The FEBS Journal

FEBS PRESS science publishing by scientists

STATE-OF-THE-ART REVIEW

Mitochondrial biogenesis and clearance: a balancing act

Christina Ploumi[1,2], Ioanna Daskalaki[1,3] and Nektarios Tavernarakis[1,2]

1 Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, Heraklion, Greece
2 Department of Basic Sciences, Faculty of Medicine, University of Crete, Heraklion, Greece
3 Department of Biology, University of Crete, Heraklion, Greece

Keywords
autophagy; mitochondrial biogenesis; mitophagy; nuclear respiratory factors; Parkin

Correspondence
N. Tavernarakis, Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, N. Plastira 100, Vassilika Vouton, PO Box 1385, Heraklion 70013, Crete, Greece
Fax: +30 2810 391067
Tel: +30 2810 391069
E-mail: tavernarakis@imbb.forth.gr

(Received 15 April 2016, revised 14 June 2016, accepted 25 July 2016)

doi:10.1111/febs.13820

Mitochondria are semi-autonomous organelles of prokaryotic origin that are postulated to have been acquired by eukaryotic cells through an early endosymbiotic event. Except for their main role in energy production, they are also implicated in fundamental cellular processes, including ion homeostasis, lipid metabolism, and initiation of apoptotic cell death. Perturbed mitochondrial function has been correlated with severe human pathologies such as type-2 diabetes, cardiovascular, and neurodegenerative diseases. Thus, proper mitochondrial physiology is a prerequisite for health and survival. Cells have developed sophisticated and elaborate mechanisms to adapt to stress conditions and alterations in metabolic demands, by regulating mitochondrial number and function. Hence, the generation of new and the removal of damaged or unwanted mitochondria are highly regulated processes that need to be accurately coordinated for the maintenance of mitochondrial and cellular homeostasis. Here, we survey recent research findings that advance our understanding and highlight the importance of the underlying molecular mechanisms.

Abbreviations
AGO2, argonaute 2; AMPK, AMP-activated protein kinase; ATFS1, activating transcription factor associated with stress 1; Atg32, autophagy-related 32; Bcl-2, B-cell lymphoma 2; BCL2L13, Bcl-2-like 13; Bnip3, Bcl-2/adenovirus E1B 19kDa-interacting protein 3; Bnip3L/Nix, Bnip3-like/NIP3-like protein X; CCCP, carbonyl cyanide m-chlorophenyl hydrazone; CerS1, ceramide synthase 1; CL, cardiolipin; COXIV, cytochorome C oxidase subunit IV; CREB, cAMP response element-binding protein; DAF-16, abnormal DAuer Formation 16; DCT-1, DAF-16/FOXO controlled germline tumor-affecting 1; DRP1, dynamin-related protein; ER, endoplasmic reticulum; ERRα, estrogen-related receptor alpha; FOXO, forkhead box O; FUNDC1, FUN14 domain-containing 1; GABARAP, GABA(A) receptor-associated protein; GABP, GA-binding protein transcription factor; GCN5L1, general control of amino acid synthesis 6-like 1; HO-1, heme oxygenase-1; ILS, insulin-like signaling; IMM, inner mitochondrial membrane; IMS, intermembrane space; KEAP1, Kelch-Like ECH-associated protein 1; LC3-II, lipidated form of LC3; LC3, light chain 3; LIR, LC3-interacting region; MAPK, mitogen-activated protein kinase; Mba1, multi-copy Bypass of AFG3; MDVs, mitochondrial-derived vesicles; MFN2, mitofusin 2; miRNA, micro RNA; MPP, mitochondrial processing peptidase; MtCK, mitochondrial creatine kinase; mtDNA, mitochondrial DNA; MTHFD2, methylenetetrahydrofolate dehydrogenase ($NADP^+$ dependent) 2; mTOR, mechanistic target of rapamycin; NAC, nascent polypeptide-associated complex; NBR1, neighbor of BRCA 1 gene 1; NDP52, nuclear dot protein 52 kDa; NDPK-D, nucleoside diphosphatate kinase-D; NFE2L, nuclear factor erythroid 2-like; NRF, nuclear respiratory factor; OMM, outer mitochondrial membrane; OPA1, optic atrophy 1; p62, nucleoporin 62; PARIS, Parkin-interacting substrate; PARL, presenilin-associated rhomboid-like protease; PGAM-5, phosphoglycerate mutase homolog-5; PGC1α, proliferator-activated receptor gamma coactivator 1 alpha; PGC1β, proliferator-activated receptor gamma coactivator 1 beta; PINK1, propeptide of phosphatase and tensin homolog-induced kinase 1; PKA, protein kinase A; PLS3, phospholipid scramblase 3; rRNA, ribosomal RNA; SAM, sorting and assembly machinery; SIRT, sirtuin; SKN-1, skinhead-1; SOD1, superoxide dismutase 1; SQSTM1, sequestosome 1; TFAM, transcription factor A mitochondrial; TFB1M, transcription factor B1 mitochondrial; TFB2M, transcription factor B2 mitochondrial; TFE3, transcription factor biding to IGHM enhancer 3; TFEB, transcription factor EB; TIM, translocase of the inner membrane; TOMM34, translocase of outer mitochondrial membrane 34kDa subunit; TOM, translocase of the outer membrane; ULK1, unc-51-like autophagy-activating kinase 1; UPR^{mt}, mitochondrial unfolded protein response; UPS, ubiquitin proteasome system; $\Delta\Psi m$, mitochondrial membrane potential.

Introduction

Mitochondria, the so-called 'powerhouses of the cell', are double-membrane organelles that form highly dynamic and multifunctional networks in the cytoplasm of eukaryotic cells. Owing to their bacterial origin, mitochondria carry multiple copies of their own circular DNA. Nevertheless, the majority of mitochondrial proteins are encoded by nuclear genes. Aside from their crucial role in energy production, mitochondria constitute major regulators of cellular homeostasis and survival. Lipid metabolism, calcium signaling, metabolite synthesis, as well as apoptotic cell death are only some of the pathways in which mitochondria play pivotal roles.

Proper mitochondrial function is maintained through mechanisms that tightly regulate the biogenesis and clearance of mitochondria. As mitochondria cannot be generated *de novo*, new organelles arise from pre-existing ones, through a multi-step process which involves both fusion and fission events. Mitochondrial biogenesis also entails replication of mitochondrial DNA (mtDNA), transcription, and translation of mtDNA-encoded genes, as well as loading of phospholipids and nuclear-encoded proteins in different mitochondrial subcompartments [1,2]. Perturbed mitochondrial function may trigger cell death and is a hallmark of various human pathologies ranging from diabetes to neurodegeneration and age-related disorders [3]. Thus, cells have developed efficient mechanisms to remove damaged or dysfunctional mitochondria, with autophagy the most prominent one. Autophagic elimination of mitochondria is achieved either nonselectively through general autophagy, or selectively, through specific, mitochondria-targeting autophagy, termed mitophagy. In this review, we discuss recent findings that shed light on the pathways that govern mitochondrial biogenesis and turnover, focusing primarily on the regulatory mechanisms underlying their crosstalk and proper coordination.

Mitochondrial biogenesis

Mitochondrial biogenesis is a tightly regulated process, dependent on the activity of both mitochondrial and nuclear factors (Fig. 1). Although almost 90% of mitochondrial proteins are nuclear-encoded, synchronous transcription and translation of nuclear and mitochondrial genes is needed for the generation of new organelles. For example, the formation of respiratory chain complexes involves both nuclear- and mitochondrial-encoded products. Imbalance in the composition of these complexes leads to proteotoxic stress and subsequent activation of mitochondrial turnover mechanisms [4,5].

Following transcription and translation, nuclear-encoded proteins are folded in a process coupled to the translocase of the outer membrane (TOM)-mediated import [6]. After being folded, mitochondrial proteins are directed by their presequence to different mitochondrial subcompartments: the outer mitochondrial membrane (OMM), the intermembrane space (IMS), the inner mitochondrial membrane (IMM), or the matrix [7]. These steps are controlled by the AMP-activated protein kinase (AMPK), the mechanistic target of rapamycin (mTOR), and insulin-like signaling (ILS) pathways, as well as, by signaling cascades triggered by calcium and nitric oxide [8–12].

Nuclear transcription regulation

Mitochondrial biogenesis is orchestrated by specific nuclear transcription factors that regulate the expression of genes encoding mitochondrial proteins. The nuclear respiratory factors (NRF1 and NRF2) are key components of the regulatory network that controls mitochondrial biogenesis. Studies in cell cultures reveal that NRF1 forms homodimers and regulates multiple target genes, encoding proteins of the respiratory complex [cytochrome C oxidase subunit IV (COXIV), cytochrome c], the mitochondrial protein import machinery (TOMM34), the detoxification response (i.e. components of glutathione biosynthesis pathway and metallothionein-1 and 2), the heme biosynthetic pathway (globin), and the transcription of mtDNA [transcription factor A mitochondrial (TFAM), transcription factor B1 mitochondrial (TFB1M), transcription factor B2 mitochondrial (TFB2M)], among others [13–15]. Notably, NRF1 transcriptional activity is methylation-dependent [16]. NRF2 [also known as GA-binding protein transcription factor (GABP)], similar to NRF1, regulates the expression of mitochondrial transcription factors (TFAM, TFB1M, TFB2M) and components of the respiratory chain (COXIV subunits) [17]. Furthermore, the GABP beta subunit 1 (GABPβ1) is deacetylated by SIRT7, a modification that triggers its binding to GABP alpha subunit (GABPα) and enhances GABP transcriptional activity in mice [18].

Nuclear factor erythroid 2-like 2 (NFE2L2) is another transcription factor implicated in the regulation of mitochondrial biogenesis. Importantly, NFE2L2 indirectly regulates NRF1 target genes by inducing NRF1 gene expression, as tested in mice and mammalian cell cultures [19]. NFE2L2 also targets several genes implicated in various mitochondrial

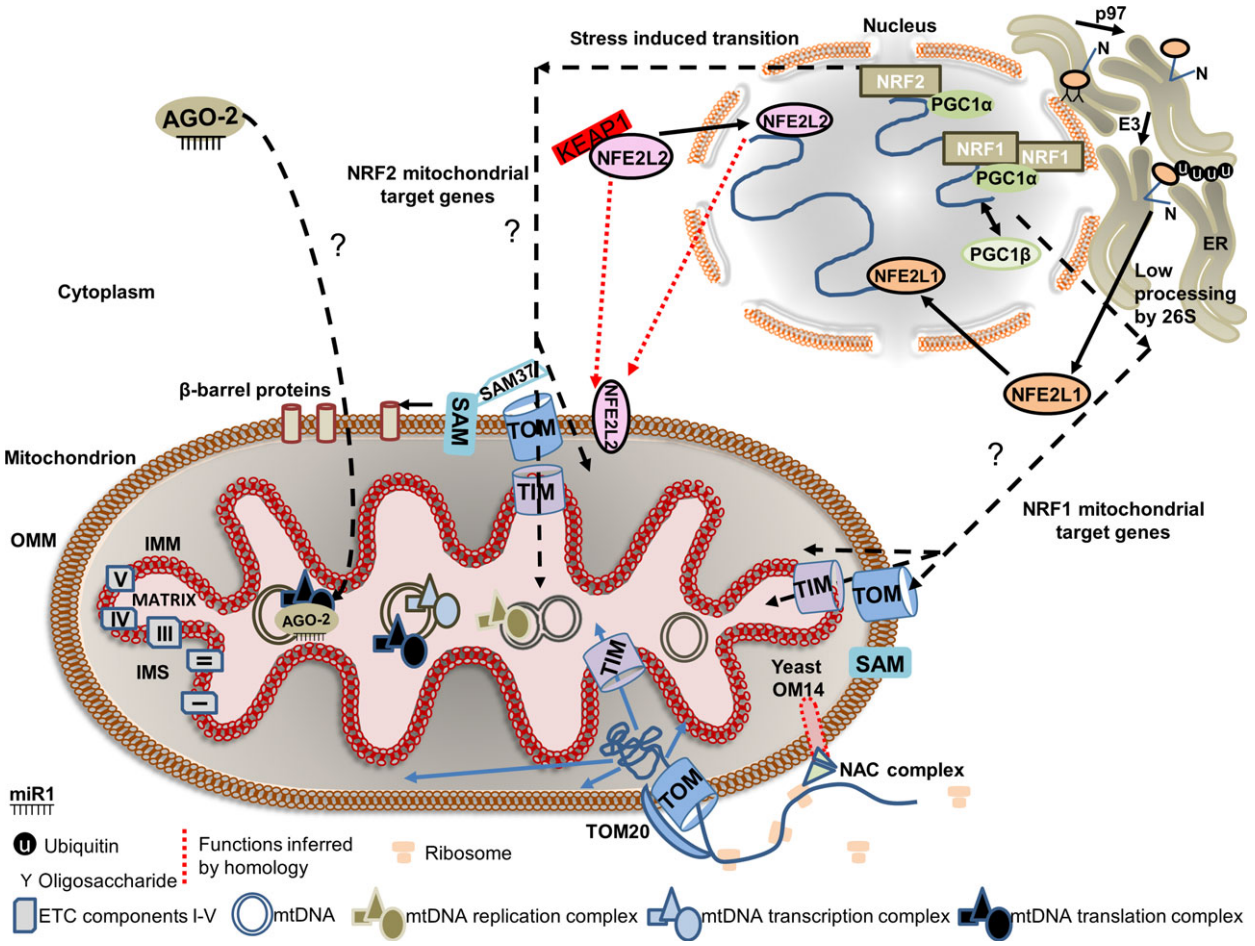

Fig. 1. Mechanisms that contribute to mitochondrial biogenesis. Transcription of mitochondrial genes by NRFs and their cofactors in the nucleus, is followed by transport of these mRNA in the mitochondrial vicinity, where mRNA translation takes place in association with the TOM20 and NAC complexes. Newly synthesized proteins are transported and positioned in the proper mitochondrial subcompartments. In parallel, mitochondria-encoded proteins are produced through transcription and translation of the mtDNA, which is maintained in many copies through replication in mitochondria. Alternatively, mitochondrial translation can be regulated by cytoplasmic factors, with the involvement of AGO2 and its associated miRNA. Except for NRFs, transcription factors NFE2Ls also target mitochondrial genes and their transcriptional activity is regulated by their subcellular localization.

processes and biosynthetic pathways such as the heme oxygenase-1 (HO-1), the methylenetetrahydrofolate $NADP^+$-dependent dehydrogenase 2 (MTHFD2), and the superoxide dismutase 1 (SOD-1) [19–21]. NFE2L2 is predominantly retained in the cytoplasm by interacting with the Kelch ECH-associated protein 1 (KEAP1). In response to stress, NFE2L2 dissociates from KEAP1 and translocates to the nucleus, where it transcriptionally regulates its target genes [22]. Similarly, the *Caenorhabditis elegans* homolog of NFE2L2, skinhead-1 (SKN-1), transcribes genes involved in mitochondrial biogenesis, including factors of the mitochondrial protein import machinery, ATP synthase subunits, as well as mitochondrial transcription factors [23]. Notably, SKN-1 has also been reported to be anchored on the OMM by interacting with the phosphoglycerate mutase homolog-5 (PGAM-5). This mitochondrial fraction of SKN-1 is released upon stress and enters the nucleus, where it regulates gene targets, distinct from those regulated by the cytoplasmic fraction. This indicates that SKN-1 may exert an, as yet unexplored mitochondrial-specific function, and further adds to the complexity of NFE2L2 regulation [24]. NFE2L1, another member of the nuclear factor erythroid 2-like family, has been implicated in the regulation of detoxification mechanisms [25]. It is also likely involved in mitochondrial biogenesis, as mitochondrial genes are among its potential targets. Post-translational modifications, such as proteolysis, ubiquitination, and deglycosylation have been

suggested to regulate NFE2L1 subcellular localization, and thus its transcriptional activity in human cell lines [26,27]. Nonetheless, elucidation of the involvement of NFE2L1 in mitochondrial-related functions requires further investigation.

PGC1α and PGC1β are tissue-specific transcriptional coactivators of the PPAR-γ coactivator-1 family, as it has been shown in goldfish, among others [28]. Both factors have been implicated in the regulation of mitochondrial biogenesis. Recently, Necdin has been identified as a novel regulator of PGC1α in studies performed in mice and extracted primary cortical neurons. Particularly, Necdin prevents the ubiquitination of PGC1α, thus leading to its stabilization. In turn, PGC1α upregulates the expression of genes encoding mitochondria-specific proteins, thereby contributing to mitochondrial biogenesis. Moreover, it is suggested that Necdin has a neuroprotective role in experimental models of human diseases [29]. The entire range of upstream regulators of PGC1α and β, in combination with their downstream effectors constitute a complex and interconnected mitochondria-related regulatory network. Indeed, PGC1α is a known partner of both NRFs and the estrogen-related factor α (ERRα), while PGC1β is a partner of NRF1, ERRα, ERRβ, and ERRγ, as indicated by studies performed in mouse myoblasts and primary hepatocytes, as well as in Huntington disease patients [30–32]. In myotubes of isolated mouse primary myoblasts, PGC1α is positively regulated by the deacetylase SIRT1 and the transcription factors TFE3 (transcription factor binding to IGHM enhancer 3), CREB (cAMP response element-binding protein), and the forkhead transcription factor FoxO1 [33]. Similarly, PGC1β is also regulated by CREB and c-MYC [34,35]. Several questions pertinent to the different transcriptional targets of PGC1α and PGC1β, as well as the full spectrum of their regulatory functions and modulation remain to be answered. An important open issue is whether PGC1α stabilization (Necdin-dependent or independent) protects somatic tissues against mitochondrial insults, as it happens in neurons. It would be relevant to investigate whether PGC1β exerts similar effects to those of PGC1α.

Mitochondria-associated regulatory mechanisms

In addition to nuclear-specific regulation, mitochondria-related mechanisms, such as mitochondrial protein import, mtDNA replication, transcription, and translation have an indispensable role in mitochondrial biogenesis. The OMM, TOM, and sorting and assembly machinery (SAM) complexes, as well as the IMM, translocase of the inner membrane (TIM) complex are the main mediators of mitochondrial protein import, and their role in mitochondrial biogenesis is well documented. TOM40 is the import pore of the OMM, and is permeable by all mitochondria-targeted precursor proteins. β-barrel precursors are translocated from TOM to the SAM complex which in turn mediates their integration to the OMM. In yeast, the SAM component Sam37, in addition to stabilizing the complex, also binds to Tom22 and facilitates the transfer of β-barrel precursors from TOM to SAM [36]. Tom20, another component of the TOM complex, anchors nuclear-encoded transcripts and stabilizes them in the mitochondrial vicinity, making them available for local translation by resident ribosomes. Tom20 also participates in their concomitant import through the TOM pore, as shown in yeast [37]. The function of Tom20 is likely fine-tuned by the nascent polypeptide-associated (NAC) complex, a ribosome-bound chaperone, which, as shown in *C. elegans*, prevents the formation of protein aggregates and thus enhances polypeptide import in mitochondria during translation [38]. In yeast, cytoplasmic ribosomes are attached to the OMM through a specific receptor, the outer membrane protein 14 (OM14). This association is NAC-dependent and possibly modulates mitochondrial biogenesis [39].

The processes of mtDNA replication, transcription, and translation are also major contributors to mitochondrial biogenesis. In mammals, mitochondrial transcription requires the mitochondrial RNA polymerase and the transcription factors TFAM and TFB2M [40,41]. Translation is mainly executed on the matrix-faced side of the IMM. In yeast, mitochondrial ribosomes are tethered to the IMM by two contact sites. One involves the 21S rRNA and the other a novel membrane receptor, the multi-copy bypass of AFG3 (Mba1), which is integrated in the IMM, facing the matrix [42]. In addition to ribosomes, tRNA, and coactivators, the mitochondrial translation initiation factors mIF2 and mIF3 (the AIM23 homolog in *Saccharomyces cerevisiae*) are critical for the translation of mtDNA-encoded transcripts [43].

Importantly, cytoplasmic factors also translocate to mitochondria to facilitate translation in the matrix. For example, in cardiomyocytes and myoblasts, Argonaute 2 (AGO2) was reported to specifically enter mitochondria in complex with miRNA-1 during myogenesis, although, it is not clear how AGO2 and specific miRNA enter mitochondria. In mitochondria, both AGO2 and miRNA-1 enhance translation, in contrast to their typical cytoplasmic role. Association of mitochondrial AGO2 with rRNA species and specific transcripts suggests a modulatory role in mitochondrial

translation [44]. Additional emerging findings further highlight the important role of miRNA in regulating mitochondrial physiology [45,46]. Notably, miRNA-494, miRNA-149, and miRNA-761 in mice, as well as miRNA-27b in Hep2 HeLa derivatives, differentially regulate mitochondrial biogenesis [47–50]. However, the mechanistic aspects of the involvement of miRNA in the regulation of mitochondrial gene expression remain largely elusive.

Mitochondrial turnover

During aging and under stress, mitochondrial function is perturbed by damage accumulating in mitochondria. As a first line of defense, cells have developed intricate mechanisms to repair damage and rescue the affected organelles. Intramitochondrial ATP- dependent proteases recognize misfolded and/or oxidized polypeptides in various mitochondrial subcompartments and trigger their proteolysis, whereas the cytosolic ubiquitin proteasome system (UPS) selectively targets OMM proteins [51]. In parallel, activation of the mitochondrial unfolded protein response (UPR^{mt}) initiates a retrograde signaling cascade to the nucleus, to ameliorate mitochondrial proteotoxic stress by upregulating the expression of specific mitochondrial chaperones [52]. Another interesting repair mechanism that recently came out is the generation of mitochondrial-derived vesicles (MDVs). MDVs are formed by the enclosure of mitochondrial components in mitochondrial membranes, which are subsequently cleaved through fission—independent mechanisms, released to the cytosol, and finally targeted to lysosomes for degradation [53]. In addition, mitochondrial dynamics, orchestrated by ongoing fission and fusion events contribute to the dilution and sequestration of mitochondrial damage. Failure of such mitochondrial quality control mechanisms leads to activation of pathways that ultimately mediate the destruction of injured organelles [51].

The most prominent mechanism of mitochondrial turnover is autophagy. Autophagy—meaning 'self-eating' in Greek—is a catabolic process, through which eukaryotic cells degrade cytoplasmic constituents, with the aim to salvage vital metabolites and also to protect from toxicity caused by damaged organelles or aggregated proteins. Three main types of autophagy have been identified in eukaryotic cells: macroautophagy, chaperone-mediated autophagy, and microautophagy, with macroautophagy (hereafter referred to as autophagy) being mostly responsible for organelle targeting [54].

Autophagy commences with the engulfment and sequestration of cytoplasmic material in double-membrane vesicles, called autophagosomes. The autophagosomes are subsequently fused with endolysosomes to form the autolysosomes, in which the engulfed cargo is finally degraded by resident acid hydrolases [55]. Although, it was originally believed that autophagy acts in a nonselective/bulk manner, it has now become clear that it is mainly a selective process [56]. Mitophagy, the mitochondrial-specific form of autophagy, targets mitochondria for degradation through receptor-mediated mechanisms. Three main types of mitophagy receptors have been characterized: receptors that bind ubiquitinated mitochondrial proteins, receptors constitutively localized at the OMM, and receptor-like lipid molecules of mitochondrial membranes (Fig. 2).

PINK1/Parkin-mediated mitophagy

The best known pathway of mitophagy is mediated by PINK1 and Parkin and it is mainly studied in mammalian cell cultures. Under normal conditions, the nuclear-encoded propeptide of phosphatase and tensin homolog-induced kinase 1 (PINK1) is rapidly translocated to the mitochondrial matrix of healthy mitochondria, where it is cleaved by the mitochondrial processing peptidase (MPP) [57]. The remaining transmembrane fragment of PINK1 is subsequently cleaved by the IMS presenilin-associated rhomboid-like (PARL) protease, and released in the cytoplasm, where it is finally degraded by the UPS [58]. Notably, cleaved PINK1 fragments act as mitophagy inhibitors in the cytosol, by binding directly to the E3 ubiquitin ligase Parkin and blocking its translocation and stabilization on the OMM [59].

In mitochondria with dissipated membrane potential ($\Delta\Psi m$), PINK1 remains stably associated with the TOM complex and thus escapes MPP- and PARL-dependent processing [60]. Upon homodimerization, PINK1 is autophosphorylated in T257, S228, and S402; modifications that render it capable of recruiting Parkin in the OMM [61]. PINK1-mediated phosphorylation of ubiquitin and the ubiquitin-like domain of Parkin, both at S65, are sufficient for activation of the E3 ligase Parkin [62]. Interestingly, studies in mouse embryonic fibroblasts and cardiomyocytes have shown that Mitofusin 2 (MFN2) is an additional PINK1 substrate, indicating a functional interplay between mitochondrial dynamics and mitophagy. Phosphorylated MFN2 acts as a receptor of Parkin in the OMM of damaged mitochondria [63]. However, the indispensability of MFN2 for Parkin recruitment should be further validated, given that Parkin can be efficiently stabilized on peroxisomes (organelles that lack

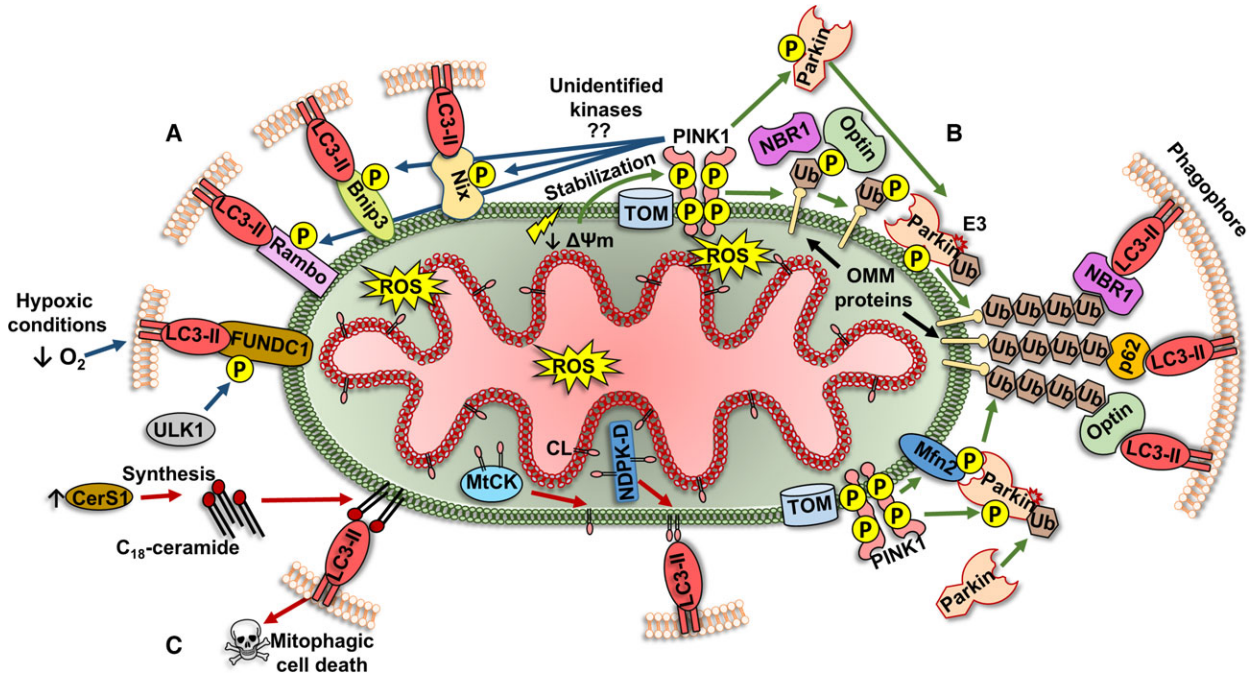

Fig. 2. Mitophagy mechanisms. Mitophagy selectively targets mitochondria for degradation through receptor-mediated mechanisms. (A) OMM receptor-mediated mitophagy. Constitutively OMM-localized proteins (Bnip3, Nix, FUNDC1, Bcl-Rambo) can directly bind to lipidated LC3 (LC3-II), through their phospho-regulated LIR domains. (B) PINK1/Parkin-mediated mitophagy. Disrupted mitochondrial membrane potential (ΔΨm) results in OMM stabilization and subsequent activation of PINK1. Targets of phosphorylated PINK1 (Ubiquitin/Ub and MFN2) recruit the E3 Ligase Parkin in the OMM. Upon PINK1-dependent activation, Parkin ubiquitinates numerous OMM proteins that, in turn, attract specific autophagy receptors (p62/SQSTM1, NBR1, optineurin/optin) to mediate mitochondrial engulfment. (C) Lipid-mediated mitophagy. OMM-localized lipids can also function as mitophagy receptors. In damaged mitochondria, cardiolipin (CL) is translocated to the OMM through specific transporters (MtCK, NDPK-D) and directly binds to LC3-II on the phagophore. Furthermore, induced synthesis of the sphingolipid C_{18}-ceramide leads to its integration in the OMM, triggering a mitophagic type of cell death.

MFN2), in which PINK1 is ectopically expressed [60]. It is likely that phosphorylated ubiquitin acts as a Parkin receptor in this case; however, the involvement of alternative Parkin interactors cannot be excluded. Upon efficient stabilization and activation on the OMM, Parkin ubiquitinates numerous OMM proteins, which in turn attract specific autophagy receptors to trigger engulfment by autophagosomes. Nucleoporin 62/sequestosome 1 (p62/SQSTM1), the neighbor of BRCA 1 (NBR1), and optineurin are such receptors, encompassing an ubiquitin-binding domain and a light chain 3 (LC3)-interacting region (LIR), which mediate interaction with the phagophore. Recent studies in HeLa cells, deficient for some autophagy receptors, have shown that optineurin and nuclear dot protein 52 kDa (NDP52) can directly interact with PINK1-dependent phospho-ubiquitin chains at the OMM of damaged mitochondria, even in the absence of Parkin [64]. These findings suggest that additional E3 ligases participate in the ubiquitination of OMM proteins and the induction of mitophagy.

OMM receptor-mediated autophagy

In addition to ubiquitin-binding receptors, proteins localized constitutively to the OMM, including the Bcl-2/Adenovirus E1B 19 kDa-interacting protein 3 (Bnip3), the Bnip3-like/NIP3-like protein X (Bnip3L/Nix), Bcl-2-like 13 (Bcl2L13), and the FUN14 domain-containing protein 1 (FUNDC1) are capable of recruiting the autophagic machinery to mitochondria. These receptors contain phosphorylation-regulated LIR motifs, which mediate direct interaction with the LC3-II-labeled phagophore. Bnip3 and its homolog Bnip3L/Nix are pro-apoptotic members of the Bcl-2 (B-cell lymphoma 2) family with a putative Bcl-2 homology 3 domain. Aside from their pro-apoptotic role, both proteins have been implicated in autophagy induction. They serve as selective mitophagy receptors, by directly binding to LC3 and GABA(A) receptor-associated proteins (GABARAP) of the phagophore and are ultimately degraded through mitophagy, as shown in mouse embryonic fibroblasts and HeLa cells

[65–67]. Recent findings in mouse embryonic fibroblasts indicate that during mitophagy, the anti-apoptotic proteins Bcl-2 and FKBP38 escape degradation, by translocating from mitochondria to the ER [68]. Rescue of anti-apoptotic, combined with degradation of pro-apoptotic proteins, likely serve prosurvival functions that prevent initiation of cell death upon mitophagy induction. Similarly, in mammalian cells, the S17 and S24 residues of Bnip3 LIR domain can serve as phosphorylation targets, resulting in the induction of mitophagy and inhibition of apoptotic cell death [69]. However, the relevant kinases and/or phosphatases remain unidentified, and PINK1 might be involved.

Recent studies have shown that DCT-1 (DAF-16/FOXO controlled, germline tumor affecting-1), the *C. elegans* homolog of Bnip3/Nix, acts downstream of PINK1/Parkin and is ubiquitinated in a PINK1-dependent manner under oxidative stress. Evidence suggests that DCT-1 ubiquitination does not induce its proteasomal degradation but likely promotes its mitophagic activity [23]. These findings are consistent with a recent study in *Drosophila melanogaster,* demonstrating that ubiquitination of BNIP3L/Nix by Parkin results in the recruitment of ubiquitin-binding receptor NBR1 to mitochondria, thereby promoting mitophagy [70]. BCL2L13, also known as Bcl-Rambo, is another member of the Bcl-2 family, which was recently identified as the mammalian functional homolog of autophagy-related 32 (Atg32) in yeast. BCL2L13 can function as an efficient mitophagy receptor in Atg32 deficient yeast cells, and promotes mitochondrial fragmentation and mitophagy in mammalian cell cultures [71]. FUNDC1 is an additional OMM mitophagy receptor in mammalian cells, which is activated under hypoxic conditions. Dephosphorylation of Y18 as well as ULK1 (UNC-51-like autophagy-activating kinase 1)-dependent phosphorylation of S17 are modifications in the LIR motif of FUNDC1 that have recently been suggested to induce its interaction with LC3 [72,73]. Interestingly, a recent study in HeLa cells showed that FUNDC1 interacts with both dynamin-related protein 1 (DRP1) and optic atrophy 1 (OPA1) factors, thus indicating that mitochondrial fission and fusion are coordinated during mitophagy [74].

Lipid-mediated mitophagy

In addition to protein receptors, OMM-localized lipids can function as efficient baits for the recruitment of the mitophagic machinery. In eukaryotic cells, cardiolipin (CL), a dimeric phospholipid, predominantly localized on the IMM, is translocated to the OMM, in damaged mitochondria, where it directly interacts with the lipidated form of LC3 (LC3-II). Several enzymes participate in CL externalization to the OMM. The nucleoside diphosphate kinase-D (NDPK-D) and the mitochondrial creatine kinase (MtCK) are IMS proteins that mediate CL transfer from the IMM to the IMS-facing side of the OMM. Subsequently, the phospholipid scramblase 3 (PLS3) facilitates transmigration of CL to the cytoplasm-facing side of the OMM, where it functions as an 'eat-me' signal [75,76]. In addition to CL, ceramide is a sphingolipid that can also interact with LC3-II. Notably, overexpression of ceramide synthase 1 (CerS1), or exogenous supplementation with C_{18}-ceramide initiates a mitophagic type of cell death in various cell carcinomas [77]. Relevant to the upstream signals that regulate intracellular levels of ceramide, overexpression of the FLT3 tyrosine kinase receptor suppresses expression of CerS1 and reduces levels of ceramide in acute myeloid leukemia cells, impinging on tumor progression. In this case, mitophagy appears to be DRP1-dependent, indicating that mitochondrial fission predisposes mitochondria for engulfment by autophagosomes [78].

Coordination of mitochondrial biogenesis and clearance

To uphold mitochondrial homeostasis, cells maintain a dynamic balance between the opposing processes of mitochondrial biogenesis and clearance. Coordination of the underlying mechanisms is primarily achieved through transcriptional and post-translational regulation of key factors (Fig. 3).

The cAMP is one of the upstream signals that regulate both mitochondrial biogenesis and mitophagy. Increased cAMP levels lead to the protein kinase A (PKA)-dependent activation of the cAMP response element-binding protein (CREB), which in turn upregulates the expression of PGC1α, the master regulator of mitogenesis [79]. In addition to its nuclear function, CREB transcriptionally activates expression of mtDNA-encoded genes as shown by *in vitro* experiments [80]. The cAMP pathway has also been suggested to negatively regulate mitophagy, as its effector, PKA, phosphorylates and inhibits LC3-II, as shown in HeLa cells [81].

The *C. elegans* NFE2L2 homolog, SKN-1, can also stimulate mitophagy, in addition to its role in mitogenesis. Indeed, accumulation of dysfunctional mitochondria activates the oxidative stress-sensitive SKN-1, which in turn initiates a retrograde response, promoting the coordinated activation of both mitophagy and mitogenesis-related genes, in the nematode.

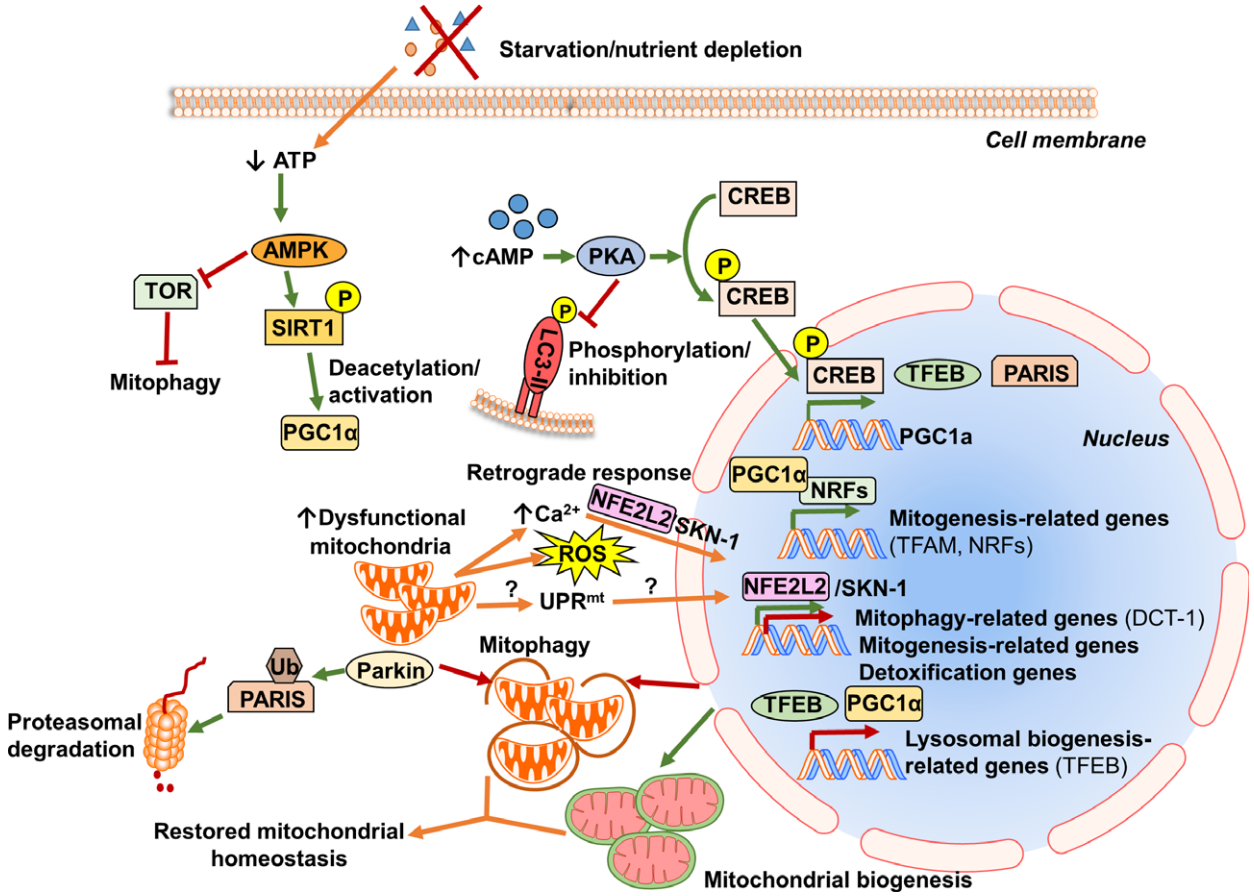

Fig. 3. Coordination of mitochondrial biogenesis and turnover. Mitochondrial homeostasis is maintained through proper coordination of both mitochondrial biogenesis and turnover. cAMP signaling tilts the balance in favor of mitogenesis as its effector, PKA, transcriptionally upregulates PGC1α and in parallel inhibits lipidated LC3 (LC3-II). In this context, the AMPK pathway has a dual role: it induces autophagy through mTOR inhibition and in parallel promotes mitochondrial biogenesis by activating PGC1α. The mitogenesis-promoting program of PGC-1α also interfaces with mitophagy by regulating lysosomal biogenesis-genes (TFEB). In *C. elegans*, excessive mitochondrial damage activates the NFE2L2 homolog SKN-1. This, in turn, initiates a retrograde response (likely also engaging UPR^{mt}) that coordinates the upregulation of mitophagy and mitogenesis-related genes. The E3 ligase Parkin is an additional regulator of mitochondrial homeostasis: Aside from triggering mitophagy, Parkin enhances mitochondrial renewal, by inhibiting PARIS, a PGC1α repressor.

Importantly, the BNIP3L/Nix homolog DCT-1 is among SKN-1 targets, and its expression is nonredundantly coregulated by the DAF-16/FOXO transcription factor [23]. Part of the retrograde response from mitochondria to the nucleus is mediated by the induction of UPR^{mt} upon mitochondrial proteotoxic stress. In this context, activation of the transcription factor associated with stress-1 (ATFS-1), which is normally located in the mitochondrial matrix, translocates to the nucleus, under mitochondrial stress conditions, in *C. elegans*. In turn, ATFS-1 transcriptionally activates genes encoding mitochondrial chaperones to restore homeostasis [82]. Whether UPR^{mt} and mitophagy function synergistically or in a mutually exclusive manner to alleviate mitochondrial damage is not fully understood. Unidentified factors could sense the inability of UPR^{mt} to fully restore mitochondrial homeostasis, and in turn lead to removal of proteotoxically damaged mitochondria through upregulation of mitophagy-related genes.

In addition to its well-known mitogenesis-promoting functions, PGC1α positively regulates general autophagy and mitophagy by directly inducing the expression of transcription factor EB (TFEB), which mediates lysosomal biogenesis [83]. In turn, TFEB upregulates expression of PGC1α, establishing a positive feedback loop that maintains the balance between mitochondrial biogenesis and degradation. Furthermore, it was recently shown that the general control of amino acid synthesis 5-like 1 (GCN5L1) functions to negatively regulate both mitochondrial biogenesis and degradation pathways, acting on both PGC1α

and TFEB, as shown in mouse embryonic fibroblasts [84].

AMP-activated protein kinase is an additional regulator of mitochondrial homeostasis, which in response to nutrient depletion, promotes mitophagy through inhibition of mTOR and activation of ULK1. By contrast, in rat hepatocytes, AMPK-mediated phosphorylation of SIRT1 results in the deacetylation and subsequent activation of PGC1α, likely as an adaptive response to compensate for enhanced mitochondrial turnover [85]. In mice, the MEK/ERK pathway and specifically the activity of p38 mitogen-activated protein kinase (p38 MAPK) have been correlated with increased mitochondrial mass through the positive regulation they exert on PGC1α [86]. Notably, the same pathway has also been implicated in the regulation of mitochondrial degradation. In this case, MAPK1 and MAPK14, instead of p38 MAPK, promote both starvation- and hypoxia-induced mitophagy in HeLa cells [87].

Intriguingly, studies in mice showed that in addition to its well-characterized role in carbonyl cyanide m-chlorophenyl hydrazone-induced mitochondrial clearance, Parkin is also involved in the induction of mitochondrial biogenesis, via ubiquitination and UPS-dependent elimination of the Parkin-interacting substrate (PARIS), a transcription factor that negatively regulates PGC1α and its target NRF1 [88]. Additionally, Parkin enhances transcription of mtDNA-encoded genes through direct association with TFAM in proliferating cells [89].

Concluding remarks

Perturbations in mitochondrial number and function severely impair cellular homeostasis and trigger the onset of disease. Thus, cells have developed multiple and well-conserved mechanisms that regulate mitochondrial biogenesis and degradation. Precise coordination of these processes promotes longevity and stress resistance, whereas disruption of their balance accelerates aging and eventually leads to cellular and organismal death. Indeed, the study and validation of possible pharmacological targets that would restore the coordination of both pathways in related pathologies has recently received particular attention.

While significant progress has been attained toward elucidating the specifics of mitochondrial content homeostasis, important questions still remain. How cells shape and adapt their mitochondrial network in response to various physiological or stress cues is not fully understood. The molecular origin of the mitochondrial network specialization in different cell types also remains obscure. Moreover, the interface between signaling pathways implicated in diverse pathologies, or known to influence longevity, and the mechanisms that modulate mitochondrial biogenesis and turnover is in need of further characterization. These are just some of the issues that require attention. Ongoing research efforts are poised to reveal new components and shed light on the mechanistic aspects of the complex regulatory network that manages cellular bioenergetics by fine-tuning mitochondrial content.

Acknowledgements

Work in the authors' laboratory is funded by grants from the European Research Council (ERC), the European Commission Framework Programmes, and the General Secretariat for Research and Technology of the Greek Ministry of Education.

Author contributions

CP and ID summarized the literature, created the initial figures, and wrote the paper. NT edited the paper, revised the figures, and contributed to the writing.

Conflict of interest

The authors declare no conflict of interests.

References

1 Zhu J, Wang KZ & Chu CT (2013) After the banquet: mitochondrial biogenesis, mitophagy, and cell survival. *Autophagy* **9**, 1663–1676.

2 Ventura-Clapier R, Garnier A & Veksler V (2008) Transcriptional control of mitochondrial biogenesis: the central role of PGC-1alpha. *Cardiovasc Res* **79**, 208–217.

3 Vafai SB & Mootha VK (2012) Mitochondrial disorders as windows into an ancient organelle. *Nature* **491**, 374–383.

4 McKenzie M, Lazarou M & Ryan MT (2009) Chapter 18 Analysis of respiratory chain complex assembly with radiolabeled nuclear- and mitochondrial-encoded subunits. *Methods Enzymol* **456**, 321–339.

5 Matsuda N, Sato S, Shiba K, Okatsu K, Saisho K, Gautier CA, Sou YS, Saiki S, Kawajiri S, Sato F *et al.* (2010) PINK1 stabilized by mitochondrial depolarization recruits Parkin to damaged mitochondria and activates latent Parkin for mitophagy. *J Cell Biol* **189**, 211–221.

6 Fraga H & Ventura S (2015) Influence of cytoplasmatic folding on mitochondrial import. *Curr Med Chem* **22**, 2349–2359.

7 Schulz C & Rehling P (2014) Remodelling of the active presequence translocase drives motor-dependent mitochondrial protein translocation. *Nat Commun* **5**, 4349.
8 Zong H, Ren JM, Young LH, Pypaert M, Mu J, Birnbaum MJ & Shulman GI (2002) AMP kinase is required for mitochondrial biogenesis in skeletal muscle in response to chronic energy deprivation. *Proc Natl Acad Sci USA* **99**, 15983–15987.
9 Cunningham JT, Rodgers JT, Arlow DH, Vazquez F, Mootha VK & Puigserver P (2007) mTOR controls mitochondrial oxidative function through a YY1-PGC-1alpha transcriptional complex. *Nature* **450**, 736–740.
10 Lagouge M, Argmann C, Gerhart-Hines Z, Meziane H, Lerin C, Daussin F, Messadeq N, Milne J, Lambert P, Elliott P *et al.* (2006) Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. *Cell* **127**, 1109–1122.
11 Wu H, Kanatous SB, Thurmond FA, Gallardo T, Isotani E, Bassel-Duby R & Williams RS (2002) Regulation of mitochondrial biogenesis in skeletal muscle by CaMK. *Science* **296**, 349–352.
12 Nisoli E, Clementi E, Paolucci C, Cozzi V, Tonello C, Sciorati C, Bracale R, Valerio A, Francolini M, Moncada S *et al.* (2003) Mitochondrial biogenesis in mammals: the role of endogenous nitric oxide. *Science* **299**, 896–899.
13 Yang ZF, Drumea K, Mott S, Wang J & Rosmarin AG (2014) GABP transcription factor (nuclear respiratory factor 2) is required for mitochondrial biogenesis. *Mol Cell Biol* **34**, 3194–3201.
14 Satoh J, Kawana N & Yamamoto Y (2013) Pathway analysis of ChIP-Seq-based NRF1 target genes suggests a logical hypothesis of their involvement in the pathogenesis of neurodegenerative diseases. *Gene Reg Syst Biol* **7**, 139–152.
15 Biswas M & Chan JY (2010) Role of Nrf1 in antioxidant response element-mediated gene expression and beyond. *Toxicol Appl Pharmacol* **244**, 16–20.
16 Domcke S, Bardet AF, Adrian Ginno P, Hartl D, Burger L & Schubeler D (2015) Competition between DNA methylation and transcription factors determines binding of NRF1. *Nature* **528**, 575–579.
17 Bruni F, Polosa PL, Gadaleta MN, Cantatore P & Roberti M (2010) Nuclear respiratory factor 2 induces the expression of many but not all human proteins acting in mitochondrial DNA transcription and replication. *J Biol Chem* **285**, 3939–3948.
18 Ryu D, Jo YS, Lo Sasso G, Stein S, Zhang H, Perino A, Lee JU, Zeviani M, Romand R, Hottiger MO *et al.* (2014) A SIRT7-dependent acetylation switch of GABPbeta1 controls mitochondrial function. *Cell Metab* **20**, 856–869.
19 Piantadosi CA, Carraway MS, Babiker A & Suliman HB (2008) Heme oxygenase-1 regulates cardiac mitochondrial biogenesis via Nrf2-mediated transcriptional control of nuclear respiratory factor-1. *Circ Res* **103**, 1232–1240.
20 Malhotra D, Portales-Casamar E, Singh A, Srivastava S, Arenillas D, Happel C, Shyr C, Wakabayashi N, Kensler TW, Wasserman WW *et al.* (2010) Global mapping of binding sites for Nrf2 identifies novel targets in cell survival response through ChIP-Seq profiling and network analysis. *Nucleic Acids Res* **38**, 5718–5734.
21 Jaramillo MC & Zhang DD (2013) The emerging role of the Nrf2-Keap1 signaling pathway in cancer. *Genes Dev* **27**, 2179–2191.
22 Taguchi K, Motohashi H & Yamamoto M (2011) Molecular mechanisms of the Keap1-Nrf2 pathway in stress response and cancer evolution. *Genes to Cells* **16**, 123–140.
23 Palikaras K, Lionaki E & Tavernarakis N (2015) Coordination of mitophagy and mitochondrial biogenesis during ageing in C. elegans. *Nature* **521**, 525–528.
24 Paek J, Lo JY, Narasimhan SD, Nguyen TN, Glover-Cutter K, Robida-Stubbs S, Suzuki T, Yamamoto M, Blackwell TK & Curran SP (2012) Mitochondrial SKN-1/Nrf mediates a conserved starvation response. *Cell Metab* **16**, 526–537.
25 Zhao R, Hou Y, Xue P, Woods CG, Fu J, Feng B, Guan D, Sun G, Chan JY, Waalkes MP *et al.* (2011) Long isoforms of NRF1 contribute to arsenic-induced antioxidant response in human keratinocytes. *Environ Health Perspect* **119**, 56–62.
26 Wang W & Chan JY (2006) Nrf1 is targeted to the endoplasmic reticulum membrane by an N-terminal transmembrane domain. Inhibition of nuclear translocation and transacting function. *J Biol Chem* **281**, 19676–19687.
27 Sha Z & Goldberg AL (2014) Proteasome-mediated processing of Nrf1 is essential for coordinate induction of all proteasome subunits and p97. *Current Biol* **24**, 1573–1583.
28 LeMoine CM, Genge CE & Moyes CD (2008) Role of the PGC-1 family in the metabolic adaptation of goldfish to diet and temperature. *J Exp Biol* **211**, 1448–1455.
29 Hasegawa K, Yasuda T, Shiraishi C, Fujiwara K, Przedborski S, Mochizuki H & Yoshikawa K (2016) Promotion of mitochondrial biogenesis by necdin protects neurons against mitochondrial insults. *Nat Commun* **7**, 10943.
30 Shao D, Liu Y, Liu X, Zhu L, Cui Y, Cui A, Qiao A, Kong X, Liu Y, Chen Q *et al.* (2010) PGC-1 beta-regulated mitochondrial biogenesis and function in myotubes is mediated by NRF-1 and ERR alpha. *Mitochondrion* **10**, 516–527.

31 Lustig Y, Ruas JL, Estall JL, Lo JC, Devarakonda S, Laznik D, Choi JH, Ono H, Olsen JV & Spiegelman BM (2011) Separation of the gluconeogenic and mitochondrial functions of PGC-1{alpha} through S6 kinase. *Genes Dev* **25**, 1232–1244.
32 Taherzadeh-Fard E, Saft C, Akkad DA, Wieczorek S, Haghikia A, Chan A, Epplen JT & Arning L (2011) PGC-1alpha downstream transcription factors NRF-1 and TFAM are genetic modifiers of Huntington disease. *Mol Neurodegener* **6**, 32.
33 Salma N, Song JS, Arany Z & Fisher DE (2015) Transcription factor Tfe3 directly regulates Pgc-1alpha in muscle. *J Cell Physiol* **230**, 2330–2336.
34 Chang CY & McDonnell DP (2012) Molecular pathways: the metabolic regulator estrogen-related receptor alpha as a therapeutic target in cancer. *Clin Cancer Res* **18**, 6089–6095.
35 Herzig S, Long F, Jhala US, Hedrick S, Quinn R, Bauer A, Rudolph D, Schutz G, Yoon C, Puigserver P *et al.* (2001) CREB regulates hepatic gluconeogenesis through the coactivator PGC-1. *Nature* **413**, 179–183.
36 Wenz LS, Ellenrieder L, Qiu J, Bohnert M, Zufall N, van der Laan M, Pfanner N, Wiedemann N & Becker T (2015) Sam37 is crucial for formation of the mitochondrial TOM-SAM supercomplex, thereby promoting beta-barrel biogenesis. *J Cell Biol* **210**, 1047–1054.
37 Eliyahu E, Pnueli L, Melamed D, Scherrer T, Gerber AP, Pines O, Rapaport D & Arava Y (2010) Tom20 mediates localization of mRNAs to mitochondria in a translation-dependent manner. *Mol Cell Biol* **30**, 284–294.
38 Kirstein-Miles J, Scior A, Deuerling E & Morimoto RI (2013) The nascent polypeptide-associated complex is a key regulator of proteostasis. *EMBO J* **32**, 1451–1468.
39 Lesnik C, Cohen Y, Atir-Lande A, Schuldiner M & Arava Y (2014) OM14 is a mitochondrial receptor for cytosolic ribosomes that supports co-translational import into mitochondria. *Nat Commun* **5**, 5711.
40 Kanki T, Ohgaki K, Gaspari M, Gustafsson CM, Fukuoh A, Sasaki N, Hamasaki N & Kang D (2004) Architectural role of mitochondrial transcription factor A in maintenance of human mitochondrial DNA. *Mol Cell Biol* **24**, 9823–9834.
41 Yakubovskaya E, Guja KE, Eng ET, Choi WS, Mejia E, Beglov D, Lukin M, Kozakov D & Garcia-Diaz M (2014) Organization of the human mitochondrial transcription initiation complex. *Nucleic Acids Res* **42**, 4100–4112.
42 Pfeffer S, Woellhaf MW, Herrmann JM & Forster F (2015) Organization of the mitochondrial translation machinery studied in situ by cryoelectron tomography. *Nat Commun* **6**, 6019.
43 Kuzmenko A, Derbikova K, Salvatori R, Tankov S, Atkinson GC, Tenson T, Ott M, Kamenski P & Hauryliuk V (2016) Aim-less translation: loss of *Saccharomyces cerevisiae* mitochondrial translation initiation factor mIF3/Aim23 leads to unbalanced protein synthesis. *Sci Rep* **6**, 18749.
44 Zhang X, Zuo X, Yang B, Li Z, Xue Y, Zhou Y, Huang J, Zhao X, Zhou J, Yan Y *et al.* (2014) MicroRNA directly enhances mitochondrial translation during muscle differentiation. *Cell* **158**, 607–619.
45 Das S, Bedja D, Campbell N, Dunkerly B, Chenna V, Maitra A & Steenbergen C (2014) miR-181c regulates the mitochondrial genome, bioenergetics, and propensity for heart failure in vivo. *PLoS One* **9**, e96820.
46 Li P, Jiao J, Gao G & Prabhakar BS (2012) Control of mitochondrial activity by miRNAs. *J Cell Biochem* **113**, 1104–1110.
47 Yamamoto H, Morino K, Nishio Y, Ugi S, Yoshizaki T, Kashiwagi A & Maegawa H (2012) MicroRNA-494 regulates mitochondrial biogenesis in skeletal muscle through mitochondrial transcription factor A and Forkhead box j3. *Am J Physiol Endocrinol Metab* **303**, E1419–E1427.
48 Mohamed JS, Hajira A, Pardo PS & Boriek AM (2014) MicroRNA-149 inhibits PARP-2 and promotes mitochondrial biogenesis via SIRT-1/PGC-1alpha network in skeletal muscle. *Diabetes* **63**, 1546–1559.
49 Shen L, Chen L, Zhang S, Du J, Bai L, Zhang Y, Jiang Y, Li X, Wang J & Zhu L (2016) MicroRNA-27b regulates mitochondria biogenesis in myocytes. *PLoS One* **11**, e0148532.
50 Xu Y, Zhao C, Sun X, Liu Z & Zhang J (2015) MicroRNA-761 regulates mitochondrial biogenesis in mouse skeletal muscle in response to exercise. *Biochem Biophys Res Commun* **467**, 103–108.
51 Tatsuta T & Langer T (2008) Quality control of mitochondria: protection against neurodegeneration and ageing. *EMBO J* **27**, 306–314.
52 Pellegrino MW, Nargund AM & Haynes CM (2013) Signaling the mitochondrial unfolded protein response. *Biochim Biophys Acta* **1833**, 410–416.
53 Sugiura A, McLelland GL, Fon EA & McBride HM (2014) A new pathway for mitochondrial quality control: mitochondrial-derived vesicles. *EMBO J* **33**, 2142–2156.
54 Boya P, Reggiori F & Codogno P (2013) Emerging regulation and functions of autophagy. *Nat Cell Biol* **15**, 713–720.
55 Mizushima N & Komatsu M (2011) Autophagy: renovation of cells and tissues. *Cell* **147**, 728–741.
56 Mizumura K, Choi AM & Ryter SW (2014) Emerging role of selective autophagy in human diseases. *Front Pharmacol* **5**, 244.
57 Greene AW, Grenier K, Aguileta MA, Muise S, Farazifard R, Haque ME, McBride HM, Park DS & Fon EA (2012) Mitochondrial processing peptidase

regulates PINK1 processing, import and Parkin recruitment. *EMBO Rep* **13**, 378–385.
58 Meissner C, Lorenz H, Hehn B & Lemberg MK (2015) Intramembrane protease PARL defines a negative regulator of PINK1- and PARK2/Parkin-dependent mitophagy. *Autophagy* **11**, 1484–1498.
59 Fedorowicz MA, de Vries-Schneider RL, Rub C, Becker D, Huang Y, Zhou C, Alessi Wolken DM, Voos W, Liu Y & Przedborski S (2014) Cytosolic cleaved PINK1 represses Parkin translocation to mitochondria and mitophagy. *EMBO Rep* **15**, 86–93.
60 Lazarou M, Jin SM, Kane LA & Youle RJ (2012) Role of PINK1 binding to the TOM complex and alternate intracellular membranes in recruitment and activation of the E3 ligase Parkin. *Dev Cell* **22**, 320–333.
61 Okatsu K, Oka T, Iguchi M, Imamura K, Kosako H, Tani N, Kimura M, Go E, Koyano F, Funayama M *et al.* (2012) PINK1 autophosphorylation upon membrane potential dissipation is essential for Parkin recruitment to damaged mitochondria. *Nat Commun* **3**, 1016.
62 Koyano F, Okatsu K, Kosako H, Tamura Y, Go E, Kimura M, Kimura Y, Tsuchiya H, Yoshihara H, Hirokawa T *et al.* (2014) Ubiquitin is phosphorylated by PINK1 to activate parkin. *Nature* **510**, 162–166.
63 Chen Y & Dorn GW 2nd (2013) PINK1-phosphorylated mitofusin 2 is a Parkin receptor for culling damaged mitochondria. *Science* **340**, 471–475.
64 Lazarou M, Sliter DA, Kane LA, Sarraf SA, Wang C, Burman JL, Sideris DP, Fogel AI & Youle RJ (2015) The ubiquitin kinase PINK1 recruits autophagy receptors to induce mitophagy. *Nature* **524**, 309–314.
65 Novak I, Kirkin V, McEwan DG, Zhang J, Wild P, Rozenknop A, Rogov V, Lohr F, Popovic D, Occhipinti A *et al.* (2010) Nix is a selective autophagy receptor for mitochondrial clearance. *EMBO Rep* **11**, 45–51.
66 Rikka S, Quinsay MN, Thomas RL, Kubli DA, Zhang X, Murphy AN & Gustafsson AB (2011) Bnip3 impairs mitochondrial bioenergetics and stimulates mitochondrial turnover. *Cell Death Differ* **18**, 721–731.
67 Hanna RA, Quinsay MN, Orogo AM, Giang K, Rikka S & Gustafsson AB (2012) Microtubule-associated protein 1 light chain 3 (LC3) interacts with Bnip3 protein to selectively remove endoplasmic reticulum and mitochondria via autophagy. *J Biol Chem* **287**, 19094–19104.
68 Saita S, Shirane M & Nakayama KI (2013) Selective escape of proteins from the mitochondria during mitophagy. *Nat Commun* **4**, 1410.
69 Zhu Y, Massen S, Terenzio M, Lang V, Chen-Lindner S, Eils R, Novak I, Dikic I, Hamacher-Brady A & Brady NR (2013) Modulation of serines 17 and 24 in the LC3-interacting region of Bnip3 determines pro-survival mitophagy versus apoptosis. *J Biol Chem* **288**, 1099–1113.
70 Gao F, Chen D, Si J, Hu Q, Qin Z, Fang M & Wang G (2015) The mitochondrial protein BNIP3L is the substrate of PARK2 and mediates mitophagy in PINK1/PARK2 pathway. *Hum Mol Genet* **24**, 2528–2538.
71 Murakawa T, Yamaguchi O, Hashimoto A, Hikoso S, Takeda T, Oka T, Yasui H, Ueda H, Akazawa Y, Nakayama H *et al.* (2015) Bcl-2-like protein 13 is a mammalian Atg32 homologue that mediates mitophagy and mitochondrial fragmentation. *Nat Commun* **6**, 7527.
72 Wu W, Tian W, Hu Z, Chen G, Huang L, Li W, Zhang X, Xue P, Zhou C, Liu L *et al.* (2014) ULK1 translocates to mitochondria and phosphorylates FUNDC1 to regulate mitophagy. *EMBO Rep* **15**, 566–575.
73 Liu L, Feng D, Chen G, Chen M, Zheng Q, Song P, Ma Q, Zhu C, Wang R, Qi W *et al.* (2012) Mitochondrial outer-membrane protein FUNDC1 mediates hypoxia-induced mitophagy in mammalian cells. *Nat Cell Biol* **14**, 177–185.
74 Chen M, Chen Z, Wang Y, Tan Z, Zhu C, Li Y, Han Z, Chen L, Gao R, Liu L *et al.* (2016) Mitophagy receptor FUNDC1 regulates mitochondrial dynamics and mitophagy. *Autophagy* **12**, 689–702.
75 Li XX, Tsoi B, Li YF, Kurihara H & He RR (2015) Cardiolipin and its different properties in mitophagy and apoptosis. *J Histochem Cytochem* **63**, 301–311.
76 Kagan VE, Jiang J, Huang Z, Tyurina YY, Desbourdes C, Cottet-Rousselle C, Dar HH, Verma M, Tyurin VA, Kapralov AA *et al.* (2016) NDPK-D (NM23-H4)-mediated externalization of cardiolipin enables elimination of depolarized mitochondria by mitophagy. *Cell Death Differ* **23**, 1140–1151.
77 Sentelle RD, Senkal CE, Jiang W, Ponnusamy S, Gencer S, Selvam SP, Ramshesh VK, Peterson YK, Lemasters JJ, Szulc ZM *et al.* (2012) Ceramide targets autophagosomes to mitochondria and induces lethal mitophagy. *Nat Chem Biol* **8**, 831–838.
78 Dany M & Ogretmen B (2015) Ceramide mediated lethal mitophagy: a novel cell death mechanism in FLT3 targeted therapy for acute myeloid leukemia. *FASEB J* **29** (147), 1.
79 Chowanadisai W, Bauerly KA, Tchaparian E, Wong A, Cortopassi GA & Rucker RB (2010) Pyrroloquinoline quinone stimulates mitochondrial biogenesis through cAMP response element-binding protein phosphorylation and increased PGC-1alpha expression. *J Biol Chem* **285**, 142–152.
80 De Rasmo D, Signorile A, Roca E & Papa S (2009) cAMP response element-binding protein (CREB) is imported into mitochondria and promotes protein synthesis. *FEBS J* **276**, 4325–4333.
81 Cherra SJ 3rd, Kulich SM, Uechi G, Balasubramani M, Mountzouris J, Day BW & Chu CT (2010) Regulation

of the autophagy protein LC3 by phosphorylation. *J Cell Biol* **190**, 533–539.
82 Nargund AM, Pellegrino MW, Fiorese CJ, Baker BM & Haynes CM (2012) Mitochondrial import efficiency of ATFS-1 regulates mitochondrial UPR activation. *Science* **337**, 587–590.
83 Settembre C, Di Malta C, Polito VA, Arencibia MG, Vetrini F, Erdin S, Erdin SU, Huynh T, Medina D, Colella P *et al.* (2011) TFEB links autophagy to lysosomal biogenesis. *Science* **332**, 1429–1433.
84 Scott I, Webster BR, Chan CK, Okonkwo JU, Han K & Sack MN (2014) GCN5-like protein 1 (GCN5L1) controls mitochondrial content through coordinated regulation of mitochondrial biogenesis and mitophagy. *J Biol Chem* **289**, 2864–2872.
85 Rodgers JT, Lerin C, Haas W, Gygi SP, Spiegelman BM & Puigserver P (2005) Nutrient control of glucose homeostasis through a complex of PGC-1alpha and SIRT1. *Nature* **434**, 113–118.
86 Akimoto T, Pohnert SC, Li P, Zhang M, Gumbs C, Rosenberg PB, Williams RS & Yan Z (2005) Exercise stimulates Pgc-1alpha transcription in skeletal muscle through activation of the p38 MAPK pathway. *J Biol Chem* **280**, 19587–19593.
87 Hirota Y, Yamashita S, Kurihara Y, Jin X, Aihara M, Saigusa T, Kang D & Kanki T (2015) Mitophagy is primarily due to alternative autophagy and requires the MAPK1 and MAPK14 signaling pathways. *Autophagy* **11**, 332–343.
88 Shin JH, Ko HS, Kang H, Lee Y, Lee YI, Pletinkova O, Troconso JC, Dawson VL & Dawson TM (2011) PARIS (ZNF746) repression of PGC-1alpha contributes to neurodegeneration in Parkinson's disease. *Cell* **144**, 689–702.
89 Kuroda Y, Mitsui T, Kunishige M, Shono M, Akaike M, Azuma H & Matsumoto T (2006) Parkin enhances mitochondrial biogenesis in proliferating cells. *Hum Mol Genet* **15**, 883–895.