17]. Accordingly, adipose-specific cardiolipin synthase 1 (*Crls1*) knockout mice exhibited decreased mitochondrial mass, abnormal cristae structure, reduced respiratory chain complex and supercomplex assembly and diminished uncoupled respiration/thermogenesis that led to whole-body metabolic defects and insulin resistance[17]. Humans also accumulate phosphatidylglycerol species such as Lyso-PG 18:0; Lyso-PG 18:1, and PG 20:0/22:5 in serum following 1 hour cold challenge consistent with observations in mice[236], underscoring the requirement of brown adipose tissue cardiolipin synthesis to thermogenesis across mammals.

Cancer cells dynamically regulate their lipidome, including that of their mitochondria, to adapt to nutrient limitations and the tumour environment[242]. One recent demonstration of this is remodelling of mitochondrial lipid composition under nucleotide limitation, which sustains respiration and division in proliferative cells[18]. Specifically, reduction of pyrimidine synthesis led to the change in the make-up of phosphatidylethanolamine and phosphatidylcholine in the mitochondria, with the substitution of normally occurring diacyl species with their ether-lipid counterparts[18]. Ether lipids are peroxisome-derived phospholipids where the acyl chain at the *sn-1* position **[G]** is attached through an ether linkage in contrast to an ester bond of diacyl phospholipids[243]. This structural change leads to the formation of non-lamellar inverted hexagonal structures, which

facilitate membrane fusion events and the formation of lipid microdomains[243,244]. In mitochondria, ether phospholipid accumulation promoted the formation of active respiratory supercomplexes that enabled proliferation under low nucleotide conditions[18]. This link between mitochondrial lipid composition and respiratory efficiency has also been reported in the context of the Barth syndrome **[G]**, where cells are defective in cardiolipin remodelling and contain low levels of ether phospholipids and reduction in supercomplexes[245,246]. Regarding carcinogenesis, ether phospholipids promote hypoxia tolerance[239] and aggressiveness of certain cancers[247–249]. Thus, modulation of ether phospholipid ratios within cellular membranes to, in part, regulate mitochondrial function, represents a strategy exploited by cancers to adapt to environmental and metabolic stress. A better understanding of their upstream regulatory control will enable us more efficiently target phospholipids in health and disease.

Conclusions and perspective

Mammalian cells use a variety of regulatory mechanisms for mitochondrial respiratory adaptations in response to intracellular or extracellular signals. One of the regulatory levels encompasses transcriptional control of mitochondrial gene expression, which, in large part, depends on chromatin complexes containing PGC1α — a program that promotes mitochondrial biogenesis and increased activity of the respiratory chain, and occurs in physiological adaptations to lower temperatures, physical activity or in certain types of tumours. Another level of control is executed at the translation of mitochondrial transcripts encoded in the nuclear genome, whereby rates of protein synthesis increase steady-state levels of subsets of mitochondrial proteins. Components of this control include the mTORC1–4E-BP1/2 axis as well as the RNA-binding protein CLUH that mediates formation of RNA granules for stability and translation of specific mRNAs. Post-translational regulatory mechanisms include various signalling-dependent protein chemical modifications that modulate protein interactions and activities. Assembly of respiratory complexes and supercomplexes, mitochondrial protein import, MICOS and OPA1-dependent cristae formation, and mitochondrial lipid composition coordinate with PTMs to adjust mitochondrial respiration and satisfy cellular energetic demands. These different levels of regulation are ultimately linked, to allow the cells to respond adequately to various cues. For example, adaptation to lower temperatures in mammalian brown adipocytes synchronizes transcriptional and translational programs of nuclear and mitochondrial encoded genes that reorganizes mitochondrial architecture and metabolic function to ensure adaptive thermogenesis.

Although the general regulatory mechanisms for mitochondrial adaptation are now known in some detail, the future challenge will be to address how these mechanisms operate in different pathological and physiological contexts. Mitochondrial energetic adaptations, such as physical activity or cold exposure, confer significant health benefits including cellular fitness and protection against metabolic diseases (Box 3). The inability of mitochondria to adapt results in chronic bioenergetic defects and cumulative cellular damage that causes inflammation which can contribute to neurodegenerative pathologies (Box 3). To comprehensively understand mitochondrial adaptive mechanisms, essential questions still need to be addressed. For example, the transcription coactivator PGC1α increases

mitochondrial respiration and biogenesis. However, it is largely unknown how the induction of mitochondrial proteins by PGC1α in different stoichiometries across multiple tissues drives remodelling of the mitochondrial proteome and ultimately adapts respiration. Another important area of future research includes dissecting the regulatory mechanisms and components that change mitochondrial organelle architecture to stimulate respiration. For instance, a complete understanding on how cristae are formed in different cell and tissue types is unknown. In response to lower temperatures, brown adipocytes increase MICOS complex assembly, but how new cristae membranes arise from coordination of MICOS and phospholipid trafficking from the ER and mitochondria is unclear. At the molecular level, it is still open how mitochondrial protein import machineries, respiratory complexes and mitochondrial cristae components assemble to regulate mitochondrial respiration during adaptive responses in different physiological or pathological contexts. Addressing these questions will provide new mechanistic insights into how exercise, nutrition and environmental stressors adapt cellular respiration to bioenergetic needs with therapeutical implications in metabolic and chronic diseases. Novel approaches combining biochemistry, physiology, cell biology, and emerging high resolution microscopy techniques will be needed to address these questions. Finally, integration of physiological and biochemical data with quantitative proteomics, PTM mapping, and metabolomics will further clarify the mechanisms governing mitochondrial function and adaptive capacity.

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Glossary

cristae
Invaginations of the inner mitochondrial membrane that increase the surface area of respiratory reactions and harbour the respiratory complexes

brown and beige adipose tissues
Thermogenic fat cells with abundant mitochondria that oxidize glucose, fatty acids and branched-chain amino acids to generate heat

uncoupling protein 1 (UCP1)
Inner mitochondrial membrane protein expressed in thermogenic tissues that dissipates membrane potential from the respiratory complexes and sustains thermogenesis

Haem
porphyrin group coordinating an iron atom required for electron transfer or oxygen transport

iron-sulfur clusters
iron sulphide molecules that transfer electrons across respiratory complexes

ER stress

a condition that occurs when the capacity of the ER lumen to fold proteins is saturated. ER stress transduces signals to other organelles such as mitochondria to adapt cellular metabolism to satisfy energy demands

mediator complex
a multiprotein complex that transduces signals from transcription factors to RNA polymerase II in order to control gene expression

catecholamines
Group of chemical neurotransmitters including dopamine, epinephrine, and norepinephrine that are released into the blood upon stress and modulate beige and brown fat tissue activity

mTOR
mammalian Target Of Rapamycin, a nutrient-sensor kinase involved in the control of cellular growth, survival, metabolism, and immunity

AMPK
AMP-activated protein kinase that senses fluctuations in ATP/AMP ratio

Translation Initiator of Short 5′ UTR (TISU) elements
Sequence elements that are downstream of transcription start sites and regulate both transcriptional and translational initiation and present in mRNAs with very short 5’ UTRs

PINK1-Parkin mitophagy pathway
a quality control pathway that marks damaged mitochondria to promote their autophagy-mediated destruction

branched-chain amino acids (BCAAs)
amino acids including valine, leucine and isoleucine that can be oxidized in the cell to obtain energy

unfolded protein response (UPR)
A process that regulates a transcriptional and translational response to ER protein folding stress

internal mitochondrial targeting sequence-like signals (i-MTSLs)
peptide sequences present within proteins destined for mitochondria that interact with import receptors and increase import competence

SEC translocon
Protein complex embedded in the endoplasmic reticulum membrane that transports proteins from and through the ER lumen

AAC carrier
ADP/ATP carrier is an inner mitochondrial membrane transporter that exchanges ATP/ADP

PIC carrier
Phosphate carrier, is an inner mitochondrial membrane transporter of phosphate

AAA+ proteases
subset of ATPase proteases that participate in diverse quality control mechanisms in mitochondria and cytosol (26S proteasome)

AKT
(a.k.a., Protein Kinase B, PKB) Group of serine/threonine kinases that respond to a myriad of external stimuli and include AKT1, AKT2 and AKT3

substrate channelling
Biochemical phenomena whereby the intermediate product from one enzyme is shuttled as a substrate for the next enzyme; e.g.: reduced CoQ from respiratory complex I is transferred and oxidized by respiratory complex III_2 to reduce cytochrome C. Reduced cytochrome C is oxidized by respiratory complex IV. As a result of this substrate channelling, protons are pumped to intermembrane space through each complex to perform cellular respiration

sphingolipids
Class of phospholipids containing a sphingosine backbone. Sphingolipids facilitate mitochondrial function by stabilizing respiratory complexes but their accumulation correlates with mitochondrial dysfunction and chronic metabolic diseases such as type 2 diabetes

***sn-1* position**
First stereochemical position on a glycerol moiety to which a fatty acid is attached

Barth syndrome
A rare X-linked genetic disorder of cardiolipin metabolism that presents with cardiomyopathy, neutropenia and muscle weakness

nuclear receptors
ligand-regulated transcriptional factors that are activated by steroid hormones and other lipid-related molecules

substantia nigra
Basal ganglia structure in the brain that plays important roles in behaviour-reward neuronal programs

Sengers syndrome
rare autosomal condition that courses with myocardiopathy, lactic acidosis, muscle weakness and short life expectancy

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Box 1 |

Mitochondrial respiratory chain and bioenergetics.

The respiratory chain is composed of five multisubunit complexes that ultimately generate ATP, the energy equivalent of the cell. These complexes are defined as complex I (NADH-UQ oxidoreductase), complex II (succinate dehydrogenase), complex III (UQH_2–cyt *c* oxidoreductase), complex IV (cytochrome *c* oxidase), and complex V (ATP synthase). In addition to electron transport function, complexes I, III, and IV are oxidation-reduction proton pumps that produce the proton gradient across the mitochondrial inner membrane. The mobile electron carriers in the electron transport chain are ubiquinone/ubiquinol (UQ/UQH_2) also known as Coenzyme Q10 (CoQ10) that diffuse within the inner membrane and cytochrome *c* that is located on the intermembrane space side of the inner membrane. UQH_2 donates electrons to complex III, while cytochrome *c* donates to complex IV.

From complexes I-IV, electrons are transferred from a redox potential span of 1.1V from the NAD^+/NADH redox couple to the $O_2/2H_2O$ couple[257]. NADH with a midpoint potential of −320 mV, serves as electron shuttle between the matrix dehydrogenases of the TCA cycle and complex I of the respiratory chain[257]. Many other enzymes with a more positive redox potential cannot thermodynamically donate electrons to NADH and instead, donate electrons directly to the UQ/UQH_2 pool, bypassing complex I[257]. These include the flavoproteins succinate dehydrogenase, glycerol-3-phosphate dehydrogenase, dihydroorotate dehydrogenase (pyrimidine synthesis), and ETF–ubiquinone oxidoreductase (fatty acid oxidation). The redox components within the respiratory chain are flavoproteins, cytochromes containing haem, iron–sulfur (Fe-S) proteins, ubiquinone, and copper that is tightly bound to certain protein subunits. Electron transport paths across the respiratory complexes are reviewed elsewhere[257–259], however, recent cryo-EM structural information on respiratory complexes and supercomplexes are enabling us to address unresolved and novel questions on the structure-function relations that drive mitochondrial respiratory activity[105,178,250,260–262].

The rates of electron transport are regulated by multiple mechanisms in cells. Downstream targets include i) allosteric regulation of respiratory enzymes, ii) enzyme content (e.g. respiratory complex abundance), iii) cofactor levels (e.g. NAD^+/NADH, $FAD/FADH_2$, $CoQ/CoQH_2$, cytochrome *c*) mitochondrial density or membrane morphology such as cristae folding. Allosteric control of respiratory enzymes occurs by NAD^+/NADH, ATP and AMP, CoA/acetyl CoA, and Ca^{2+}. For example, NADH is an allosteric inhibitor of TCA dehydrogenases[263] and ATP is of pyruvate dehydrogenase, isocitrate dehydrogenase[263], and cytochrome-*c* oxidase/complex IV[264]. Thus, when a cell enters a high energy state defined by high NADH and ATP levels, TCA and respiratory chain activity is attenuated. By contrast, when a cell requires ATP defined by high ADP/ATP ratio or increased AMP levels, TCA and respiratory chain activity are induced.

Box 2 |

Mitochondrial-ER contacts and mitochondrial functions.

A crosstalk between the endoplasmic reticulum (ER) and mitochondria regulates mitochondrial dynamics. Communication between these two organelles controls fission and fusion events[22], Ca^{2+} release[12] and cristae formation[21]. Lack of mitochondrial–ER communication is associated with the development of age-related diseases including obesity[265] and type 2 diabetes[266].

Physical contact between the ER and mitochondria constitutes the Mitochondrial-ER Contacts (MERCs). MERCs provide a tethering system that stabilizes communication between organelles. Mitofusins are proteins anchored at the outer mitochondrial membrane and the ER that actively participate in the formation of MERCs[267]. Mitofusins interact with each other[267] and have been shown to establish contacts with ER stress sensors such as PERK[268], which is an important regulator of ER stress responses, mitochondrial protein import and cristae formation[21]. Mitochondrial fission and fusion events occur at the MERCs where mitofusin 1 (MFN1), MFN2 and Mitochondrial Dynamin Like GTPase OPA1 actively participate to define mitochondrial dynamics[196,269].

MERCs regulate Ca^{2+} trafficking between the ER and mitochondria. Ca^{2+} entry into mitochondria defines cellular fitness and the ability to generate ATP by the OXPHOS complexes. Ca^{2+} activates pyruvate dehydrogenase, oxoglutarate dehydrogenase, and isocitrate dehydrogenase, which increase NADH levels and ETC activity[12,270]. Ca^{2+} is provided by the ER to mitochondria through the ER component inositol 3-phosphate receptor (IP3R) which forms a complex with DJ-1, GRP75, and mitochondrial voltage-dependent anion channel (VDAC) to regulate the transfer of Ca^{2+} from the ER to the mitochondrial matrix via the mitochondrial calcium uniporter (MCU)[271]. ER-resident vesicle-associated membrane protein B (VAPB) interacts with outer mitochondrial membrane protein tyrosine phosphatase-interacting protein-51 (PTPIP51) to promote the assembly of a tether complex that allows MERC formation[272]. Loss of this interaction results in improper Ca^{2+} trafficking and mitochondrial dysfunction[272–274]. Other components such as mitochondrial import receptor TOM70 of the outer membrane[152] and mitofusins[275] are implicated in ER-mitochondrial Ca^{2+} trafficking via interaction with IP3R and stabilization of MERCs, respectively.

Lipid trafficking between the ER and mitochondria is an open paradigm. The ER is the major supplier of lipids for mitochondria by means that are nebulous. Aside from vesicular lipid diffusion between organelles, the ERMES system in yeast[225] and VPS13D in mammals[226] participate in the transfer of lipids to mitochondria, indicating an enzymatic-driven machinery that can dictate lipid fluxes to adapt mitochondrial structure (e.g.: cristae biogenesis) and respiratory function. MERCs are a central node for lipid trafficking between the ER and mitochondria.

Box 3 |

Examples of tissue-specific physiological/pathological implications of defective adaptation of mitochondrial respiratory function

Mitochondrial respiratory chain activity is required for normal function in tissues with high energy demand such as brain, heart, and skeletal muscle. Diseases caused by underlying mitochondrial dysfunction often affect these organ systems. For example, mitochondrial diseases are genetically inherited disorders caused by mutations in nuclear- or mitochondrial-encoded mitochondrial genes. These mutations impact respiratory chain function either directly (mutation in genes encoding respiratory chain subunits) or indirectly (mutation in genes encoding mitochondrial DNA replication, transcription, translation, or membrane fission/fusion (OPA1/DRP1) machineries). One hallmark of mitochondrial DNA (mtDNA) mutations is exercise intolerance and muscle fatigue, which is characterized by an inability to maintain force during repeated or sustained muscle contractions, resulting from progressive dysfunction of the respiratory chain[276]. This stems from a mosaic impairment of mitochondrial quality control via autophagy (mitophagy) in muscle fibres, driving accumulation of mitochondria with pathogenic (mutated) mtDNA and concomitant respiratory chain deficiency, resulting from the inability of such mitochondria to generate functional respiratory complexes[277].

Impairment of transcriptional regulation of mitochondrial function has considerable pathological implications. In brown fat, the inability to increase mitochondrial genes during cold or overnutrition results in defects on thermoregulation, energy balance, and diabetes[278,279]. In skeletal muscle, loss of PGC1α affects exercise capacity and/or causes increased fatigue due to the loss of slow twitch muscle fibres, which are highly enriched in mitochondria[26,35]. Other physiological conditions where mitochondrial energetic adaptation is required are perinatal cardiac adaptation[280] or kidney function[281]. For example, defects in the transcriptional regulation of mitochondrial biogenesis cause acute renal injury and fibrosis[282]. Certain parts of the brain such as the *substantia nigra* **[G]** are especially sensitive to mitochondrial deficits[283]. Loss of dopaminergic neurons occur in Parkinson disease and is associated with defects in the Parkin-mediated mitophagy regulatory pathway that promotes PGC1α activity[284]. Other neurodegenerative diseases associated with mitochondrial failures are Alzheimer disease and Huntington disease[285]. Although less explored, emerging data also implicate the important role of mitochondrial respiratory adaptation in immune cells, such as during T cell or NK cells activation in response to infection or cancer cells[286,287].

Defects in other processes indirectly linked with mitochondrial respiratory function also lead to human disease. The mitochondrial phospholipid cardiolipin is essential for the assembly and stability of protein import complexes, metabolite carriers, and the respiratory chain. Consequently, mutations in the cardiolipin fatty-acid-chain remodelling protein TAZ-1 leads to dilated cardiomyopathy, skeletal myopathy, and neutropenia in Barth syndrome, while mutations in cardiolipin biosynthetic enzyme AGK cause hypertrophic cardiomyopathy in Sengers syndrome **[G]**[288]. In addition, mutations in mitochondrial protein import machinery such as DDP1/Tim8 and DNAJC19/Tim14 cause the neurodegenerative disorder human deafness dystonia syndrome and dilated

cardiomyopathy with ataxia, respectively[289]. Interestingly, DNAJC19 also participates in cardiolipin remodelling by interacting with prohibitins, a group of mitochondrial proteins that act as scaffolds that define membrane domains[288].

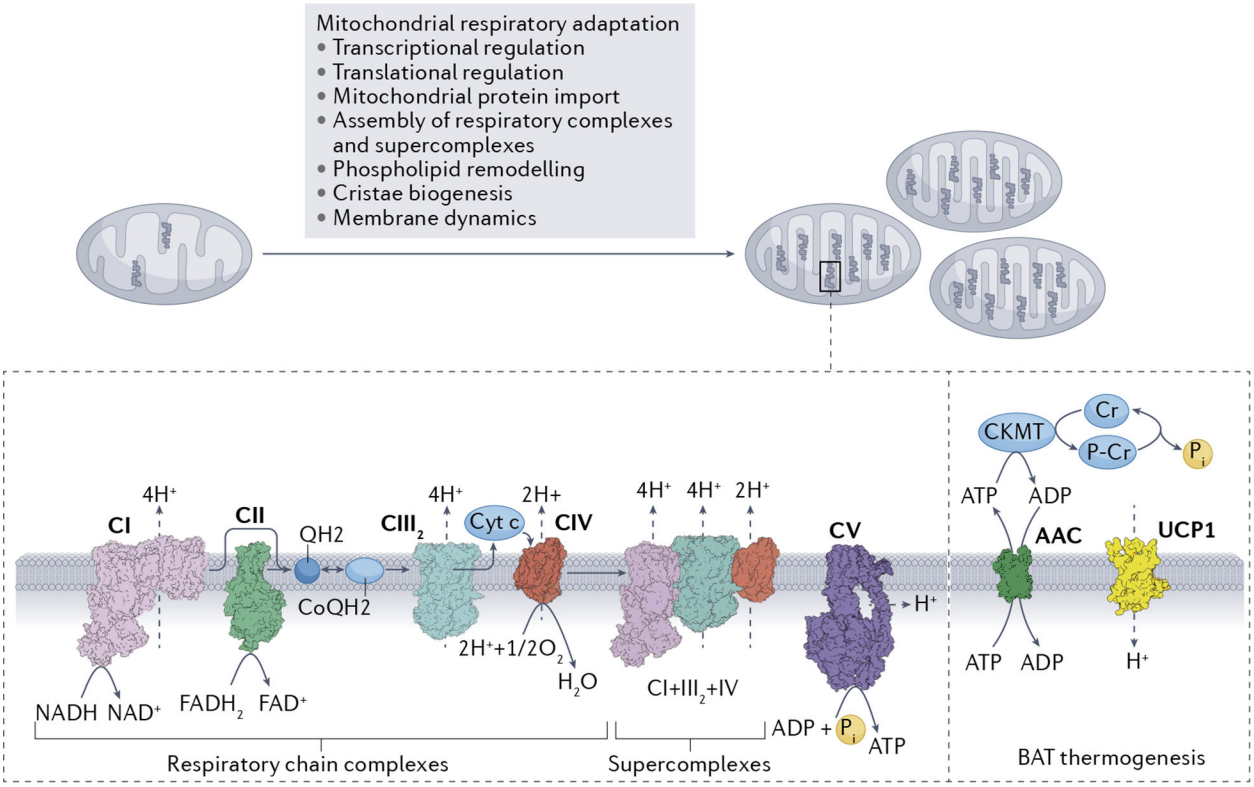

Figure 1 |. Regulation of mitochondrial respiratory capacity.

Cells and tissues experience different external and internal conditions that require mitochondrial adaptation to support ATP generation or heat production. Most cells utilize the mitochondrial respiratory chain for ATP generation, whereby ATP synthesis at respiratory complex V (CV, ATP synthase) is coupled to the electron transport through the respiratory complexes I-IV (CI, CII, $CIII_2$, CIV); however, certain cells such as brown and beige adipocytes produce heat through oxidation of metabolic substrates paired with uncoupled respiration. Multiple levels of regulation of mitochondrial respiration: from transcriptional and translation control to protein (post-translational) control. These processes increase mitochondrial biogenesis — depicted by an increase in mitochondrial number — and respiratory capacity. In the latter case, cellular pathways influence precursor protein import, membrane/cristae dynamics, respiratory chain assembly and superassembly, and phospholipid composition or remodelling to either increase the concentration of respiratory complexes in the inner mitochondrial membrane and/or their activity. Protein structures used in this figure are sourced from PDB: 5gup[250] (CI+$CIII_2$+CIV), 1zoy[251] (CII), 6zpo[252] (CV), 1okc[253] (AAC), 2lck[254] (UCP2). AAC, ADP/ATP carrier; CKMT, mitochondrial creatine kinase; Cr, creatine; P-Cr, creatine phosphate; Pi, inorganic phosphate; Q, ubiquinone; QH_2, ubiquinol.

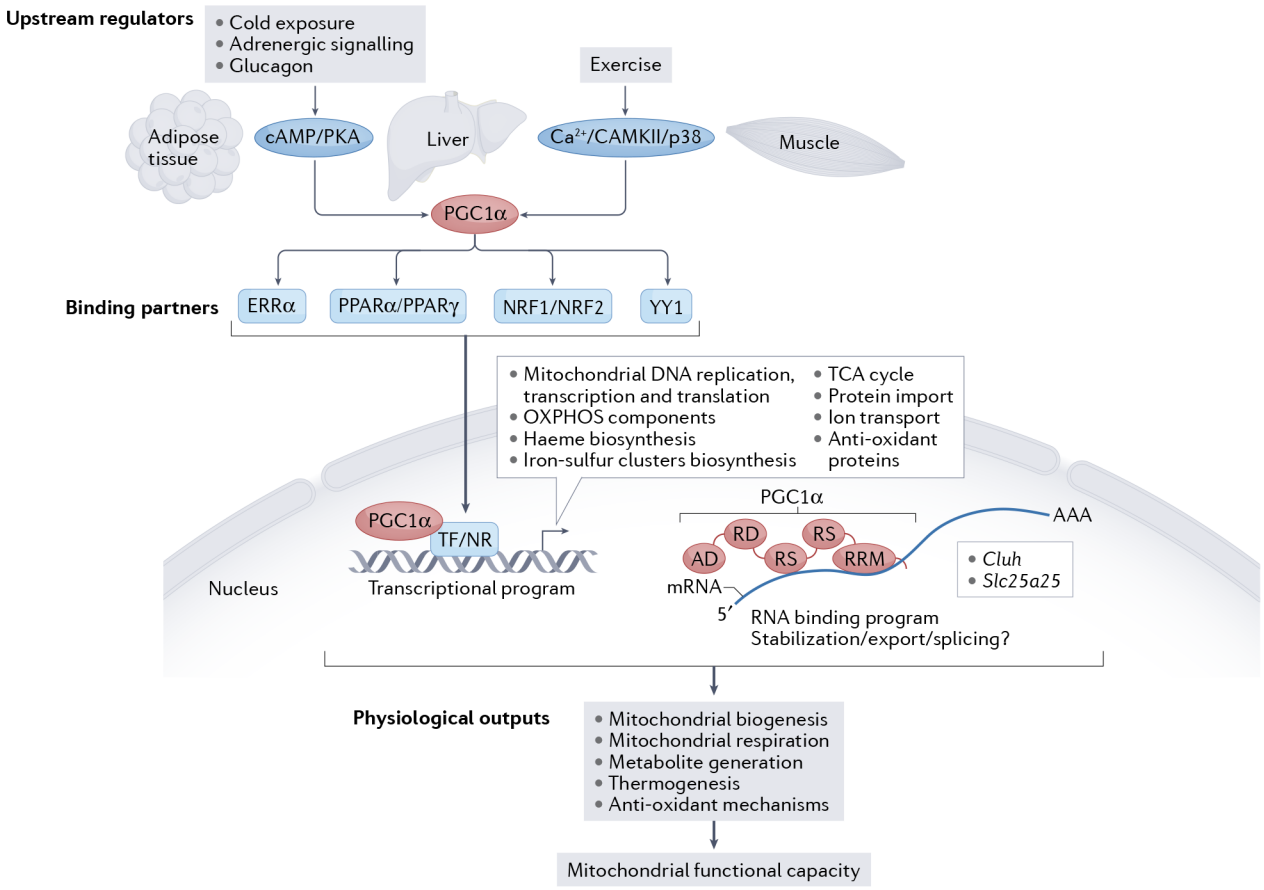

Figure 2 |. Transcriptional control of mitochondrial biogenesis through PGC1α.

PPARγ coactivator-1α (PGC1α) is a major transcriptional regulator of mitochondrial function. It is activated through multiple upstream stimuli including cold exposure and exercise. These signals converge on PGC1α either through the upregulation of *PPARGC1A* mRNA or stabilization of the protein. Once PGC1α accumulates, it interacts with various transcription factors (TFs), including several nuclear receptors **[G]** to promote mitochondrial gene expression that together increase mitochondrial functional capacity. The transcriptional network of PGC1α is complex, but one intriguing model is that specificity of gene expression programs is designated through interaction with specific transcription factors to control diverse aspects of mitochondrial function and biogenesis across numerous cell types. Another emerging mechanism of PGC1α-mediated regulation is its direct binding to various mRNAs (including *Cluh* involved in RNA granules as well as *Slc25a25*, which encodes a mitochondrial solute carrier (see also. Fig. 3)) through Ser/Arg-rich (RS) domains and RNA recognition motif (RRM). How PGC1α mRNA binding controls mRNA expression, processing, or export is unclear and an exciting area of future discovery. CAMKII, Calcium/calmodulin-dependent protein kinase II; PKA, protein kinase A; AD, activation domain; RD, repression domain.

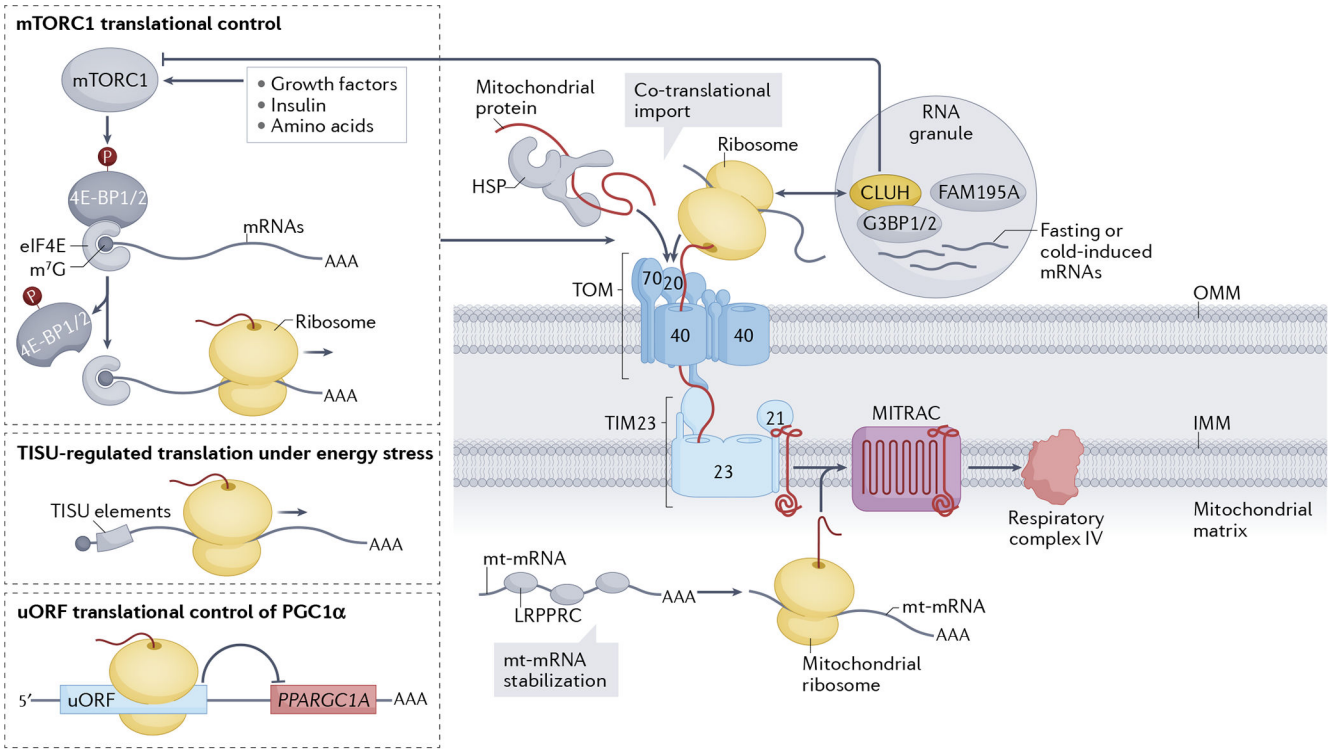

Figure 3 |. Translational control of mitochondrial respiratory chain assembly.

Multiple mechanisms regulate translation of nuclear-encoded mitochondrial mRNAs. mTORC1 is the primary driver of translation through inhibitory phosphorylation of 4E-BP1/2, releasing its interaction from the cap-binding protein eIF4E. This stimulates eIF4F formation on the 5' cap of mRNAs, recruitment of the 40S ribosome to the mRNA, formation of the AUG-48S initiation complex, and finally the 80S initiation complex preceding translation. Many mRNAs important for energy generation, including mRNAs encoding mitochondrial proteins are highly sensitive to the phosphorylation status of 4E-BPs. mTORC1 also controls energy metabolism by stimulating the activity of several transcriptional regulators such as PPARγ coactivator-1α (PGC1α). Hence mitochondrial energetics is coupled to nutritional status via mTORC1. Nevertheless, certain mRNAs — heavily enriched for mitochondrial mRNAs — contain short 5' UTRs and in many cases translation initiator elements termed TISU. TISU elements enable efficient translation initiation of short 5' UTR mRNAs, even when global protein synthesis is impaired from energetic defects. PGC1α is also regulated at the translational level through upstream open reading frame (uORF)-mediated translational repression. If this mechanism occurs under certain physiological conditions or negatively regulated by mTORC1, similar to the uORF-mediated regulation of ER-stress factor ATF4[255], is not yet known. Translation of the majority of mRNAs for mitochondrial proteins occurs in the cytosol and resulting peptides are stabilized by heat shock proteins (HSPs) before delivery to the TOM40 channel. For a subset of mRNAs, co-translational import at the outer mitochondrial membrane (OMM) through interaction with TOM70 occurs. Specific mRNAs involved in fasting or cold responses are also stabilized in RNA granules, supporting their translational capacity. One class of RNA granules is formed by CLUH, which associates with mitochondrial

mRNAs and also acts to sequester mTORC1 and RNA binding proteins G3BP1/2 to promote mitophagy. Another, termed stress granules, can contain the RNA binding protein FAM195A critical for branched-chain amino acid metabolism and thermogenesis. Paired with the influx of nuclear-encoded proteins, mitochondria adapt their protein synthesis rates via sensor proteins and complexes such the mitochondrial RNA binding protein LRPPRC and mitochondrial translation regulation assembly intermediate of cytochrome c oxidase (MITRAC). These mechanisms coordinate respiratory chain assembly through the assembly of imported peptides with mitochondrial-encoded peptides (all of which encode components of the respiratory chain). IMM, inner mitochondrial membrane.

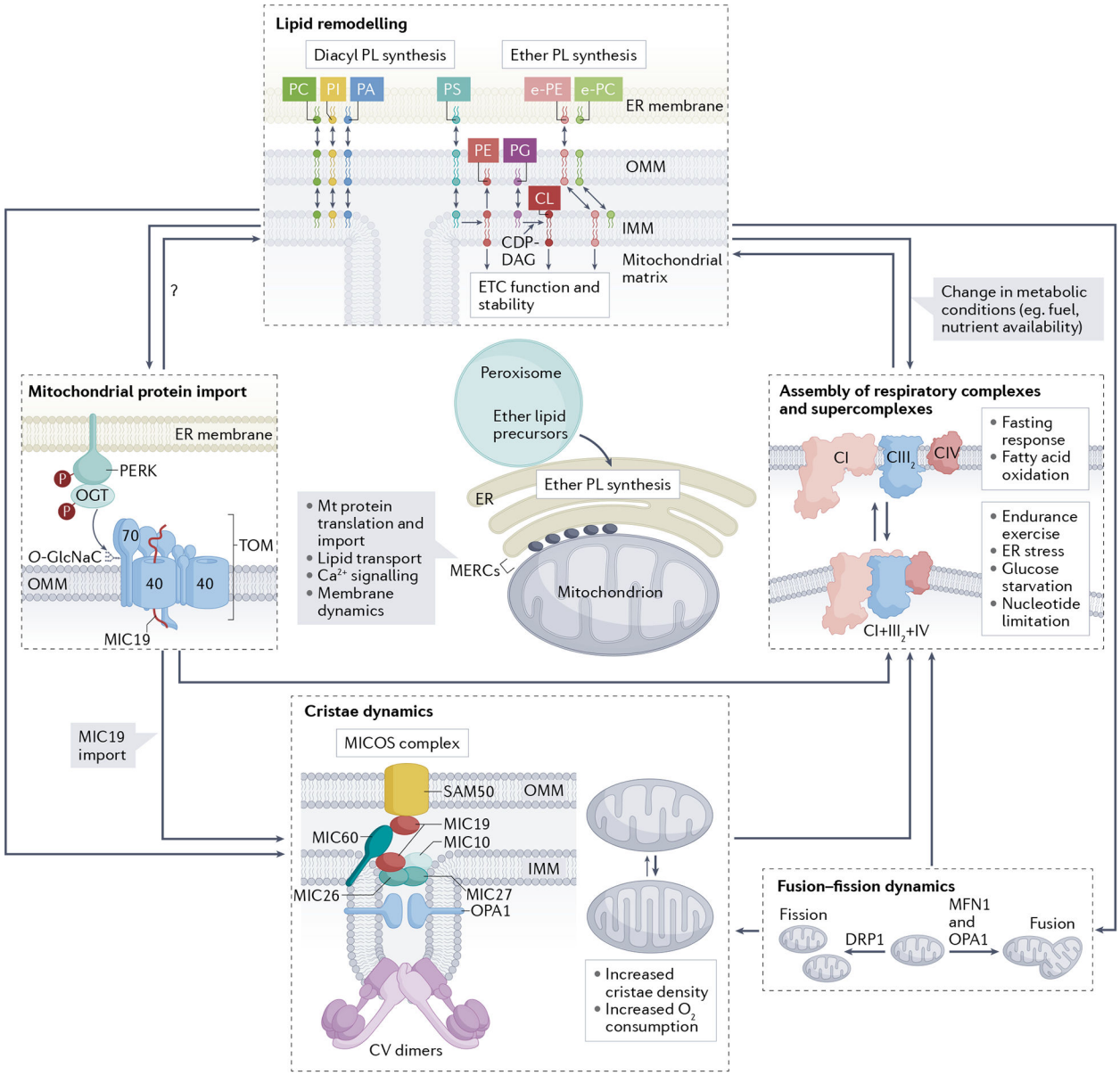

Figure 4 |. Post-translational mechanisms governing respiratory control and the role of mitochondrial membrane dynamics.

Multiple interconnected levels of post-translational control, involving inter-organelle crosstalk, promote mitochondrial respiration. Under certain metabolic conditions such as thermogenesis in beige/brown adipose tissue, exercise or insulin/IGF1 stimulation of muscle tissue, or nucleotide limitation in cancer cells, phospholipid (PL) synthesis of cardiolipin (CL), phosphatidylethanolamine (PE), or ether lipid PE are augmented to facilitate respiratory chain stability, activity, and supercomplex assembly. Synthesis of ether lipids initiates in the peroxisome and terminates in the ER, linking these organelles to mitochondrial respiratory function. Stoichiometric shifts in respiratory chain complexes towards superassembly occur under physiological conditions such as exercise and cellular stress including glucose or nucleotide limitation to enhance mitochondrial

respiration. Increased respiratory capacity of mitochondria is also promoted by cristae remodelling, which is supported by the mitochondrial contact site and cristae organizing system (MICOS). MIC60 subcomplex promotes the invagination of the inner mitochondrial membrane (IMM), which is facilitated by stabilization of the membrane via outer mitochondrial membrane (OMM) components such as SAM50. MIC10 subcomplex promotes cristae elongation. The coordination of the MICOS subcomplexes relies on the MICOS component MIC19, which bridges MIC60 and MIC10 subcomplexes. Along with the MICOS complex dynamin-like GTPase and mitochondrial fusion factor, OPA1, maintains cristae junction stability and cristae architecture[15,16]. Formation of complex V (CV; ATP synthase) dimers are an important structural element at the tip of cristae that provides proper curvature[213,216,256]. Mitochondrial–ER contacts (MERCs) — where OMM is in close apposition to the ER membrane — regulate various aspects of mitochondrial structure and function, including protein translation and import, lipid transport, membrane dynamics and Ca^{2+} signalling. During ER stress (which can result from, for example, glucose deprivation or cold stimulation), ER-localized PERK kinase activates *O*-GlcNAc transferase (OGT), which results in *O*-GlcNAcylation of the receptor TOM70. This promotes TOM70-dependent protein import, including import of MIC19, supporting cristae formation and oxygen consumption (at least in brown adipocyte mitochondria). Cristae biogenesis is also correlated with respiratory chain superassembly. Mitochondrial fusion and fission also impact mitochondrial respiratory capacity, tying with regulation of the assembly and organization of respiratory complexes and cristae dynamics. Fusion–fission events are themselves regulated by lipid remodelling. PA, phosphatidic acid; PC, phosphatidylcholine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; DRP1, dynamin-related protein 1; MFN1, mitofusin 1.